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]

April 11, 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, suggesting 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 example, 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 between 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 spectrum 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 phenological 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 diffuse 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 relationships 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 functional 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
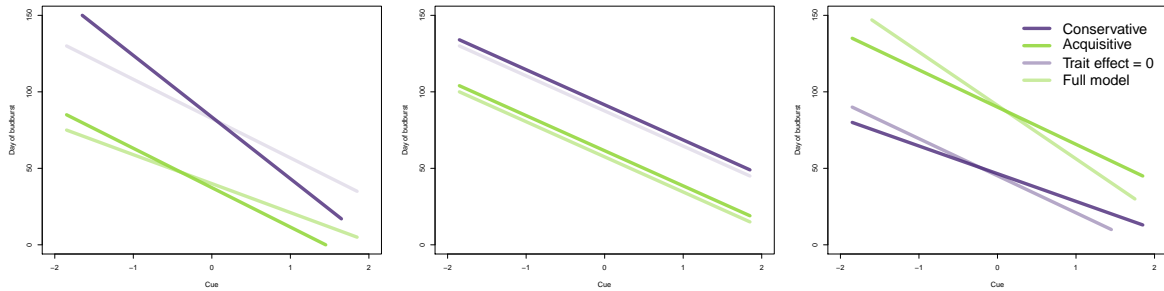145 SLA, low LNC, greater heights and heavier seeds.
146

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.

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.

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.

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

209 Joint model of trait and phenology

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

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

221 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:

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

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

225

226 The second part of the joint model is a hierarchical linear model where the normally distributed
227 response variable $Z_{i,k}$ is the day of budburst for species i experiencing forcing (F_k), chilling (C_k), and
228 photoperiod (P_k). This sub-model is linked to the trait sub-model via the shared parameters $\alpha_{\text{trait},i}$,
229 representing the "grand" trait values of species that are independent of study. The overall structure
230 of the phenology sub-model is similar to that of Ettinger et al. (https://doi.org/10.1038/s41558-020-
231 00917-3), except species' responses to forcing ($\beta_{\text{force},i}$), chilling ($\beta_{\text{chill},i}$), and photoperiod ($\beta_{\text{photo},i}$)
232 are treated not as single parameters but as a combination of parameters, a species-specific response
233 that is independent of its trait value (e.g., $\alpha_{\text{force},i}$) and an effect of its trait value (e.g., $\beta_{\text{trait.force}}$)
234 that is multiplied by $\alpha_{\text{trait},i}$ and does not differ between species. In other words, species responses to
235 cues interact with their "grand" trait values, and we assume this interaction is independent of species
236 identity. The phenology sub-model can thus be written as:

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

237 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 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 estimated 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. In using the normal distribution, our model may produce estimations of ecologically unrealistic zero or negative values. The use of the normal distribution in complex models such as ours, however, 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, and in doing so account for variation in trait data due to differences in observation frequency, datasources, as well as inherent differences in habitats, measurement protocols, and observer error. Of our four traits, we have the most data for species height. The breadth of variation in the raw height data reflects the high number of height measurements we have for most species. For example, *Acer saccharum* has 3000 observations from single study, producing a range of height values, with the model posterior estimate corresponding to the data mean. In contrast, our dataset only includes six observations of *Juglans regia* from four studies, producing a greater study level effect and is reflected by the difference between the posterior and mean estimates 2. Our seed mass data originates from only five studies, with most data coming from a single study, as such there is very little study level

280 variation. This is reflected in the high degree of overlap between the posterior estimates and the mean
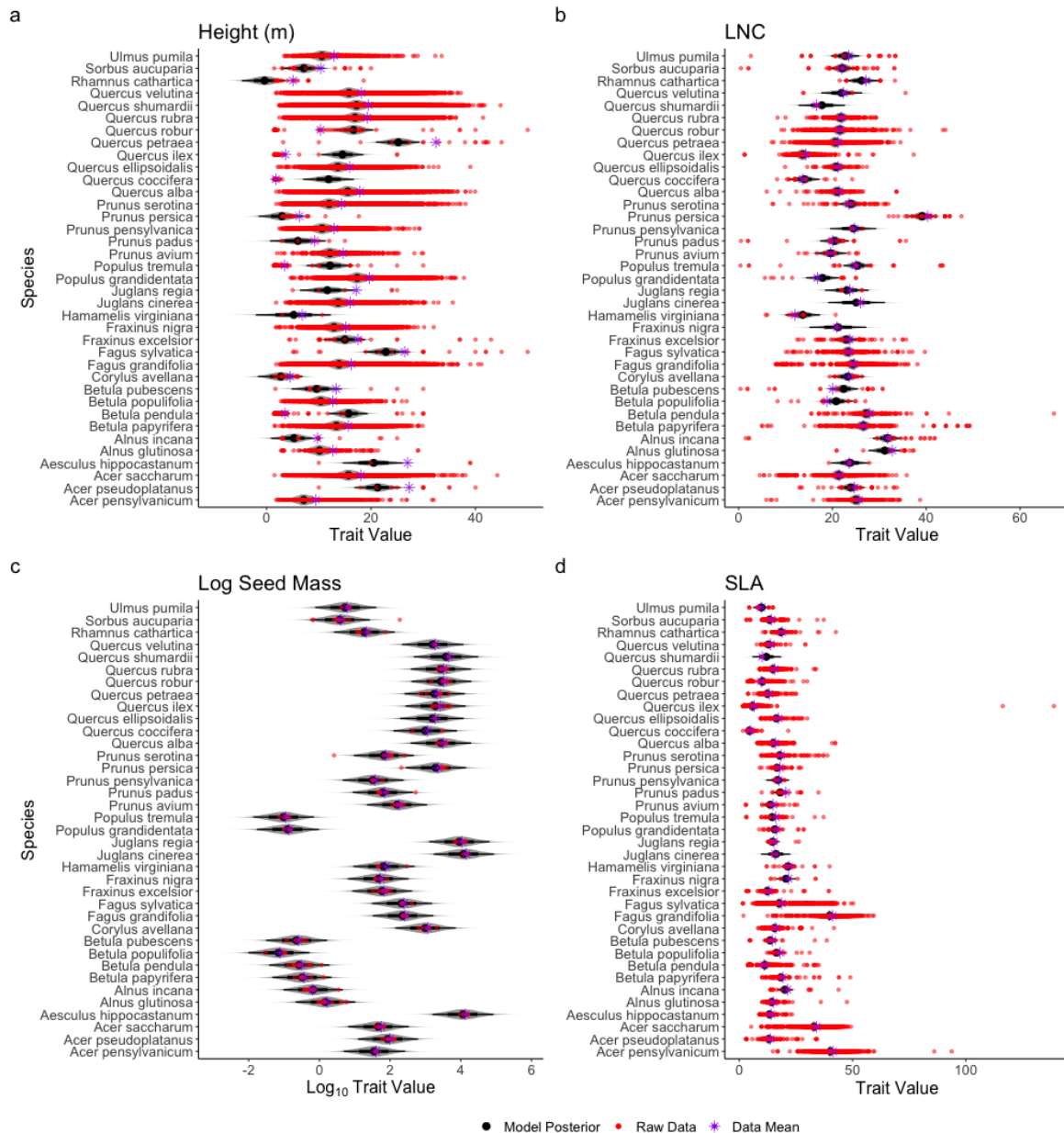281 seed mass for most species.

Figure 2: Comparisons of estimated model fits and raw data from joint models of a. height,
b. SLA, c. eed mass, and d. LNC trait effects on budburst phenological cues for 37 species of
woody deciduous plants. Trait were modeled individually, with the calculated trait value being
used to jointly model species responses to standardized chilling, forcing, and photoperiod cues.
Model posteriors are shown in black, with the thicker line depicting the 66% interval and the
thinner black line the 97% interval. Overall species level model posterior distributions were well
aligned with the raw data, shown in red, and the speceis level means from the raw data, denoted
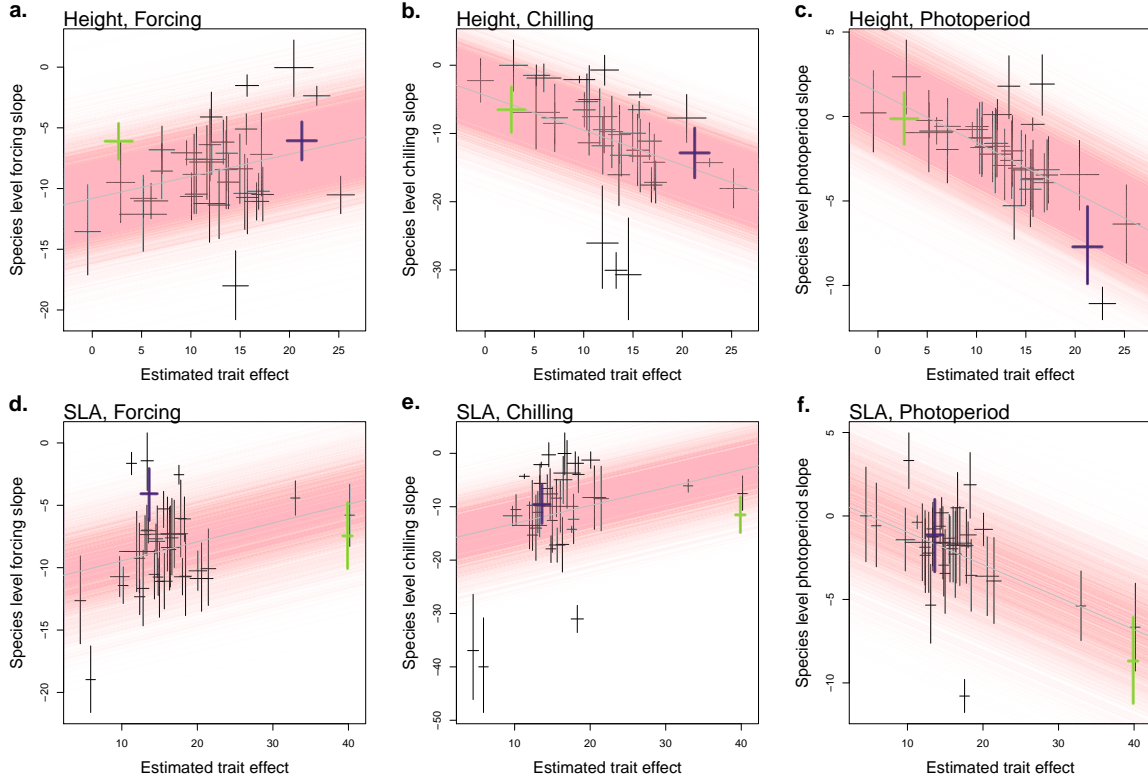as purple astricks.

Figure 3: Estimated trait values for height (a-c) and SLA (d-f) traits, correlated against species level cue responses. Parameters were estimated using our joint trait-phenology model, with the grey line depicting the mean linear relationship between these two estimates and the pink shading the distribution of the posterior estimates. Our model of tree heights estimated a positive correlation between height values and the response to forcing cues in panel a. The responses in both chilling and photoperiod cue responses (panel b and c), however were negative. The responses to cues our SLA model contrasted those of the height model, with the estimated SLA values positively corrleating with the response in forcing and chilling, but a negative correlation in the response of photoperiod cues. The species used in our illustrative examples in Fig 4 are highlighted in each panel, with the relative short species, *Corylus avellana* shown in green, and the taller species, *Acer pseudoplantanus* shown in purple in panels a to c. In panels d to f, the species with small SLA values, *Fagus grandifolia* is shown in green, and the species with large SLA values, *Quercus ilex* shown in purple.

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. 3). 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.4). 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. 3), with increasing 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.4). 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.4).

As we predicted, species log seed mass had a negative relationship with all three cue responses 3. 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 relatively 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 4. 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. S**??**). 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. S**??**).

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.3). 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 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.S**??**). 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.S**??**).

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 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 influenced plant responses to phenological cues. Species associated with acquisitive growth strategies, producing high SLA and LNC leaves, shorter heights, and small seeds, showed advances in their budburst dates in response to increasing chilling cues. 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 show 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 advance 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. Species that produced leaves with high LNC, small seeds, and were shorter, had lower
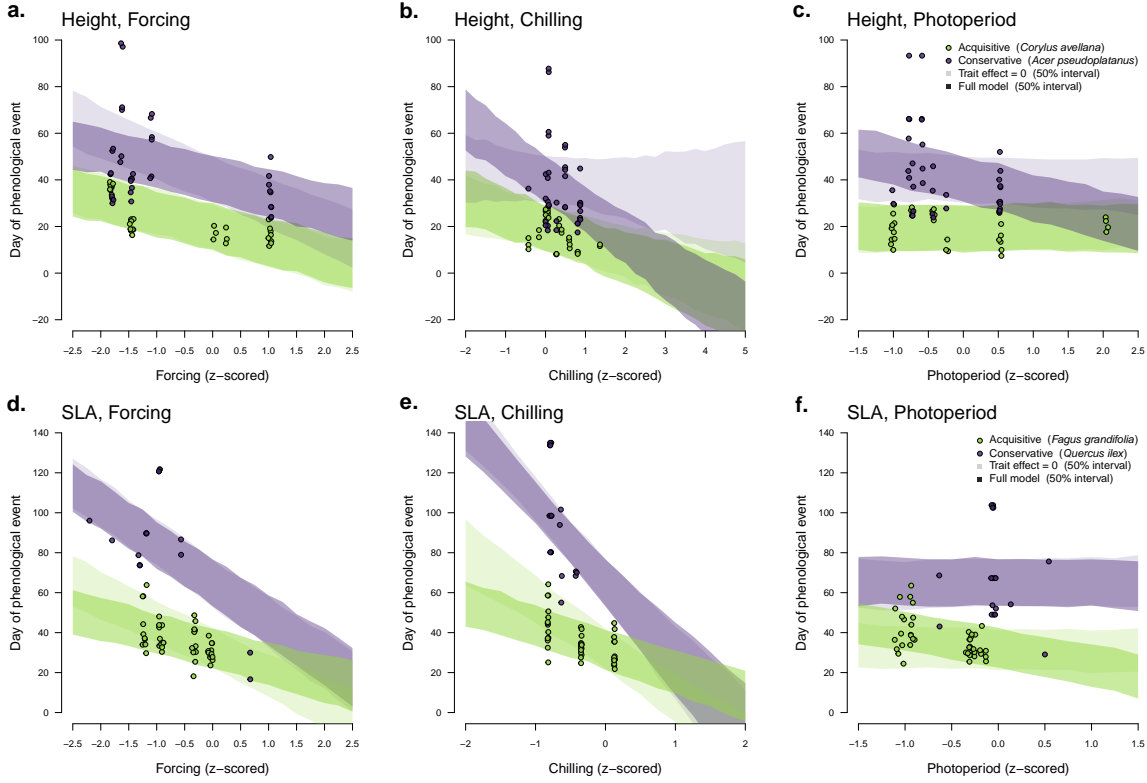
Figure 4: Estimated cue responses for acquisitive and conservative spp.

344 photoperiod cue responses and earlier budburst. These results suggest that phenology does at least
345 partially align with established gradients in traits variation, with most traits following shifts from
346 acquisitive and early budburst to more conservative, later budburst.

347 The deviation from our predicted trait-cue relationships we observed for the influence of height on
348 forcing cues and SLA on photoperiod cues may proivide interesting insight into the variable roles and
349 trade-offs that select plant phenotypes and cue use. While height is often associated with compeition
350 for light, spring temperature is also an important factor influencing other woody traits that may
351 confound resource investment into height. For example, spring temperature influences growth of the
352 cambial meristem, with spring frost events having the potential to severely affect the growth and
353 branch architecture of a tree (Clements et al., 1972; Dy and Payette, 2007; Augspurger, 2009). Early
354 season damage could pose a stronger selective pressure for later budburst on shorter species to ensure
355 cambial meristem development is not affected. Exploring additional traits that relate to height and
356 wood structure may offer a more mechanistic explanation of the observed response in forcing cue use.
357 Alternativley, our results could be the result of interactions between cue responses not accounted for
358 in our analysis. (??) for example, observed compensatory interactions between species budburst and
359 forcing and chilling cues, indicating that greater forcing cues can offsetting the effects of low chilling
360 on the timing of budburst. The positive relationship we observed between height and forcing might
361 reflect underlying complexity a model without interactions can not differentiate. Unfortunatley we were
362 unable to include such interactions in our currently model given its complexity, but further exploration
363 into assocations between height and spring temperatures may provide novel insights.

364 Of the three cues commonly included in growth chamber studies, we consistently observed the strongest
365 responses to increases in chilling, while the effects of photoperiod cues were weakest. This is in line
366 with previous growth chamber studies, which also found chilling cues to have the strongest effect on
367 budburst dates (citations?). The accumulation of chill units determines the transition of plants from

endodormancy, the phase in which woody plants are in a internally controlled state of inactivity during the coldest winter months, to ecodormancy, during which time growth inactivity is environmentally controlled and reflects limitations in resource availability (**?**). The high fitness costs of premature growth during false spring events supports our finding of stronger responses chilling cues as fitness costs to poor timing are so great. Responses in photoperiod cues, in contrast, contribute less to the timing of budburst or are more species specific (citation). We also found the effect of functional traits on photoperiod cue use to be relatively less than that of forcing and chilling. But we were surprised to find the relationship between LNC and photoperiod was similar to that of other functional traits. Leaf nitrogen content is generally thought of as a proxy for photosynthetic potential and therefore we expected it to to influence photoperiod cue use. The associations we observed between our functional traits, and their relative strength, further supports the importance of winter chilling, forcing, and photoperiod in defining both species phenologies and greater growth stragies.

In relating functional traits to phenological cues, our study is unique from the previous studies, however there are general similarities across species and phenological events. In their study of deciduous woody plants in Japan, (Osada, 2017) also observed correlations in budburst dates and leaf and wood traits, with a positive correlation between species leaf mass area (the inverse of SLA) and later budburst (Osada, 2017). In their study of *Quercus dentata*, (**?**), however, detected no correlations between the leaf traits studied and budburst. Research on other flowering events similarly did not find strong evidence for acqusisitive or conservative growth strategies to strongly relate to earlier or later events, but for there to be greater variation in how growth relates to phenology (**?**). To better understand the generality of our results, more direct studies of species cue responses and functional traits needed to further detect trade-offs in species growth and phenology.

A challenge of inferring the role of functional traits in relation to abiotic cues are a traits likelihood of having multiple roles in species interactions and in mediating environmental conditions. Such diversity in the role a trait plays could facilitate or limit the adaptive potential of species to future climates. Warming winter and spring temperatures are expected to result in species chilling requirements being met earlier (**?**), which would result in earlier release from endodormancy. As warmer springs allowing forcing temperature thresholds to also be met earlier, our results suggest species with acquisitive growth strategies will best be able to respond to lower cues to advance phenologically and budburst earlier in the spring. Species that are most able to initiate growth in the spring and tolerate late frost events, will be more likely to track these changes in temperature. This could favour species that produce less costly leaves, with high SLA, as loss of tissue to late season frosts would incurr a lower fitness cost. Similarly species that produce smaller seeds will have greater dispersal and be more likely to extend their range and migrate polward as new communities at their range edge become hospitable. Selection on the timing of budburst may also be constrained by species traits. Leaves with low LNC and less photosynthetic machinery for example, may cause as species to be limited by photoperiod length and their photosynthetic capacity. As daylengths remain constant, species with strong photoperiod responses will be constrained in their ability to advance with rising temperatures. These effects of traits on future changes in species phenologies may alter species temporal niche and ultimately change the compeitive landscape and species interations within deciduous forests.

In addition to allowing us to infer ways in which traits and phenology may alter native plant communities, our findings provide insights into the potential influence of invasive species as well. The same traits that allow native species to track changes in temperature will infer a greater fitness and adaptive advantage to invasive species as well. Invasive species are generally thought to be more sensitive to seasonal variation, with a greater ablity to shift their phenologies (**???**). Our results imply that invasive species with traits associated with high growth rates, such as SLA, greater LNC and photosynthetic potential, and less investment in wood anatomy and reproduction through seed mass, are most likely to benefit from less chilling and warmer forcing temperatues. Invasive species with this phenotype are most likely to benefit from early seasonal priority effects and the less competitve temporal niche at this time. Evidence of non-native woody species having higher rates of leaf production and differences in wood anatomy traits, like vessel diameter, have been observed in previous studies (**?**). Through these greater growth rates, invasive species will alter the compeitive dynamics within a forest, with

work in grassland communities finding the greatest impacts of earlier invasive specie phenologies on less compeitive species (**?**). In this system, for example, large seeded species were not impacted by the presence of the invader, while small seeded species were (**?**). The relative importance of phenology in determining resource availability and coexistence of native and non-native species within a community is likely limited by functional traits and the more complex processes they are proxies for.

Despite the merit of our analysis, there are several key assumptions and limitations in our data worth noteing. Our analysis is based on the assumption that stronger environmental cues will result in earlier budburst dates, as has been observed in several growth chamber studies Flynn and Wolkovich (2018); **?**. We therefore selected the priors of the phenological portion of our model to reflect this, centering these priors on negative values. We were also limited by the availability of diverse and sufficient trait data. In our attempt to find data for species trait measurements across the leaf and wood economic specturm, we were only able to find a relatively small number of species with at least one trait measurement across diverse traits. The subset of species included in this study therefore reflects the diversity of available trait data, but is a small subset of the diversity of woody plant species and most indicative of the responses of commonly measured. The trait and budburst data we used were also collected independently of one another, and therefore may reflect a greater amount of variation than would be expected if measurements were taken from the sample populations or individuals. By including study effects in our trait model, we were able to better account for these sources of variability. Furthermore, the relationships we observed are more likely to reflect general trends in trait relationships that scale across populations and species. Future studies should aim to include a greater diversity of species and functional traits. For example including traits related to wood structure and defense compounds could provide new insights into the influence of temperature on vessel development and the potential for biotic pressures from herbivory to also influence phenology. To further understand if the trends we observed occur at finer ecological scales, future studies should also aim to include trait and phenological data that are measured from within the same populations. The findings of our study provide exciting insights that can be built on and applied in future research into phenologies role as a functional trait. Areas of future study - will seed germination also shift with changing cues under climate change or will large seeded species seedlings get competitively excluded?

Advances in late budbursting speices, which are on average taller and stronger competitors for light, would lead to earlier canopy closure and negatively impact understory species that are not also advnacing phenologically.

Low sla - associated with aridity Low SLA = thicker denser leaves that conserve resources better - Ackerley et al 2002 - Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses.

Lenz, Hoch, Korner, 2012 Early season temperature controls cambial activity and total tree ring width at the alpine treeline

- How might traits constrain/facilitate future shifts in phenology?

- our findings do support the idea that phenology is an important functional trait

- traits and relationships to increased frsot risk, increased drought stress, increased herbivore pressure

- novel competitive communities

- 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

- What do our results suggest for the relationship between cue use and traits?

- – 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 and evergreen spp; Deciduous alone: bb positively correlated with leaf mass, area, vessel diam in cross spp comparisons Kitamura et al 2007 - found no relationship between budburst of their focal oak species and SLA, LNC, etc. Low sla - associated with aridity - Sun, S., D. Jin, and R. Li. 2006. LMA neg correl with leafout; larger LMA = earlier

- What do our results suggest for the bigger picture?

  - – How might traits constrain/facilitate future shifts in phenology? - our findings do support the idea that phenology is an important functional trait - traits and relationships to increased frsot risk, increased drought stress, increased herbivore pressure - novel competitive communities

  - – 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.

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.