Variation in the demographic effects of grass-endophyte symbiosis along an aridity gradient

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Abstract

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, 2021*a,b*). Although context depen-
dence, along with spatiotemporal variations in abiotic environmental conditions may reduce the
effectiveness of the benefits provided by the symbiont to the host species, our understanding of
the mechanisms that alter host-symbiont interactions across species geographic range remains
limited.

Ecological studies of plant-microbe symbiosis typically examine interactions from the plant's
perspective and rarely manipulate symbiont frequency to explore how symbiont response to
environmental variation might translate to effect on host demographic performance across host's
range. Symbionts promotes their own fitness by influencing their host's life history traits and
resistance to abiotic and biotic stress (Giauque et al., 2019; Kazenel et al., 2015; Saikkonen et al.,
1998). Therefore overlooking the role of symbionts and their potential cascading effects on the
eco-evolutionary population dynamics of host species could lead to inaccurate prediction of host
response to global change. Moreover, studies on plant-microbe symbiosis often fail to account
for the synergistic effects of abiotic and biotic factors on host dynamics across species ranges.
Finally, studies of plant-microbe symbiosis have relied on methods such as inoculating sterile
soil (Peacher and Meiners, 2020), excluding endophyte fungal hyphae using fine mesh or rotating
cores (Chung et al., 2019), and applying fungicide (Bennett and Groten, 2022). While valuable,
these approaches are often challenging to implement in field settings or on large scales. As
a result, the mechanisms by which symbionts drive host range limitation and expansion are
poorly understood, hindering our understanding of the potential cascading effects of symbionts
on eco-evolutionary species demography and range limitation in the context of global change.

Working across a precipitation gradient in the south-central US, we investigated how the de-
mographic effects of endophyte symbiosis varied from core to edge of the host range. To answer
these questions, we studied the symbiotic association between cool-season grass species (*Agrostis hyemalis*, *Elymus virginicus* and *Poa arachnifera*) and its vertically transmitted fungal symbiont
Epichloë elymi. [Describe ecology and natural history of grass-endophyte interactions][1]. Our

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[1]*I am not sure If we need this*

experiment was design to test the following hypotheses[2] :

1. We hypothesized that stress associated with aridity and low precipitation would strengthen the plant-fungal mutualism, such that the fitness benefits of endophyte symbiosis are maximized at the range edge.

2. We hypothesized that fungal growth in planta varied from range core to range edge. If endophyte growth is limited by host photosynthesis, then environments that are stressful for hosts may correspond to poor endophyte growth. Alternatively, if active regulation by the host is required to keep symbionts "in check", then environments that are stressful for hosts may correspond to high endophyte growth.

Materials and methods

Study species

Elymus virginicus (Poaceae) is a cool season perennial grass native to woodland and prairie habitats of eastern North America (Shaw, 2011). The westernmost range limits of this species correspond to the longitudinal aridity gradient in the central and southern Great Plains (fig. 1). Throughout its range, the species is symbiotic with the seed-transmitted fungal endophyte (Epichloë spp.) (Rudgers and Swafford, 2009). Across natural populations in Texas, endophyte prevalence (fraction of plants that are endophyte-symbiotic) in *Elymus virginicus* ranged from 10% to 100%, with a mean of 53% (Sneck et al., 2017). Fungal genotyping indicated that the endophytes are capable of synthesizing secondary compounds such as peramine, loline, and ergot alkaloids, which may confer resistance against drought and herbivory (Beaudry, 1951). In addition, the species is capable of both self-pollination and outcrossing (Church, 1958).

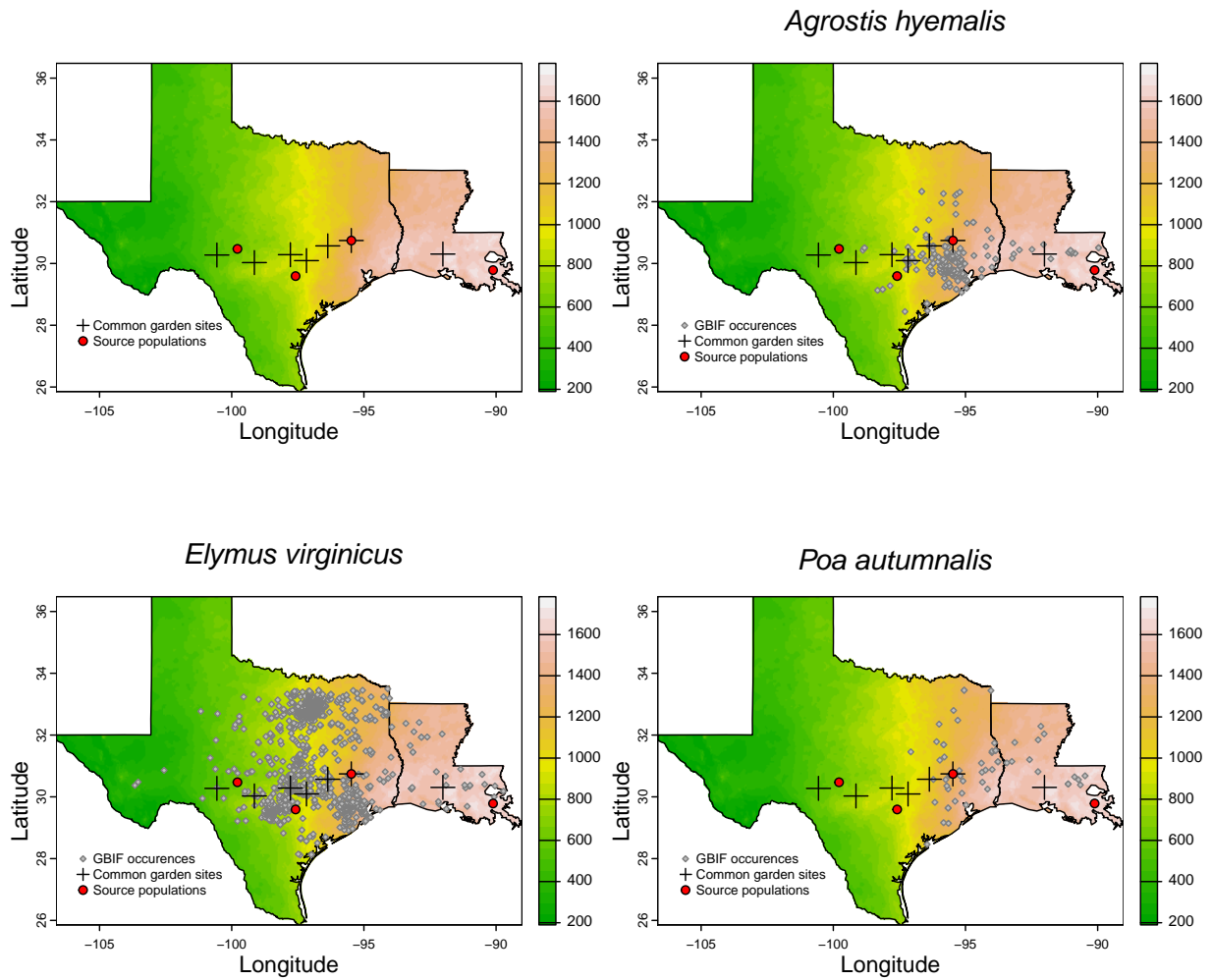[2] *I need to update these hypotheses by adding the herbivory effect*

Figure 1: Distribution of common garden sites across the longitudinal aridity gradient in the central and southern Great Plains. Red dots represent the locations of source populations, while grey dots represent the GBIF locations of the species across the study area.

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. 1). Experimental sites spanned an aridity gradient (temperature gradi-

6

ent). 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%)[3] 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. We stationed one HOBO MX2307 data logger at each site to collect temperature and volumetric water content in the soil every hour.

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 (fig.1, Table X[4]). 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

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

[4]*We need this table in the Appendix*

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.

Climatic data

To characterize the stress gradient with respect to climatic conditions, we collected the hourly temperature and soil moisture at each site using the HOBO MX2307 data loggers. We used this hourly variable to calculate the daily mean temperature (°C) and soil moisture (%)(fig. 3). We calculated the mean soil moisture and the coefficient of variation from the time the plants were placed on the ground to the time we collected demographic data[5]. The coefficient of variation of soil moisture was estimated to capture season variability in climatic data (Medvigy and Beaulieu, 2012; Meshram et al., 2017).

Demographic data

We collected demographic data including survival, growth, and reproduction during June 2023, which coincided with the flowering season of *E. virginicus*. On each individual, survival of plants was recorded as a binary (death or alive) and the size of the plant was recorded as the number of living tillers, indicated by the presence of green coloration. We recorded the number of inflorescences per plant and the number of spikelets on up to three inflorescences from three reproducing plants. We limited the spikelets count to three reproducing tillers per plot due to the time consuming nature of this measurement process. We used the number of spikelets for these three tillers to estimate the number the average number of spikelets per plants.

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[5]*I will change this later*

Figure 2: Climate variation across common garden sites. (a) Daily temperature, estimated from average hourly data collected by HOBO data loggers. (b) Daily soil moisture, estimated from average hourly data collected by HOBO data loggers.

¹²⁶ *Models building and models selection*

¹²⁷ To assess how stress associated with aridity affect plant-fungal mutualism, we developed four
¹²⁸ candidates models for each vital rate (survival, growth, flowering, fertility). Each vital rate was

¹²⁹ modeled with the grand mean intercept (β_0), slopes for variation in each covariate ($\beta_1...\beta_3$) as well

¹³⁰ as the interaction between covariates ($\beta_4...\beta_6$): Each model includes normally distributed random

¹³¹ effects for site-to-site variation ($\phi \sim N(0, \sigma_{site})$), plot to plot variation ($\rho \sim N(0, \sigma_{site})$), and

¹³² source-to-source variation that is related to the provenance of the transplants used to establish

¹³³ the common garden ($\omega \sim N(0, \sigma_{source})$) (Eq.1).

$$model1 = \beta_0 + \beta_1 T_{mean} + \beta_2 E + \beta_3 H + \beta_4 E * T_{mean} + \beta_5 H * E + \beta_6 H * T_{mean} + \phi + \omega + \rho$$

$$model2 = \beta_0 + \beta_1 T_{CV} + \beta_2 E + \beta_3 H + \beta_4 E * T_{CV} + \beta_5 H * E + \beta_6 H * T_{CV} + \phi + \omega + \rho$$

$$model3 = \beta_0 + \beta_1 M_{mean} + \beta_2 E + \beta_3 H + \beta_4 E * M_{mean} + \beta_5 H * E + \beta_6 H * M_{mean} + \phi + \omega + \rho$$

$$model4 = \beta_0 + \beta_1 M_{CV} + \beta_2 E + \beta_3 H + \beta_4 E * M_{CV} + \beta_5 H * E + \beta_6 H * M_{CV} + \phi + \omega + \rho$$

(1)

¹³⁴ We modeled survival using a Bernoulli distribution, growth with a Gaussian distribution,

¹³⁵ flower with a negative binomial and fertility (number of spikelet) with a negative binomial dis-

¹³⁶ tribution. To check whether the fitted models are compatible with the observed data, we used

¹³⁷ the posterior predictive checks (Berkhof et al., 2000; Gelman et al., 2000). All models do a good

¹³⁸ job of capturing relevant aspects of the data, such as means, standard deviations, and quantiles

¹³⁹ (fig.A1, fig.A2, fig.A3, fig.A4).

¹⁴⁰ To select the best model for each vital rate, we compared the four models using the leave-

¹⁴¹ one-out cross-validation (LOOCV)[6] (Vehtari et al., 2017). LOOCV combines both validation and

¹⁴² training methods. In this approach, one observation is used for validation while the training

¹⁴³ set consists of n-1 observations. This process is repeated for each observation, resulting in n

¹⁴⁴ estimated models (Silva and Zanella, 2024). The estimate of test error from LOOCV is calculated

¹⁴⁵ by averaging the errors across these n models (Eq.2).
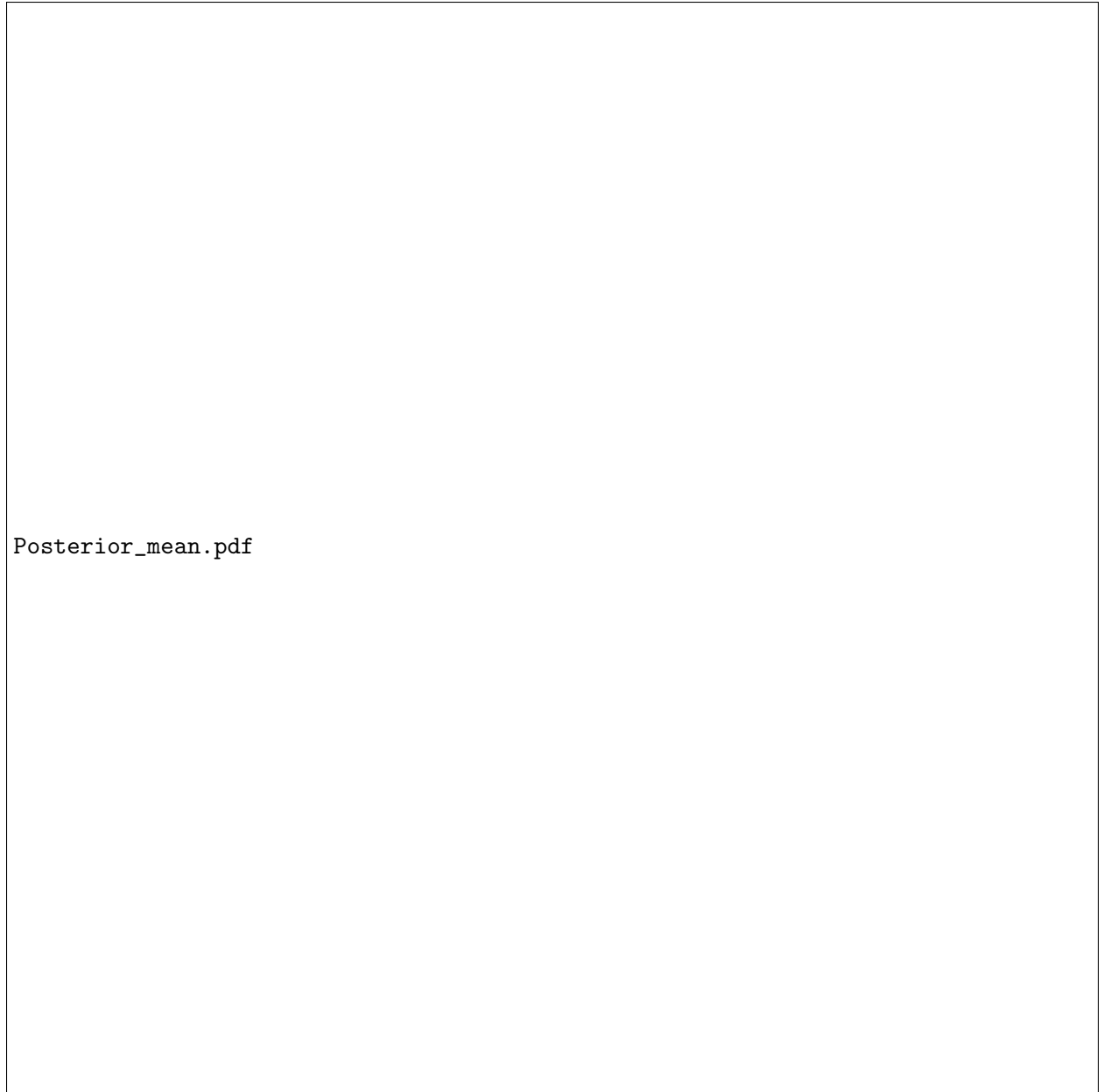
$$CV_n = \frac{1}{n} \sum_{i=1}^{n} (y_I - \hat{y}_I)^2$$

(2)

¹⁴⁶ All models were performed in R (R Core Team, 2023) and Stan (Stan Development Team,

¹⁴⁷ 2024).

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[6]*I need to add something about difference in models*

Results

Table 1: Candidate models of *E. virginicus* vital rates (growth, flowering, spikelet and survival).

Vital rate	Model	Δelpd	Δse
Survival	**model3**	**0.0**	**0.0**
Survival	model2	-0.5	0.5
Survival	model4	-0.9	0.9
Survival	model1	-2.2	1.9
Growth	**model2**	**0.0**	**0.0**
Growth	model4	0.0	0.8
Growth	model3	-0.3	0.4
Growth	model1	-0.4	0.4
Flowering	**model2**	**0.0**	**0.0**
Flowering	model4	-4.3	1.0
Flowering	model3	-6.5	1.4
Flowering	model3	-6.5	1.4
Spiket	**model2**	**0.0**	**0.0**
Spiket	model3	-0.5	0.6
Spiket	model1	-0.7	1.2
Spiket	model4	-1.2	1.2

Posterior_mean.pdf

Figure 3: Posterior mean estimates for each vital rate. (a) Daily temperature, estimated from average hourly data collected by HOBO data loggers. (b) Daily soil moisture, estimated from average hourly data collected by HOBO data loggers.

Fig_temp_mean.pdf

Figure 4: Variation in vital rates across a temperature gradient.

Acknowledgments

Statement of Authorship

Data and Code Availability

13

Appendix A

Figure A1: Comparison of the observed survival data with the posterior predictions from the survival models for each climate variable

Figure A2: Comparison of the observed growth data with the posterior predictions from the growth models for each climate variable

Figure A3: Comparison of the observed spikelet data with the posterior predictions from the spikelet models for each climate variable

Figure A4: Comparison of the observed flowering data with the posterior predictions from the flowering models for each climate variable

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