Effects of grass–endophyte symbiosis and herbivory on population demography across climatic and geographic gradients

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Abstract

Interactions between plants and fungi are ubiquitous in nature and have significant effects on plant fitness. However, the extent to which variation in fungal symbiosis affects plant demography across environmental gradients remains understudied. Using three cool-season grass species that host seed-transmitted fungal endophytes, we combined geographically-distributed field experiments across an aridity gradient and Bayesian analysis of demographic performance to test whether plant-fungi interactions constrain host demography[1] and, consequently, geographic range limits. We found that all vital rates decreased toward the species' range edges, indicating reduced demographic performance in these regions. This decline was more pronounced in individuals hosting endophytic symbionts, suggesting that plant-fungi interactions that are beneficial under benign conditions may become parasitic under stressful conditions. Our results highlight that range expansion[2] may be hindered by the presence of a mutualist.

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[1] *Not really sure what you mean by this.*

[2] *Let's think about this a bit more. I am not sure range expansion is the right framing here, but it may be more relevant to think about resilience at range limits, since this is likely a trailing edge not a leading edge.*

Introduction

Plant-microbe symbioses are widespread and ecologically important. These interactions are famously context-dependent, where the direction and strength of the interaction outcome depends on the environment in which it occurs (Bronstein, 1994; Fowler et al., 2023; Hoeksema and Bruna, 2015). Plant-microbe interactions that are beneficial under stressful conditions may become neutral or parasitic under benign conditions (Giauque et al., 2019). Under biotic stress (e.g., herbivory), endophyte symbiosis can benefit host plants by facilitating the production of secondary compounds that deter feeding or exert direct toxicity, thereby reducing insect growth, survival, and oviposition (Atala et al., 2022; Bastias et al., 2017; Vega, 2008). Similarly under abiotic stress (e.g., drought), symbionts can increase their host tolerance[3] (Clay and Schardl, 2002). However, in many plant-microbe interactions, host protection is not guaranteed solely by the presence of a symbionts; rather, the density of the symbiont can determine the effectiveness of this protection (Laughton et al., 2014). Higher endophyte densities may lead to increased resource exploitation by the symbiont, potentially imposing costs on the host, such as reduced growth or reproduction (Faeth, 2009).[4]

Context dependence raises the hypothesis that plant-microbe interactions are likely to vary across environmental gradients, from range core to range edge, with significant implications for host range expansion at leading edges or resilience at trailing edges[5]. If microbial symbiosis provides greater benefits under environmental stress, then symbionts could enhance the suitability of range-edge environments[6], potentially extending host range limits (Allsup et al., 2023; Rudgers et al., 2020). For instance, fungal endophytes improve the survival of *Bromus laevipes* populations in dry conditions, increasing their drought resistance at the range edge and thereby extending the species' geographic range (Afkhami et al., 2014; David et al., 2019). Even if the symbiont does not directly improve host survival, it may still enhance host population growth over time by increasing relative growth and reproduction, potentially offsetting the negative effects of lower survival rates (Yule et al., 2013). Conversely, if microbial symbiosis is costly for the host at range edge, symbionts could constrain host range[7] (Bennett and Groten, 2022; Benning and Moeller, 2021a,b).

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[3]*Perhaps you do this below, but I think it is worth expanding this idea and explaining more about how symbionts 'increase host tolerance', since this concept is so central to our experiment.*

[4]*I like these sentences and the idea is really interesting and important – but if we are not using the endo density data in this paper then I would remove.*

[5]*This is the idea I was suggesting in the abstract. You can see how well it fits here.*

[6]*It is implied here that range edge environments are stressful, and I think it is worth making this more explicit.*

[7]*This alternative hypothesis is important to the study but the rationale for this is not very clear. The preceding paragraph emphasizes the idea that benefits are stronger under stress. That would be a place to develop the alternative hypothesis that benefits might be strongest under favorable conditions.*

Despite growing interest in the ecological and evolutionary roles of symbiosis, most studies focus on the host plant's perspective and rarely manipulate symbiont prevalence to evaluate how environmental variation shapes the symbiont's effects on host demography across geographic ranges. Yet, symbionts promote their own fitness by influencing host life history traits and enhancing resistance to stress (Giauque et al., 2019; Kazenel et al., 2015; Saikkonen et al., 1998). Theory predicts that exclusive vertical transmission fosters mutualism and leads to high symbiont prevalence in host populations (Fine, 1975). Field studies suggest that, under stressful conditions, beneficial symbionts may increase in prevalence and even reach fixation if their fitness advantages outweigh reproductive costs (Donald et al., 2021). However, populations with lower prevalence may experience higher symbiont loss among offspring (Afkhami and Rudgers, 2008). Thus, overlooking symbiont dynamics could result in inaccurate predictions of host population responses to global change.[8]

Additionally, few studies address the synergistic effects of abiotic and biotic stressors on host-symbiont dynamics across species' ranges. For example, herbivory not only affects the fitness benefits of symbiosis but can also increase endophyte prevalence through enhanced vertical transmission[9] (Agrawal et al., 1999; Gundel et al., 2020). However, these findings are primarily based on greenhouse studies, which limits our understanding of how herbivory shapes natural population dynamics, especially under variable field conditions. If the ecological context in which herbivory occurs alters plant fitness, then interactions between herbivory and abiotic stress (e.g., drought) may substantially influence population dynamics and species range limitation.[10]

Working across a precipitation gradient in the south-central US, we investigated how the demographic effects of endophyte symbiosis vary from core to edge of the host range. To answer that question, we studied the symbiotic association between three native cool-season grass species (*Agrostis hyemalis*, *Elymus virginicus* and *Poa autumnalis*) and their vertically transmitted fungal symbionts (*Epichloë* spp.). Our experiment was design to test the following hypotheses[11]:

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[8]*Again, I really like this paragraph, but we need to think about whether and how the symbiont density data will be used here. Is that what you are pointing towards here? Otherwise I am not sure why you are bringing up symbiont prevalence, since I did not think the prevalence data were going into this paper.*

[9]*It is not clear how this illustrates synergy of abiotic and biotic stressors.*

[10]*I think the herbivory dimension of the study should be better developed. This paragraph is pretty light. I think fundamentally what we are asking is: which stressor is more important for eliciting benefits of endophyte symbiosis? And to what extent do these stressors interact?*

[11]*Technically, these are predictions, not hypotheses. As a narrative strategy, I generally prefer stating these as questions rather than either hypotheses or predictions. I find it a little boring if we know what to expect, but it is more exciting if we have questions and we are trying to find the answers.*

1. Because endophytes often produce defensive compounds (e.g., alkaloids), plants with endophytes will experience less herbivore damage and maintain higher vital rate compared to plants without endophytes when grazed[12].

2. Because endophytes can improve drought tolerance[13] (e.g., by enhancing water use efficiency or root growth), plants with endophytes will outperform plants without endophytes more in dry conditions than in wet conditions.

3. The combined stresses of herbivory and low precipitation will create conditions where the mutualistic benefits of endophytes are most pronounced, giving plants with endophytes a strong performance advantage over plants without endophytes.

4. The combined stresses of herbivory and low precipitation will amplify the physiological demands on the host plant, such that the additional resource costs of maintaining endophyte associations will reduce overall plant performance resulting in endophyte-infected plants performing worse than uninfected plants under extreme stress conditions[14].

Materials and methods

Study system

Our study was conducted across seven sites along a geographic and climatic gradient spanning Louisiana and Texas in the United States (Fig. 1; Fig.S-1). At each site, we established eight plots (1.5 m × 1.5 m), with each plot containing 18 individuals of a single species. We used three cool-season perennial grasses native to woodland and prairie habitats of eastern North America (Shaw, 2011): *Agrostis hyemalis*, *Elymus virginicus*, and *Poa autumnalis* (subfamily Pooideae). These species are capable of both self-pollination and outcrossing (Church, 1958). They typically host seed-transmitted fungal endophytes. In summer–fall 2021, seeds were collected from naturally symbiotic populations (source populations) of the three focal host species (Fig. 1). These seeds were either heat-treated to eliminate symbionts or left untreated to generate symbiont-free (E^-) and symbiotic (E^+) plants from the same genetic lineages as the source populations.

Across all sites, each plot was randomly assigned one of four initial endophyte frequency treatments (80%, 60%, 40%, or 20%) and one of two herbivory treatments (herbivore exclusion

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[12]*We do not have much direct information about 'grazing'. I would keep the focus on herbivore access or exclusion, since that is what we directly measured.*

[13]*I would lead with the abiotic stressors, since that is more central to the experimental design.*

[14]*I am confused here because this prediction seems to contradict the preceding prediction. But either way, I still think it would be more compelling to phrase these as questions.*

or herbivore access). We ensured that all plots received comparable quantities of source plant material. This experimental design was informed by natural patterns of endophyte prevalence observed in Texas, where the proportion of symbiotic individuals has been reported as 86.55% in *A. hyemalis* (ranging from 77.16% to 93.5%) (Donald et al., 2021), 53% in *E. virginicus* (ranging from 10% to 100%) (Sneck et al., 2017), and 96% in *P. autumnalis* (Rudgers et al., 2009). Additionally, fungal genotyping has confirmed the presence of biosynthetic pathways for secondary metabolites such as peramine, loline, and ergot alkaloids, which may enhance host resistance to drought and herbivory (Beaudry, 1951)[15]. Full details of the study design are provided in the supplementary materials (Supplementary Method S.1).

Demographic data collection

We collected demographic data including survival, growth, and reproduction in May and June of 2023, 2024 and 2025, which coincided with the flowering season of *Agrostis hyemalis*, *Elymus virginicus*, and *Poa autumnalis*. For each individual, plant survival was recorded as a binary variable (alive or dead), and plant size was measured as the number of living tillers. We recorded the number of inflorescences per plant and counted the number of spikelets on up to three inflorescences from three reproducing plants. Spikelet counts were limited to three reproducing tillers per plot due to the time-consuming nature of this measurement. We used the number of spikelets from these three tillers to estimate the average number of spikelets per plant.

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[15]*I am trying to justify why we choose the starting endophyte prevalence*
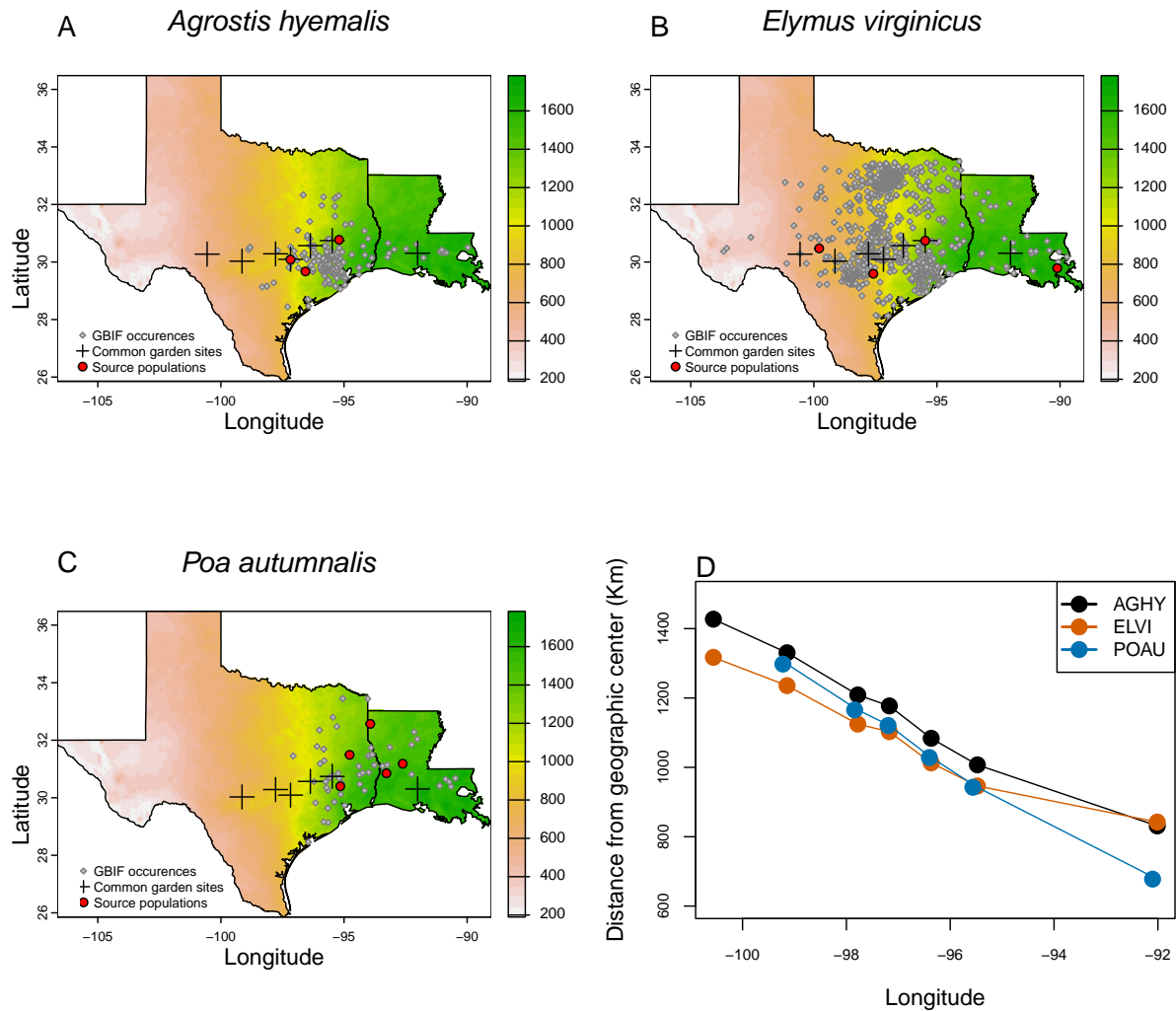
Figure 1: Distribution of common garden sites along the longitudinal aridity gradient in the central and southern Great Plains for: A) *Agrostis hyemalis* (AGHY), B) *Elymus virginicus* (ELVI), and C) *Poa autumnalis* (POAU). Red dots indicate the locations of source populations, while grey dots represent the Global Biodiversity Information Facility (GBIF) occurrence records for each species within the study area. D) Relationship between the distance from the range center and longitude for each species.

Models building and models selection

To assess how stress associated with aridity and herbivory affects plant–fungal interactions, we developed four candidate models for each vital rate (survival, growth, and fertility). Each vital rate was modeled using a grand mean intercept (β_0), slopes for variation in each covariate (β_1, β_2 and β_3), and interaction terms between covariates (β_4, β_5 and β_6). Each model included normally distributed random effects to account for site-to-site variation ($\phi \sim N(0, \sigma_{site})$),

plot-to-plot variation ($\rho \sim N(0, \sigma_{plot})$), and source-to-source variation associated with the provenance of transplants used in the common garden ($\omega \sim N(0, \sigma_{source})$) (Eq. 1).

$$
\begin{aligned}
\text{Model 1:} \quad \mu = {} & \beta_0^{\text{sp}} + \beta_{\text{P}}^{\text{sp}}P + \beta_{\text{endo}}^{\text{sp}}\text{endo} + \beta_{\text{herb}}^{\text{sp}}\text{herb} \\
& + \beta_{\text{endo}\times\text{P}}^{\text{sp}}\text{endo}\cdot P + \beta_{\text{herb}\times\text{endo}}^{\text{sp}}\text{herb}\cdot\text{endo} + \beta_{\text{herb}\times\text{P}}^{\text{sp}}\text{herb}\cdot P \\
& + \beta_{\text{P}^2}^{\text{sp}}P^2 + \beta_{\text{endo}\times\text{P}^2}^{\text{sp}}\text{endo}\cdot P^2 + \phi^{\text{sp}} + \omega^{\text{sp}} + \rho^{\text{sp}} \\
\text{Model 2:} \quad \mu = {} & \beta_0^{\text{sp}} + \beta_{\text{GD}}^{\text{sp}}\text{GD} + \beta_{\text{endo}}^{\text{sp}}\text{endo} + \beta_{\text{herb}}^{\text{sp}}\text{herb} \\
& + \beta_{\text{endo}\times\text{GD}}^{\text{sp}}\text{endo}\cdot\text{GD} + \beta_{\text{herb}\times\text{endo}}^{\text{sp}}\text{herb}\cdot\text{endo} + \beta_{\text{herb}\times\text{GD}}^{\text{sp}}\text{herb}\cdot\text{GD} \\
& + \phi^{\text{sp}} + \omega^{\text{sp}} + \rho^{\text{sp}} \\
\text{Model 3:} \quad \mu = {} & \beta_0^{\text{sp}} + \beta_{\text{MD}}^{\text{sp}}\text{MD} + \beta_{\text{endo}}^{\text{sp}}\text{endo} + \beta_{\text{herb}}^{\text{sp}}\text{herb} \\
& + \beta_{\text{endo}\times\text{MD}}^{\text{sp}}\text{endo}\cdot\text{MD} + \beta_{\text{herb}\times\text{endo}}^{\text{sp}}\text{herb}\cdot\text{endo} + \beta_{\text{herb}\times\text{MD}}^{\text{sp}}\text{herb}\cdot\text{MD} \\
& + \phi^{\text{sp}} + \omega^{\text{sp}} + \rho^{\text{sp}}
\end{aligned}
\tag{1}
$$

where $sp \in \{1,2,3\}$ represents the species index, P denotes Precipitation, GD refers to distance from geographic center, and MD stands for mahalanobis distance, endo indicates Endophyte status (absence or present), and herb represents Herbivory (fenced and unfenced). The terms $\phi^{(sp)}$, $\omega^{(sp)}$, and $\rho^{(sp)}$ are plot-level, population-level, and site-level random effects, respectively, for species sp.

Survival was modeled using a Bernoulli distribution, growth with a Gaussian distribution, flowering with a negative binomial distribution, and fertility (number of spikelets) also with a negative binomial distribution. To assess the goodness-of-fit of the models, we performed posterior predictive checks (Berkhof et al., 2000; Gelman et al., 2000). All models effectively captured key aspects of the data, including means, standard deviations, and quantiles (Fig. **??**, Fig. **??**, Fig. **??**, Fig. **??**). To determine the best model for each vital rate, we compared the four models using leave-one-out cross-validation (LOOCV) (Vehtari et al., 2017). LOOCV combines validation and training by using one observation for testing and the remaining $n-1$ observations for training. This process is repeated n times once for each observation resulting in n fitted models (Silva and Zanella, 2024). The test error estimate from LOOCV is obtained by averaging the prediction errors across all n models (Eq. 2).

$$
CV_n = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2
\tag{2}
$$

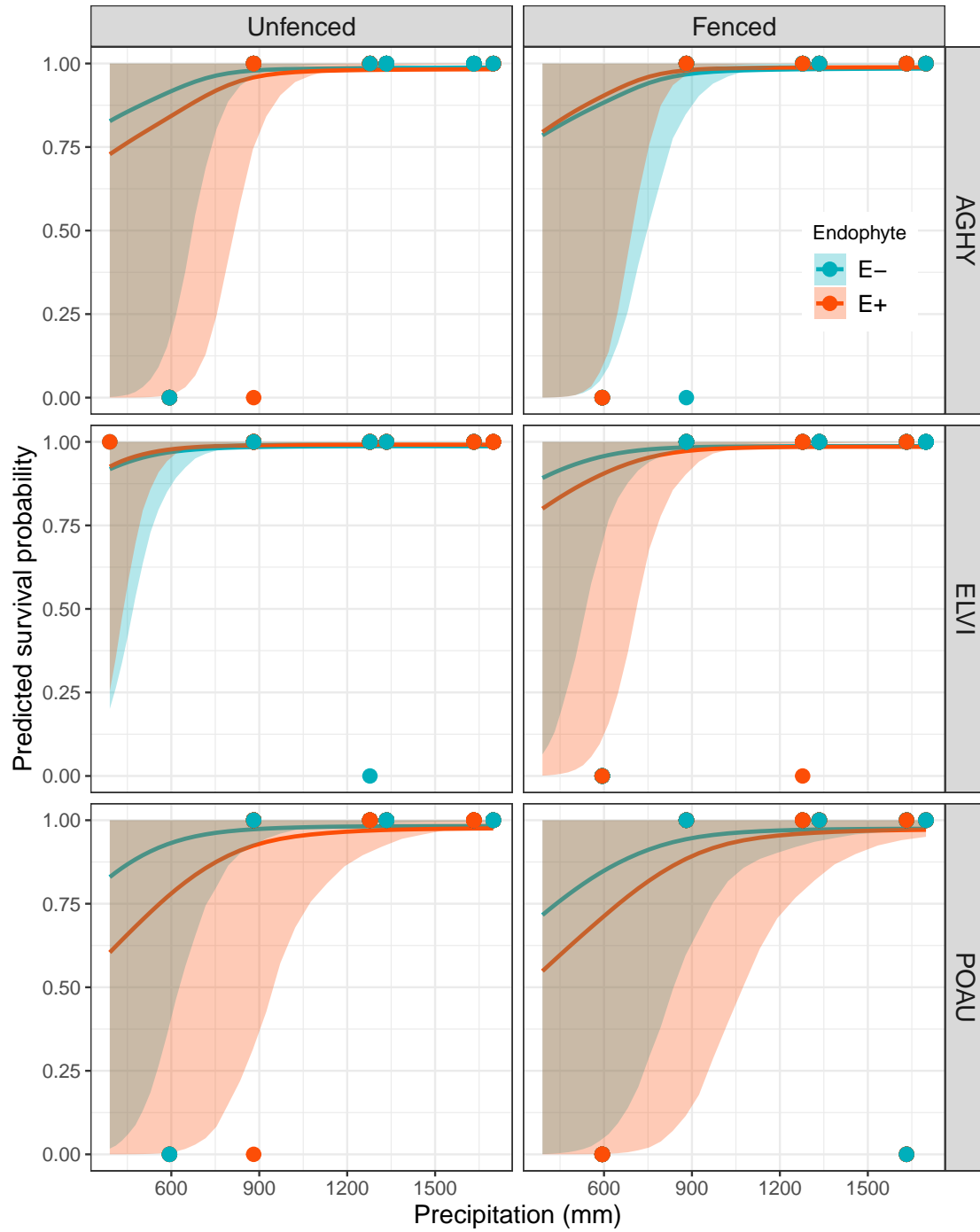All models were implemented in R (R Core Team, 2023) using Stan via the RStan interface (Stan Development Team, 2024).

8

Results

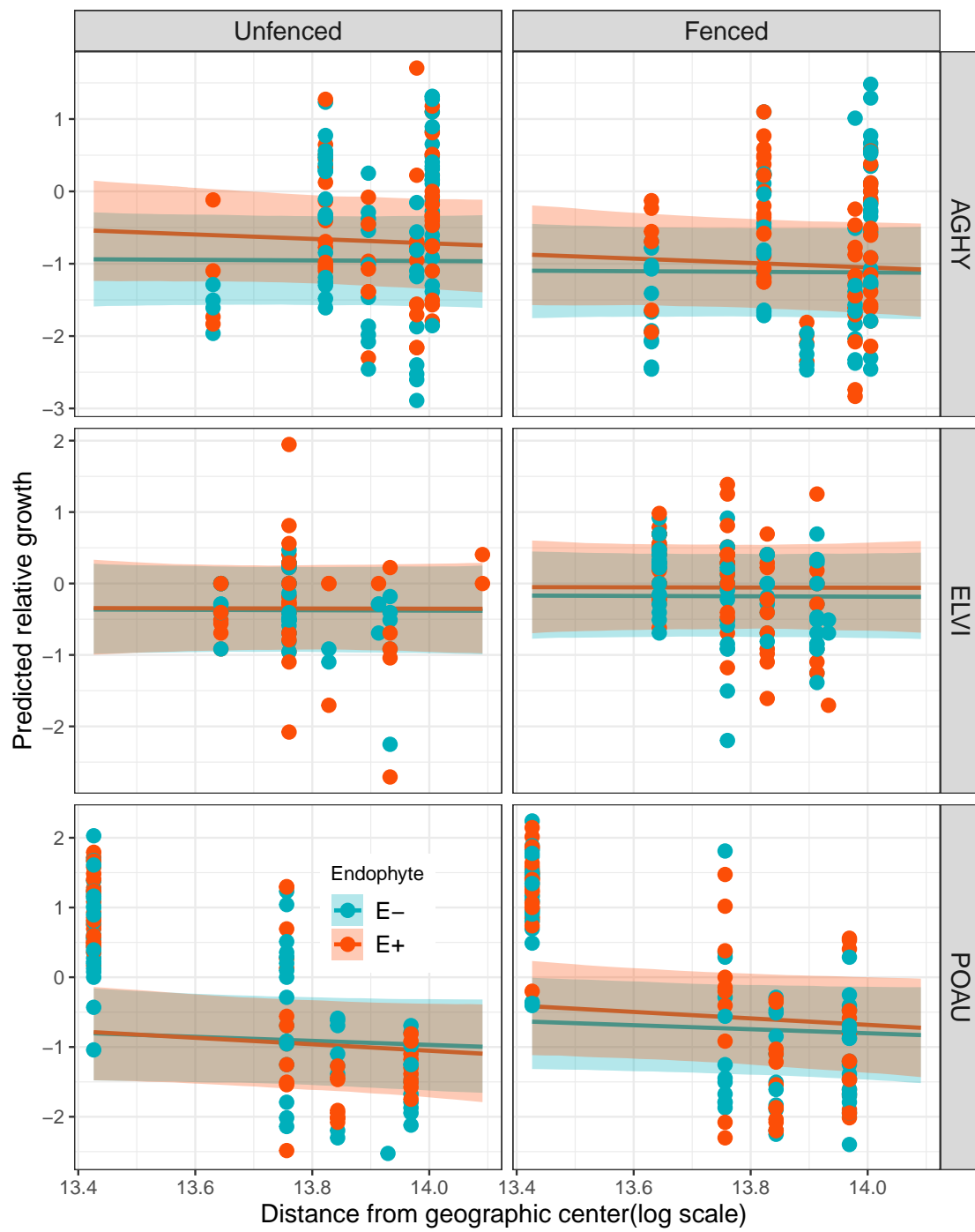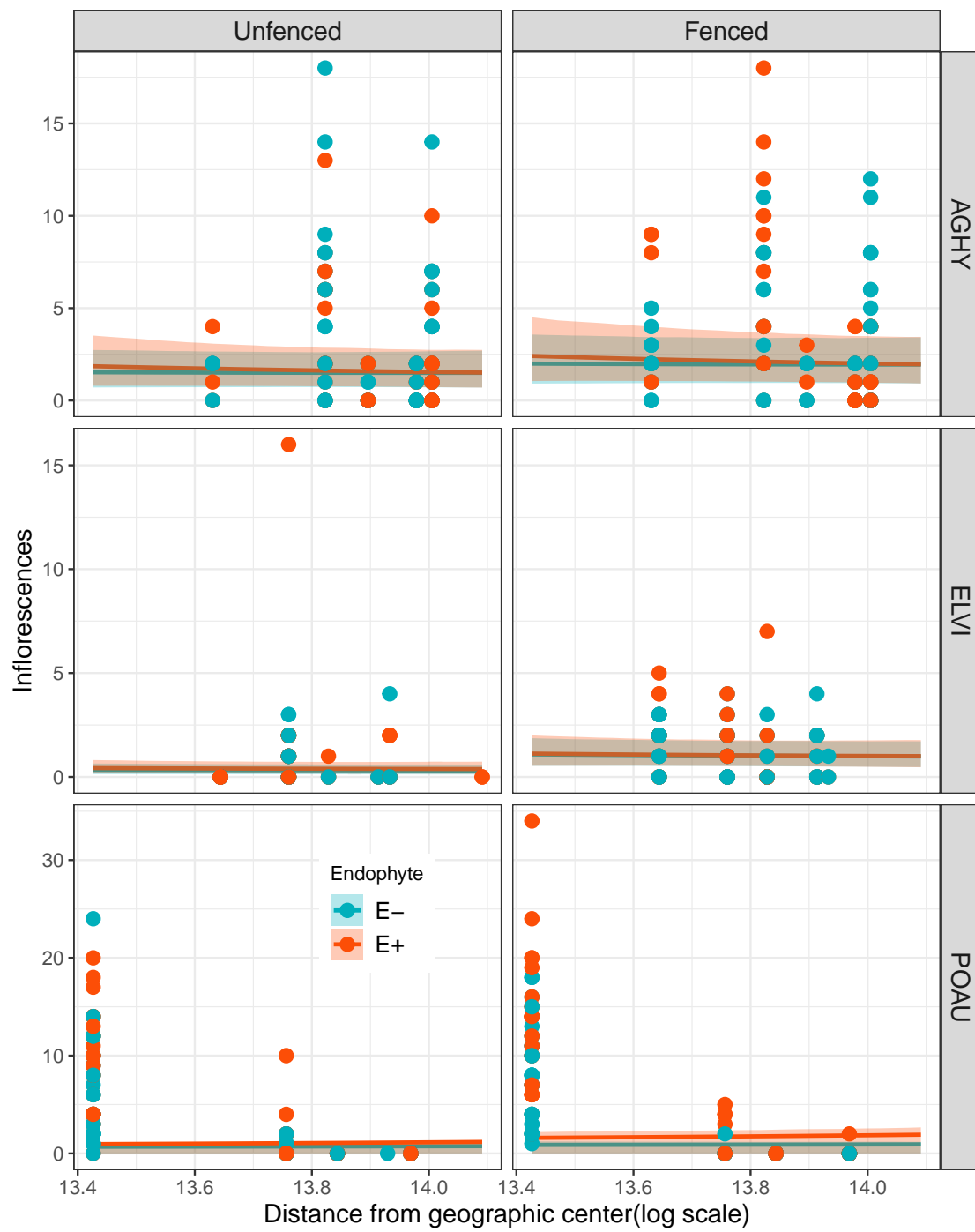**Figure 2:** *XXX*

Figure 3: *XXX*

Figure 4: *XXX*

Acknowledgements

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Supporting Information

S.1 Supporting methods

Study design

Experimental Design To understand the demographic effects of endophyte symbiosis from core to edge of the host range, we established common gardens at 7 sites across the geographic range of *Elymus virginicus* (Fig. S-1). Experimental sites spanned an aridity gradient (temperature gradient). Common gardens were established in 8 plots per site. Plots were 1.5m * 1.5m and the area was tilled of existing vegetation to control for native plant competition. Plots were also selected in shaded areas under tree canopy or near shrubs to mimic the natural environmental of the species. In each plot, we planted 15 individuals of *E. virginicus* approximately 15 cm deep in an evenly spaced 4*4 grid pattern, with positions randomly assigned. For each plot, we randomly assigned a starting endophyte frequency (80%, 60%, 40%, 20%)[16] and herbivory treatment (herbivores exclusion and herbivores accessibility). We ensured that all plots had comparable quantities of source populations. After establishing the plots, we watered the plants and recorded initial tiller counts, flowering status and plot position, endophyte status, source population of each individual plant. For herbivory exclusion plots, we enclosed them with 1.2m tall mesh fencing to prevent browsing by vertebrate herbivores and sprayed the plots with insecticide. For herbivores accessibility plots (control treatment), we half enclosed the plots with the mesh netting.

Source populations and Identification of individual endophyte status Plants used in the common garden experiment were derived from natural populations throughout the native range in the south-central US. At each of these natural populations we collected seeds. Some of the seeds of *E. virginicus* were heat treated to produce endophyte negative plants (E^-) . To do so, we placed these seeds in a drying oven set at 60℃ for approximately five days (120 hours). While this method eliminates the endophytes from all individuals, it does not affect seed viability. All seeds (both heat-treated and non-heat-treated) were planted in the Rice University greenhouse. Seedlings were regularly fertilized every two weeks. The seedlings were then vegetatively propagated to produce enough individuals for your experiment (N = 840). Before planting in the field, we confirmed the endophyte status (E^+ or E^-) of all seedlings using either microscopy or an immunoblot assay. This was necessary due to the varying success of the heat treatment and differences in the prevalence of endophytes between

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[16]*Do we need a schematic of one replicate of the experimental design?*

the natural populations. Leaf tissues were stained with aniline blue lactic acid and viewed
under a compound microscope at 200x-400x to identify fungal hyphae. The immunoblot
assay (Phytoscreen field tiller endophyte detection kit, Agrinostics Ltd. Co.) uses monoclonal
antibodies that target proteins of Epichloë spp. and chromagen to visually indicate presence
or absence. Both methods yield similar detection rates.

S.2   Supporting Tables

Table S-1: Populations and Coordinates

Population	Location	Coordinates	NE+ / NE-
Sam Houston State University Field Station	Huntsville, TX	30°44′30.5″N, 95°28′28.2″W	NE+ = 39, NE- = 50
Texas Tech University	Junction, TX	30°28′18.2″N, 99°47′01.7″W	NE+ = 49, NE- = 83
Palmetto State Park	Palmetto, TX	29.591623, -97.584781	NE+ = 250, NE- = 192
Jean Lafitte National Historical Park and Preserve	Jean Lafitte, LA	29.785105, -90.114933	NE+ = 83, NE- = 94

Table S-2: Model comparison by vital rate using ELPD (Expected Log Pointwise Density) differences. The values in the **elpd_diff** column represent the difference in the model's ELPD relative to the best model for each vital rate, with positive values indicating a worse model fit. The **se_diff** column shows the standard error of the ELPD difference. Best models for each vital rate are bolded. Model selection is based on the ELPD difference and associated standard error, with smaller ELPD differences and lower standard errors indicating better model fit.

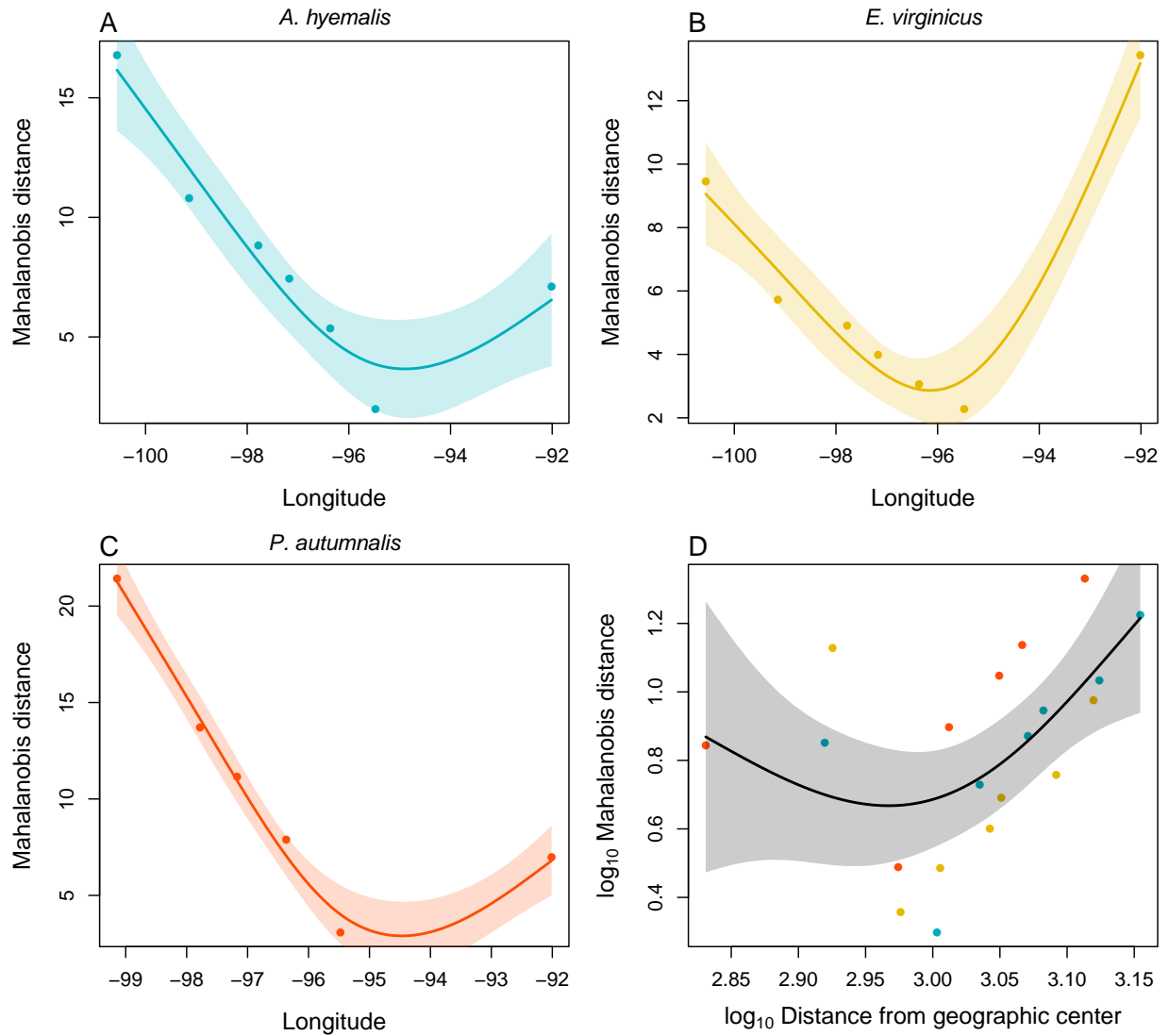| Vital rate	Model	elpd_diff	se_diff
Survival	**model1**	**0.0**	**0.0**
	model2	-1.0	1.5
	model3	-1.0	1.5
Growth	**model3**	**0.0**	**0.0**
	model2	-1.3	2.0
	model1	-2.1	2.2
Flowering	**model2**	**0.0**	**0.0**
	model3	0.0	3.2
	model1	-1.3	3.9

S.3 Supporting Figures

Figure S-1: (A-C) Relationship between distance from geographic center and longitude for the focal species (*Agrostis hyemalis*, *Elymus virginicus*, and *Poa autumnalis*). A generalized additive model (GAM) with a smooth term was used to capture the non-linear trends in these relationships for each species. The solid lines represent the fitted GAM with shaded regions showing the 95% confidence interval. D) displays the relationship between the distance from geographic center and the distance from niche centroid for all species also fitted with a GAM on a log scale. The p-value for the smooth term in the GAM model is p=0.0166**, indicating a significant relationship between log-transformed geographic distance and Mahalanobis distance.
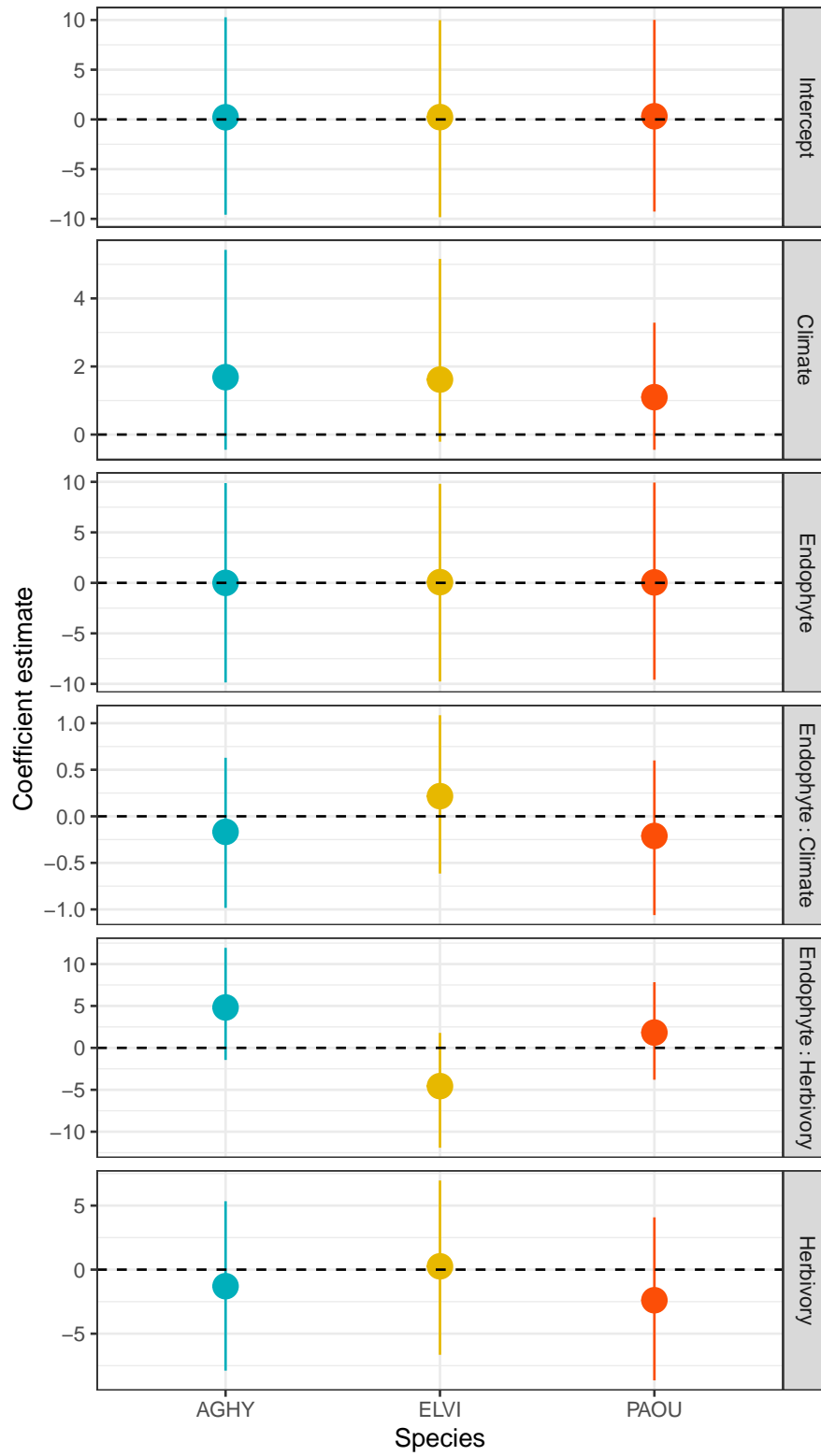
Figure S-2: Coefficient estimates from Bayesian models linking survival and geographic distance. Points represent posterior means and lines indicate 95% credible intervals. Species abbreviations: *Agrostis hyemalis* (AGHY), *Elymus virginicus* (ELVI), and *Poa autumnalis* (PAOU). Horizontal dashed lines denote zero effect.
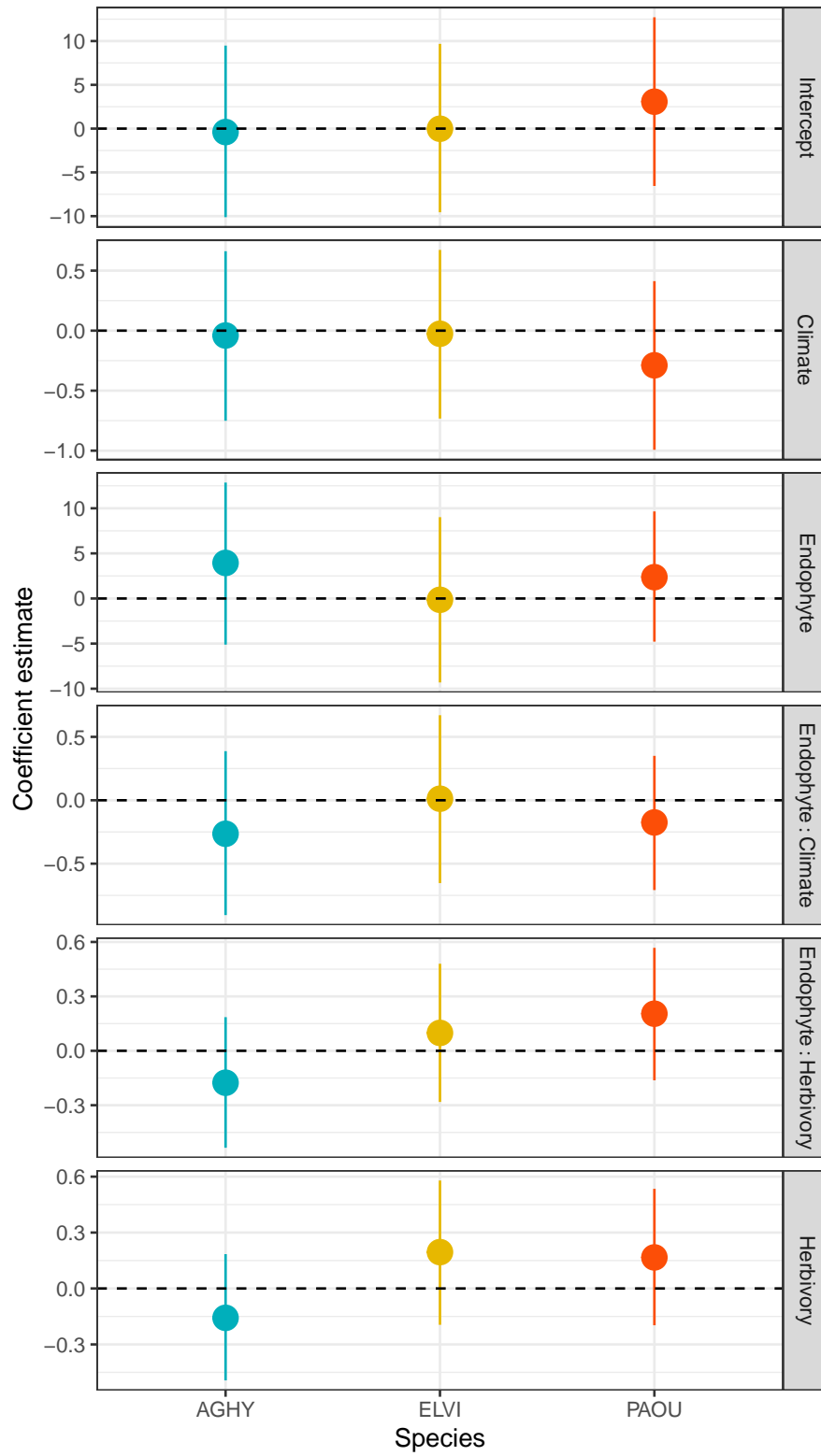
Figure S-3: Coefficient estimates from Bayesian models linking growth and geographic distance. Points represent posterior means and lines indicate 95% credible intervals. Species abbreviations: *Agrostis hyemalis* (AGHY), *Elymus virginicus* (ELVI), and *Poa autumnalis* (PAOU). Horizontal dashed lines denote zero effect.
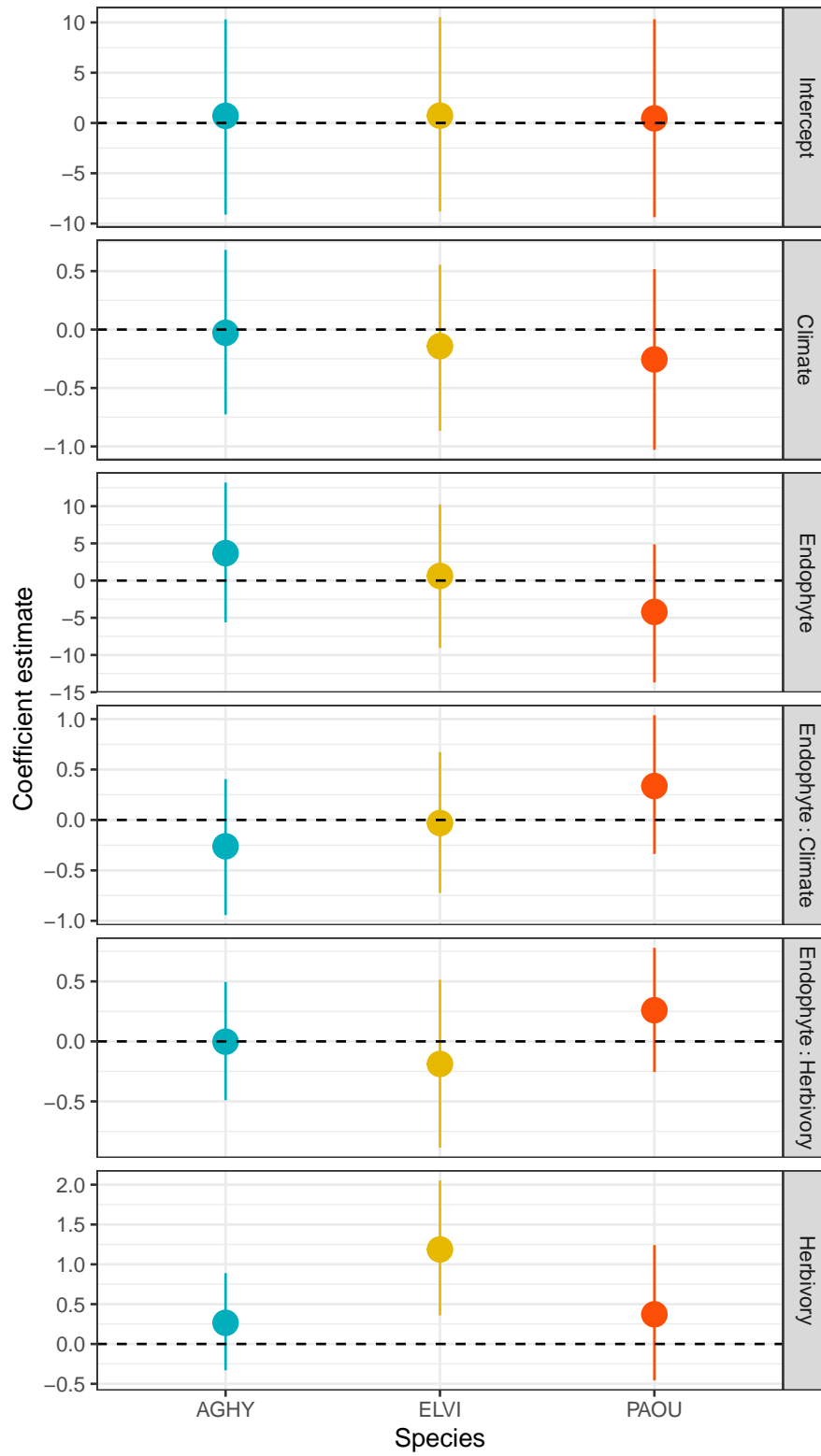
Figure S-4: Coefficient estimates from Bayesian models linking flowering and geographic distance. Points represent posterior means and lines indicate 95% credible intervals. Species abbreviations: *Agrostis hyemalis* (AGHY), *Elymus virginicus* (ELVI), and *Poa autumnalis* (PAOU). Horizontal dashed lines denote zero effect.