

Synergistic 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).

Ecological studies of plant-microbe symbiosis typically focus on interactions from the plant's perspective and rarely manipulate symbiont prevalence to assess how symbiont responses to environmental variation may influence host demographic performance across the host's range. Symbionts promote their own fitness by affecting host life history traits and enhancing resistance to abiotic and biotic stress (Giauque et al., 2019; Kazenel et al., 2015; Saikkonen et al., 1998). Theory predicts that exclusive vertical transmission promotes mutualism and leads to a high prevalence of symbiosis within host populations (Fine, 1975). Field-based experiments on plant-fungal symbiosis and population fitness suggest that,

within populations, beneficial symbionts may become more prevalent and eventually reach fixation when the recruitment advantages they provide outweigh their reproductive costs under stressful conditions (Donald et al., 2021). However, populations with lower endophyte prevalence may experience higher rates of symbiont loss among offspring (Afkhami and Rudgers, 2008). Therefore, overlooking the role of symbionts and their potential cascading effects on the eco-evolutionary dynamics of host populations could lead to inaccurate predictions of host responses to global change.

Studies on plant–microbe symbiosis often overlook the synergistic effects of abiotic and biotic factors on host dynamics across species’ ranges. Previous research on biotic factors, such as herbivory, has shown that herbivory not only influences the reproductive fitness benefits of symbiosis but also increases symbiont prevalence by enhancing vertical transmission of the fungus to the next generation (Agrawal et al., 1999; Gundel et al., 2020). While informative, these studies have primarily been conducted in greenhouse conditions rather than in field settings or at larger spatial scales, limiting our understanding on how herbivory affect natural populations fitness. If the ecological context in which herbivory occurs affects population fitness, then the interaction between herbivory and abiotic stressors (e.g., drought) could significantly alter host population dynamics and, consequently, influence species range limitations.

Working across a precipitation gradient in the south-central US, we investigated how the demographic 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 autumnalis*) and their vertically transmitted fungal symbiont *Epichloë* sp.. Our experiment was design to test the following hypotheses:

1. We hypothesized that stress associated with aridity and low precipitation would strengthen plant-fungal mutualism, such that the fitness benefits of endophyte symbiosis (survival, growth and fecundity) are maximized at the range edge.
2. We hypothesized that the stress associated with herbivory would reduce 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 species

Agrostis hyemalis, *Elymus virginicus*, and *Poa autumnalis* (subfamily Pooideae) are cool-season perennial grasses native to woodland and prairie habitats in eastern North America (Shaw, 2011). The westernmost range limits of these species correspond to the longitudinal aridity

gradient in the central and southern Great Plains (Fig. 1). Throughout their range, these species are symbiotic with seed-transmitted fungal endophytes (*Epichloë* sp. and *E. typhina* subsp. *poae*) (Rudgers and Swafford, 2009). However, *Poa autumnalis* may also acquire endophytes through horizontal transmission (Gundel et al., 2020). Across natural populations in Texas, endophyte prevalence (the fraction of plants that are endophyte-symbiotic) is 86.55%, ranging from 77.16% to 93.5% in *Agrostis hyemalis* (Donald et al., 2021), 53% (ranging from 10% to 100%) in *Elymus virginicus* (Sneck et al., 2017), and 96% in *Poa autumnalis* (Rudgers et al., 2009). Fungal genotyping indicates 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). Additionally, these species are capable of both self-pollination and outcrossing (Church, 1958).

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 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. 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

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

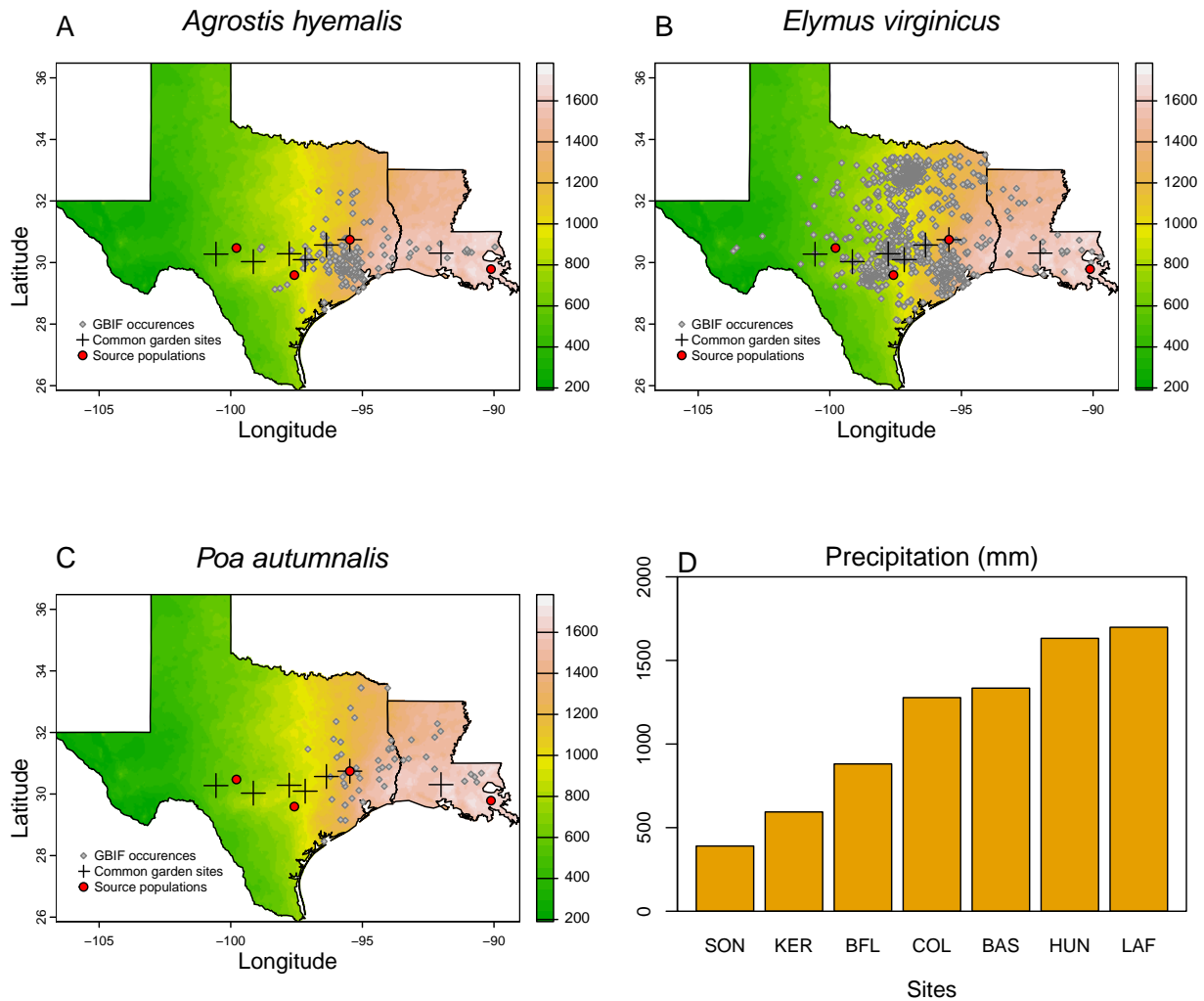


Figure 1: Distribution of common garden sites across the longitudinal aridity gradient in the central and southern Great Plains for A) *Agrostis hyemalis*, B) *Elymus virginicus* and C) *Poa autumnalis*. Red dots represent the locations of source populations, while grey dots represent the GBIF locations of each species across the study area. D) Cumulative precipitation during the demographic data collection period.

native range in the south-central US (fig.1, Table X²). 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

²We need this table in the Appendix

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.

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 ($^{\circ}\text{C}$) and soil moisture (%) (fig. ??). 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 data³. 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 average number of spikelets per plants.

Models building and models selection

To assess how stress associated with aridity affect plant-fungal mutualism, we developed four candidates models for each vital rate (survival, growth, flowering, fertility). Each vital rate was modeled with the grand mean intercept (β_0), slopes for variation in each covariate ($\beta_1 \dots \beta_3$) as well as the interaction between covariates ($\beta_4 \dots \beta_6$): Each model includes normally distributed

³I will change this later

random effects for site-to-site variation ($\phi \sim N(0, \sigma_{site})$), plot to plot variation ($\rho \sim N(0, \sigma_{site})$), and source-to-source variation that is related to the provenance of the transplants used to establish the common garden ($\omega \sim N(0, \sigma_{source})$) (Eq.1).

$$\begin{aligned} model1 &= \beta_0 + \beta_1 T_{mean} + \beta_2 E + \beta_3 H + \beta_4 E * T_{mean} + \beta_5 H * E + \beta_6 H * T_{mean} + \phi + \omega + \rho \\ model2 &= \beta_0 + \beta_1 T_{CV} + \beta_2 E + \beta_3 H + \beta_4 E * T_{CV} + \beta_5 H * E + \beta_6 H * T_{CV} + \phi + \omega + \rho \\ model3 &= \beta_0 + \beta_1 M_{mean} + \beta_2 E + \beta_3 H + \beta_4 E * M_{mean} + \beta_5 H * E + \beta_6 H * M_{mean} + \phi + \omega + \rho \\ model4 &= \beta_0 + \beta_1 M_{CV} + \beta_2 E + \beta_3 H + \beta_4 E * M_{CV} + \beta_5 H * E + \beta_6 H * M_{CV} + \phi + \omega + \rho \end{aligned} \quad (1)$$

We modeled survival using a Bernoulli distribution, growth with a Gaussian distribution, flower with a negative binomial and fertility (number of spikelet) with a negative binomial distribution. To check whether the fitted models are compatible with the observed data, we used the posterior predictive checks (Berkhof et al., 2000; Gelman et al., 2000). All models do a good job of capturing relevant aspects of the data, such as means, standard deviations, and quantiles (fig.??, fig.??, fig.??, fig.??).

To select the best model for each vital rate, we compared the four models using the leave-one-out cross-validation (LOOCV)⁴ (Vehtari et al., 2017). LOOCV combines both validation and training methods. In this approach, one observation is used for validation while the training set consists of n-1 observations. This process is repeated for each observation, resulting in n estimated models (Silva and Zanella, 2024). The estimate of test error from LOOCV is calculated by averaging the errors across these n models (Eq.2).

$$CV_n = \frac{1}{n} \sum_{i=1}^n (y_I - \hat{y}_I)^2 \quad (2)$$

All models were performed in R (R Core Team, 2023) and Stan (Stan Development Team, 2024).

Results

Acknowledgements

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⁴I need to add something about difference in models

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Supporting Information