

¹ Increasing prevalence of plant-fungal symbiosis across two
² centuries of environmental change

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⁷ *Manuscript elements:* Figure 1, figure 2, table 1, appendix A (for print; including figure A1,
⁸ figure A2, and table A1), supplemental PDF. Figure 2 is to print in color.

⁹ *Keywords:* .

¹⁰ *Manuscript type:* Article.

¹¹ Prepared using the suggested L^AT_EX template for *Am. Nat.*

Abstract

Species' distributions and abundances are shifting in response to climate change. Most species harbor microbial symbionts that have the potential to influence these responses. Mutualistic microbial symbionts may provide resilience to environmental change by protecting their hosts from increasing stress. Alternatively, environmental change disrupts these interactions may lead to declines in hosts or symbionts. Microbes can be preserved within the dried plant tissue of herbarium specimens presenting an opportunity to quantify changes across broad temporal and spatial scales. We ask how the prevalence of symbionts of grasses, *Epichloë* fungal endophytes, which can protect hosts from drought, have changed over time in response to climate change, and how these changes vary across hosts' ranges. Specifically, we took seed samples from herbarium specimens of three grass host species collected over the last two centuries, quantified fungal hyphae presence within the seeds using microscopy, and evaluated spatial and temporal trends. Overall, endophytes have increased in prevalence over the last two centuries from 25% prevalence to 75% prevalence on average across the three hosts. We also found that changes in prevalence were associated with observed changes in annual and seasonal climate drivers corresponding with each hosts' peak growing season. Thus we provide novel evidence for a cryptic biological response to climate change that may contribute to the resilience of the symbiosis through ongoing environmental change.

Introduction

31 Understanding how biotic interactions are altered by global climate change is a major goal of
32 basic ecological research and conservation management (Blois et al., 2013; Gilman et al., 2010).
33 Documented responses to environmental change, such as shifts in species' distributions (Aitken
34 et al., 2008) and phenology (Piao et al., 2019), are typically blind to concurrent changes in associ-
35 ated biotic interactions. Empirically evaluating these biotic changes, whether interacting species
36 shift in tandem with their partners or not (HilleRisLambers et al., 2013), is crucial to predicting
37 future climate change responses. Such evaluations have been limited because data on species
38 interactions are limited, with few datasets extending over the long time scales of contemporary
39 climate change (Poisot et al., 2021).

40 Natural history collections, which were originally collected to study and preserve taxonomic
41 diversity, present a unique opportunity to explore long-term changes in ecological interactions
42 across broad spatial scales (Meineke et al., 2018). Natural history collections, built and main-
43 tained by the efforts of thousands of scientists, are invaluable time machines, primarily com-
44 prised of physical specimens of organisms along with information about the time and place of
45 their collection. These specimens are samples preserving physical legacies of ecological processes
46 and species' interactions from dynamically changing environments across time and space. Re-
47 searchers have used plant collections to document climate change responses, including shifts in
48 phenology (Berg et al., 2019; Park et al., 2019; Willis et al., 2017), as well as in rates of pollination
49 (Duan et al., 2019; Pauw and Hawkins, 2011), and herbivory (Meineke et al., 2019). However,
50 focus has been lacking on long-term changes in a particularly common type of biotic interaction,
51 microbial symbioses.

52 Microbial symbionts are common to all macroscopic organisms and can have important ef-
53 ffects on their hosts' survival, growth and reproduction (McFall-Ngai et al., 2013; Rodriguez et al.,
54 2009). Many microbial symbionts act as mutualists, engaging in reciprocally beneficial interac-
55 tions with their hosts that ameliorate environmental stress. For example, bacterial symbionts of

56 insects, such as *Wolbachia*, can improve their hosts' thermal tolerance (Renoz et al., 2019; Truitt
57 et al., 2019), and arbuscular mycorrhizal fungi, documented in 70-90% of families of land plants
58 (Parniske, 2008), allow their hosts to persist through drought conditions by improving water and
59 nutrient uptake (Cheng et al., 2021). On the other hand, changes in the mean and variance of
60 environmental conditions may disrupt microbial mutualisms by changing the costs and benefits
61 of the interaction for each partner, leading the interaction to deteriorate (Aslan et al., 2013). Coral
62 bleaching (the loss of symbiotic algae) due to temperature stress (Sully et al., 2019) is perhaps the
63 best known example, but this phenomenon is not unique to corals. Lichens exposed to elevated
64 temperatures experienced loss of photosynthetic function along with changes in the composition
65 of their algal symbiont community (Meyer et al., 2022). How commonly and under what condi-
66 tions microbial mutualisms deteriorate or strengthen under climate change remain unanswered
67 questions, but previous work suggests that these alternative responses may depend on the inti-
68 macy and specialization of the interaction as well as the physiological tolerances of the mutualist
69 partners (Rafferty et al., 2015; Toby Kiers et al., 2010; Warren and Bradford, 2014).

70 Understanding of how microbial symbioses are affected by climate change is additionally
71 complicated by spatial heterogeneity in the direction and magnitude of environmental change
72 (IPCC, 2021). Beneficial symbionts are likely able to shield their hosts from environmental stress
73 in locations that experience a small degree of change, but symbionts in locations that experience
74 changes of large magnitude may be pushed beyond their physiological limits (Webster et al.,
75 2008). Additionally, symbionts are often unevenly distributed across their hosts' distribution.
76 Facultative symbionts may be absent from portions of the host range (Afkhami et al., 2014),
77 and hosts may engage with a diversity of partners (different species, or different locally-adapted
78 strains of one species) across their environments (Frade et al., 2008; Rolshausen et al., 2018). Iden-
79 tifying broader spatial trends in symbiont prevalence is therefore an important step in developing
80 predictions for where to expect changes in the symbiosis in future climates.

81 *Epichloë* fungal endophytes are specialized symbionts of cool-season grasses, which have been
82 documented in ~ 30% of cool-season grasses (Leuchtmann, 1992) They are transmitted vertically

83 from maternal plants to offspring through the grasses' seeds. Vertical transmission creates a feed-
84 back between the fitness of host and symbiont (Douglas, 1998; Fine, 1975; Rudgers et al., 2009).
85 Over time, endophytes that act as mutualists for their hosts should rise in prevalence within a
86 population. *Epichloë* are known to improve their hosts' drought tolerance (Decunta et al., 2021),
87 and protect their hosts against herbivores (Crawford et al., 2010) and pathogens (Xia et al., 2018)
88 likely through the production of a diverse suite of alkaloids and other secondary metabolites. The
89 fitness feedback induced by vertical transmission leads to the prediction that endophyte preva-
90 lence should be high in populations where these fitness benefits are most important. Previous
91 survey studies have documented large-scale spatial patterns in endophyte prevalence structured
92 by environmental gradients (Afkhami, 2012; Bazely et al., 2007; Granath et al., 2007; Sneck et al.,
93 2017). We predicted that prevalence should track temporal changes in environmental drivers
94 that elicit these fitness benefits. For example, endophyte-mediated drought tolerance should
95 lead prevalence to increase in regions where precipitation declines over time. Early research on
96 *Epichloë* used herbarium specimens to describe the broad taxonomic diversity of hosts (White
97 and Cole, 1985). Grasses are commonly identified and collected based on the presence of their
98 reproductive structures meaning that collected specimens typically contain seeds, conveniently
99 preserving the fungi along with their host plants on herbarium sheets. This creates the oppor-
100 tunity to leverage the unique spatio-temporal sampling of herbarium collections to examine the
101 response of the symbiosis to historical climate change.¹

102 In this study, we assessed for the first time the long-term responses of endophyte symbiosis to
103 climate change through the use of herbarium specimens of three *Epichloë* endophyte host species,
104 *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*. We first address questions describing
105 spatial and temporal trends in endophyte prevalence: (i) How has endophyte prevalence changed
106 over time? and (ii) How spatially heterogeneous are temporal trends in endophyte prevalence? We
107 then address how climate change may be driving these trends in endophyte prevalence by asking:
108 (iii) What is the relationship between variation in temporal trends in endophyte prevalence and

¹Volker's suggestions included moving all material specific to study system into methods, but it seems a bit difficult to do.

¹⁰⁹ changes in climate drivers? We examined 2,346 specimens collected across eastern North
¹¹⁰ America between 1824 and 2019.

¹¹¹ **Methods**

¹¹² *Focal species*

¹¹³ Our surveys focused on three endophyte hosts, *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus*
¹¹⁴ *virginicus*. Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001; Leuchtmann et al.,
¹¹⁵ 2014), while *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl, 2002). These C₃
¹¹⁶ grass species are commonly represented in natural history collections with broad distributions
¹¹⁷ covering much the Eastern United States. *A. hyemalis* is a small short-lived perennial species
¹¹⁸ that germinates in the spring and typically blooms between March and July (most common
¹¹⁹ collection month: May). *A. perennans* is of similar stature but is longer lived than *Agrostis hyemalis*
¹²⁰ and blooms in late Summer and early Autumn (most common collection month: September).
¹²¹ This species is relatively more sparsely distributed, tending to be found in shadier and more
¹²² moist habitats while *A. hyemalis* is often found in open and recently disturbed ground. Both
¹²³ *Agrostis* species are recorded from throughout the Eastern US, but *A. perennans* has a slightly
¹²⁴ more northern distribution, whereas *A. hyemalis* is found rarely as far north as Canada and is
¹²⁵ listed as a rare plant in Minnesota. *E. virginicus* is a larger and relatively longer-lived species that
¹²⁶ is more broadly distributed than the *Agrostis* species. It begins flowering as early as March or
¹²⁷ April but continues throughout the Summer (most common collection month: July).

¹²⁸ *Herbarium surveys*

¹²⁹ We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens
¹³⁰ included from each collection). In total, we quantified endophyte symbiosis for 1135 *A. hyemalis*
¹³¹ specimens collected between 1824 and 2019, 357 *A. perennans* specimens collected between 1863
¹³² and 2017, and 854 *E. virginicus* specimens collected between 1839 and 2019 (Fig. 1, Fig 2A).

133 Our sampling plan was designed to minimize damage to these specimens. We chose our focal
134 species in part because they are commonly represented in herbarium collections, and produce
135 high numbers of seeds, meaning that small samples would not diminish the value of the spec-
136 imens for future studies. We collected 5-10 seeds per specimen after examining the herbarium
137 sheet under a dissecting microscope to ensure that we collected mature seeds, not florets or un-
138 filled seeds, fit for our purpose of identifying fungal endophytes with microscopy. We excluded
139 specimens for which information about the collection location and date were unavailable. Each
140 specimen was assigned geographic coordinates based on collection information recorded on the
141 herbarium sheet using the geocoding functionality of the ggmap R package (Kahle et al., 2019).
142 Many specimens had digitized collection information readily available, but for those that did
143 not, we transcribed information from pictures of the specimens. Collections were geo-referenced
144 to the nearest county centroid, or nearest municipality when that information was available. For
145 a few of the oldest specimens, only information at the state level was available, and so we used
146 the state centroid.

147 After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hy-
148 phae, which grow intercellularly, in each specimen using microscopy. We first softened seeds
149 with a 10% NaOH solution, then stained the seeds with aniline blue dye and squashed them un-
150 der a microscope cover slip. We examined the squashed seeds for the presence of fungal hyphae
151 at 100X magnification (Bacon and White, 2018). In some cases, the tissues examined during mi-
152 croscopy came from flowers or otherwise non-viable seeds, which were excluded from the seed
153 counts for that specimen. On average we scored 4.7 seeds per specimen in *A. hyemalis*, 4.2 seeds
154 per specimen in *A. perennans*, and 3.8 seeds per specimen in *E. virginicus*. Due to imperfect verti-
155 cal transmission (Afkhami and Rudgers, 2008), it is possible that symbiotic host-plants produce a
156 mixture of symbiotic and non-symbiotic seeds. We therefore designated each specimen as sym-
157 biotic if endophyte hyphae were observed in one or more seeds, or non-symbiotic if hyphae were
158 observed in zero seeds. To capture uncertainty in the identification process, we recorded both
159 a "liberal" and a "conservative" endophyte status for each plant. When we identified potential

¹⁶⁰ endophytes with unusual morphology, low uptake of stain, or a small amount of fungal hyphae
¹⁶¹ across the scored seeds, we recorded a positive liberal status (more likely to be a true endophyte)
¹⁶² and a negative conservative status (less likely to be a true endophyte). 89% of scored plants had
¹⁶³ matching liberal and conservative endophyte statuses, reflecting high confidence in endophyte
¹⁶⁴ identification. The following analyses presented in the main text used the liberal statuses, but we
¹⁶⁵ repeated all analyses with the conservative statuses which yielded qualitatively similar results
¹⁶⁶ (Figure A5).

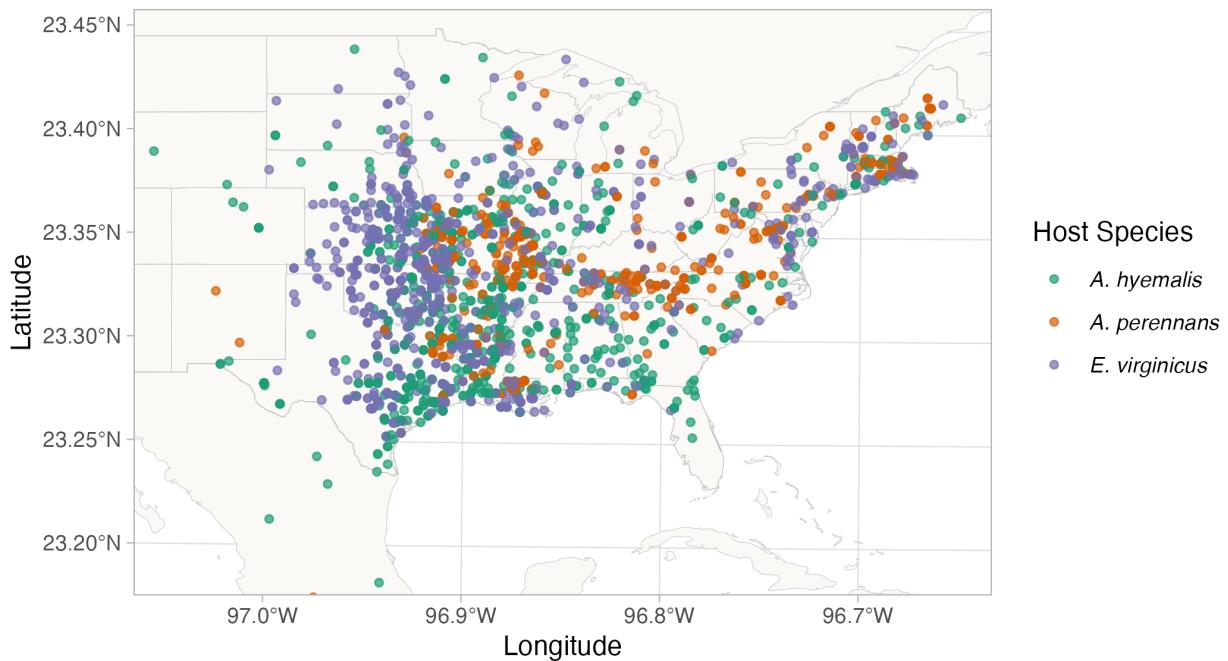


Figure 1: **Collection locations of herbarium specimens sampled for endophyte presence absence.** Color designates host species (*A. hyemalis* (green), *A. perennans* (orange), *E. virginicus* (purple))

¹⁶⁷ Assessing spatial and temporal changes in endophyte prevalence

¹⁶⁸ We assessed spatial and temporal changes in endophyte prevalence across each host distribu-
¹⁶⁹ tion, first quantifying the long-term overall trends and then examining how these trends vary

across the spatial extent of each host distribution. We used an approximate Bayesian method,
 Integrated Nested Laplace Approximation (INLA), to construct spatio-temporal models of en-
 dophyte prevalence. INLA provides a computationally more efficient method of ascertaining
 model posteriors for certain models that can be formulated as latent Gaussian Models (Rue
 et al., 2009). Many common statistical models, including structured and unstructured mixed-
 effects models, can be represented as latent Gaussian Models. Fitting models with structured
 spatial effects is possible with MCMC sampling but can require long computation times, mak-
 ing INLA an effective alternative, which has been used to model spatial patterns in flowering
 phenology (Willems et al., 2022), the abundance of bird species (Meehan et al., 2019) and but-
 terflies citecrossley2022opposing, the distribution of temperate trees (Engel et al., 2022) as well
 as the population dynamics of endangered amphibians (Knapp et al., 2016) and other ecological
 processes (Beguin et al., 2012).

To quantify overall trends in endophyte prevalence, we modeled endophyte presence/absences
 (P) as a Bernoulli response variable for each specimen s at a given location i . In all cases, models
 for each species were fit separately.

$$P_{s,i} \sim \text{Bernoulli}(\hat{P}_{s,i}) \quad (1a)$$

$$\text{logit}(\hat{P}_{s,i}) = \alpha_{s,i} + \beta_s * \text{year}_i + \chi_i + \omega_i \quad (1b)$$

The expected endophyte prevalence, \hat{P} , was modelled with spatially-indexed random inter-
 cepts α that accounts for potential spatial autocorrelation between data points, and with a slope
 β describing the overall change in endophyte prevalence. We accounted for potential biases in-
 troduced during the process of collecting specimens as well as in scoring ability by including
 random effects specific to each collector χ and to each scorer ω . Previous work has questioned
 whether the behavior of historical botanists and uneven sampling may introduce biases into eco-
 logical inferences made from historic collections (Kozlov et al., 2020). Prolific collectors who
 contribute thousands of specimens may be more or less likely to collect certain species, or spec-

imens with certain traits (Daru et al., 2018). Similarly, the process of scoring seeds for hyphae involved many individual researchers, who may vary in their likelihood of positively identifying fungal hyphae. By including a random effect for collectors and for scorers, we accounted for variance across individual researchers that may bias our predictions of changes in endophyte prevalence.

To quantify how temporal trends may vary spatially, we repeated the modelling above, but instead incorporating a spatially-varying coefficient for collection year.

$$P_{s,i} \sim Bernoulli(\hat{P}_{s,i}) \quad (2a)$$

$$\text{logit}(\hat{P}_{s,i}) = \alpha_{s,i} + \tau_{s,i} * \text{year}_i + \chi_i + \omega_i \quad (2b)$$

Note that the expected endophyte prevalence, \hat{P} , was modelled as above, and that τ describes the spatially-varying random slope for collection year. This spatially-varying random effect allowed us to flexibly estimate variation in slope at locations across the study region. All spatially-structured random intercepts and slopes were constructed using stochastic partial differential equations (SPDE) that depend on a covariance matrix according to the proximity of each collection location (Bakka et al., 2018; Lindgren et al., 2011). The covariance matrix was approximated using a Matérn covariance function, with each data point assigned a location according to the nodes of a mesh of non-overlapping triangles across our study area (Fig A2).

We performed model fitting using the `inlabru` R package (), with vague priors, and compared models with different sizes of mesh, which had little effect on the resulting model estimates. Each mesh was bounded by the predicted host distribution, described below. Posterior modes were stable indicating that numeric convergence was successful. We assessed model fit with graphical posterior predictive checks (Fig. A3). The model performed adequately at classifying the historical data, comparing the accuracy of predictions from the model with observed data (avg. AUC = 0.77; Fig. A4).

215

Modeling distributions of host species

216 Jacob needs to write this, but we built SDMs using MaxEnt and the same climate predictors
 217 described below.

218

Validating the model with an out-of-sample test

219 We evaluated the predictive ability of the model using contemporary endophyte surveys as out-
 220 of-sample test data, an important but rarely used strategy in ecological studies (Tredennick et al.,
 221 2021). We used data from contemporary surveys of endophyte prevalence in *A. hyemalis* and *E.*
 222 *virginicus*. Surveys of *E. virginicus* were conducted in 2013 as described in Sneck et al. (2017),
 223 and surveys of *A. hyemalis* took place between 2015 and 2020. Population surveys of *A. hyemalis*
 224 were initially designed to cover longitudinal variation in endophyte prevalence towards its range
 225 edge, while surveys of *E. virginicus* were designed to cover latitudinal variation along its range
 226 edge. In total, we visited 43 populations of *A. hyemalis* and 20 populations of *E. virginicus* across
 227 the central southeastern US, in Texas and neighboring states (Fig A4). During surveys, we col-
 228 lected seeds from up to 30 individuals per location (average number of plants sampled: 22.9).
 229 We quantified the endophyte status of each individual with staining microscopy as described for
 230 the herbarium surveys, and calculated the prevalence of endophytes within the population (pro-
 231 portion of symbiotic plants divided by the number of sampled plants). The contemporary survey
 232 period (2013-2020) is at the most recent edge of the time period encompassed by the historical
 233 observations used for model fitting. We compared the model's prediction for these locations to
 234 the observed population prevalence.²

²considering dropping this section because the model does a good job at predicting the mean regional prevalence, but has way less variability than the contemporary surveys

235

Assessing the role of climate drivers

236 We assessed how the magnitude of climate change may have driven changes in endophyte preva-
237 lence by assessing correlations between changes in climate and changes in endophyte prevalence
238 predicted from our spatial model at evenly spaced pixels across the study area. We first down-
239 loaded monthly temperature and precipitation rasters from the PRISM climate group (Daly and
240 Bryant, 2013) covering the time period between 1895 and 2020 using the 'prism' R package (Hart
241 and Bell, 2015). Prism provides reconstructions of historic climate variables across the United
242 States by spatially-interpolating weather station data (Di Luzio et al., 2008). We calculated 30-
243 year climate normals for annual and seasonal mean temperature and cumulative precipitation
244 for the recent (1990 to 2020) and historic (1895 to 1925) periods. We used three four-month
245 seasons within the year (Spring: January, February, March, April; Summer: May, June, July, Au-
246 gust; Autumn: September, October, November, December). This division of seasons allowed us
247 to quantify differences in climate associated with the two "cool" seasons that shoulder summer
248 when we expect our focal species to be most biologically active (*A. perennans*: Spring; *E. virginicus*:
249 Spring and Summer; *A. perennans*: Fall). In addition to mean climate conditions, environmental
250 variability in and of itself can influence population dynamics (Tuljapurkar, 1982) and changes in
251 variability are a key prediction of climate change models (IPCC, 2021; Stocker et al., 2013). So we
252 calculated the coefficient of variation during each period for each annual and seasonal climate
253 driver as the interannual standard deviation divided by the mean across each 30-year period.
254 We then took the difference between recent and historic periods for the mean and coefficient of
255 variation for each climate driver (Fig. A5). Because initial analyses indicated a high degree of
256 collinearity between seasonal and annual changes in temperature, we used annual temperature
257 only, along with annual and seasonal precipitation, in the subsequent analysis. All together, this
258 left us with measurements of change in 10 potential climate drivers: the mean and coefficient
259 of variation of annual temperature, as well as the mean and coefficient of variation of cumula-
260 tive annual precipitation, cumulative spring precipitation, cumulative summer precipitation, and

261 cumulative autumn precipitation (Fig A8-A9).

262 To evaluate whether areas that have experienced high degrees of change in climate have
263 experienced the greatest changes in endophyte prevalence, we explored correlations between
264 these climate drivers and the predicted spatially-varying trends with a Gaussian model:

$$\tau_i \sim Normal(\hat{\mu}_i, \sigma^2) \quad (3a)$$

$$\hat{\mu}_i = \beta * \mathbf{X}_{env} \quad (3b)$$

265 where the trend at a given location (τ_i) is predicted by our environmental covariates \mathbf{X}_{env}
266 according to the vector of parameters β . Calculating correlations from many pixels across the
267 study region risks artificially inflating confidence in our results due to large sample sizes, and
268 so we repeated this calculation using only a random subsample of 100 pixels across the study
269 region.

270 Results

271 *How has endophyte prevalence changed over time?*

272 We found that endophyte prevalence increased within the examined specimens over the last
273 two centuries for all three host species (Fig. 4). On average, *A. hyemalis* and *E. virginicus* both
274 increased from 30 % to over 70% prevalence across the study region, and *A. perennans* increased
275 from 15% to over 70% prevalence. Our model indicates a higher certainty that overall temporal
276 trends are positive for *A. hyemalis* and *A. perennans* than for *E. virginicus* (99% probability of a
277 positive overall year slope in *A. hyemalis*, 89% probability of a positive overall year slope in *A.*
278 *perennans*, and 58% probability of a positive overall year slope in *E. virginicus*).³

³These numbers are currently outdated. I am making some adjustments to models, and will update with final model

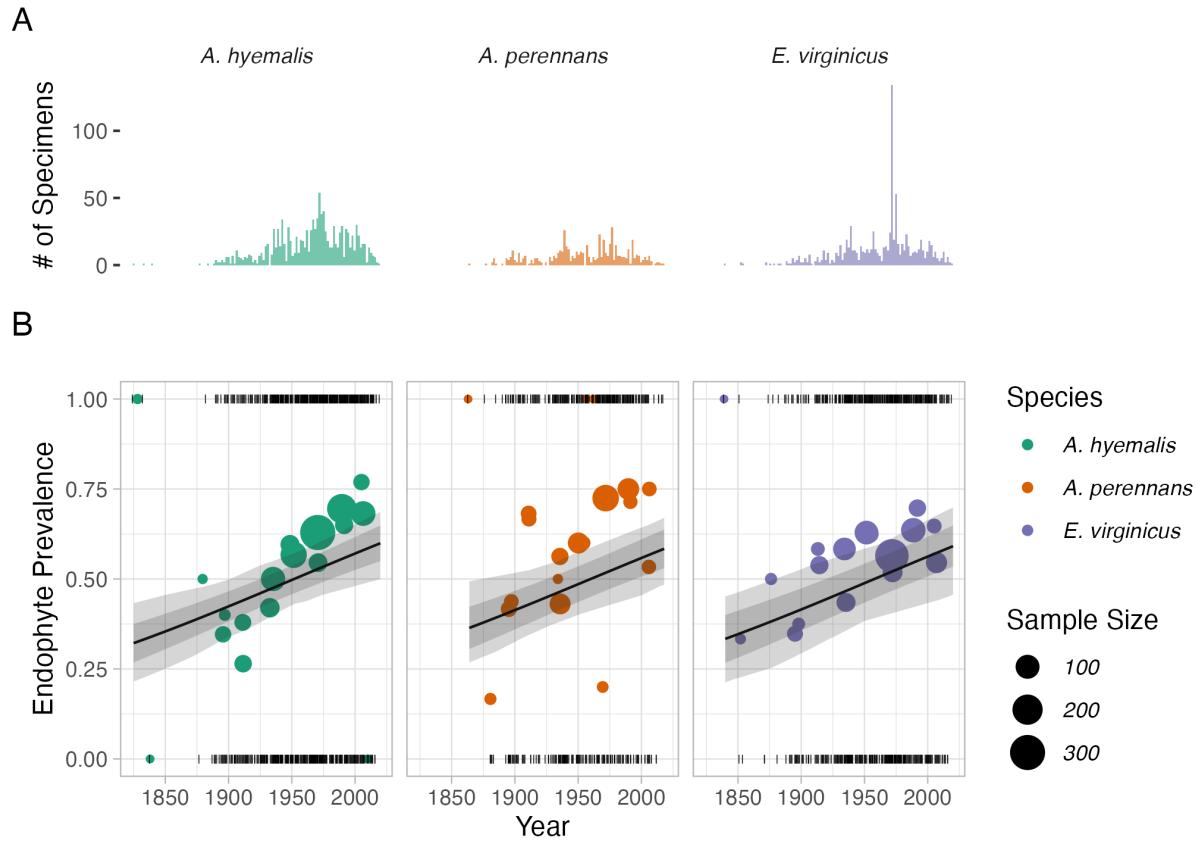


Figure 2: Temporal trends in endophyte prevalence. (A) Histograms show the frequency of collection through time for each host species. (B) Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species and point size is determined by the number of specimens. Lines show predicted mean endophyte prevalence over the study period along with the 50% and 95% CI bands.

279 *How spatially heterogenous are temporal trends in endophyte prevalence?*

280 Our model revealed hotspots of change in endophyte prevalence . While there was an overall
 281 increase in endophyte prevalence, these changes varied across the host species' ranges (Fig. 3).
 282 In some regions, posterior estimates of our spatially varying temporal trends, τ , indicate that *A.*
 283 *hyemalis* and *A. perennans* experienced increases in percent prevalence by as much as 4% per year

284 over the study period, while *E. virginicus* experienced increases up to around 1.5 % per year. In
285 other regions, there were negligible changes. Notably, the symbionts of *E. virginicus* experienced
286 only slight increases in prevalence, and were less spatially variable than the other two species.
287 Regions that start with low endophyte prevalence, as in the southwestern portion of the range
288 of *A. hyemalis*(Fig. A1), also experienced negligible change, suggesting that this may be driven
289 more by the absence of the endophyte.⁴ Predicted trends for *A. perennans* show certain areas of
290 both large increase and of large decrease, however this species, for which we have the fewest
291 samples, has the largest uncertainty. The posterior estimates of our spatially varying temporal
292 trends, indicate relatively narrow certainty (need to compute⁵).

⁴more discussion material, but putting it here for now.

5

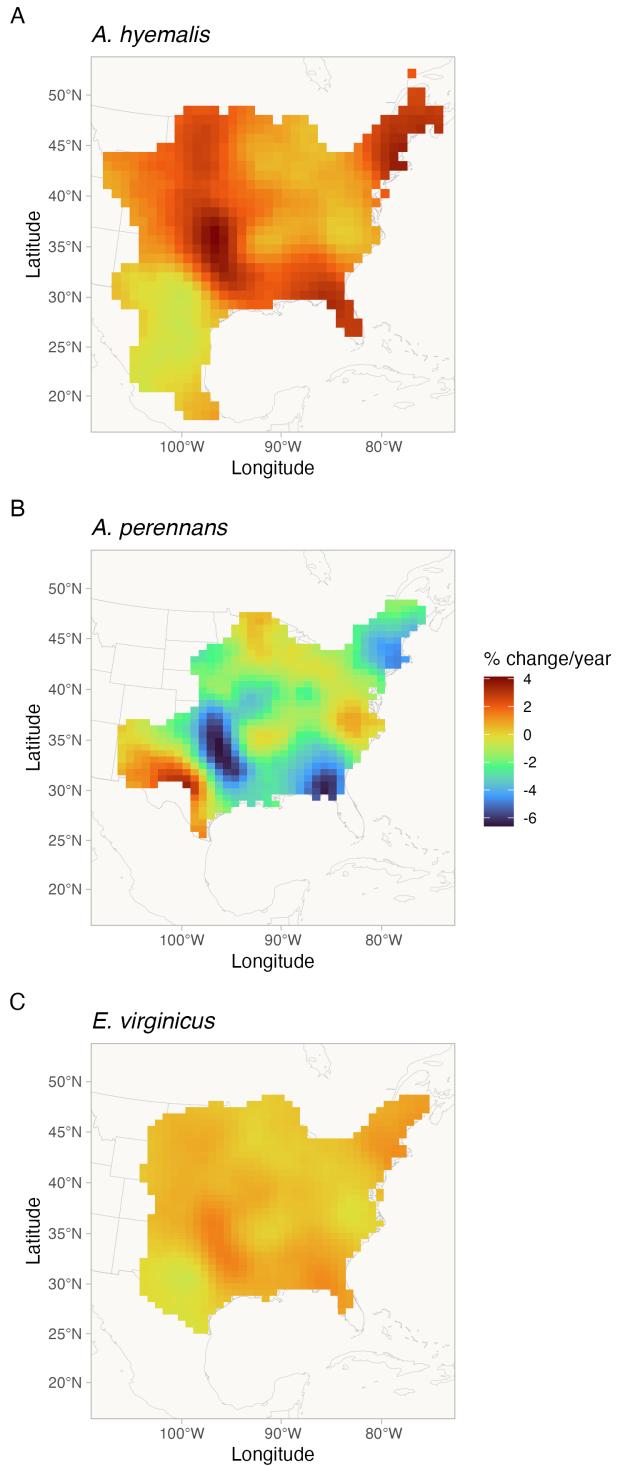


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species. Color indicates the relative change in predicted endophyte prevalence.

293 *Assessing collector and scorer influences on predicted endophyte prevalence*

294 We quantified temporal and spatial trends in endophyte prevalence while accounting for poten-
295 tial biases introduced by collectors and by individuals who quantified endophyte presence/absence
296 with the use of random effects. We found no evidence that collector biases influenced our re-
297 sults. Collector random effects were consistently small; Fig 4A, and models fit with and without
298 this random effect provide qualitatively similar results. The identity of individual scorers did
299 contribute to observed patterns in endophyte prevalence. For example, 3 of the 16 scorers were
300 more likely than average to assign positive endophyte status, as indicated by 95% credible in-
301 tervals that do not overlap 0) (Fig 4B). However, this may have been driver by differences in
302 scorers biases during the seed scoring process, or by unintended spatial clustering of the speci-
303 mens scored by each scorer. Interpreting our models with the inclusion of the scorer effect thus
304 provides conservative estimates of the absolute magnitude of changes in endophyte prevalence.

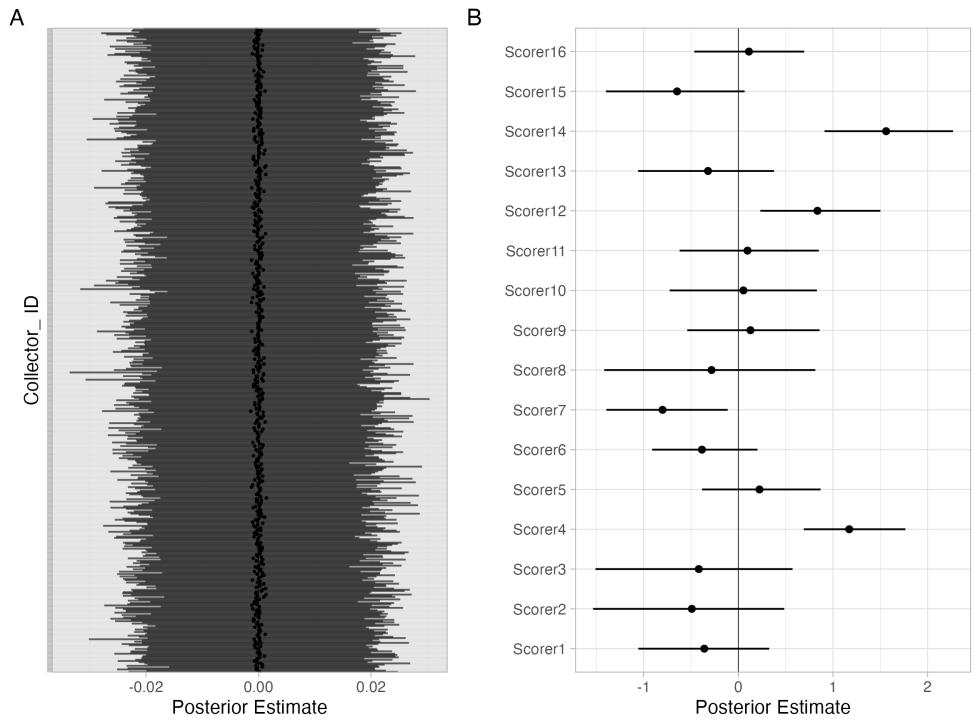


Figure 4: Posterior estimates of (A) collector and (B) scorer random effects. Points show the posterior mean along with 95% CI for random effects estimate from 532 collectors and 16 scorers.

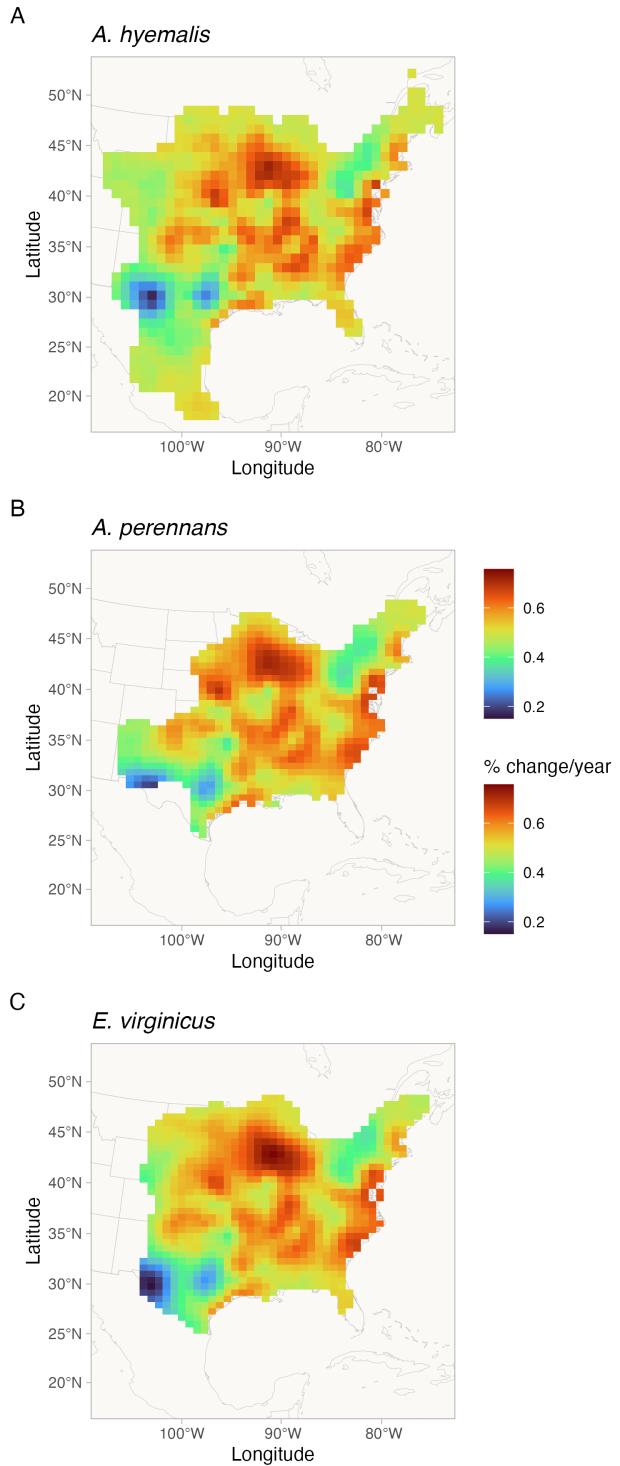


Figure 5: Mean predicted endophyte prevalence for each host species (columns) in 1925 (top row) and 2020 (bottom row). Color indicates mean predicted rate of endophyte prevalence across the predicted distribution of each species.

305 What is the relationship between variation in temporal trends in endophyte
306 prevalence and changes in climate drivers?

307 We found that trends in endophyte prevalence were strongly associated with seasonal climate
308 change drivers (Fig. 6). For the majority of the study region, the climate has become wetter and
309 cooler over the last century (Fig. A7-A8), a consequence of regional variation in global climate
310 change (IPCC, 2021). Within the study region, spatially heterogeneous environmental changes
311 were predictive of changes in endophyte prevalence. For example, strong increases in prevalence
312 within *E. virginicus* were most associated with declines in Summer precipitation (a negative cor-
313 relation in Fig. 7) as well as with increases in the year-to-year variability of annual temperature
314 (a positive correlation in Fig. 7). Changes were also associated with reductions in average an-
315 nual temperatures, and increases in year-to-year temperature variability. *A. perennans* endophyte
316 prevalence increased most strongly in regions that experienced reduced spring precipitation and
317 reduced variability in annual temperature. Although these correlations were weaker, changes
318 in *A. perennans* endophyte prevalence were also associated with increased in increases in annual
319 precipitation and increasing autumn precipitation. For *A. hyemalis*, endophyte prevalence in-
320 creased most strongly in regions that experienced reductions in autumn precipitation variability.
321 Correlations using only a subsampling of pixels were qualitatively similar to these results (Fig.
322 A11), suggesting that the patterns we find are not spurious associations.

323 6

⁶Only have plotted results for AGHY right now.

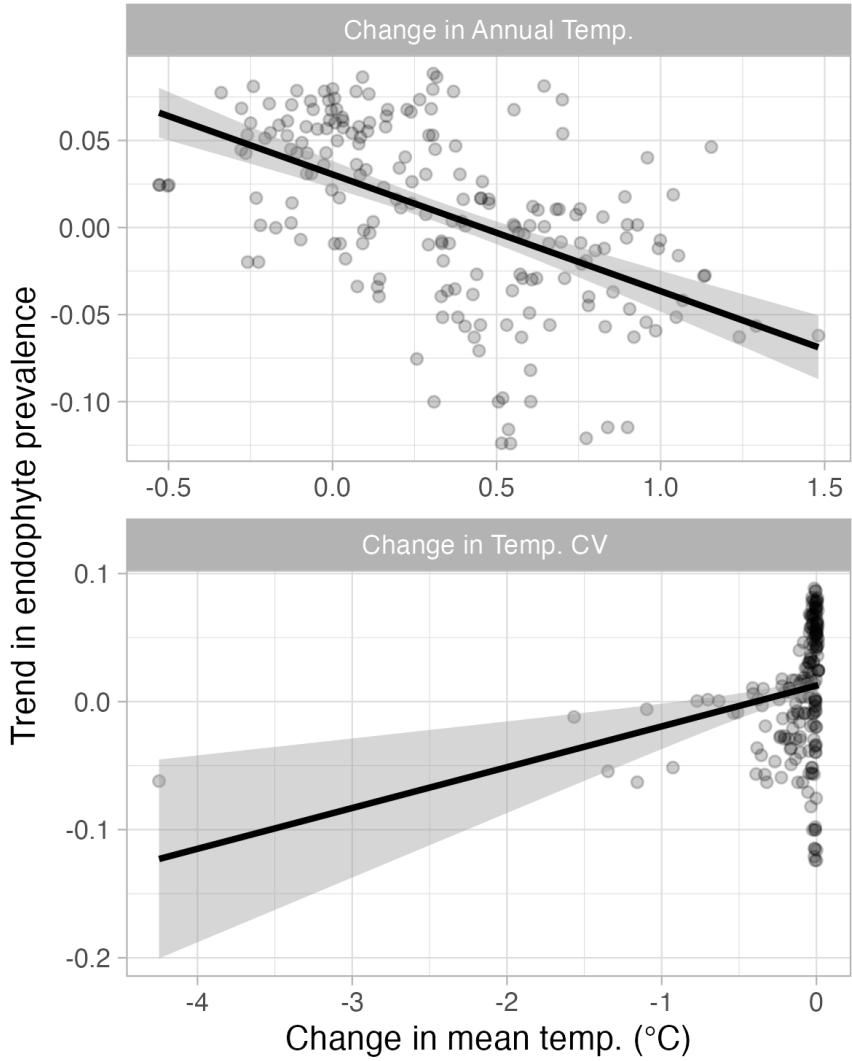


Figure 6: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (mm), as well as the change in the coefficient of variation of each climate driver. Positive correlation coefficients indicate that greater increases in a climate driver were associated with larger increases in endophyte prevalence, while negative values indicate that . Asterisks denote correlation coefficients $> .3$ or $< -.3$.

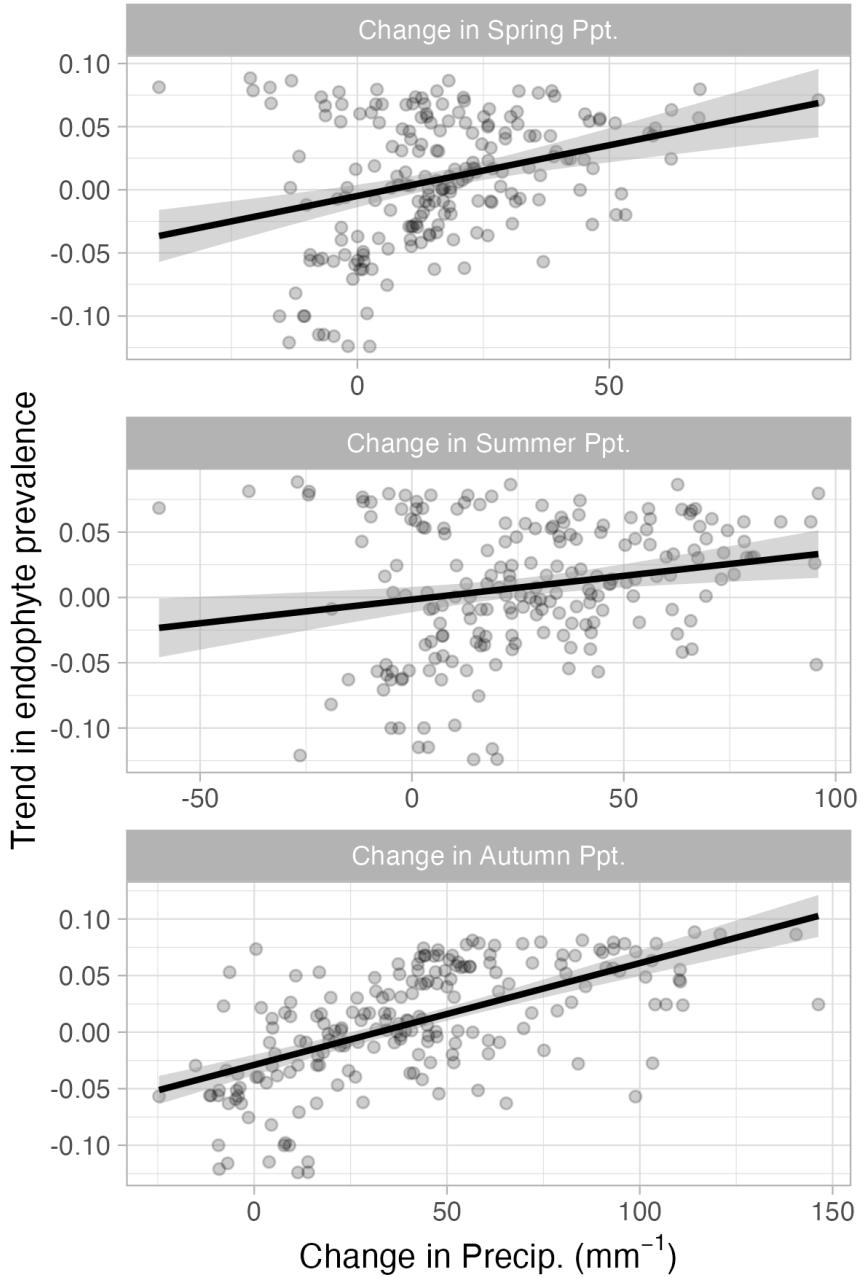


Figure 7: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (mm), as well as the change in the coefficient of variation of each climate driver. Positive correlation coefficients indicate that greater increases in a climate driver were associated with larger increases in endophyte prevalence, while negative values indicate that .
 Asterisks denote correlation coefficients $> .3$ or $< -.3$.

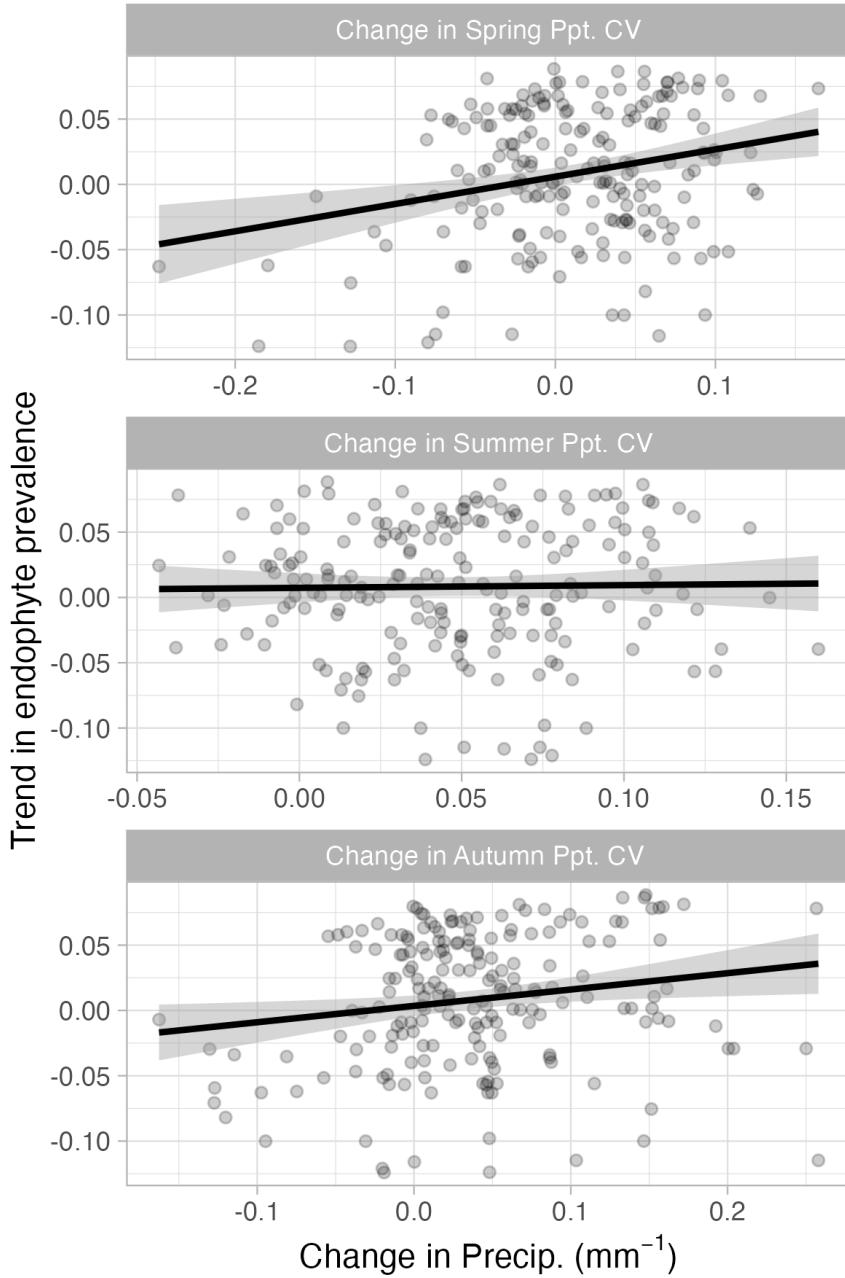


Figure 8: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (mm), as well as the change in the coefficient of variation of each climate driver. Positive correlation coefficients indicate that greater increases in a climate driver were associated with larger increases in endophyte prevalence, while negative values indicate that .
23 Asterisks denote correlation coefficients $> .3$ or $< -.3$.

324 *Performance on test data*

325 We found that while the model predicts broader regional trends in endophyte prevalence present
 326 in the contemporary survey data such as declining endophyte prevalence towards western longi-
 327 tudes in *A. hyemalis* (Fig. 6 B-C), however the contemporary data contains additional variability
 328 at smaller scales not captured by our sampling of herbarium specimens. We interpreted this to
 329 mean that the model captured regional spatial dynamics, but underpredicts local scale dynamics.
 330 We discuss potential model improvements in the Discussion.

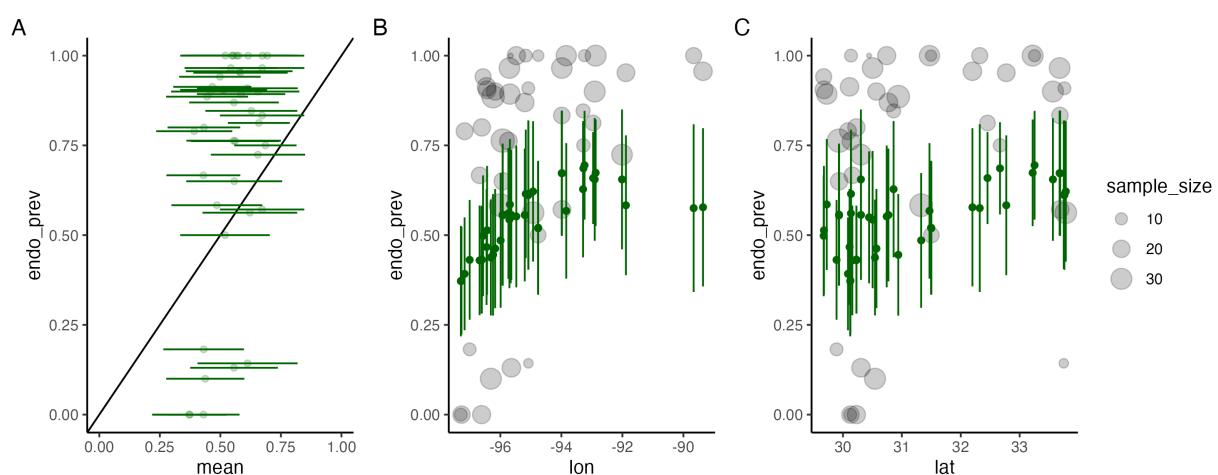


Figure 9: **Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporaneous endophyte prevalence in *A. hyemalis*, as indicated by some overlap of predicted 95% CI with the 1:1 line, however contemporary test data generally had more variance between populations than model predictions. The model did recapitulate broader regional trends across (B) longitude and (C) latitude. Point size in panels B and C reflect sample sizes of contemporary endophyte population surveys.

Discussion

332 Our examination of historic plant specimens revealed a cryptic biotic reponse to climate change.
333 For the three host species we examined, there have been clear increases in fungal endophyte
334 prevalence over the last two centuries. Increases in prevalence of *Epichloë*, which are vertically
335 transmitted, can potentially be interpreted as adaptive changes that improve the fitness of their
336 hosts under stressful conditions. This interpretation is in line with theory predicting that the
337 positive fitness feedback caused by vertical transmission leads beneficial symbionts to rise in
338 prevalence within a population (Fine, 1975). We found that trends in endophyte prevalence var-
339 ied across the distribution of each species in assocation with observed changes in climate drivers,
340 suggesting that the endophytes have contributed to host resilience under environmental change.
341 Taken together, this suggests a strengthening of the mutualism over the last two centuries.

342 Differences between the responses of each host species underscore that while all of these
343 C₃ grasses share similar broad-scale distributions, each engages in unique biotic interactions
344 and has unique niche requirements. We identified hotspots of change for *A. perennans*, which
345 experienced the strongest absolute changes in endophyte prevalence (Fig. 5). Declines in the
346 southern portion of its range and increases in the north suggest a potential poleward range shift
347 of endophytic plants. Based on previous work demonstrating that endophytes can shield their
348 hosts from drought stress (Decunta et al., 2021), we generally predicted that drought conditions
349 could be a driver of increasing endophyte prevalence. In line with this expectation, increas-
350 ing prevalence for this species was associated with decreasing precipitation, most strongly with
351 autumn-season declines (Fig. 7). *A. perennans* typically blooms in the autumn. Endophytes could
352 be playing a role helping hosts weather autumn-season droughts while the species is dormant.
353 It may be useful to investigate whether lagged climate effects are important predictors of host
354 fitness in this system (Evers et al., 2021). To our knowledge, the response of the symbiosis in
355 *A. perennans* to drought has not been examined experimentally, but in a greenhouse experiment,
356 endophytes had a positive effect on host reproduction under shaded, low-light conditions (Davitt

357 et al., 2010). *Epichloë* endophytes have been connected to a suite of non-drought related fitness
358 benefits including herbivore protection (Brem and Leuchtmann, 2001), salinity resistance (Wang
359 et al., 2020), and mediation of the soil microbiome (Roberts and Ferraro, 2015) These effects are
360 potentially mediated by the diverse bioactive alkaloids and other signaling compounds they pro-
361 duce (Saikkonen et al., 2013). The strong increase in symbiotic *A. perennans* could be explained,
362 at least in part, by these diverse benefits. *A. hyemalis* experienced more consistently positive in-
363 creases in endophyte prevalence related to changes in spring temperature and precipitation. This
364 result is in line with previous work demonstrating drought benefits in a greenhouse manipula-
365 tion with this species (Davitt et al., 2011) that led us to expect that endophyte prevalence should
366 similarly increase at a greater rate in regions that have experienced increasing drought. For *E.*
367 *virginicus*, which experienced the most modest changes in endophyte prevalence overall, we found
368 a strong relationship between temporal trends and changes in the mean and variability of tem-
369 perature, as well as with decreases in summer precipitation. Surveys by Sneck et al. (2017), used
370 as part of the test data in this study, identified a drought index (SPEI) that integrates precipitation
371 with estimated evapotranspiration as an important predictor of endophyte prevalence. While we
372 show consistent increasing trends in prevalence between the three species, the mechanisms that
373 explain these changes may be diverse and idiosyncratic.

374 Our spatially-explicit model predicted regions of both high and low endophyte prevalence,
375 suggesting that symbiotic and non-symbiotic host plants have overlapping, but non-identical
376 niche requirements. Endophytes fitness benefits potentially explain the spatial distribution of
377 prevalence by allowing their hosts to persist in environments where they otherwise could not
378 (Afkhami et al., 2014; Kazenel et al., 2015). For example, fitness benefits of the symbiosis could
379 explain high predicted prevalence in *E. virginicus* towards the north or in *A. hyemalis* towards
380 its range center coinciding with strong environmental gradients. Previous population surveys
381 for endophytes, which were used as test data for our model, found similar latitudinal trends
382 in prevalence in these species (Rudgers and Swafford, 2009; Sneck et al., 2017), but at smaller
383 scales. While the model recreated these large-scale spatial trends, test data was more variable.

384 Using test data to validate our model predictions allows us to evaluate places to improve the
385 model's ability to perform well at out-of-sample prediction, which will be particularly important
386 for predicting host and symbiont niche-shifts under future climate change. Lack of information
387 on local variability may simply be a feature of data derived from herbarium specimens. Even
388 though they are samples from local populations, they are single specimens that are aggregated
389 over in broad-scale model estimates. Poor predictive ability at local scales in this grass-endophyte
390 system is not surprising, as previous studies have found that local variation, even to the scale of
391 hundreds of meters can structure endophyte-host niches (Kazenel et al., 2015). Sneck et al. (2017)
392 also identified host genotype as an important predictor of endophyte prevalence in *E. virginicus*.
393 Other studies have found factors including land-use history (Vikuk et al., 2019) and the biotic
394 environment, including herbivory (Rudgers et al., 2016), to be important predictors of endophyte
395 ecology. Incorporating available climatic and soil layers as covariates is an obvious first step that
396 could improve predictions. Towards the goal of predicting the dynamics of microbial symbioses
397 under climate change, models that integrate data from local and regional scales would be an
398 important step to bridge the gap that often exists between large but broad bioclimatic and bio-
399 diversity data and small but local data on biotic interactions. (Isaac et al., 2020; Miller et al.,
400 2019)

401 Our analysis advances the use of herbarium specimens in global change biology in two ways.
402 First and foremost, this is the first study to link long-term changes in microbial symbioses to
403 changes in climate using specimens from natural history collections. The responses of micro-
404 bial symbioses are a rich target for future studies within museum specimens, particularly those
405 that take advantage of advances in sequencing technology. While we used relatively coarse
406 presence/absence data based on fungal morphology, other studies have examined historic plant
407 microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these
408 studies have so far been limited to relatively few specimens at limited spatial extents (Bieker
409 et al., 2020; Bradshaw et al., 2021; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al.,
410 2015). Continued advances in capturing historic DNA and in filtering out potential contami-

411 nation during specimen storage (Bakker et al., 2020; Daru et al., 2019; Raxworthy and Smith,
412 2021) will be imperative in the effort to scale up these efforts. This scaling up will be essential
413 to be able to quantify changes not just in the prevalence of symbionts, but also in symbionts'
414 intraspecific variation and evolutionary responses to climate change, as well as in changes in the
415 wider microbial community. Answering these questions as well as the unknown questions that
416 future researchers may ask also reiterates the value in capturing meta-information during ongo-
417 ing digitization efforts at herbaria around the world and during the accession of newly collected
418 specimens (Lendemer et al., 2020). Second, we accounted for several potential biases in the data
419 observation process that may be common to many collections-based research questions by using
420 a spatially-explicit random effects model. Spatial autocorrelation (Willems et al., 2022), potential
421 biases introduced by the sampling habits of collectors (Daru et al., 2018), and variation between
422 contemporary researchers during the collection of trait data, if not corrected for could lead to
423 over-confident inference about the strength and direction of historic change. Previous studies
424 that have quantified the effects of collector biases typically find them to be small (Davis et al.,
425 2015; Meineke et al., 2019), and we similarly did not find that collector has a strong effect on the
426 results of our analysis. Fitting this model in a Bayesian framework allows for full propagation of
427 uncertainty.

428 Ultimately, a central goal of global change biology is to generate predictive insights into the
429 future of natural systems. While this survey of historic endophyte prevalence is necessarily cor-
430 relative, it serves as a foundation to develop better predictive models of the response of microbial
431 symbioses to climate change. Combining the insights from this type of regional-scale survey with
432 field experiments and physiological data could be invaluable. While we found that climate is
433 strongly correlated with endophytes' temporal responses, we do not know why trends in preva-
434 lence were weak in some areas or how endophytes would respond to more extreme changes in
435 climate. For example, transplanting symbiotic and non-symbiotic plants beyond the range edge
436 of *A. hyemalis* could tell us whether persistent low endophyte prevalence in that area is a result of
437 environmental conditions that lead the symbiosis to negative fitness consequences, or is a result

438 of some historical contingency or dispersal limitation that has thus far limited the presence of
439 symbiotic hosts from a region where they would otherwise flourish and provide resilience. While
440 we observed evidence of mutualism resilience, more extreme environmental changes than those
441 observed in our study could potentially push one or both partners beyond their physiological
442 limit, leading to the collapse of the mutualism. Our analysis thus far is agnostic to changes in the
443 distributions of hosts. Mechanistic models could connect the responses of both host and sym-
444 bionts to abiotic climate drivers integrating dispersal processes. Beyond host-microbe symbioses,
445 building these types of models would work towards quantitatively attributing biotic responses
446 to anthropogenically driven climate change, similar to methods in climate science and economics
447 (Carleton and Hsiang, 2016; Stott et al., 2010).

448 Acknowledgments

449 We thank Jessica Budke for help in drafting our initial destructive sampling plan, and to the
450 many members of herbarium staff who facilitated our research visits, as well as to the hundreds
451 of collectors who contributed to the natural history collections. Several high schooler and un-
452 dergraduate researchers contributed to data collection, including A. Appio-Riley, P. Bilderback,
453 E. Chong, K. Dickens, L. Dufresne, B. Gutierrez, A. Johnson, S. Linder, E. Scales, B. Scherick,
454 K. Schrader, E. Segal , G. Singla, and M. Tucker. This research was supported by funding from
455 National Science Foundation (grants 1754468 and 2208857) and by funding from the Texas Ecolab
456 Program.

457 Statement of Authorship

458 Data and Code Availability

459 On initial submission, you may use this section to provide a URL for editors and reviewers that
460 is ‘private for peer review’. After acceptance, this section must be updated with correct, working

⁴⁶¹ DOIs for data deposits (typically on the Dryad Digital Repository,) and code deposits (such as
⁴⁶² in Zenodo).

463

Appendix A

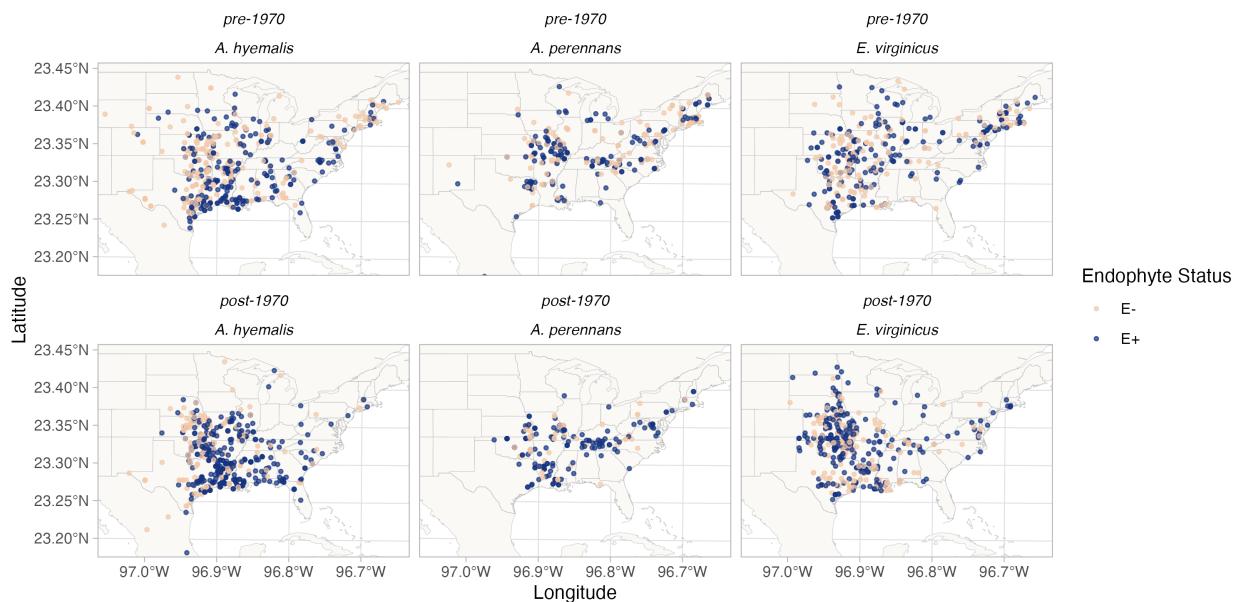


Figure A1: **Endophyte presence/absence in specimens of each host species.** Points show collection locations colored according to whether the specimen contained endophytes (E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.

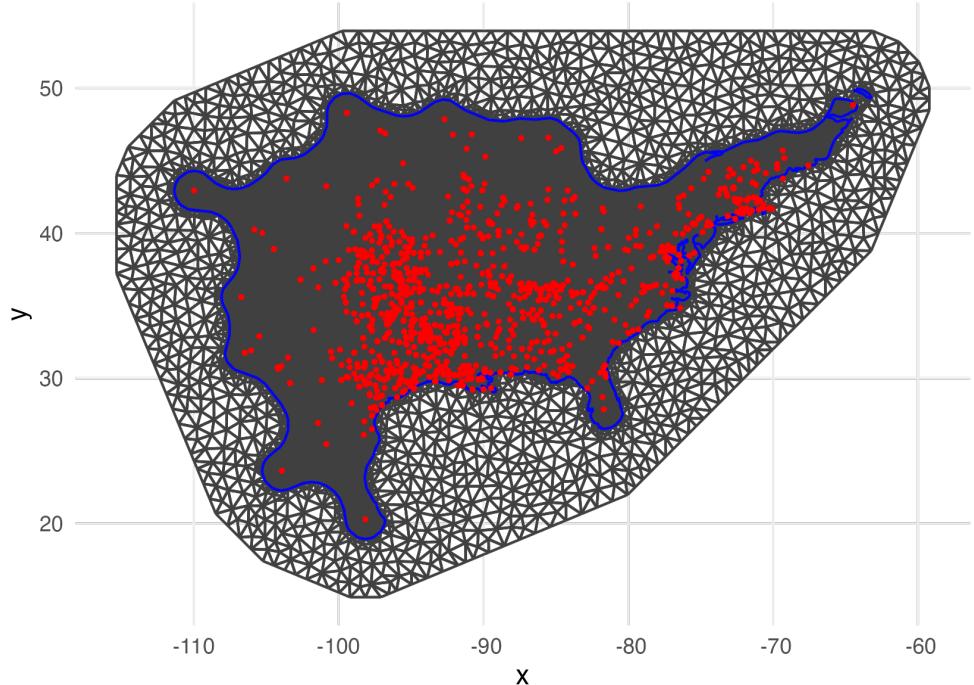


Figure A2: Delauney triangulation mesh used to estimate spatial dependence between data points. Grey lines indicate edges of triangles used to define distances between observations. Red points indicate locations of sampled herbarium specimens, and the blue outlines show the international borders used to define the edge of the mesh along coastlines.

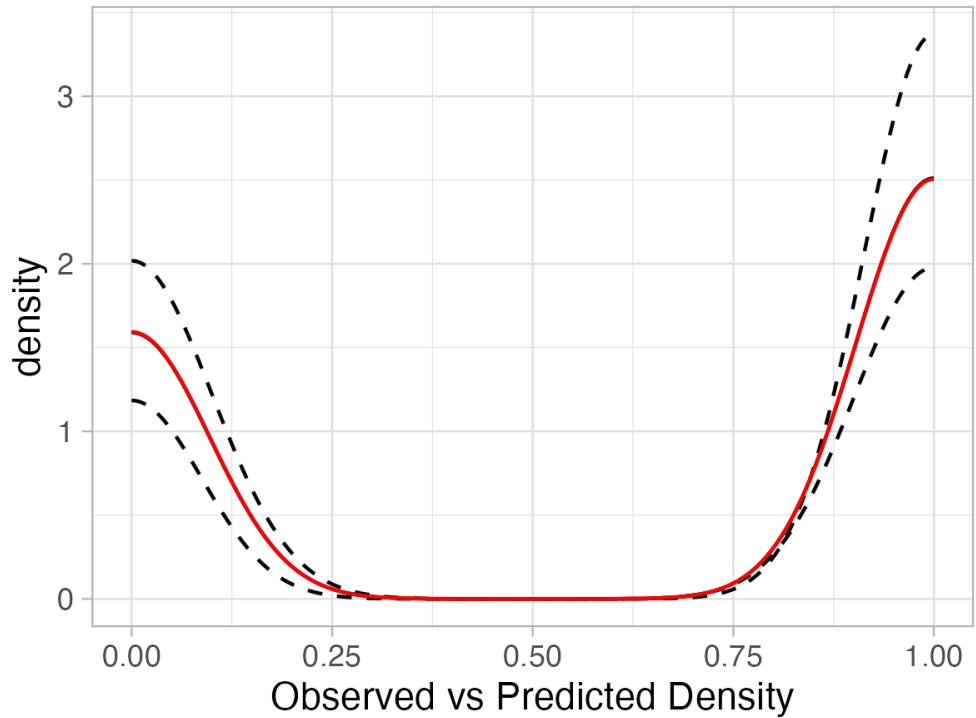


Figure A3: Consistency between real data and simulated values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (red) along with the mean(solid) and 95% CI (dashed) of simulated values (black).

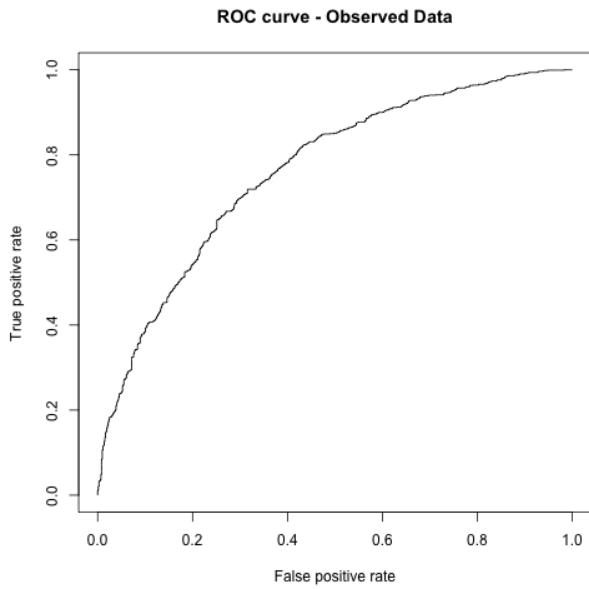


Figure A4: ROC plot showing model performance classifying observations according to endophyte status. The curves show adequate model performance for observed (top) and test (bottom) data. The AUC for each is 0.77.

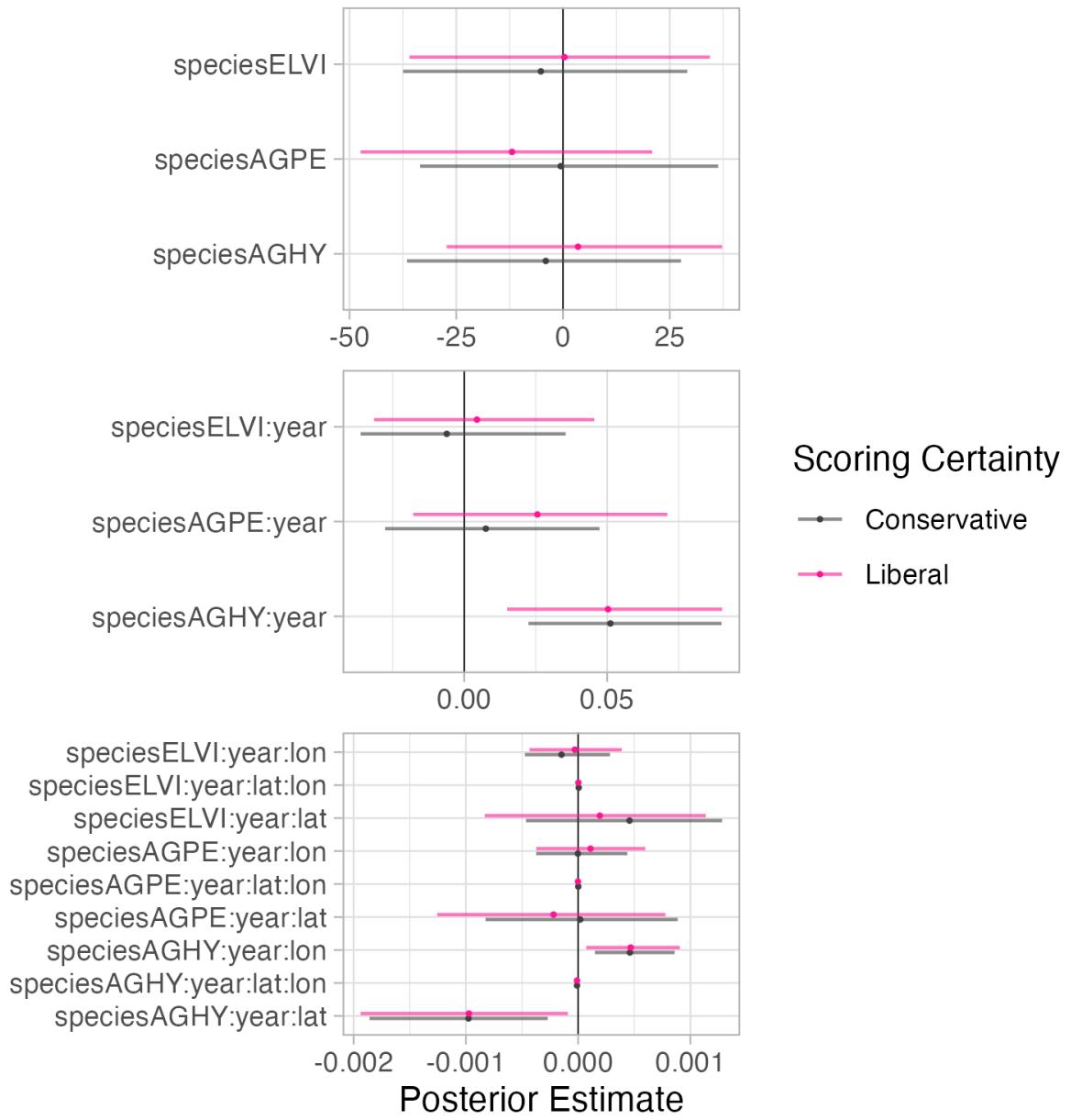


Figure A5: Comparison of posterior estimates of fixed effects when using Liberal or Conservative endophyte scores.

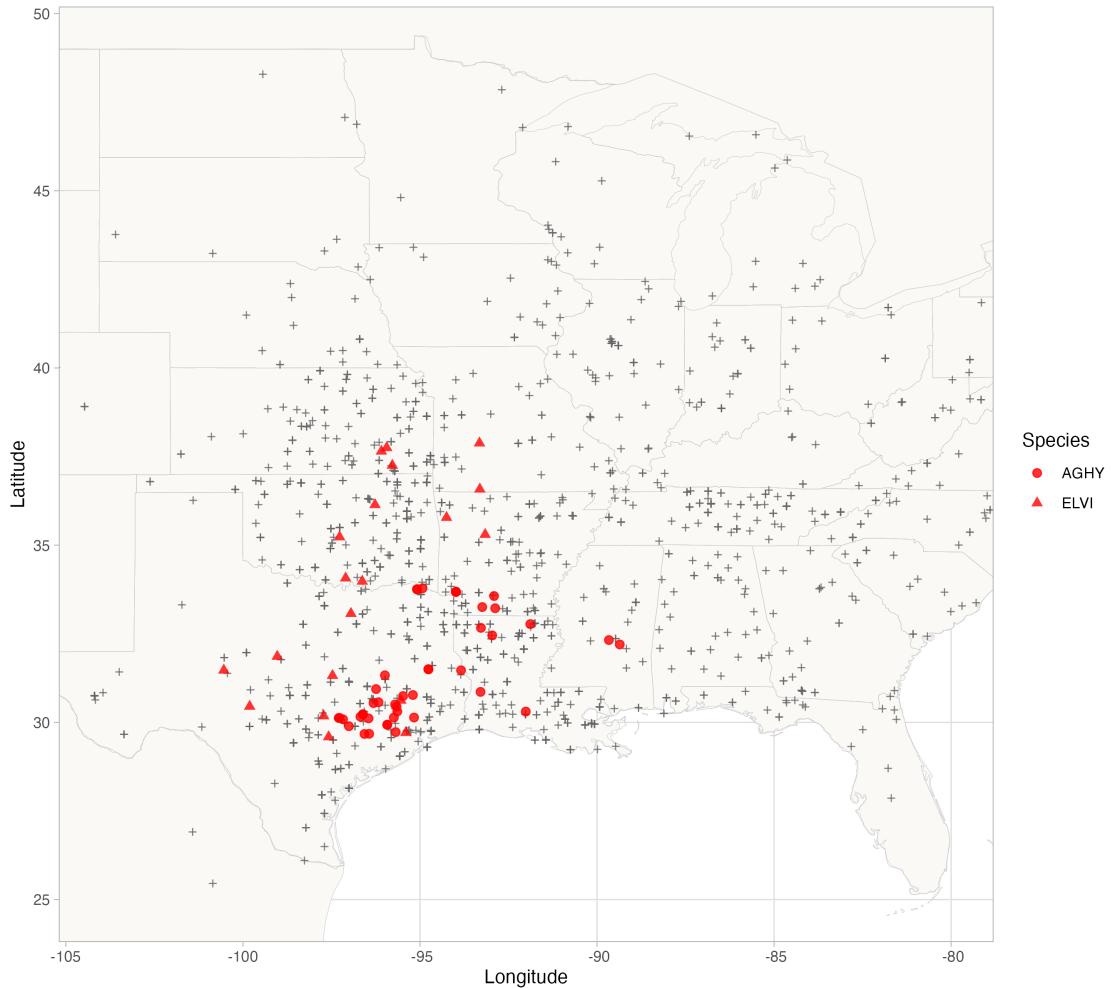


Figure A6: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).

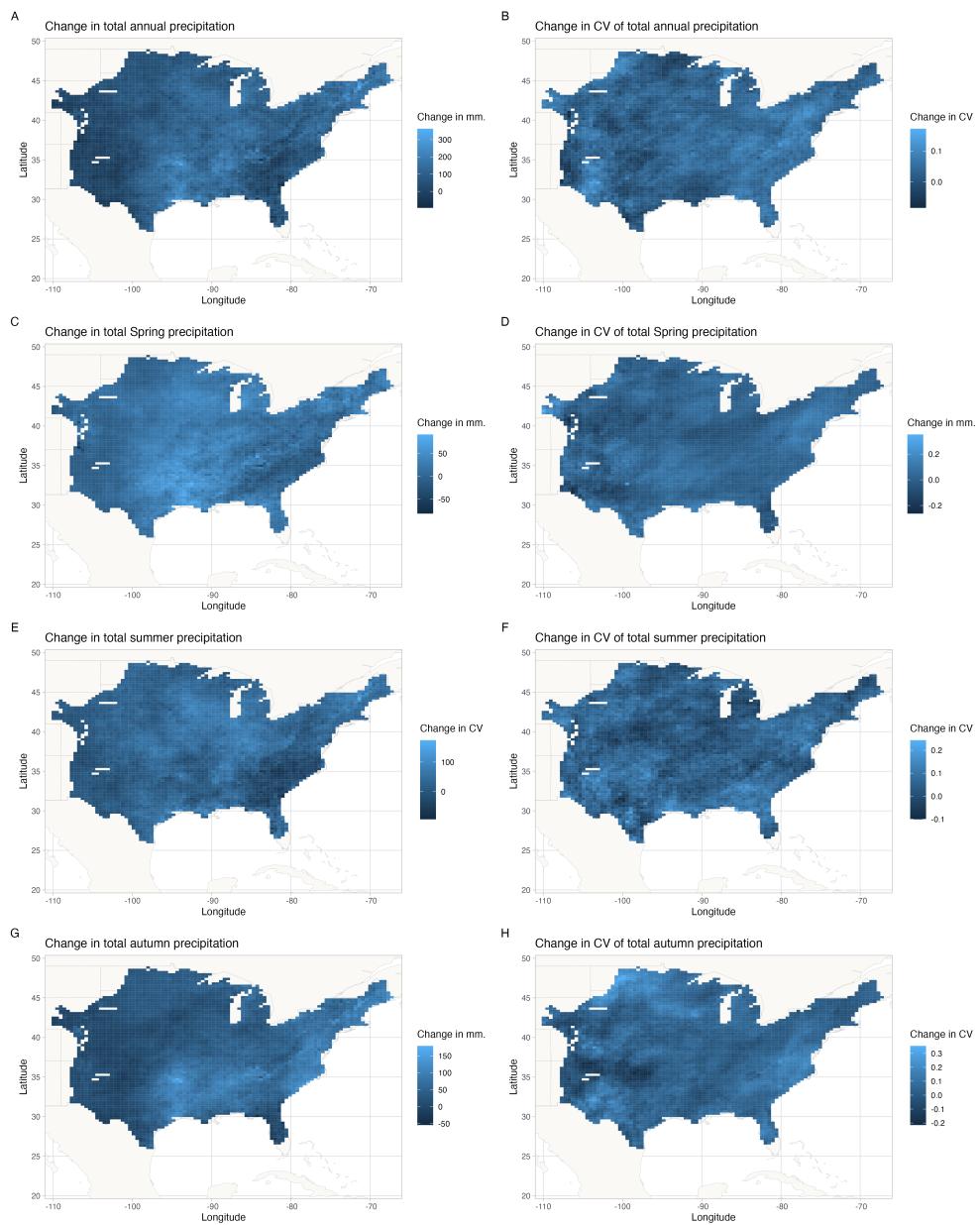


Figure A7: Change in precipitation between the periods 1895-1925 and 1990-2020. Color represents change in annual or seasonal total precipitation (A,C,E,G) and in the coefficient of variation of annual or seasonal total precipitation (B,D,F,H). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change were pulled from studies areas specific to each host species.

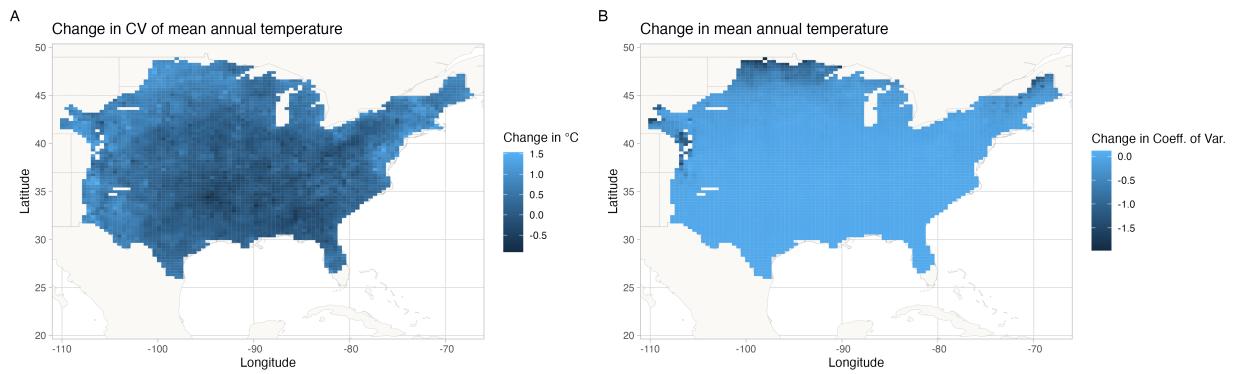


Figure A8: Change in temperature between the periods 1895-1925 and 1990-2020. Color represents change in annual mean temperature (A) and in the coefficient of variation of annual mean temperature (B). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change were pulled from studies areas specific to each host species.

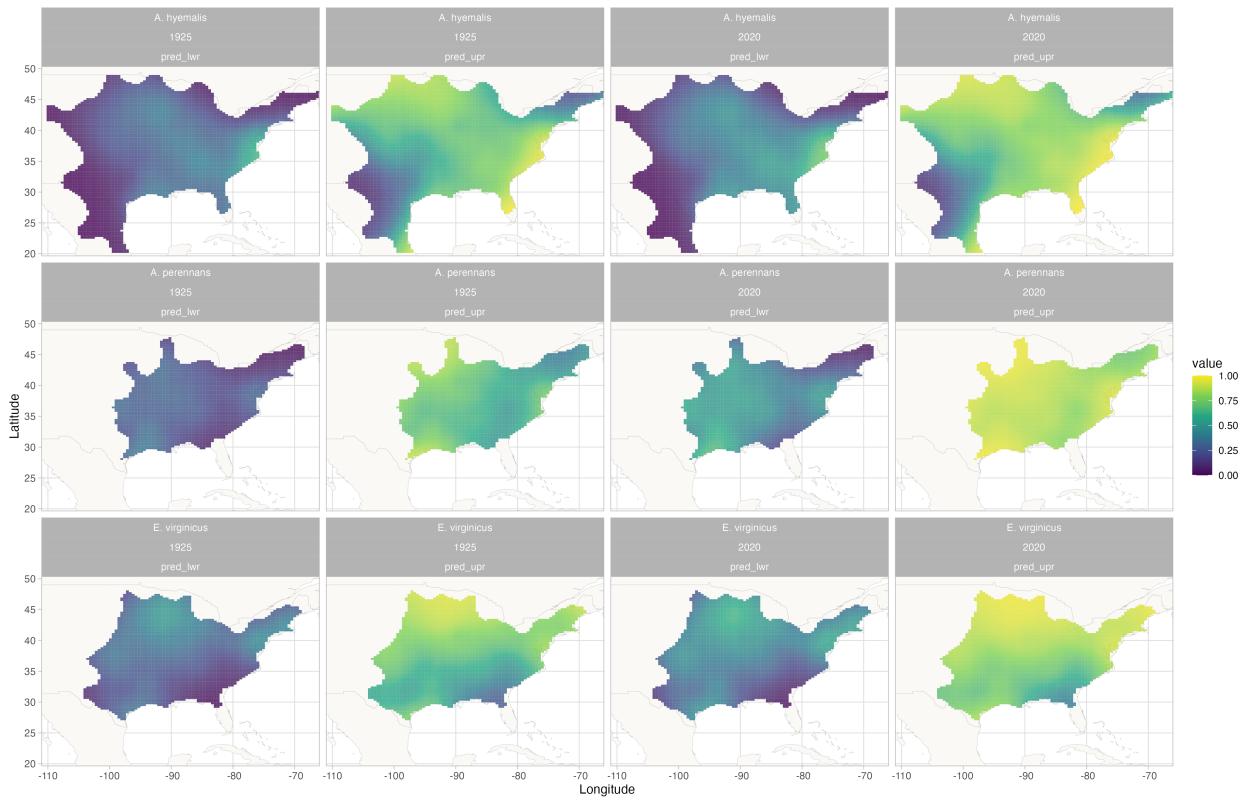


Figure A9: Uncertainty associated with spatial trends in endophyte prevalence. Color represents change in predicted endophyte prevalence. Panels show upper and lower 95% posterior probability for each host species between 1925 and 2020.

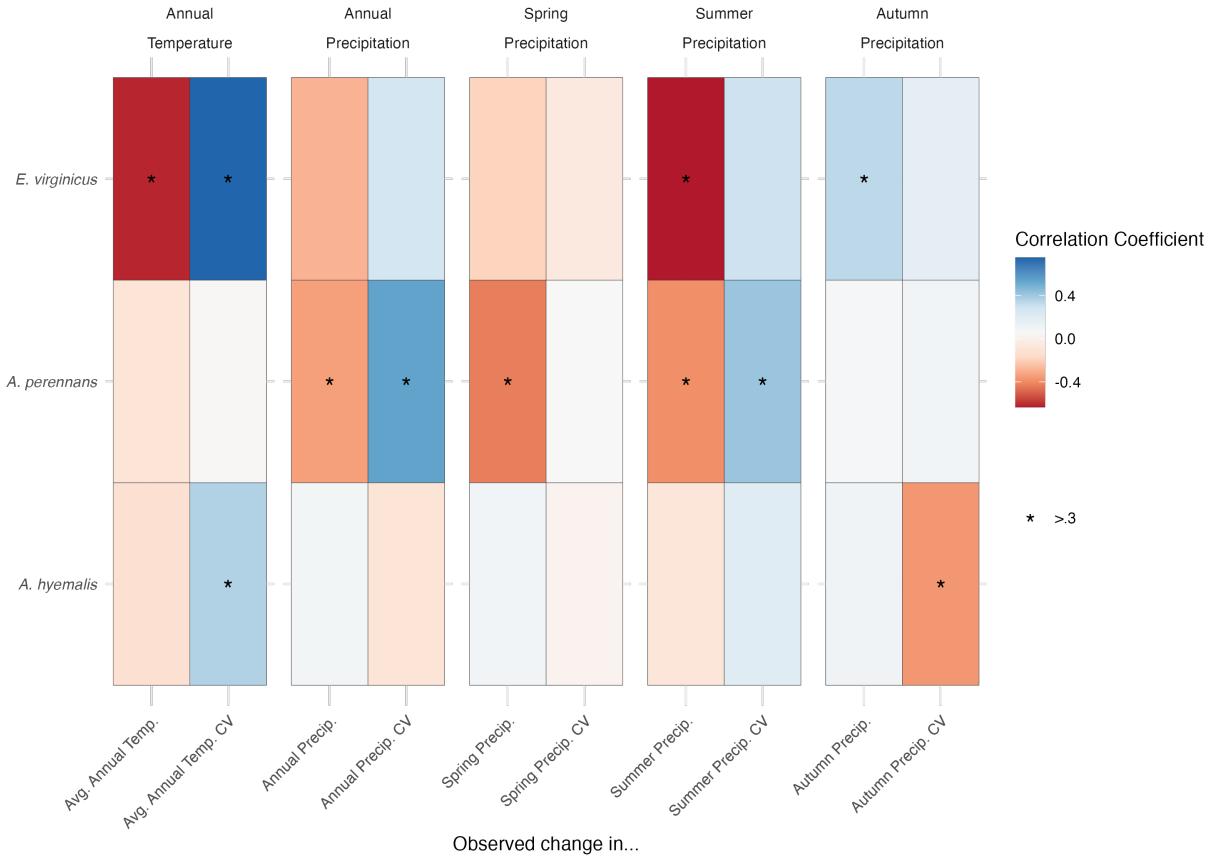


Figure A10: Correlations between changes in climate drivers and changes in endophyte prevalence from a random sample of 100 pixels across the study region. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (mm), as well as the change in the coefficient of variation of each climate driver. Positive correlation coefficients indicate that greater increases in a climate driver were associated with larger increases in endophyte prevalence, while negative values indicate that . Asterisks denote correlation coefficients $> .3$ or $< -.3$.

Table A1: Summary of herbarium samples across collections

Herbarium Collection	AGHY	AGPE	ELVI
Botanical Research Institute of Texas	341	189	176
Louisiana State University	71	–	61
Mercer Botanic Garden	3	–	6
Missouri Botanic Garden	78	39	31
Texas A&M	73	–	49
University of Kansas	134	–	20
University of Oklahoma	65	30	91
University of Texas & Lundell	169	41	99
Oklahoma State University	30	–	69

464

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