

Effect of grass-endophyte symbiosis and herbivory on population demography across a climatic and geographic gradient

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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 frequency affects plant distribution remains understudied. Using three cool-season grass species, we combined field experiments on plant populations with variation in leaf endophytic fungi and Bayesian statistics to test whether plant-fungi interactions constrain host demography 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 may be hindered by the presence of a mutualist.

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 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 (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).

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. If microbial symbiosis provides greater benefits under environmental stress, then symbionts could enhance the suitability of range-edge environments, 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 (Bennett and Groten, 2022; Benning and Moeller, 2021a,b).

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 incomplete predictions of host population responses to global change.

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 (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.

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 cool-season grass species (*Agrostis hyemalis*, *Elymus virginicus* and *Poa autumnalis*) and their vertically transmitted fungal symbiont *Epichloë* spp. Our experiment was design to test the following hypotheses:

1. Stress associated with aridity and low precipitation enhances plant–fungal mutualism, such that the fitness benefits of endophyte symbiosis (survival, growth, and fecundity) are maximized at the range edge.
2. Stress associated with herbivory weakens plant–fungal mutualism, such that the fitness costs of endophyte symbiosis (survival, growth, and fecundity) are maximized at the range edge.

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) containing individuals of 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. Seeds were collected in summer–fall 2006 from naturally symbiotic

populations (source populations) of the three focal host species. These seeds were either heat-treated to eliminate symbionts or left untreated, thereby generating 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 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). Full details of the study design are provided in the supplementary materials.

Demographic data collection

We collected demographic data including survival, growth, and reproduction in May and June of 2023 and 2024, 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, indicated by the presence of green coloration. 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.

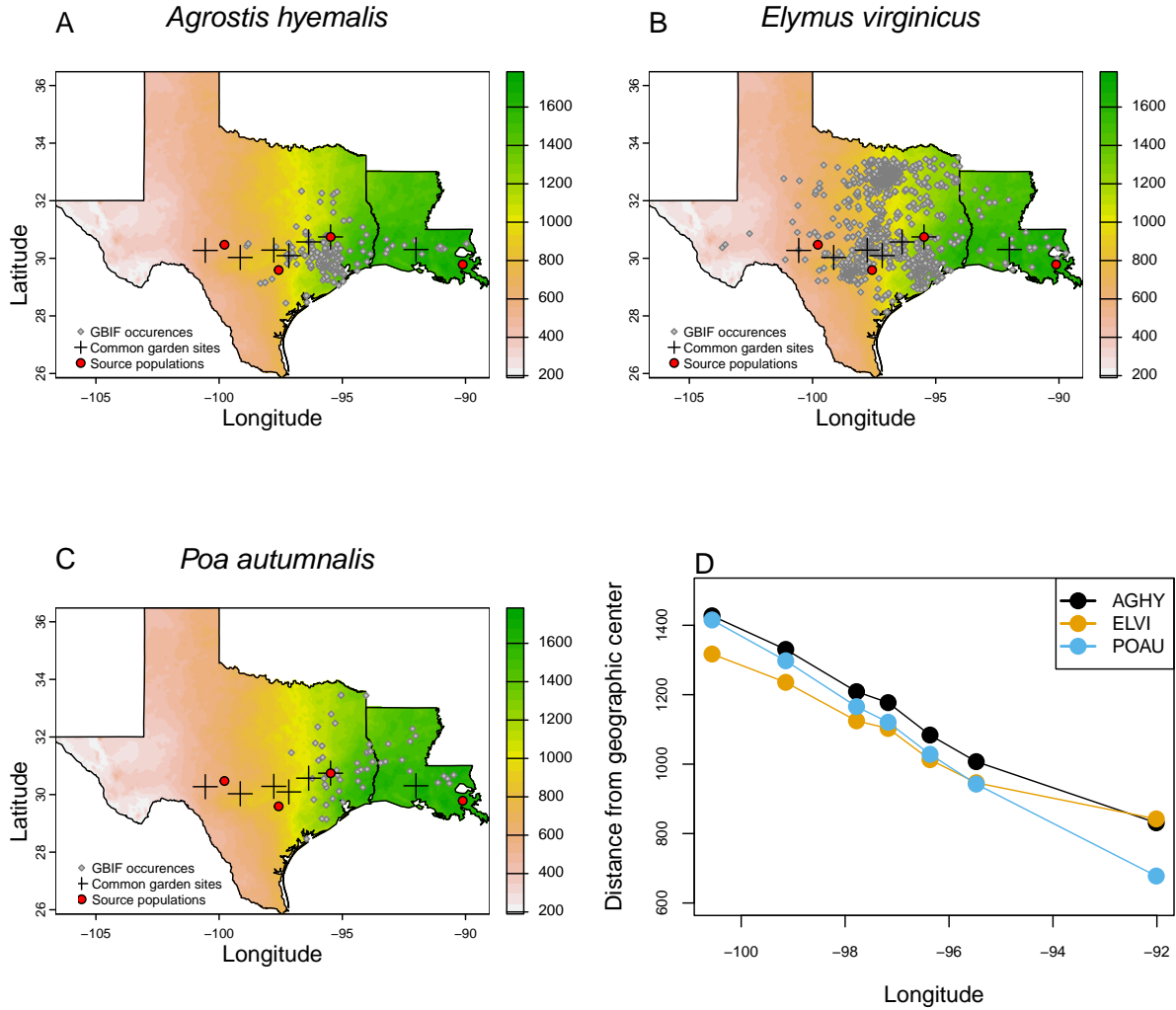


Figure 1: Distribution of common garden sites along the longitudinal aridity gradient in the central and southern Great Plains for: A) *Agrostis hyemalis*, B) *Elymus virginicus*, and C) *Poa autumnalis*. Red dots indicate the locations of source populations, while grey dots represent GBIF occurrence 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 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: } \mu &= \beta_0^{\text{sp}} + \beta_P^{\text{sp}} P + \beta_{\text{endo}}^{\text{sp}} \text{endo} + \beta_{\text{herb}}^{\text{sp}} \text{herb} \\
&\quad + \beta_{\text{endo} \times P}^{\text{sp}} \text{endo} \cdot P + \beta_{\text{herb} \times \text{endo}}^{\text{sp}} \text{herb} \cdot \text{endo} + \beta_{\text{herb} \times P}^{\text{sp}} \text{herb} \cdot P \\
&\quad + \beta_{P^2}^{\text{sp}} P^2 + \beta_{\text{endo} \times P^2}^{\text{sp}} \text{endo} \cdot P^2 + \phi^{\text{sp}} + \omega^{\text{sp}} + \rho^{\text{sp}} \\
\text{Model 2: } \mu &= \beta_0^{\text{sp}} + \beta_{\text{SPEI}}^{\text{sp}} \text{SPEI} + \beta_{\text{endo}}^{\text{sp}} \text{endo} + \beta_{\text{herb}}^{\text{sp}} \text{herb} \\
&\quad + \beta_{\text{endo} \times \text{SPEI}}^{\text{sp}} \text{endo} \cdot \text{SPEI} + \beta_{\text{herb} \times \text{endo}}^{\text{sp}} \text{herb} \cdot \text{endo} + \beta_{\text{herb} \times \text{SPEI}}^{\text{sp}} \text{herb} \cdot \text{SPEI} \\
&\quad + \beta_{\text{SPEI}^2}^{\text{sp}} \text{SPEI}^2 + \beta_{\text{endo} \times \text{SPEI}^2}^{\text{sp}} \text{endo} \cdot \text{SPEI}^2 + \phi^{\text{sp}} + \omega^{\text{sp}} + \rho^{\text{sp}} \\
\text{Model 3: } \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} \\
&\quad + \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} \\
&\quad + \phi^{\text{sp}} + \omega^{\text{sp}} + \rho^{\text{sp}} \\
\text{Model 4: } \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} \\
&\quad + \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} \\
&\quad + \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, SPEI is the Standardized Precipitation Evapotranspiration Index, GD refers to distance from geographic center, and MD stands for mahalanobis distance, endo indicates Endophyte presence (binary), and herb represents Herbivory (binary). 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).

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

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

132 **Results**

133 **Acknowledgements**

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Supporting Information

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%)¹ 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.

¹Do we need a schematic of one replicate of the experimental design?

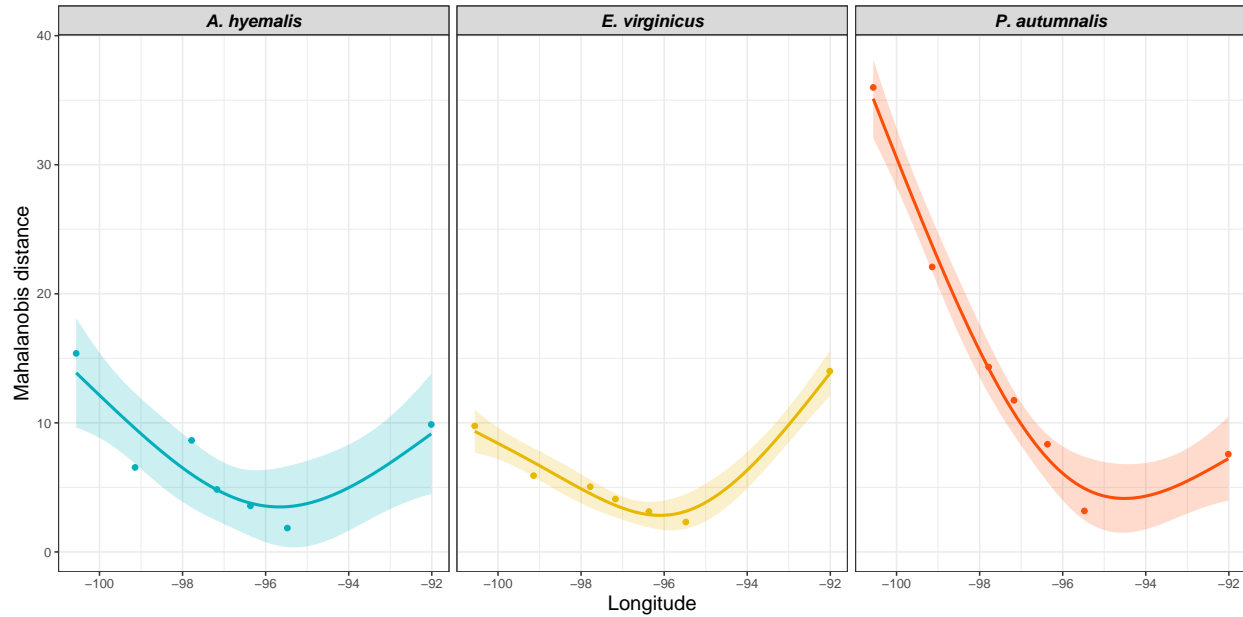


Figure S-1: Distribution of common garden sites along the longitudinal aridity gradient in the central and southern Great Plains for: A) *Agrostis hyemalis*, B) *Elymus virginicus*, and C) *Poa autumnalis*. Red dots indicate the locations of source populations, while grey dots represent GBIF occurrence records for each species within the study area. D) Relationship between the distance from the range center and longitude for each species.

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°C 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 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.

Table S-1: 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