

Variation in the demographic effects of grass-endophyte symbiosis and endophyte hyphal density across an aridity gradient

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Open Research statement: All data X used in this paper are publicly available. Should the paper be accepted, all computer scripts supporting the results will be archived in a Zenodo package, with the DOI included at the end of the article. During peer review, our data and code are available at <https://github.com/jmoutouama/ELVI-endophyte-density>.

1

Abstract

2

Keywords

3 density-dependence, ecotones, woody encroachment, shrubs, integral projection model,
4 dispersal, Allee effects

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 (Fowler et al. 2023). On the one hand endophyte symbiosis can benefit host plants by enhancing host resistance to abiotic stress such as drought, competitive ability, and resistance to herbivory and diseases. On the other hand, endophyte symbiosis can also be detrimental to host species by reducing host biomass or reproduction under nutrient poor conditions. These context-dependent costs and benefits may ultimately underlie the observed distribution of host species.

P2: Context-dependence raises the hypothesis that plant-microbe interactions are likely to vary across environmental gradients spanning range-core to range-edge. If the benefits of microbial symbiosis strengthen under environmental stress then symbionts could make range-edge environments more suitable, possibly extending the host's range limits.

P3. Ecological studies of plant-microbe symbiosis usually study the interaction from the plant's perspective. Much less is known about how dynamics of the symbiont respond to environmental variation, and how this might translate to its influence on host performance.

P4: We studied the symbiotic association between a cool-season grass species (*Elymus virginicus*) and its vertically transmitted fungal symbiont *Epichloë elymi*. [Describe ecology and natural history of grass-endophyte interactions]

P5: Working across a precipitation gradient in the south-central US, we asked how the demographic effects of endophyte symbiosis varied from core to edge of the host

29 range. We also quantified fungal growth in planta from range core to range edge. Our
30 experiment was design to test the following hypotheses:

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

34 We hypothesized that fungal growth in planta varied from range core to range edge.
35 If endophyte growth is limited by host photosynthesis, then environments that are stress-
36 ful for hosts may correspond to poor endophyte growth. Alternatively, if active regula-
37 tion by the host is required to keep symbionts “in check”, then environments that are
38 stressful for hosts may correspond to high endophyte growth.

39 **Materials and methods**

40 *Study species*

41 *Elymus virginicus* (Virginia wildrye) is a C3 perennial bunchgrass native to woodland
42 and prairie habitats of eastern North America. The westernmost range limits of this
43 species correspond to the longitudinal aridity gradient in the central and southern Great
44 Plains (Figure 1). The life cycle of *E. virginicus* is typical of cool-season grasses, with
45 seed germination in winter, growth in spring, and seed production in early summer. We
46 measure plant size as a count of vegetative tillers. Reproducing plants produce spikelet-
47 bearing inflorescences, and each spikelet typically produces 2-3 seeds. This species is
48 capable of both self-pollination and outcrossing (Sneck et al. 2019). Throughout its
49 range, *E. virginicus* is commonly symbiotic with the seed-transmitted fungal endophyte
50 *Epichloë elymi* (Clavipitaceae). In a prior study across natural populations in Texas,
51 endophyte prevalence (fraction of plants that are endophyte-symbiotic) ranged from 10%

52 to 100 %, with a mean of 53 % (Sneck et al. 2017). Fungal genotyping indicated that the
53 endophytes are capable of synthesizing peramine, loline, and ergot alkaloids, which may
54 confer resistance against drought and herbivory (Sneck et al. 2017).

55 *Common garden experiment*

56 *Source material, identification of individual endophyte status and experimental Design.* We es-
57 tablished a common garden experiment at 7 sites across the geographic range of *Elymus*
58 *virginicus* (fig.1). Experimental sites spanned a aridity gradient (temperature gradient
59 in addition to the soil moisture gradient) From the time the plants were placed on the
60 ground to the time we collected demographic data, hourly temperature and soil mois-
61 ture at each site using the HOBO MX2307 data loggers. We used this hourly variable
62 to calculate the daily mean temperature (degC) and soil moisture %(fig.3). The coeffi-
63 cient of variation of soil moisture was estimated to capture season variability in climatic
64 data. The common garden experiment used *E. virginicus* plants that were derived from
65 natural populations throughout the native range in the south-central US (fig.1, Table X).
66 At each of these natural populations we collected seeds. These seeds were planted at
67 Rice University greenhouse on Happy Frog Potting Soil (Samoa, CA) in 3.8 cm 14 cm
68 (107 ml volume) containers. Seedlings were regularly fertilized (Miracle-Gro Liquid All
69 Purpose Plant Food Concentrate) every two weeks. To reach the target numbers for each
70 population, we opted to employ vegetative cloning of the greenhouse-grown plants in
71 order to achieve our desired quantity (N = 840).

72 Before planting in the field, we confirmed the endophyte status of all individuals
73 using either microscopy or an immunoblot assay. This was necessary due to the varying
74 success of the heat treatment and differences in the prevalence of endophytes between the
75 natural populations. Leaf tissues were stained with aniline blue lactic acid and viewed

76 under a compound microscope at 200x-400x to identify fungal hyphae. The immunoblot
77 assay (Phytoscreen field tiller endophyte detection kit, Agrinostics Ltd. Co.) uses mono-
78 clonal antibodies that target proteins of *Epichloë* spp. and chromagen to visually indicate
79 presence or absence. Both methods yield similar detection rates.

80 Common gardens were established in 8 plots per site. Plots were 1.5m X 1.5m and
81 the area was tilled of existing vegetation to control for native plant competition. Plots
82 were also selected in shaded areas under tree canopy or near shrubs to mimic the natural
83 environmental of the species. For each plot, we randomly assigned a starting endophyte
84 frequency (80%, 60%, 40%, 20%, N = 2 for each endophyte frequency, N = 15 plants
85 per plot) and herbivory treatment (herbivory excluded and control, N = 4 for each her-
86 bivory treatment). In each plot, we planted the *E. virginicus* approximately 15 cm deep
87 in an evenly spaced 44 grid pattern, with positions randomly assigned. We ensured that
88 all plots had comparable quantities of populations and genotypes between endophyte
89 statuses and herbivory treatments and that plants had reached similar growth stages.
90 The vegetatively cloned plants were distributed across all sites intentionally to allow for
91 comparison of endophyte hyphae densities. After establishing the plot, we watered the
92 plants and recorded initial tiller counts, flowering status and plot position so that the
93 endophyte status, source population, and genotype of each individual plant was docu-
94 mented. For herbivory exclusion plots, we enclosed them with 1.2m tall mesh fencing to
95 prevent browsing by vertebrate herbivores and sprayed the plots with insecticide (Sevin
96 Insect Killer Concentrate). For herbivory control plots, we half enclosed the plots with
97 the mesh netting to control for the presence of fencing. We stationed one HOBO MX2307
98 data logger at each site to collect temperature and volumetric water content in the soil
99 every hour.

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.

Endophyte density measurements

We collected leaf samples from E+ genotypes that were clonally replicated across two or more sites to quantify endophyte hyphal density and test its associations with host genotype and environmental factors. Relying on clonally replicated genotypes for this analysis allowed us to observe the same genetic individuals in different environments, and partition variation in symbiont density between genetic and environmental sources.

There were seven unique genotypes of E+ hosts (three PALM, three JLP, one SHS) that were clonally replicated across two or more experimental sites. We collected leaf samples from clonal replicates of these genotypes at up to five sites (excluding COL and SON, where no samples were taken) at the time of the demographic census. Samples were taken opportunistically based on survival status and size (we avoided leaf collection from small individuals with few leaves that were vulnerable to mortality). We collected 20 leaf samples in total, consisting of one genotype replicated at five sites, three genotypes replicated at three sites, and three genotypes replicated at two sites.

124 Samples were placed in a cold cooler in the field and then stored in a -20 freezer until
125 processing. In the lab, we examined sections of inner leaf sheath for hyphal density.
126 For each sample, four “peels” of the leaf sheath were placed on a single gridded glass
127 slide (including xmm cells), stained with an aniline blue-lactic acid solution, and viewed
128 under a light microscope at X magnification, following methods in [Sneck et al. 2019](#). We
129 randomly sub-sampled cells on the gridded slide, skipping those with less than 25% leaf
130 peel coverage, until we reached 10 cells for each sample, which amounted to a subsample
131 totaling up to 12mm² per leaf. We took digital images of each grid cell. Using ImageJ,
132 we set the scale using the microscope reticle to measure the visible area of the leaf peel
133 within the cell and the total length of the hyphae fragments within the leaf peel. This
134 yielded an estimate of endophyte density in the units of length (mm) per area (mm²).

135 *Host demography analysis*

136 To assess how stress associated with aridity and low precipitation affect plant-fungal
137 mutualism, we developed five mixed-effects models for each vital rate. Each vital rate
138 was modeled with 10 candidates models. These included XXX

139 In each of the mixed-effects models, we specified two random effects to account
140 for heterogeneity among plot within site and heterogeneity among host source popu-
141 lations. The first random effect was a nested random effect (plot within site) and the
142 second one was a random intercept effect (population). We modeled growth with a
143 Gaussian distribution using the package `lmerTest` ([Source](#)). We modeled fertility (num-
144 ber of spikelet) with a zero-inflated negative binomial distribution using the package
145 `glmmTMB` ([Source](#)). We preferred the negative binomial in which the variance was
146 modeled as a non-linear function of the mean (variance = $m(1 + m/k)$). For each vital
147 rate, the first model was the intercept only model which is the null model. The second

148 and the third models regressed vital rate against the additive effect of endophyte status
149 and mean of soil moisture or coefficient of variation of soil moisture. The fourth and fifth
150 models regressed vital rates against the interaction effect of endophyte status and mean
151 of soil moisture or coefficient of variation of soil moisture (Appendix S1). We compared
152 the five models using the Akaike Information Criterion (AIC) to select the best model
153 ([Source](#)).

154 *Symbiont density analysis*

155 For the subset of E+ plants sampled for endophyte hyphal density, we used AIC-based
156 model selection to quantify support for genetic and environmental influences on sym-
157 biont density. We fit mixed models with a Gaussian error distribution using the natural
158 log of endophyte density as the response variable. All sampled plants had been pre-
159 viously assessed as E+ but a few leaf peel samples had no detectable endophytes. We
160 therefore added an arbitrarily small constant value to the density measurements (0.1) to
161 avoid $\log(0)$. All models used the 10 subsamples as raw data and included the individual
162 ID as a random effect.

163 We analyzed endophyte density with two complementary approaches. In the first
164 set of four candidate models we included host-symbiont genotype, experimental site,
165 neither, or both as explanatory fixed-effect variables (Table). Because we did not have
166 consistent representation of all genotypes at all sites we were not able to fit a geno-
167 type:site interaction, as would arise if different genotypes performed better at different
168 sites. We assessed support for the candidate models using AIC, and for the model that
169 included both genotype and site we partitioned the variance explained by each factor
170 by deriving the marginal R^2 using package `partR2` ([cite](#)). Models including site capture
171 all possible differences across sites. To explore the specific influence of soil moisture,

172 we fit a second set of eight candidate models that included genotype and/or mean soil
173 moisture, and genotype and/or the CV of soil moisture, including interactions.

174 All analyzes were conducted in R 4.1.3 ([Source](#)).

175 **Results**

176 All the best-supported stress vital rate models included endophyte status, suggesting a
177 strong effect of fungal mutualism on plant demography. Soil aridity (change in water
178 content per day) had a large influence on individual growth rate, number of inflores-
179 cences, and number of spikelets (Fig X, Table X). However, that influence was different
180 for endophyte-free individuals and endophyte colonized. Endophyte colonized indi-
181 viduals had a size advantage in a more arid condition than endophyte-free individuals
182 in higher seasonal variation (Fig. A). In contrast, endophyte free individuals produced
183 more spikelet under more arid conditions than endophyte colonized individuals (Fig. C).
184 There was no difference between endophyte-free individuals and endophyte-colonized
185 individuals in flower number (Fig. C).

186 **Discussion**

187 **Acknowledgements**

188 **Author contributions**

189 All authors contributed to study design. THD and TEXM led data analysis, modeling,
190 and writing early drafts of the manuscript. All authors participated in preparing the
191 manuscript for submission.

Figure legends

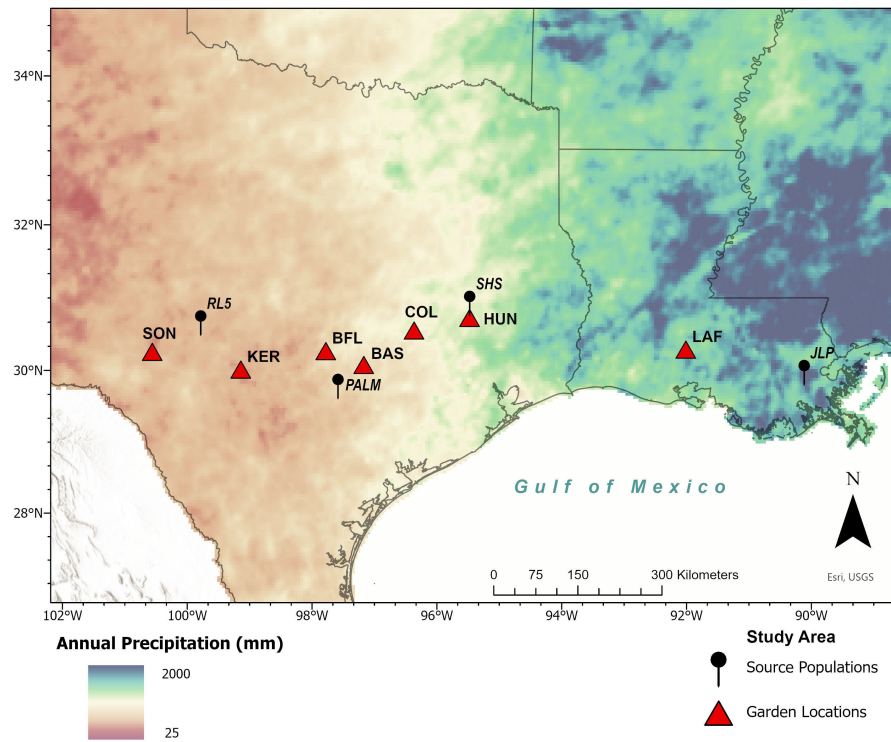


Figure 1

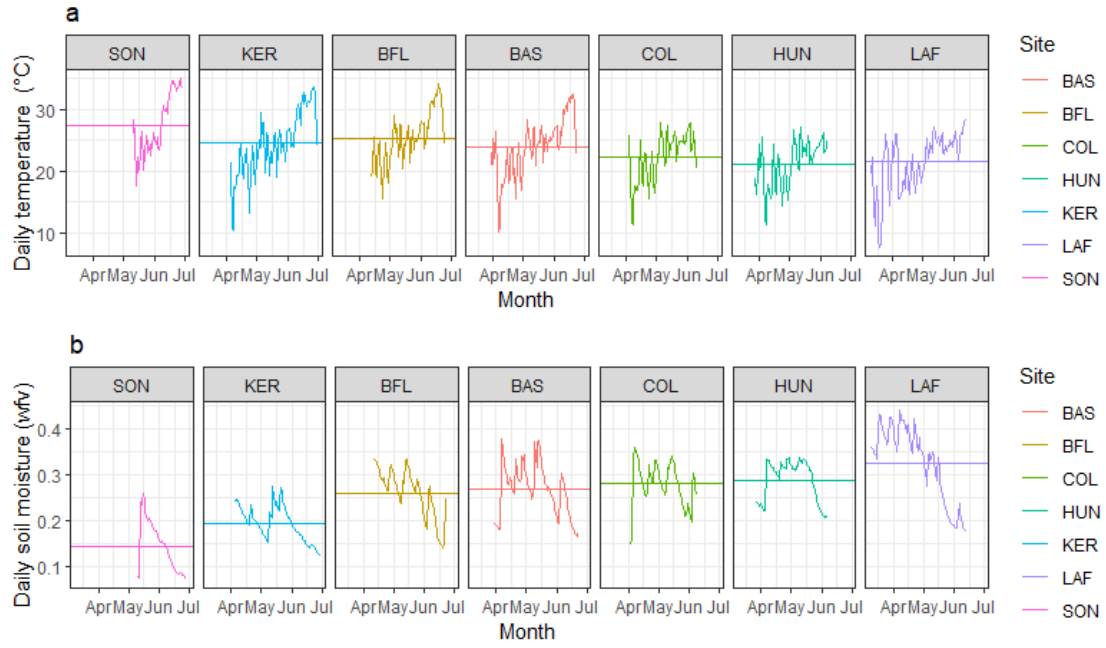


Figure 2

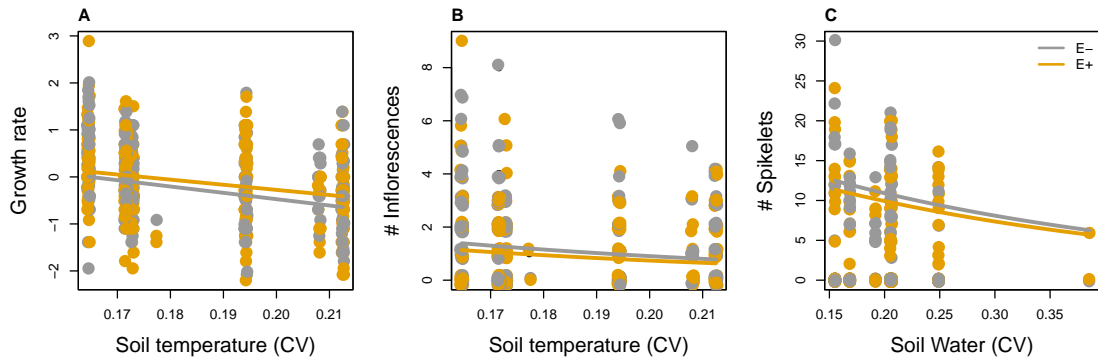


Figure 3