Differences in leaf and wood traits predict phenological sensitivity to daylength more than temperature

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Research questions

1. Do phenological cue-trait relationships change across transects? Populations?
2. How do budburst cues relate to functional traits in temperate woody species?
3. How do shrub and tree species differ in their cue-trait relationships?

Materials and Methods

Field sampling

We combined *in situ* trait data with budburst data from two growth chamber cutting experiments conducted across eastern and western deciduous forests in North America. We collected both the trait and budburst data from populations that span latitudinal gradient of 4-6° for eastern and western communities respectively. Our trait measurements were taken across eight populations, of which there were four eastern populations — Harvard Forest, Massachusetts, USA (42.55°N, 72.20°W), White Mountains, , USA (44.11°N, XXX°W)), Second College Grant, , USA (44.79°N, XXX°W), and St. Hippolyte, Quebec, Canada (45.98°N, 74.01°W) and four western population—E.C. Manning Park (49.06°N, 120.78°W), Sun Peaks (50.88°N, 119.89°W), Alex Fraser Research Forest (52.14°N, 122.14°W), and Smithers, British Columbia (BC), Canada (54.78°N, 127.17°W). For the growth chamber studies, cuttings were collected from the most southern and northern populations in each transect (n_{pop}=4).

Across all eight populations, we measured a diverse assemblage of species from the understory and canopy layers. We selected the angiosperm species that were most abundant in our forest communities, with an aim to maximize the number of closely related species and congeners between our eastern and western communities. We focused on angiosperm species only, as these species are more likely to have similar environmental controls to their leafout phenology, to provide better traits comparisons that would be possibel if including gymnosperm species.

Functional traits

We measured all traits in the summer prior to each growth chamber study. For our eastern transect, traits were measured from May 28 to August 12, 2015, and from May 29 to July 30, 2019 for our western transect. At each population, we measured a total of six traits: height, diameter at breast height (DBH), stem specific density (SSD), type of ring porosity, leaf mass area (LMA), and the percent leaf nitrogen content (LNC). Each trait was measured for each species present at a populations, resulting in us measuring 1-10 healthy, adult, individuals per species at each population.

We measured traits in accordance to the methods discusseed by Pérez-Harguindeguy et al. (2013). We calculated tree height using trigonometric methods, using a TruePulse 200L rangefinder and measured DBH 1.42m from the ground. For shrub heights, we measured the distance from the ground to the height of the top foliage and measured stem diameters at approximately 1 cm above the ground level. To measure stem specific density, we collected a 10cm sample, taken close to the base of the stem. All samples were kept cool and we measured wood volume using the water displacement method within 12 hours of sample collection. We dried our stem samples upon returning from the field at 105°C for 24h before being immediately weighed. Stem specific density was calculated as the mass of the sample over its volume. Using the WSL xylem database, we recorded the type of ring porosity for 72.3% of our species.

For our two leaf traits, we haphazardly selected five, fully expanded, and hardened leaves. We kept leaves cool during sampling and for each leaf, we took high resolution scans using a Canon flatbed scanner (CanoScan Lide 220) within 12 hours of collection. We estimated leaf area using the ImageJ software (version X). Upon returning from the field, we dried the leaves for 48h at 70° and weighed each leaf using a precision balance. Leaf mass area was calculated as the ratio of the leaf mass over it's area.

Growth chamber study

For our growth chamber study, we collected branch cuttings from our highest and lowest latitude populations in each transect. With each study, we included two temperature treatments and daylength, for a total of eight distinct treatments. Treatments included two levels of chilling—with our eastern study having no additional chilling or 30 days at 4°C, and 30 days or 70 days of chilling at 4°C for our western study, under dark conditions. A forcing treatment included either a cool regime of 15:5°C or a warm regime of 20:10°C, and finally, photoperiods of either 8 or 12 hours. We recorded budburst stages of each sample every 1-3 days for up to four months. For a more detailed discussion of study sample collection and methods see Loughnan and Wolkovich (In prep).

Statistical Anlaysis

Our analysis combined the trait data with the budburst data from the growth chamber study. For each trait, we developed a joint Bayesian model, in which the relationship between traits and cues is used to estimate budburst. This statistical approach improves upon previous analyses of multiple traits, as it allows us to carry through uncertainty between trait and phenology data—combining observational trait data with phenology data from a controlled environment study.

Our joint model consists of two parts. The first is a hierarchical linear model, in which we have partitioned the variation of individual observations (i) of a given trait value ($y_{\text{trait}[i]}$) to account for the effects of species (*sp id*), population-level differences arising from transects (*transect id*), as well as the interaction between transects and latitude (*transect* $\times$ *latitude*), and finally, residual variation or 'measurement error' (σ_{trait}). We included transect as a categorical variable and latitude as a continuous variable. A model of this parameterization was ran individually for each of our field collected traits (height, DBH, SSD, LMA, LNC). These traits were modeled using their natural units, with the except of LMA, which was rescaled by 100. Comparisons across species ring-porosity were made using the posterior estimates of our height model, allowing us to best account for inherent differences in species wood anatomy and growth form.

$$\begin{aligned}
\mu_{trait} &= \alpha_{\text{grand trait}} + \alpha_{\text{sp[sp id]}} + \beta_{\text{transect}} \times transect + \beta_{\text{transect}\times\text{latitude}} \times (transect \times latitude) \\
\alpha_{Sp} &\sim normal(0, \sigma_{sp}) \\
y_{trait} &\sim normal(\mu_{trait}, \sigma_{trait})
\end{aligned} \tag{1}$$

The second part of our joint model models the relationship between traits and phenological cues, estimating their effects on budburst. In this portion of the model, we used partial pooling to uniquely estimate species-level variance ($\alpha_{\text{sp[sp id]}}$)—which controls for variation in the number of trait estimates per species and trait variability—which are then used as predictors of species-level estimates of each cue ($\beta_{\text{force[sp]}}$, $\beta_{\text{chill[sp]}}$, $\beta_{\text{photo[sp]}}$). In addition to the species-level estimates, our model also estimates the overall effect of each trait on each cue ($\beta_{\text{trait.chill}}$, $\beta_{\text{trait.force}}$, $\beta_{\text{trait.photo}}$). From this we can estimate how well trait effects explain species-level differences by estimating the the species-level cue variation not explained by traits ($\alpha_{\text{chill}[sp]}$, $\alpha_{\text{force}[sp]}$, $\alpha_{\text{photo}[sp]}$) and individual species responses to cues (C_i, F_i, P_i, respectively). Finally, our model estimates the residual variation across species ($\alpha_{\text{pheno[sp]}}$) and observations ($\sigma_{\text{pheno}}$). Chilling was included as chill portions, and both forcing and photoperiod as continuous variable, with all cues standardized by z-scoring each cue to allow comparisons (see Loughnan and Wolkovich (In prep) for more details).

$$\begin{aligned}
\mu_{pheno} &= \alpha_{\text{pheno[sp]}} + \beta_{\text{chill[sp]}} \times C_i + \beta_{\text{force[sp]}} \times F_i + \beta_{\text{photo[sp]}} \times P_i \\
y_{\text{pheno[i]}} &\sim normal(\mu_{pheno}, \sigma_{\text{pheno}})
\end{aligned} \tag{2}$$

trait-cue relationship:

$$\begin{aligned}
\beta_{\text{chill[sp]}} &= \alpha_{\text{chill}[sp]} + \beta_{\text{trait.chill}} \times \alpha_{\text{trait sp[sp]}} \\
\beta_{\text{force[sp]}} &= \alpha_{\text{force}[sp]} + \beta_{\text{trait.force}} \times \alpha_{\text{trait sp[sp]}} \\
\beta_{\text{photo[sp]}} &= \alpha_{\text{photo}[sp]} + \beta_{\text{trait.photo}} \times \alpha_{\text{trait sp[sp]}}
\end{aligned} \tag{3}$$

Model includes residual variation across species and variation in cues not attributed to the trait (partially pooled):

$$\begin{aligned}
\alpha_{\text{pheno}} &\sim normal(\mu_{\alpha_{\text{pheno}}}, \sigma_{\alpha_{\text{pheno}}}) \\
\alpha_{\text{force}} &\sim normal(\mu_{\alpha_{\text{force}}}, \sigma_{\alpha_{\text{force}}}) \\
\alpha_{\text{chill}} &\sim normal(\mu_{\alpha_{\text{chill}}}, \sigma_{\alpha_{\text{chill}}}) \\
\alpha_{\text{photo}} &\sim normal(\mu_{\alpha_{\text{photo}}}, \sigma_{\alpha_{\text{photo}}})
\end{aligned} \tag{4}$$

For each individually modeled trait, we used weakly informative priors unique to each trait. We validated each models priors using prior predictive checks. We coded our models in the Stan programming language and fit them using the rstan package. Each model had four chains ran for 6000-8000 total sampling iterations. All our models met basic diagnostic checks, including no divergences, high effective sample sizes (n_{eff}) that exceeded 10% of the number of iterations, and $\hat{R}$ values close to 1. Our model estimates are reported as the mean values, with the 90% uncertainty interval. Estimates are given to one decimal place, as this level of precision reflects the level we can infer from our dataset (Gelman et al., 2020).

Results

Across our eight populations, we measured 47 species of which 28 were in our eastern transect and 22 within our western transect. The species found in both transects include species dominant in both the understory and canopy layer, in our eastern communities we sampled 13 shrubs and 15 trees, while we sampled 18 shrubs and 4 trees in our western communities, and three species occurred in both transects. In total we measured traits of 1428 unique individuals between the two transects across our six traits: height (n = 1317), DBH (n = 1220), SSD (n = 1359), LMA (n = 1345), LNC (n = 1351, ring porosity (n= 34)). Across our two growth chamber studies, we made observations of 4211, with studies spanning 82 and 113 days for our eastern and western studies respectfully. Using our joint modeling approach, we were able to combine our trait data with budburst data from 4211 cuttings across the same 47 species we sampled the traits of in the field.

Most of our traits showed negligible trait variation across populations, both in terms of differences between the two transects (main effect of transect only) or by latitudes within each transect (an interactive effect between transect and latitude). Only leaf mass area (LMA) differed by latitude within transects (0.3, UI: 0.2,0.4)—with the LMA of eastern species increasing with latitude—but not western species (Fig. 1 d). Similarly, leaf nitrogen content (LNC) showed a weak interaction between latitude and transect (0.3, UI: 0.2,0.4)—with higher latitude populations in eastern transect having lower LNC than lower latitude populations and western species (Fig. 1e). Our wood and structural traits, however, showed no differences across populations or transects (Table 2 - 3). The differences we found across populations were small to negligible, especially in comparison to species-level differences, which varied considerably and up to XX fold (Fig. 2.

Only a subset of our traits related to at least one budburst cue, but responses were generally weak. Of our three cues, only photoperiod had relationships with other traits (Fig.). We found similar cue relationships for our two structural traits, finding taller plants with greater DBH to exhibit stronger photoperiod responses, producing earlier estimates of budburst under longer daylengths (-1.7, UI: -2.9, -0.5 for height and -2.3, UI: -3.4, -1.1 for DBH). We, however, found no relationship between cues and SSD (Table 3) or between cues and species different types of wood porosity 3.

Of our two leaf traits, only one showed a moderate relationship with cues. We found leaf mass area to relate to photoperiod, with low LMA species advancing their budburst timing with longer photoperiods (-7.5, UI: -10.9, -4.1). But we found no relationship between cues and LNC.

Which cues had the strongest effects on budburst varied across our trait models, with some models showing stronger responses to photoperiod than temperature (Fig. 4). For most of our trait models, chilling followed by forcing, were the strongest cues of budburst (Fig. 4). But in accounting for the relationship between LMA and cues, our model estimated stronger responses to photoperiod (-7.5, UI: -10.9, -4.1) that than forcing (-7.1, UI: -13.0, -1.6)—while effects of chilling were weak (90% UI crossing zero) (Table 4). In contrast, our LNC (Table 5) and SSD models (Table 3) both showed weak photoperiod effects on budburst (90% UI crossing zero). These finding suggest that additional traits alter the effects and relative importance of cues on budburst (Fig. 5). This—paired with strong species-level variation—may cause variation in the estimated responses of species relative budburst dates across the different trait models.

By synthesizing the effects of multiple traits across species, our results allow us to make generalizations across groups of species. Estimates of budburst using our model parameters show clear differences in species timing between trees and shrubs for some traits but not all (Fig. 6). We found height to have strong correlations between budburst timing and trait values, with earlier estimates of budbust for shrubs—especially under stronger cues—and later budburst estimates for trees (Fig. 6). Diameter at breast height showed similar trends, but this was not the case for our two leaf traits. Leaf nitrogen content, for example, showed no distinct separation between shrub and tree functional groups (Fig. 6).

References

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Figures

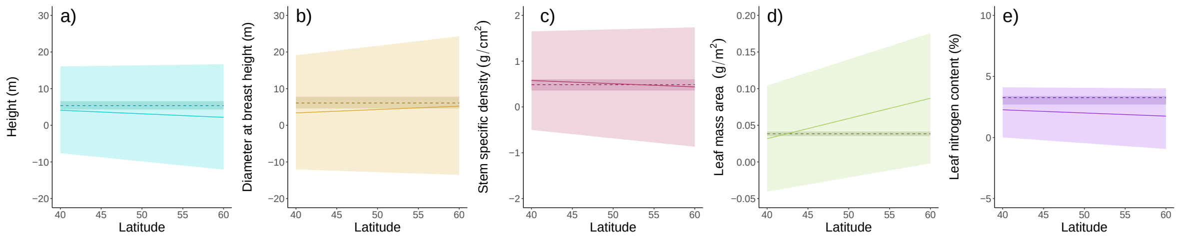

Figure 1: We found geographic differences for four of our functional traits, with the direction of the relationship varying across traits. Of our traits a. height, b. diameter at base height, c. leaf mass area, and e. leaf nitrogen content showed strong interactions between latitude and transect, while d. stem specific density showed no effects of geography. Dashed lines represent the western transect and solid lines the eastern transect.

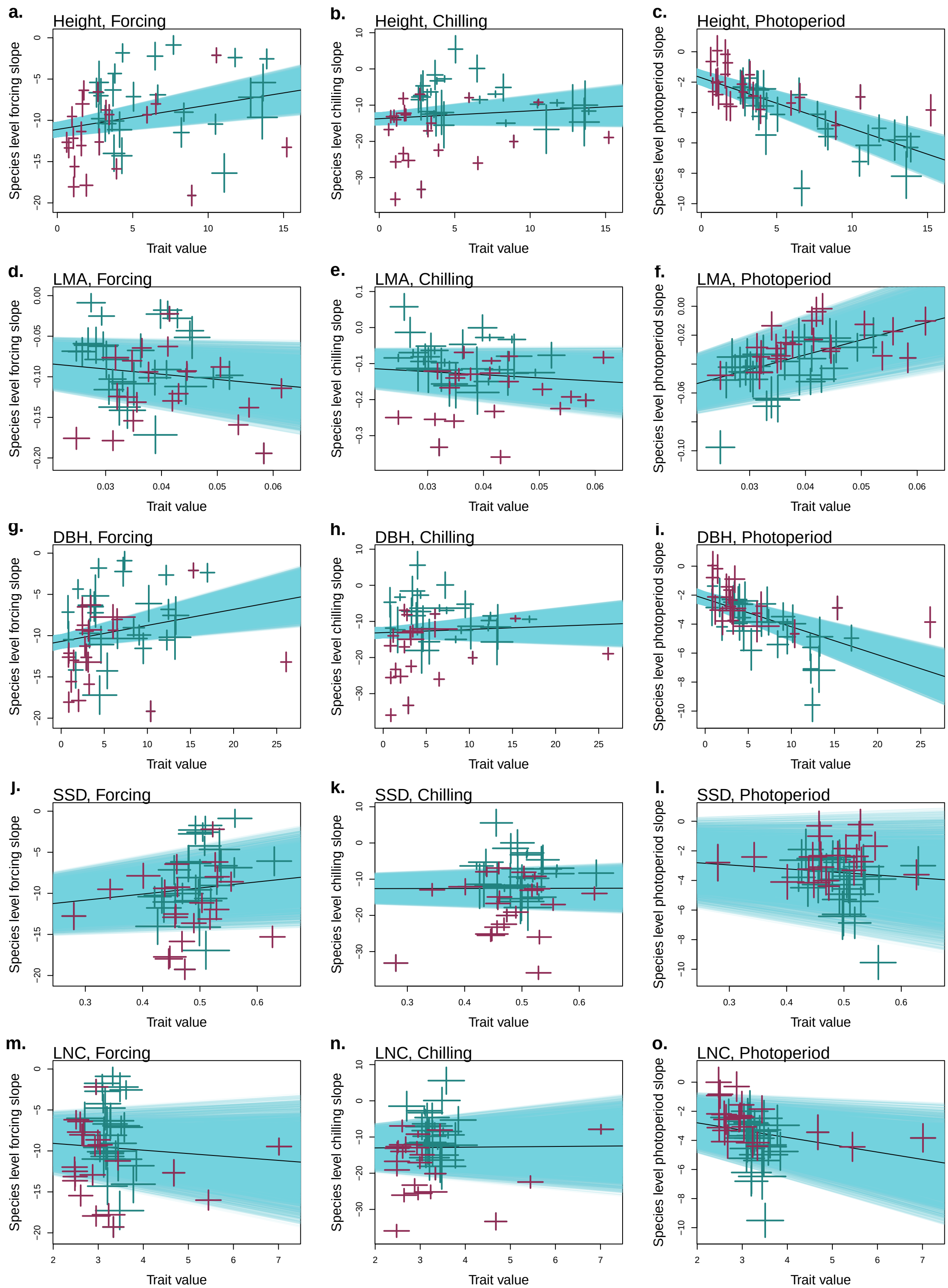

Figure 2: Relationship between species traits and cue responses, for height (a-c), leaf mass area (d-f), diameter at breast height (g-i), stem specific density (j-l), and the leaf nitrogen content (m-o). Point colours representing different species groups, with tree species are depicted in maroon and shrub species in teal.

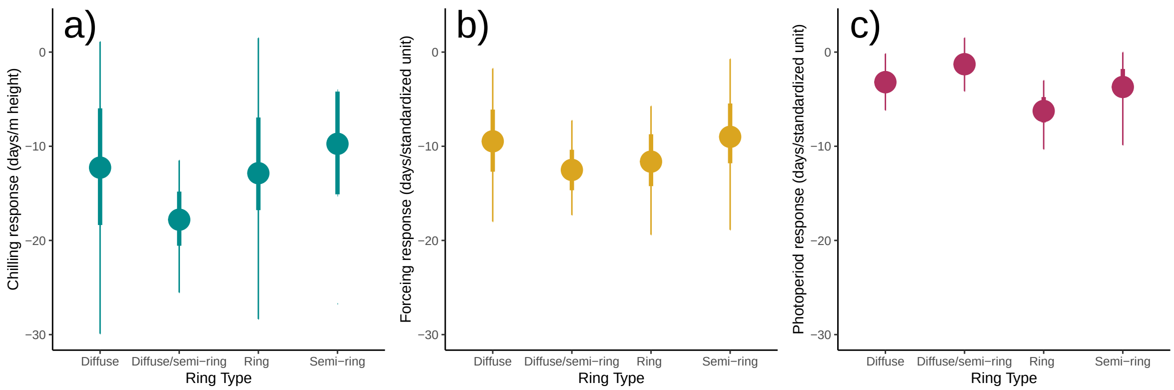

Figure 3: Despite species wood structures causing differing growth strategies, we did not find this trait to correlate with differences in cue responses across species. Thinner lines represent the 90% UI and thicker lines the 50% UI. Here we show the results for height only.

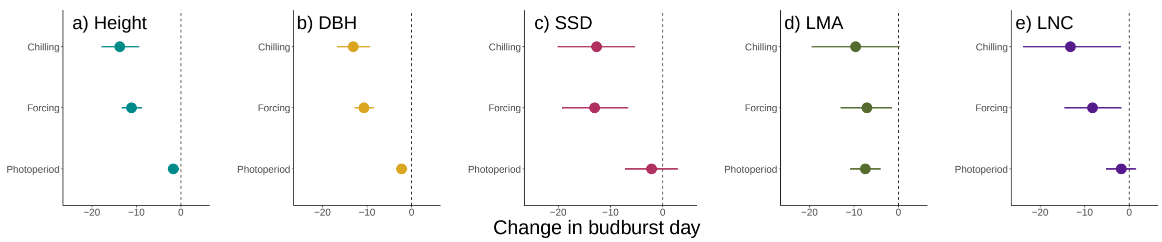

Figure 4: We found fairly consistent estimates for budburst cue responses to chilling, forcing, and photoperiod for each of our trait models: a. height, b. diameter at breast height, c. stem specific density, d. leaf mass area, and e. leaf nitrogen contnet. Lines represent 90% uncertainty intervals.

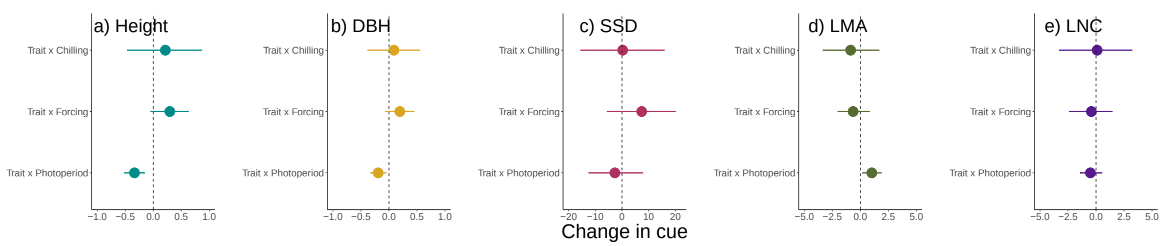

Figure 5: The relationships between traits and cue responses varied considerably across each of our trait models, a. height, b. diameter at breast height, c. stem specific density, d. leaf mass area, and e. leaf nitrogen content, and for individual cues. Lines represent 90% uncertainty intervals. Note the differences in the scale of the x-axis.

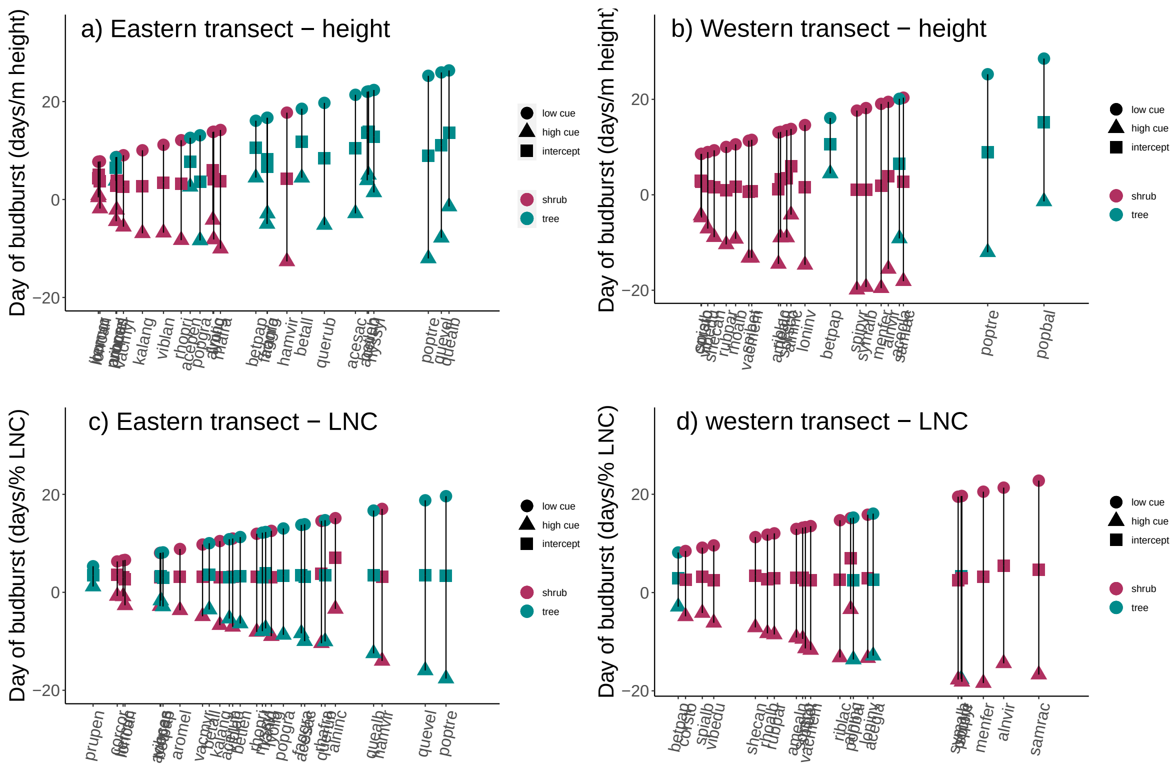

Figure 6: We found budburst estimates differed between our full model (intercept plus cues, depicted as triangles for high cues and as circles for low cues), versus the intercepts only model (without cues, shown as squares). Species are ordered in increasing budburst dates for both the eastern (a and c) and western (b and d) populations, spanning from early budbursting shrubs, in red, to late budbursting trees in blue. For traits such as height (a and b) we found distinct partitioning of budburst across shrub and tree species, but this was not the case for all traits, with our model of leaf nitrogen content showing highly mixed budburst order of shrub and tree species (c and d).

Tables

Table 1: Summary output from a joint Bayesian model of height and budburst phenology in which species are partially pooled. The effect of transect is modeled as a categorical variable and latitude as continuous in interaction term with transect. The model includes photoperiod and forcing as continuous variables, with all three cues z-scored to allow comparisons across cues.

	mean	5%	25%	75%	95%
Transect	2.60	-3.00	0.40	4.90	8.30
Transect x latitude	-0.10	-0.20	-0.10	-0.00	0.00
Forcing	-11.10	-13.30	-12.00	-10.20	-8.80
Chilling	-13.70	-17.80	-15.50	-12.10	-9.50
Photoperiod	-1.70	-2.90	-2.20	-1.20	-0.50
Trait x forcing	0.30	-0.10	0.20	0.40	0.60
Trait x chilling	0.20	-0.50	-0.00	0.50	0.90
Trait x photoperiod	-0.30	-0.50	-0.40	-0.30	-0.20

Table 2: Summary output from a joint Bayesian model of DBH and budburst phenology in which species are partially pooled. The effect of transect is modeled as a categorical variable and latitude as continuous in interaction term with transect. The model includes photoperiod and forcing as continuous variables, with all three cues z-scored to allow comparisons across cues.

	mean	5%	25%	75%	95%
Transect	-6.40	-13.70	-9.40	-3.40	1.00
Transect x latitude	0.10	-0.10	0.00	0.20	0.30
Forcing	-10.70	-12.80	-11.60	-9.90	-8.60
Chilling	-13.10	-16.70	-14.60	-11.60	-9.40
Photoperiod	-2.30	-3.40	-2.70	-1.80	-1.10
Trait x forcing	0.20	-0.10	0.10	0.30	0.40
Trait x chilling	0.10	-0.40	-0.10	0.30	0.50
Trait x photoperiod	-0.20	-0.30	-0.20	-0.10	-0.10

Table 3: Summary output from a joint Bayesian model of SSD and budburst phenology in which species are partially pooled. The effect of transect is modeled as a categorical variable and latitude as continuous in interaction term with transect. The model includes photoperiod and forcing as continuous variables, with all three cues z-scored to allow comparisons across cues.

	mean	5%	25%	75%	95%
Transect	0.40	-0.10	0.20	0.60	0.90
Transect x latitude	-0.00	-0.00	-0.00	-0.00	0.00
Forcing	-13.00	-19.30	-15.70	-10.50	-6.70
Chilling	-12.70	-20.10	-15.60	-9.70	-5.40
Photoperiod	-2.20	-7.20	-4.20	-0.00	2.80
Trait x forcing	7.40	-5.70	2.00	13.00	20.10
Trait x chilling	0.30	-15.60	-5.90	6.50	15.90
Trait x photoperiod	-2.70	-12.60	-7.00	1.50	7.80

Table 4: Summary output from a joint Bayesian model of LMA and budburst phenology in which species are partially pooled. The effect of transect is modeled as a categorical variable and latitude as continuous in interaction term with transect. The model includes photoperiod and forcing as continuous variables, with all three cues z-scored to allow comparisons across cues.

	mean	5%	25%	75%	95%
Transect	-11.70	-15.30	-13.20	-10.20	-8.00
Transect x latitude	0.30	0.20	0.20	0.30	0.40
Forcing	-7.10	-13.00	-9.30	-4.80	-1.60
Chilling	-9.60	-19.50	-13.50	-5.70	0.20
Photoperiod	-7.50	-10.90	-8.90	-6.00	-4.10
Trait x forcing	-0.60	-2.00	-1.20	-0.10	0.80
Trait x chilling	-0.90	-3.40	-1.90	0.10	1.70
Trait x photoperiod	1.00	0.20	0.70	1.40	1.90

Table 5: Summary output from a joint Bayesian model of LNC and budburst phenology in which species are partially pooled. The effect of transect is modeled as a categorical variable and latitude as continuous in interaction term with transect. The model includes photoperiod and forcing as continuous variables, with all three cues z-scored to allow comparisons across cues.

	mean	5%	25%	75%	95%
Transect	0.00	-0.80	-0.30	0.40	0.90
Transect x latitude	-0.00	-0.00	-0.00	-0.00	-0.00
Forcing	-8.20	-14.50	-10.80	-5.80	-1.80
Chilling	-13.20	-23.80	-17.70	-8.80	-2.00
Photoperiod	-1.80	-5.20	-3.10	-0.40	1.40
Trait x forcing	-0.40	-2.40	-1.20	0.40	1.40
Trait x chilling	0.10	-3.30	-1.20	1.50	3.20
Trait x photoperiod	-0.50	-1.40	-0.90	-0.10	0.50