

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

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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 that disrupts these interactions may lead to declines in hosts or symbionts. Microbes preserved within herbarium specimens offer a unique opportunity to quantify changes in microbial symbiosis across broad temporal and spatial scales. We asked how the prevalence of seed-transmitted fungal symbionts of grasses (*Epichloë* endophytes), which can protect hosts from abiotic stress, have changed over time in response to climate change, and how these changes vary across host species' ranges. Specifically, we analyzed #¹ herbarium specimens of three grass host species collected over the last two centuries (18## – 20##) for the presence or absence of endophyte symbiosis, and evaluated spatial and temporal trends in endophyte prevalence. We found that endophytes have increased in prevalence over the last two centuries from ca. 25% prevalence to ca. 75% prevalence, on average, across the three host species. We also found that changes in prevalence were associated with observed **changes in annual and seasonal climate drivers**² corresponding to each host species' peak growing season. Our results provide novel evidence for a cryptic biological response to climate change that may contribute to the resilience of host-microbe symbiosis through context-dependent benefits that confer a fitness advantage to symbiotic hosts under environmental change.

¹*Give numbers*

²*Describe "changes" – warming? drying?*

Introduction

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

41 Natural history specimens, which were originally collected to study and preserve taxonomic
42 diversity, present a unique opportunity to explore long-term changes in ecological interactions
43 across broad spatial and temporal scales (Meineke et al., 2018). Natural history collections, built
44 and maintained by the efforts of thousands of scientists, are invaluable time machines, primarily
45 comprised of physical specimens of organisms along with information about the time and place
46 of their collection. These specimens often preserve physical legacies of ecological processes and
47 species' interactions from dynamically changing environments across time and space. For exam-
48 ple, previous researchers have used plant collections (herbaria) to document shifts in phenology
49 (Berg et al., 2019; Park et al., 2019; Willis et al., 2017), pollination (Duan et al., 2019; Pauw and
50 Hawkins, 2011), and herbivory (Meineke et al., 2019) related to anthropogenic climate change.
51 However, few previous studies have leveraged biological collections to examine climate change-
52 related shifts in a particularly common type of interaction: microbial symbiosis.

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

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

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

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

84 vertically from maternal plants to offspring through seeds. Vertical transmission creates a feed-
85 back between the fitness of host and symbiont (Douglas, 1998; Fine, 1975; Rudgers et al., 2009).
86 Over time, endophytes that act as mutualists should rise in prevalence within a host population
87 (Donald et al., 2021). *Epichloë* are known to improve their hosts' drought tolerance (Decunta
88 et al., 2021) and protect their hosts against herbivores (Crawford et al., 2010) and pathogens (Xia
89 et al., 2018) likely through the production of a diverse suite of alkaloids and other secondary
90 metabolites. The fitness feedback induced by vertical transmission leads to the prediction that
91 endophyte prevalence should be high in populations where these fitness benefits are most im-
92 portant. Previous survey studies have documented large-scale spatial patterns in endophyte
93 prevalence structured by environmental gradients (Afkhami, 2012; Bazely et al., 2007; Granath
94 et al., 2007; Sneck et al., 2017). We predicted that prevalence should track temporal changes in
95 environmental drivers that elicit these fitness benefits.

96 Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic diver-
97 sity of host species that harbor these symbionts (White and Cole, 1985), establishing that signa-
98 tures of endophyte symbiosis can be recovered from long-preserved plant tissue.³ However, no
99 subsequent studies, to our knowledge, have used the vast resources of biological collections to
100 quantitatively assess spatio-temporal trends in endophyte prevalence and their environmental
101 correlates. Grasses are commonly collected and identified based on the presence of their re-
102 productive structures, meaning that preserved specimens typically contain seeds, conveniently
103 preserving the fungi along with their host plants on herbarium sheets. This creates the oppor-
104 tunity to leverage the unique spatio-temporal sampling of herbarium collections to examine the
105 response of the symbiosis to historical climate change.

106 In this study, we assessed the long-term responses of endophyte symbiosis to climate change
107 through the use of herbarium specimens of three North American host grass species (*Agrostis*
108 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*). We first address questions describing spatial
109 and temporal trends in endophyte prevalence: (i) How has endophyte prevalence changed over

³Not sure about this but I am trying to pre-empt skepticism that fungi are less detectable in older samples.

the past two centuries? and (ii) How spatially heterogenous are temporal trends in endophyte prevalence across eastern North America? We then address how climate change may be driving trends in endophyte prevalence by asking: (iii) What is the relationship between variation in temporal trends in endophyte prevalence and changes in climate drivers? We predicted that aggregate endophyte prevalence would increase over time in tandem with climate warming, and that hotspots of endophyte change would correspond spatially to hotspots of climate change. To answer these questions we examined a total of 2,346 specimens collected across eastern North America between 1824 and 2019.⁴

118 Methods

119 Focal species

Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*. Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001; Leuchtmann et al., 2014), while *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl, 2002). These C₃ grass species are commonly represented in natural history collections with broad distributions covering much the eastern United States. *A. hyemalis* is a small short-lived perennial species that germinates in the spring and typically flowers between March and July (most common collection month: May). *A. perennans* is of similar stature but is longer lived than *Agrostis hyemalis* and flowers in late Summer⁵ and early Autumn (most common collection month: September). *A. perennans* is more sparsely distributed, tending to be found in shadier and more moist habitats, while *A. hyemalis* is commonly found in open and recently disturbed ground. Both *Agrostis* species are recorded from throughout the Eastern US, but *A. perennans* has a slightly more northern distribution, whereas *A. hyemalis* is found rarely as far north as Canada

⁴I think the consensus was to keep the out-of-sample validation which should absolutely go into the Intro as an important element of novelty. Should go in the Abstract too.

⁵Are seasons capitalized?

132 and is listed as a rare plant in Minnesota. *E. virginicus* is a larger and relatively longer-lived
133 species that is more broadly distributed than the *Agrostis* species. It begins flowering as early as
134 March or April but continues throughout the Summer (most common collection month: July).

135 *Herbarium surveys*

136 We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens
137 included from each collection). We permission from herbarium staff, we acquired seed samples
138 from 1135 *A. hyemalis* specimens collected between 1824 and 2019, 357 *A. perennans* specimens
139 collected between 1863 and 2017, and 854 *E. virginicus* specimens collected between 1839 and
140 2019 (Fig. 1, Fig 2A). We chose our focal species in part because they are commonly represented
141 in herbarium collections, and produce high numbers of seeds, meaning that small samples would
142 not diminish the value of the specimens for future studies. We collected 5-10 seeds per specimen
143 after examining the herbarium sheet under a dissecting microscope to ensure that we collected
144 mature seeds, not florets or unfilled seeds, fit for our purpose of identifying fungal endophytes
145 with microscopy. We excluded specimens for which information about the collection location and
146 date were unavailable. Each specimen was assigned geographic coordinates based on collection
147 information recorded on the herbarium sheet using the geocoding functionality of the ggmap
148 R package (Kahle et al., 2019). Many specimens had digitized collection information readily
149 available, but for those that did not, we transcribed information printed on the herbarium sheet.
150 Collections were geo-referenced to the nearest county centroid, or nearest municipality when
151 that information was available. For a few of the oldest specimens, only information at the state
152 level was available, and so we used the state centroid.

153 After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hy-
154 phae, which grow intercellularly, in each specimen using microscopy. We first softened seeds
155 with a 10% NaOH solution, then stained the seeds with aniline blue⁶ dye and squashed them
156 under a microscope cover slip. We examined the squashed seeds for the presence of fungal hy-

⁶Most of our stains in the lab also have lactic acid.

157 phae at 200-400X magnification (Bacon and White, 2018). In some cases, the tissues examined
158 during microscopy came from flowers or otherwise non-viable seeds, which were excluded for
159 that specimen. On average we scored 4.7 seeds per specimen of *A. hyemalis*, 4.2 seeds per spec-
160 imen of *A. perennans*, and 3.8 seeds per specimen of *E. virginicus*; we scored # seeds in total. .
161 Due to imperfect vertical transmission (Afkhami and Rudgers, 2008), it is possible that symbiotic
162 host-plants produce a mixture of symbiotic and non-symbiotic seeds. We therefore designated a
163 specimen as endophyte-symbiotic if *Epichloë* hyphae were observed in one or more of its seeds,
164 or non-symbiotic if hyphae were observed in none of its seeds. To capture uncertainty in the
165 endophyte scoring process, we recorded both a "liberal" and a "conservative" endophyte sta-
166 tus for each plant⁷. When we identified potential endophytes with unusual morphology, low
167 uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a pos-
168 itive liberal status (more likely to be endophyte-positive) and a negative conservative status (less
169 likely to be endophyte-positive). 89% of scored plants had matching liberal and conservative
170 scores, reflecting high confidence in endophyte status. The following analyses in the main text
171 used the liberal status, but we repeated all analyses with the conservative status which yielded
172 qualitatively similar results (Figure A5)⁸.

173 *Modeling spatial and temporal changes in endophyte prevalence*

174 We assessed spatial and temporal changes in endophyte prevalence across each host distribu-
175 tion, first quantifying the "global" trends, aggregating across space, and then examining spatial
176 heterogeneity in the direction and magnitude of endophyte change (hotspots and coldspots)
177 across the spatial extent of each host's distribution. To appropriately account for the spatial
178 non-independence of geo-referenced occurrences we used an approximate Bayesian method,
179 Integrated Nested Laplace Approximation (INLA), to construct spatio-temporal models of en-
180 dophyte prevalence. INLA provides a computationally more efficient method of ascertaining

⁷Plant or seed?

⁸Use ?? for citing figures, and stay consistent between Figure and Fig.

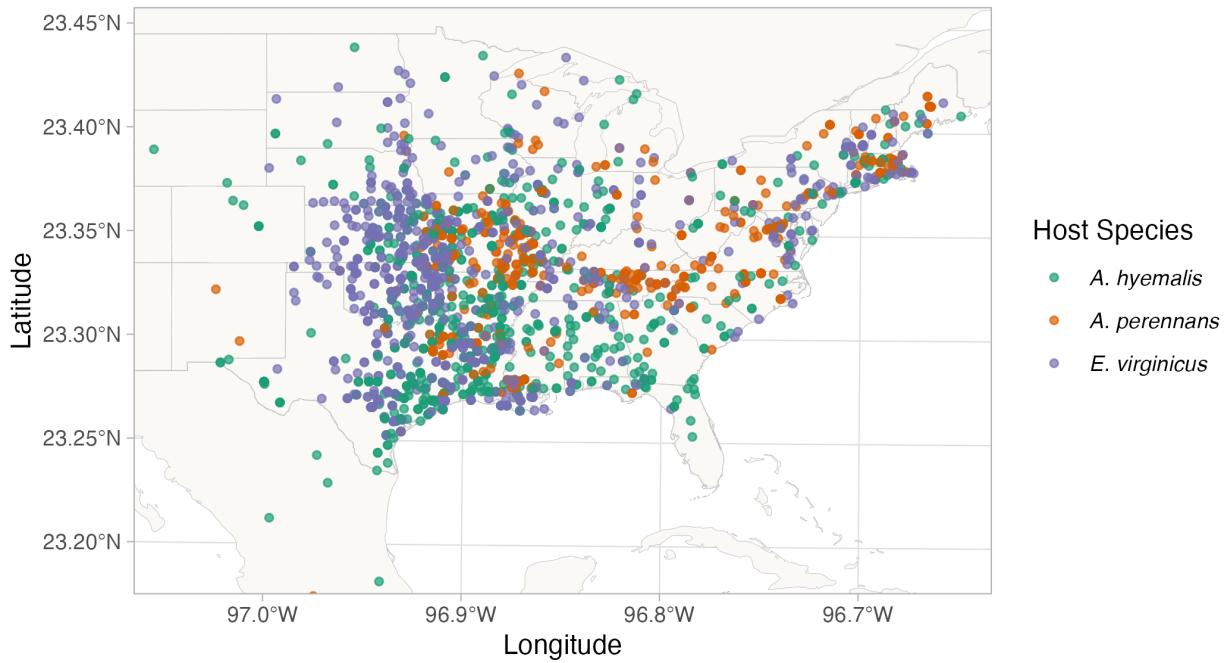


Figure 1: Collection locations of herbarium specimens of three grass host species across eastern North America that were sampled for *Epichloë* endophyte presence or absence.

181 parameter posterior distributions for certain models that can be formulated as latent Gaussian
 182 Models (Rue et al., 2009). Many common statistical models, including structured and unstruc-
 183 tured mixed-effects models, can be represented as latent Gaussian Models. Fitting models with
 184 structured spatial effects is possible with MCMC sampling but can require long computation
 185 times, making INLA an effective alternative, which has been used to model spatial patterns in
 186 flowering phenology (Willems et al., 2022), the abundance of bird species (Meehan et al., 2019)
 187 and butterflies citecrossley2022opposing, the distribution of temperate trees (Engel et al., 2022)
 188 as well as the population dynamics of endangered amphibians (Knapp et al., 2016) and other
 189 ecological processes (Beguin et al., 2012).⁹

⁹ I think we need another sentence or two in this paragraph that provides an intuitive explanation for why INLA is the right approach for our analysis, written specifically for people who don't know what INLA is and don't want to know. I know the model includes a spatial decay term that controls spatial autocorrelation, a feature that I think is worth highlighting.

First, to quantify global trends in endophyte prevalence, we modeled endophyte presence/absences of the i^{th} specimen (P_i) as a Bernoulli response variable with expected probability of endophyte occurrence \hat{P}_i . We modeled \hat{P}_i as a linear function of collection year and accounting for random effects associated with location ($l[i]$, a unique latitude-longitude combination), collector identity ($c[i]$), and scorer identity ($s[i]$) of the i^{th} specimen.

$$\text{logit}(\hat{P}_i) = \alpha_{l[i]} + \beta * \text{year}_i + \chi_{c[i]} + \omega_{s[i]} \quad (1a)$$

190 Spatially-indexed random intercepts $\alpha_{l[i]}$ account for potential spatial autocorrelation between
 191 data points, and year slope β describes the overall temporal trend in endophyte prevalence. We
 192 accounted for potential biases introduced during the process of collecting specimens as well as
 193 in scoring ability by including random effects specific to each collector χ and scorer ω . Previous
 194 work suggests that behavior of historical botanists and uneven sampling may introduce biases
 195 into ecological inferences made from historic collections (Kozlov et al., 2020). Prolific collectors
 196 who contribute thousands of specimens may be more or less likely to collect certain species,
 197 or specimens with certain traits (Daru et al., 2018). Similarly, the process of scoring seeds for
 198 hyphae involved many student researchers who, even with standardized training, may vary
 199 in their likelihood of positively identifying *Epichloë* hyphae. By including a random effect for
 200 collectors and for scorers, we accounted for variance across individual researchers that may
 201 bias our predictions of changes in endophyte prevalence. **Models for each host species were fit**
 202 **separately.**^{10 11}

Second, to quantify how temporal trends may vary spatially, we repeated the modelling above, but incorporated a spatially-varying coefficient for collection year:

$$\text{logit}(\hat{P}_i) = \alpha_{l[i]} + \beta_{l[i]} * \text{year}_i + \chi_{c[i]} + \omega_{s[i]} \quad (2a)$$

¹⁰It would be great to pull all species into one model and have them share variance terms for the random effects. I suspect such a model would give better and more stable estimates.

¹¹I updated the notation in ways that make more sense to me, but you should check that this is true to the actual model (I think it is). Also, a more complete presentation of this model would show the variance terms for α , χ , and ω . I presume the latter two are Gaussian but I don't know how to represent the distribution of α .

203 The spatially-varying year slope β_l allowed us to flexibly estimate variation in the temporal
204 trajectory of endophyte change at locations across the study region.

205 For both models, spatially-structured random intercepts (α_l) and slopes (β_l) were constructed
206 using stochastic partial differential equations (SPDE) that depend on a covariance matrix accord-
207 ing to the proximity of each collection location (Bakka et al., 2018; Lindgren et al., 2011). The
208 covariance matrix was approximated using a Matérn covariance function, with each data point
209 assigned a location according to the nodes of a mesh of non-overlapping triangles across our
210 study area (Fig A2).¹²

211 We performed model fitting using the inlabru R package (), with vague priors¹³, and com-
212 pared models with different sizes of mesh, which had little effect on the resulting model esti-
213 mates¹⁴. Each mesh¹⁵ was bounded by the predicted host distribution, described below. Posterior
214 modes were stable¹⁶ indicating that numeric convergence was successful. We assessed model fit
215 with graphical posterior predictive checks (Fig. A3). The model performed adequately at clas-
216 sifying the historical data, comparing the accuracy of predictions from the model with observed
217 data (avg. AUC = 0.77; Fig. A4).¹⁷

218 *Modeling distributions of host species*

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

¹²This paragraph would be a place to describe the variance terms for the other random effects.

¹³I thought you needed informative priors on the spatial decay parameters

¹⁴That's good but you still need to state what mesh size you used and what that means, biologically.

¹⁵You have not defined what you mean by "mesh".

¹⁶Assessed how?

¹⁷Maybe move this to validation section, and then have both in-sample and out-of-sample approaches.

221 *Validating the model with an out-of-sample test*

222 We evaluated the predictive ability of the model using contemporary endophyte surveys as out-
223 of-sample test data, **an important but rarely used strategy in ecological studies** (Tredennick et al.,
224 2021).¹⁸ We used data from contemporary surveys of endophyte prevalence in *A. hyemalis* and
225 *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted in 2013 as
226 described in Sneck et al. (2017), and **surveys of *A. hyemalis* took place between 2015 and 2020**¹⁹.
227 Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation in endo-
228 phyte prevalence towards its range edge, while surveys of *E. virginicus* were designed to cover
229 latitudinal variation along its range edge. In total, we visited 43 populations of *A. hyemalis* and 20
230 populations of *E. virginicus* across the south-central US, with emphasis on Texas and neighboring
231 states (Fig A4²⁰). During surveys, we collected seeds from up to 30 individuals per location (av-
232 erage number of plants sampled: 22.9). We quantified the endophyte status of each individual
233 with staining microscopy as described for the herbarium surveys (with 5-10 seeds scored per
234 individual), and calculated the prevalence of endophytes within the population (proportion of
235 symbiotic plants divided by the number of sampled plants). For each population, we compared
236 the observed fraction of endophyte-symbiotic hosts to the predicted probability of endophyte oc-
237 currence \hat{P} derived from the model based on location and year, with collector and scorer random
238 effects fixed at zero. **The contemporary survey period (2013-2020) is at the most recent edge of**
239 **the time period encompassed by the historical observations used for model fitting.** We compared
240 **the model's prediction for these locations to the observed population prevalence.**²¹

¹⁸This is the type of thing to emphasize in the intro? Are there any other collections-based papers that have done anything like this?? None to my knowledge.

¹⁹We have added more recent AGHY survey data. I am not sure if you have access to this but you should definitely use it. Karl or I can point you to the right file.

²⁰This is now A6. Good reminder to use the ref function.

²¹It is not clear if you are testing model 1 ("global trend") or model 2 ("spatially varying trends").

Assessing the role of climate drivers

242 We assessed how the magnitude of climate change may have driven changes in endophyte preva-
243 lence by assessing correlations between changes in climate and changes in endophyte prevalence
244 predicted from our spatial model at evenly spaced pixels across the study area. We first down-
245 loaded monthly temperature and precipitation rasters from the PRISM climate group (Daly and
246 Bryant, 2013) covering the time period between 1895 and 2020 using the 'prism' R package (Hart
247 and Bell, 2015). Prism provides reconstructions of historic climate variables across the United
248 States by spatially-interpolating weather station data (Di Luzio et al., 2008). We calculated 30-
249 year climate normals for annual and seasonal mean temperature and cumulative precipitation
250 for the recent (1990 to 2020) and historic (1895 to 1925) periods. We used three four-month sea-
251 sons within the year (Spring: January, February, March, April; Summer: May, June, July, August;
252 Autumn: September, October, November, December). This division of seasons allowed us to
253 quantify differences in climate associated with the two "cool" seasons, when we expect our fo-
254 cal species to be most biologically active (*A. hyemalis* flowering phenology: Spring; *E. virginicus*:
255 Spring and Summer; *A. perennans*: Fall). In addition to mean climate conditions, environmental
256 variability itself can influence population dynamics (Tuljapurkar, 1982) and changes in variabil-
257 ity are a key prediction of climate change models (IPCC, 2021; Stocker et al., 2013). Therefore
258 we calculated the coefficient of variation (CV) during each period for each annual and seasonal
259 climate driver as the interannual standard deviation divided by the mean across each 30-year
260 period. We then took the difference between recent and historic periods for the mean and CV for
261 each climate driver (Fig. A5)²². Because initial analyses indicated a high degree of collinearity
262 between seasonal and annual changes in temperature, we used annual temperature only, along
263 with annual and seasonal precipitation, in the subsequent analysis. All together, this left us with
264 measurements of change in 10 potential climate drivers: the mean and coefficient of variation
265 of annual temperature, as well as the mean and coefficient of variation of cumulative annual

²²This is Figure A7 – Can you make the color scale on these diverging at zero?

²⁶⁶ precipitation, cumulative spring precipitation, cumulative summer precipitation, and cumulative
²⁶⁷ autumn precipitation (Fig A8-A9).

²⁶⁸ To evaluate whether areas that have experienced the greatest changes in endophyte preva-
²⁶⁹ lence (hotspots of endophyte change) are associated with high degrees of change in climate
²⁷⁰ (hotspots of climate change), we modeled spatially varying slopes of endophyte change through
²⁷¹ time (β_l) as a linear function of environmental covariates, with a Gaussian error distribution.²³
²⁷² Calculating correlations from many pixels across the study region risks artificially inflating confi-
²⁷³ dence in our results due to large sample sizes, and so we repeated this calculation using only a
²⁷⁴ random subsample of 100 pixels across the study region²⁴.²⁵²⁶

²⁷⁵ **Results**

²⁷⁶ *How has endophyte prevalence changed over time?*

²⁷⁷ We found that endophyte prevalence increased within the examined specimens over the last
²⁷⁸ two centuries for all three host species (Fig. 4). On average, *A. hyemalis* and *E. virginicus* both
²⁷⁹ increased from 30 % to over 70% prevalence across the study region, and *A. perennans* increased
²⁸⁰ from 15% to over 70% prevalence. Our model indicates a higher certainty that overall temporal
²⁸¹ trends are positive for *A. hyemalis* and *A. perennans* than for *E. virginicus* (99% probability of a
²⁸² positive overall year slope in *A. hyemalis*, 89% probability of a positive overall year slope in *A.*
²⁸³ *perennans*, and 58% probability of a positive overall year slope in *E. virginicus*).²⁷

²³I think we need to account for uncertainty in the slopes. They are outputs of a (quasi) Bayesian model so we should be able to propagate all the uncertainty in the posterior distribution.

²⁴100 seems like a low number to me. What if we did this for all of the herbarium collection locations?

²⁵Are the methods above repeated for each species separately?

²⁶I cut the notation for the Gaussian model for now because it is a pretty simple model and the notation may be overkill, plus because I changed your tau's to beta's there were betas on both sides of the tilde, which was confusing/annoying. Happy have the notation back if you prefer it. I am also a little confused because the appendix has spearman correlations but there are no methods here for where those come from.

²⁷These numbers are currently outdated. I am making some adjustments to models, and will update with final model

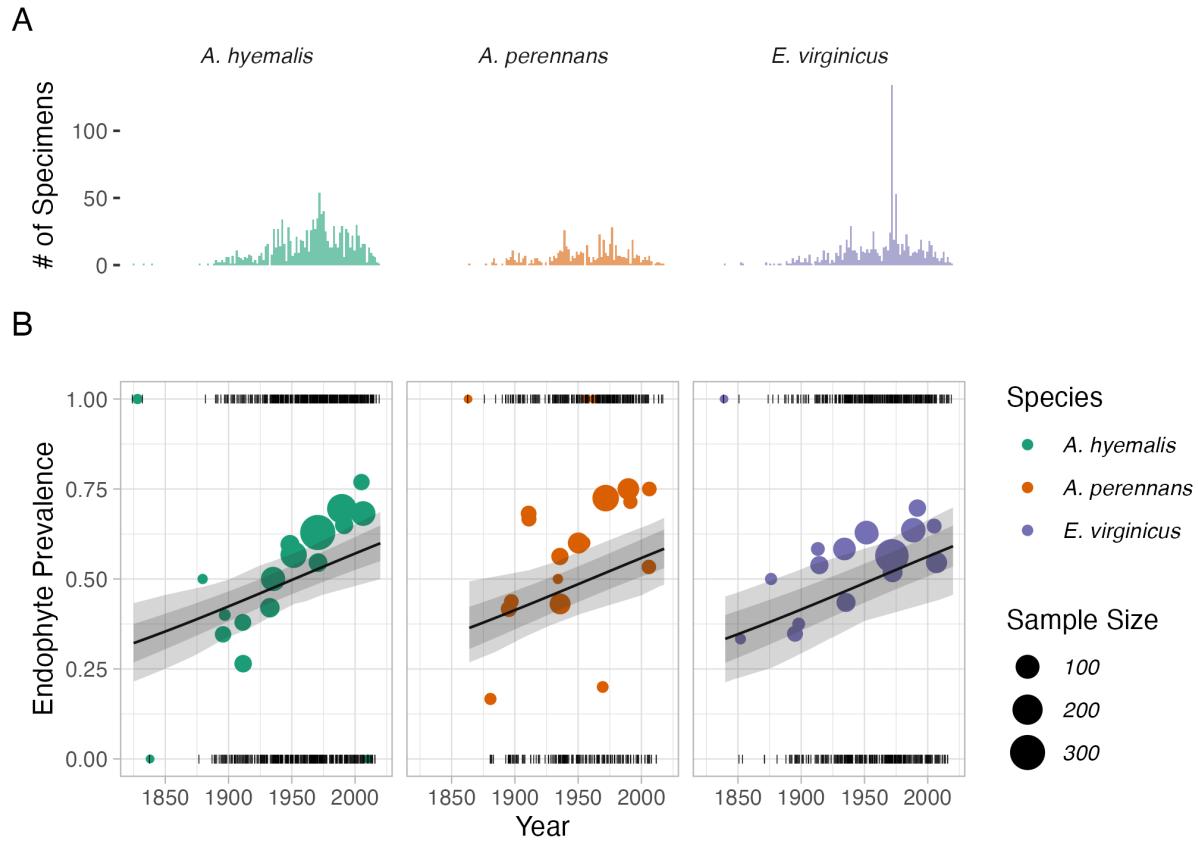


Figure 2: Temporal trends in endophyte prevalence. (A) Histograms show the frequency of collection through time for each host species. (B) Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species and point size is determined by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

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

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

289 over the study period, while *E. virginicus* experienced increases up to around 1.5 % per year. In
290 other regions, there were negligible changes. Notably, the symbionts of *E. virginicus* experienced
291 only slight increases in prevalence, and were less spatially variable than the other two species.
292 Regions that start with low endophyte prevalence, as in the southwestern portion of the range
293 of *A. hyemalis*(Fig. A1), also experienced negligible change, suggesting that this may be driven
294 more by the absence of the endophyte.²⁸ Predicted trends for *A. perennans* show certain areas
295 of both large increase and of large decrease, however this species, for which we have the fewest
296 samples, has the largest uncertainty. The posterior estimates of our spatially varying temporal
297 trends, indicate relatively narrow certainty (need to compute²⁹).

²⁸more discussion material, but putting it here for now.

²⁹

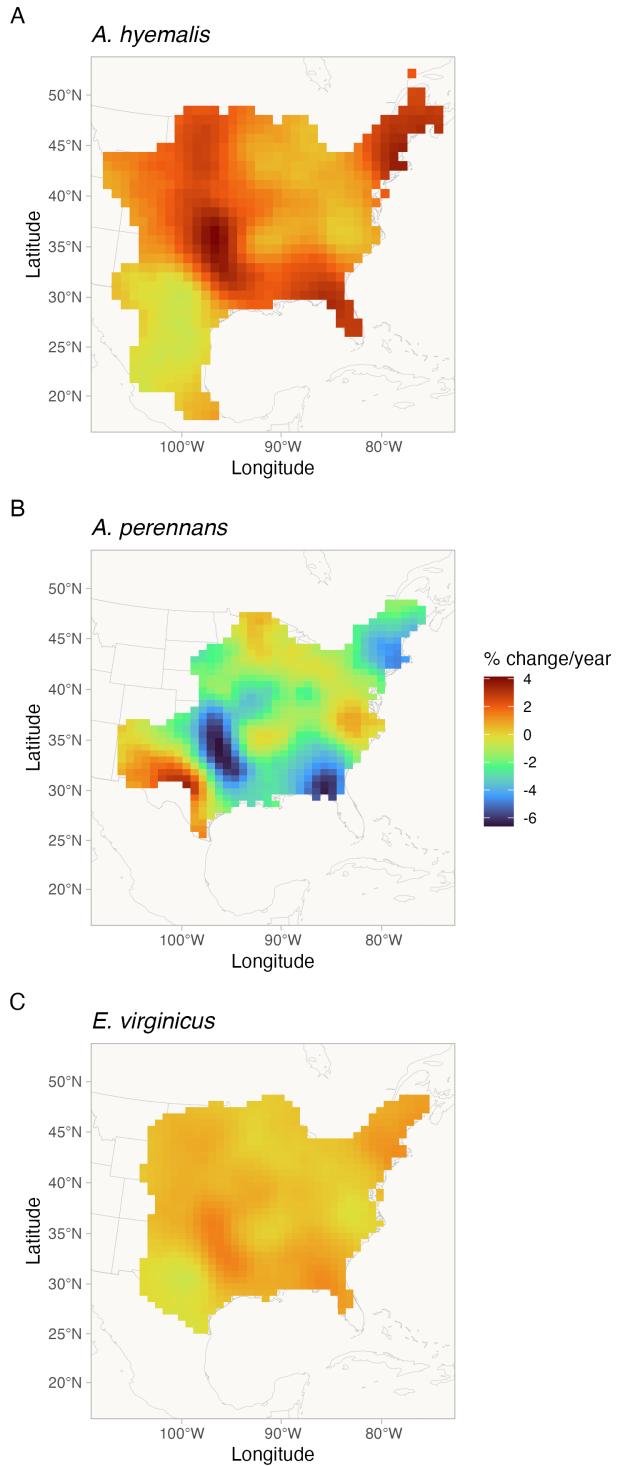


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species. Color indicates the relative change in predicted endophyte prevalence.

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

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

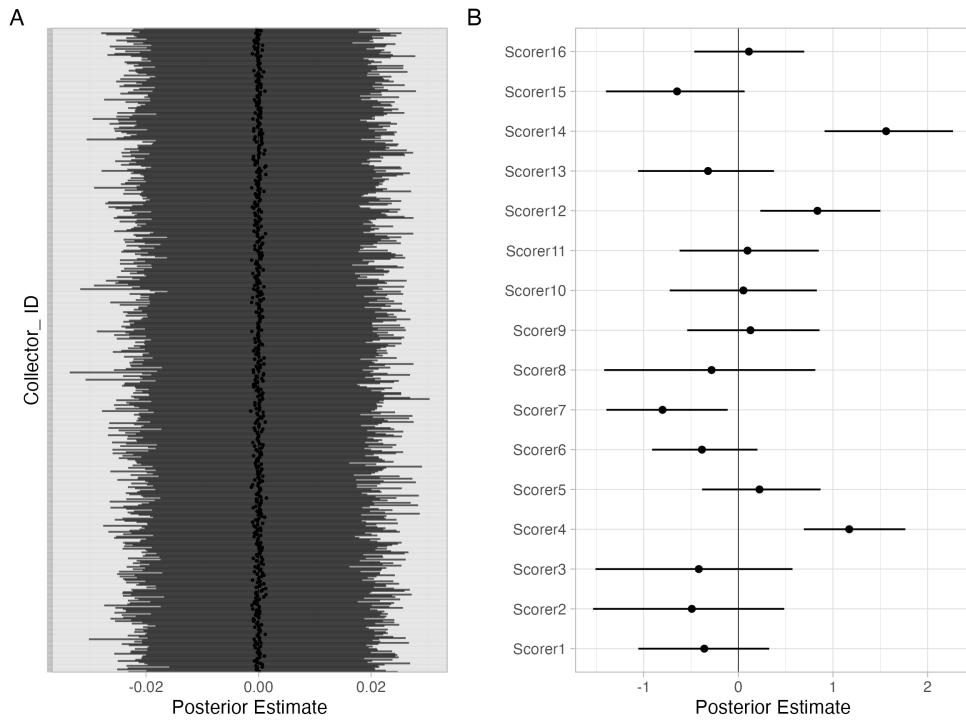


Figure 4: Posterior estimates of (A) collector and (B) scorer random effects. Points show the posterior mean along with 95% CI for random effects estimate from 532 collectors and 16 scorers.

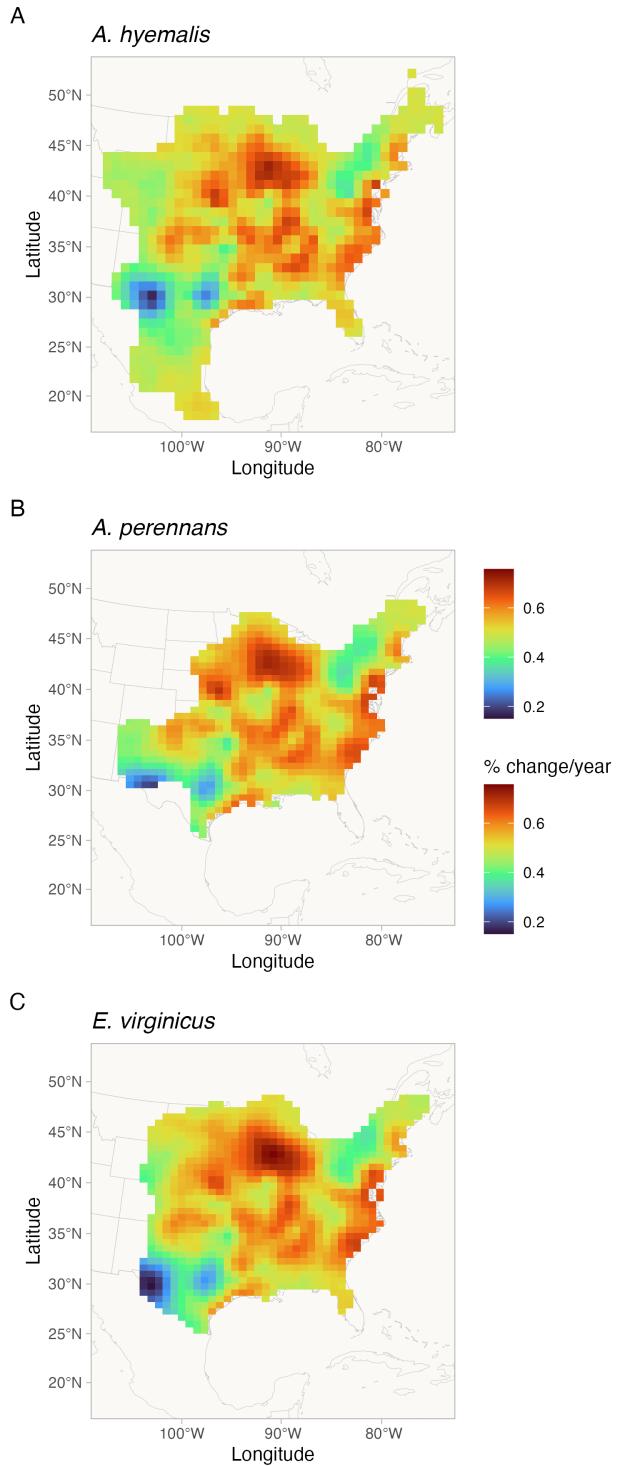


Figure 5: **Mean predicted endophyte prevalence for each host species (columns) in 1925 (top row) and 2020 (bottom row).** Color indicates mean predicted rate of endophyte prevalence across the predicted distribution of each species.

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

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

328 30

³⁰Only have plotted results for AGHY right now.

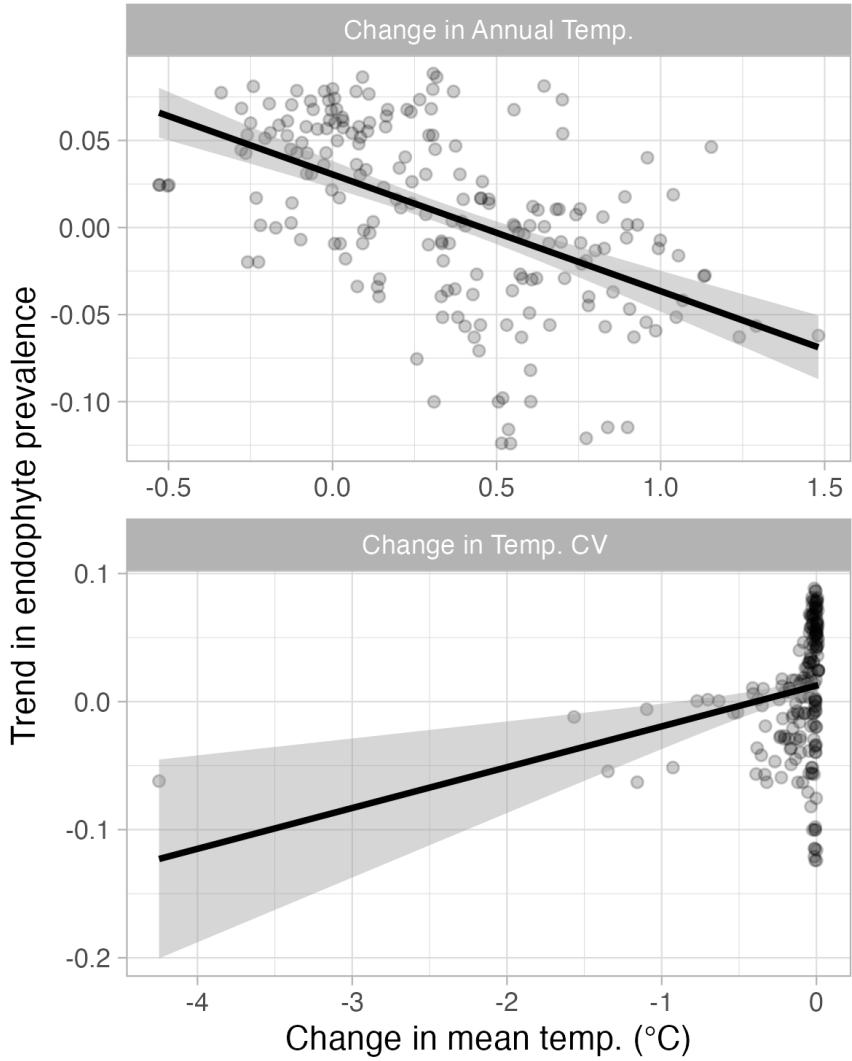


Figure 6: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (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 $< -.3$.

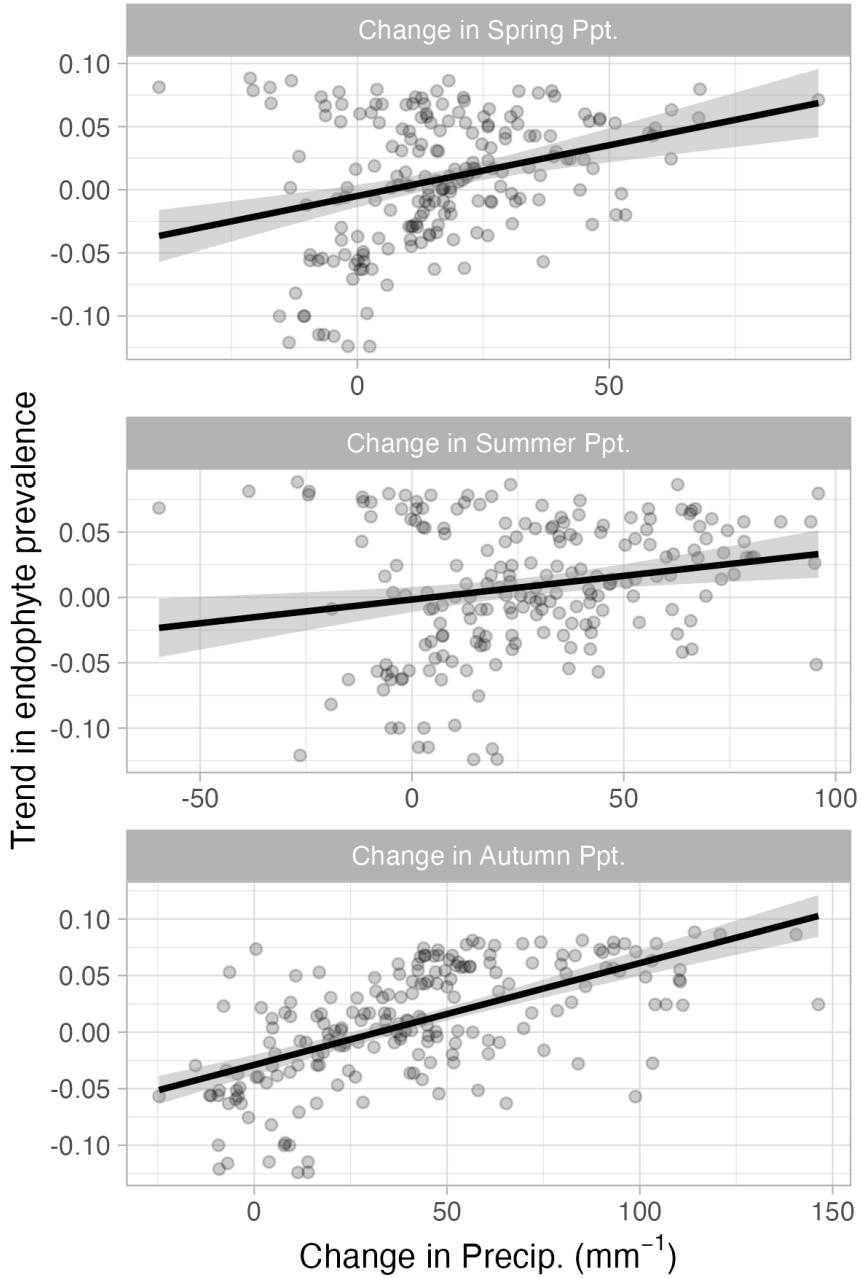


Figure 7: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (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 .
23 Asterisks denote correlation coefficients $> .3$ or $< -.3$.

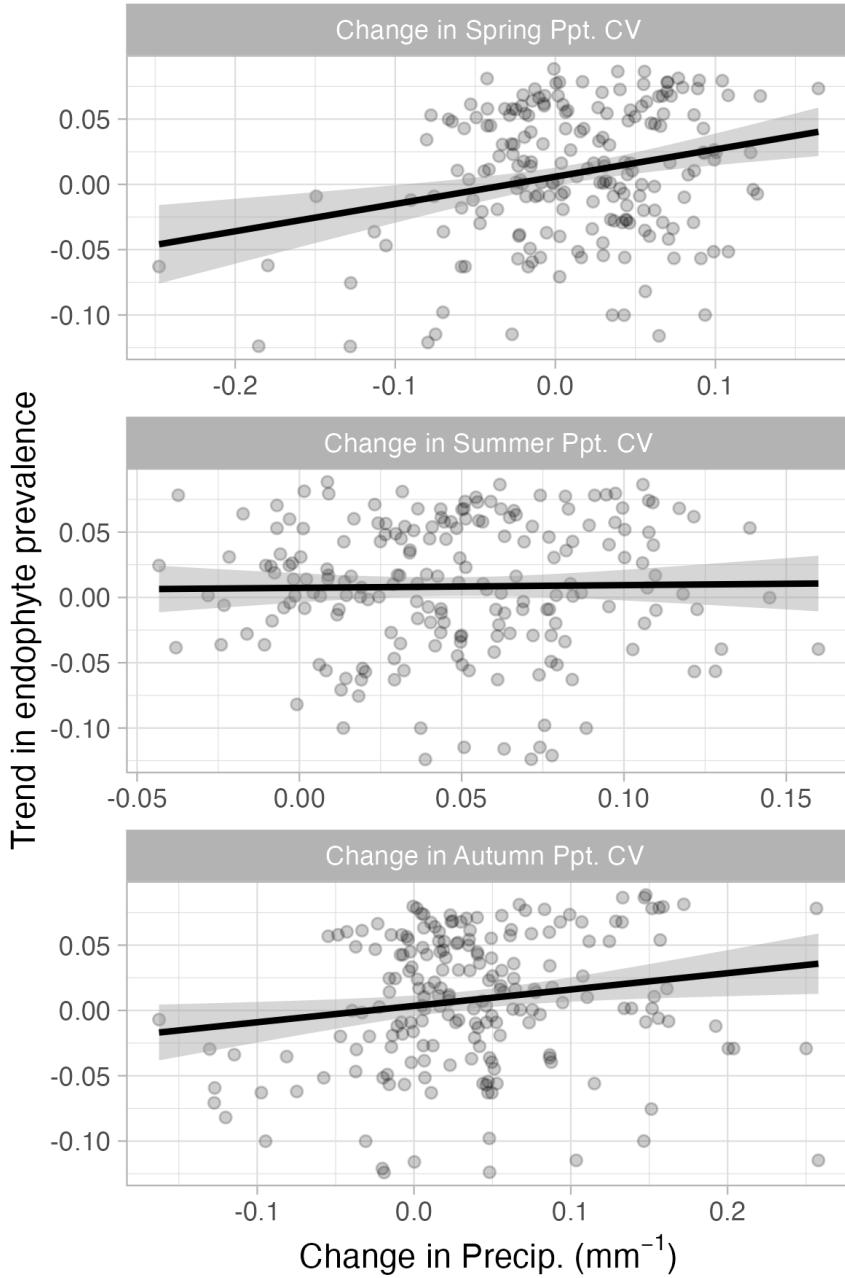


Figure 8: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (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 .
24 Asterisks denote correlation coefficients $> .3$ or $< -.3$.

329 *Performance on test data*

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

335 We discuss potential model improvements in the Discussion.

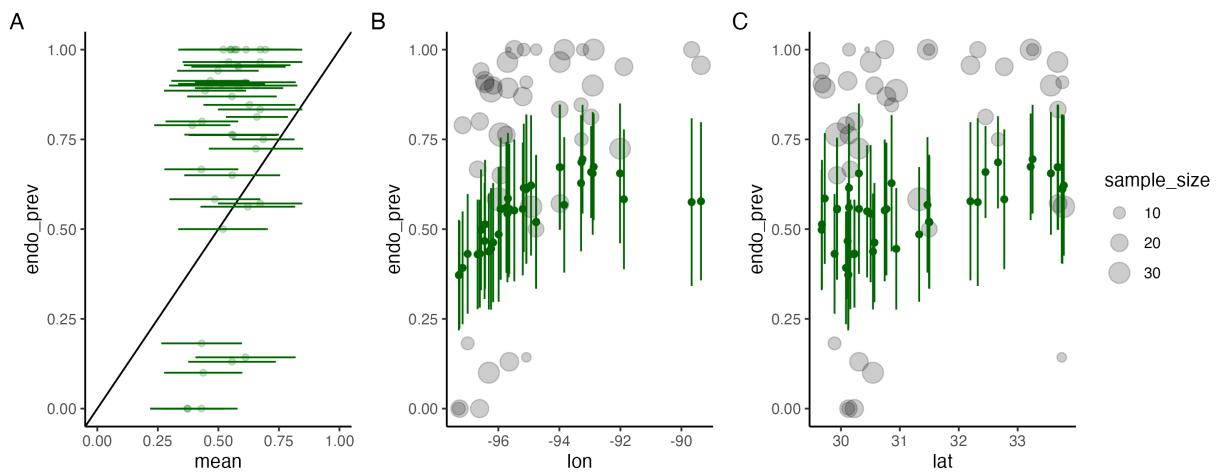


Figure 9: **Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporaneous endophyte prevalence in *A. hyemalis*, as indicated by some overlap of predicted 95% CI with the 1:1 line, 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

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

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

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

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

389 Using test data to validate our model predictions allows us to evaluate places to improve the
390 model's ability to perform well at out-of-sample prediction, which will be particularly important
391 for predicting host and symbiont niche-shifts under future climate change. Lack of information
392 on local variability may simply be a feature of data derived from herbarium specimens. Even
393 though they are samples from local populations, they are single specimens that are aggregated
394 over in broad-scale model estimates. Poor predictive ability at local scales in this grass-endophyte
395 system is not surprising, as previous studies have found that local variation, even to the scale of
396 hundreds of meters can structure endophyte-host niches (Kazenel et al., 2015). Sneck et al. (2017)
397 also identified host genotype as an important predictor of endophyte prevalence in *E. virginicus*.
398 Other studies have found factors including land-use history (Vikuk et al., 2019) and the biotic
399 environment, including herbivory (Rudgers et al., 2016), to be important predictors of endophyte
400 ecology. Incorporating available climatic and soil layers as covariates is an obvious first step that
401 could improve predictions. Towards the goal of predicting the dynamics of microbial symbioses
402 under climate change, models that integrate data from local and regional scales would be an
403 important step to bridge the gap that often exists between large but broad bioclimatic and bio-
404 diversity data and small but local data on biotic interactions. (Isaac et al., 2020; Miller et al.,
405 2019)

406 Our analysis advances the use of herbarium specimens in global change biology in two ways.
407 First and foremost, this is the first study to link long-term changes in microbial symbioses to
408 changes in climate using specimens from natural history collections. The responses of micro-
409 bial symbioses are a rich target for future studies within museum specimens, particularly those
410 that take advantage of advances in sequencing technology. While we used relatively coarse
411 presence/absence data based on fungal morphology, other studies have examined historic plant
412 microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these
413 studies have so far been limited to relatively few specimens at limited spatial extents (Bieker
414 et al., 2020; Bradshaw et al., 2021; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al.,
415 2015). Continued advances in capturing historic DNA and in filtering out potential contami-

416 nation during specimen storage (Bakker et al., 2020; Daru et al., 2019; Raxworthy and Smith,
417 2021) will be imperative in the effort to scale up these efforts. This scaling up will be essential
418 to be able to quantify changes not just in the prevalence of symbionts, but also in symbionts'
419 intraspecific variation and evolutionary responses to climate change, as well as in changes in the
420 wider microbial community. Answering these questions as well as the unknown questions that
421 future researchers may ask also reiterates the value in capturing meta-information during ongo-
422 ing digitization efforts at herbaria around the world and during the accession of newly collected
423 specimens (Lendemer et al., 2020). Second, we accounted for several potential biases in the data
424 observation process that may be common to many collections-based research questions by using
425 a spatially-explicit random effects model. Spatial autocorrelation (Willems et al., 2022), potential
426 biases introduced by the sampling habits of collectors (Daru et al., 2018), and variation between
427 contemporary researchers during the collection of trait data, if not corrected for could lead to
428 over-confident inference about the strength and direction of historic change. Previous studies
429 that have quantified the effects of collector biases typically find them to be small (Davis et al.,
430 2015; Meineke et al., 2019), and we similarly did not find that collector has a strong effect on the
431 results of our analysis. Fitting this model in a Bayesian framework allows for full propagation of
432 uncertainty.

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

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

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462 Statement of Authorship

463 Data and Code Availability

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

⁴⁶⁶ DOIs for data deposits (typically on the Dryad Digital Repository,) and code deposits (such as
⁴⁶⁷ in Zenodo).

468

Appendix A

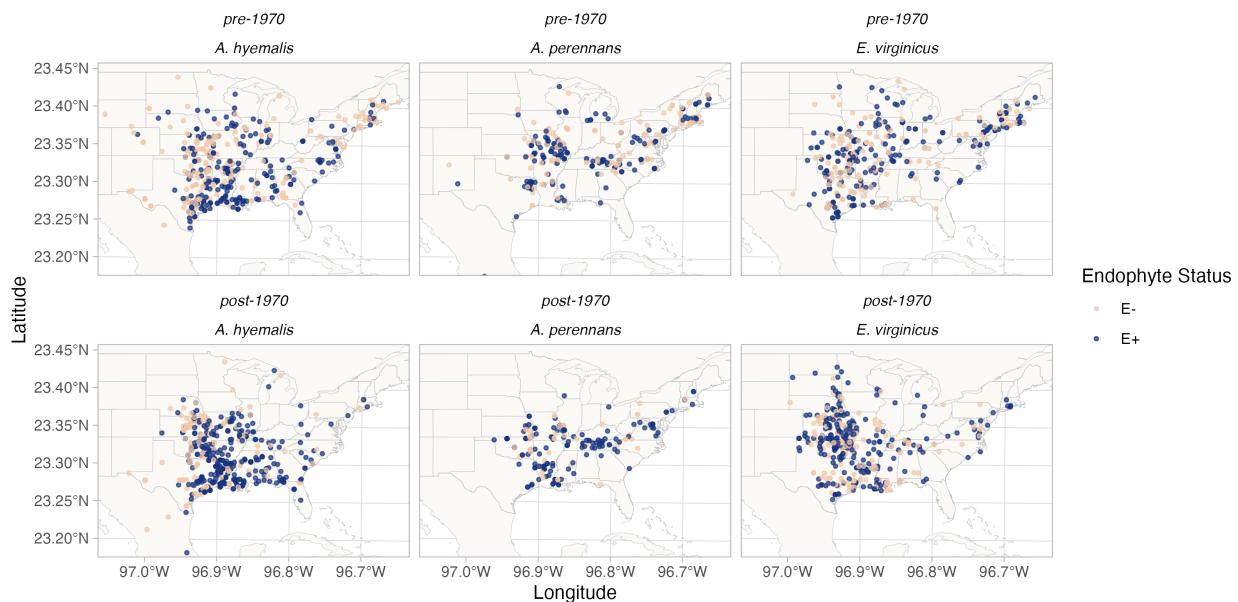


Figure A1: **Endophyte presence/absence in specimens of each host species.** Points show collection locations colored according to whether the specimen contained endophytes (E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.

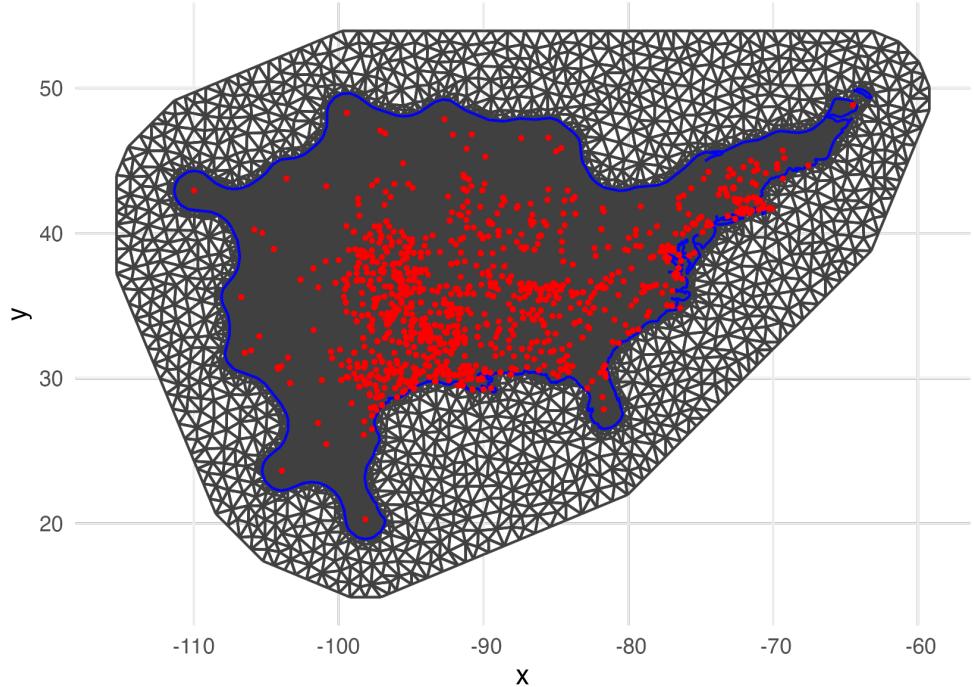


Figure A2: Delauney triangulation mesh used to estimate spatial dependence between data points. Grey lines indicate edges of triangles used to define distances between observations. Red points indicate locations of sampled herbarium specimens, and the blue outlines show the international borders used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

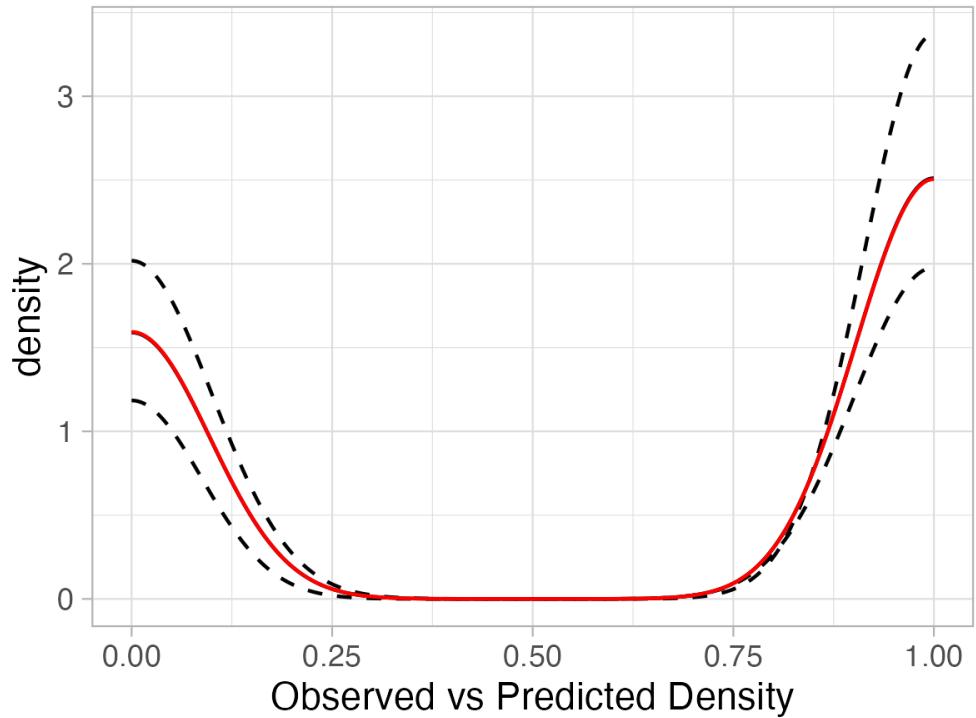


Figure A3: Consistency between real data and simulated values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (red) along with the mean(solid) and 95% CI (dashed) of simulated values (black).

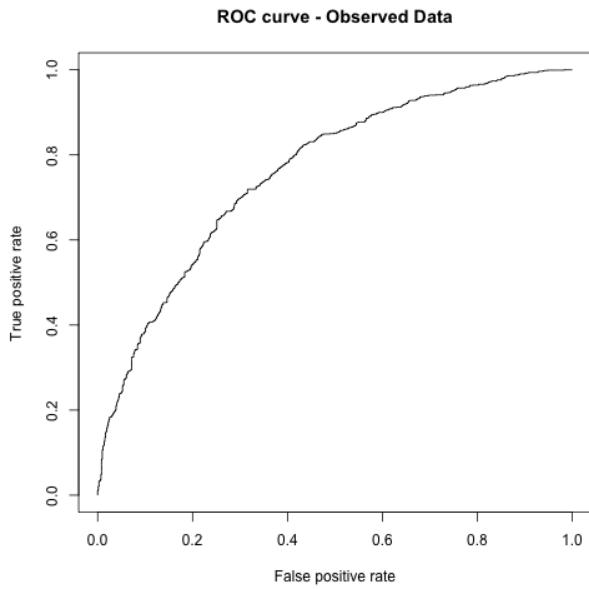


Figure A4: ROC plot showing model performance classifying observations according to endophyte status. The curves show adequate model performance for observed (top) and test (bottom) data. The AUC for each is 0.77.

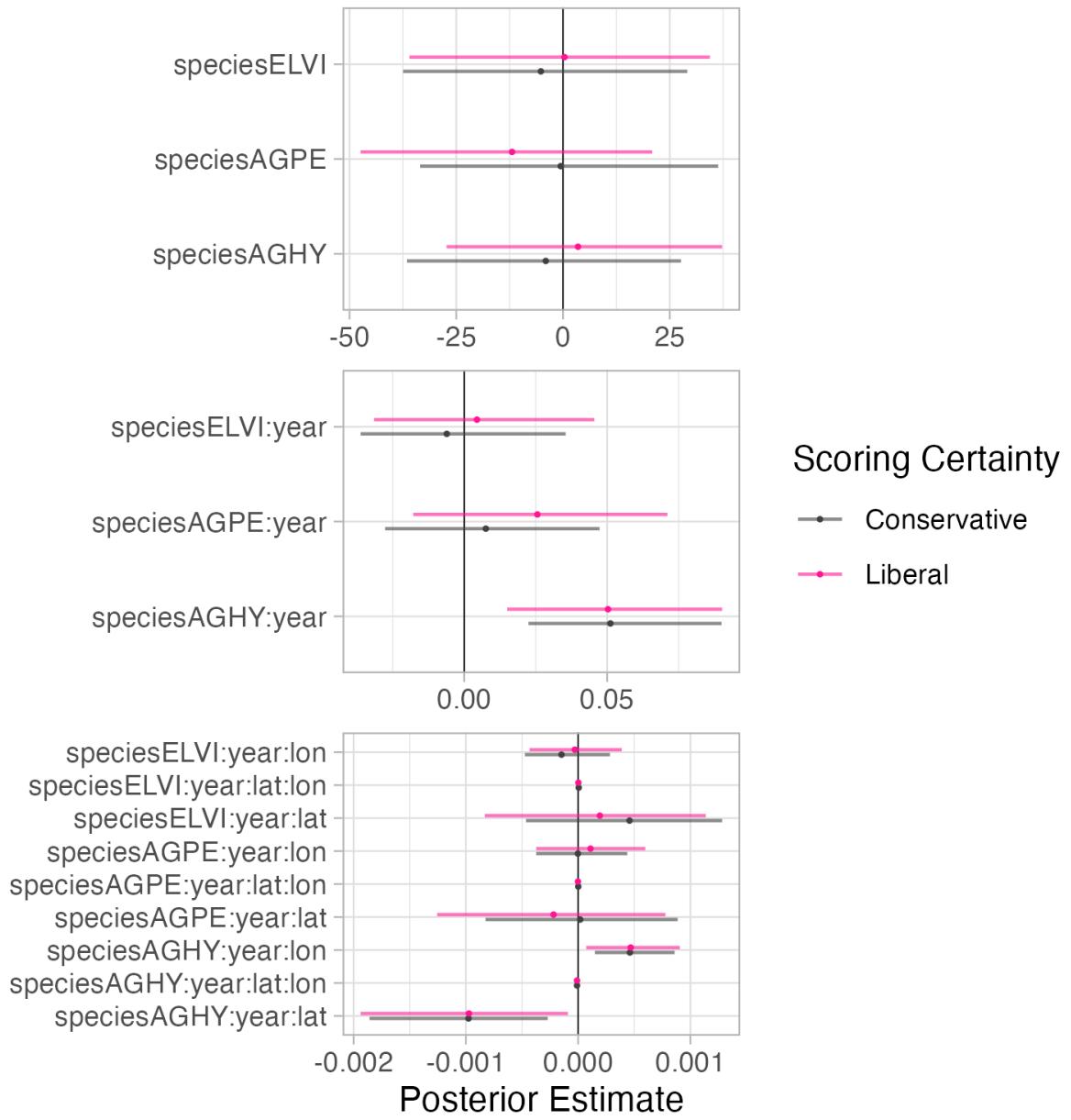


Figure A5: Comparison of posterior estimates of fixed effects when using Liberal or Conservative endophyte scores.

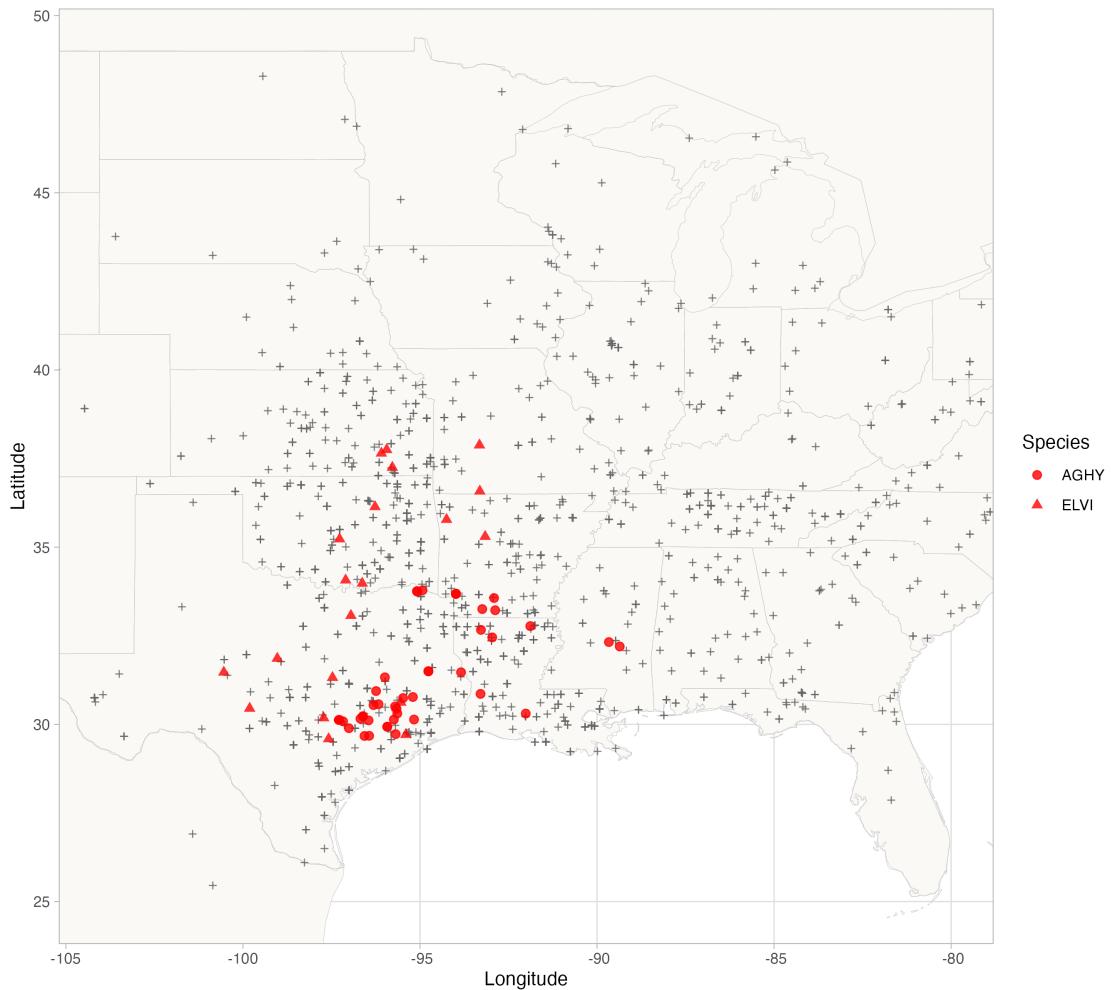


Figure A6: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).

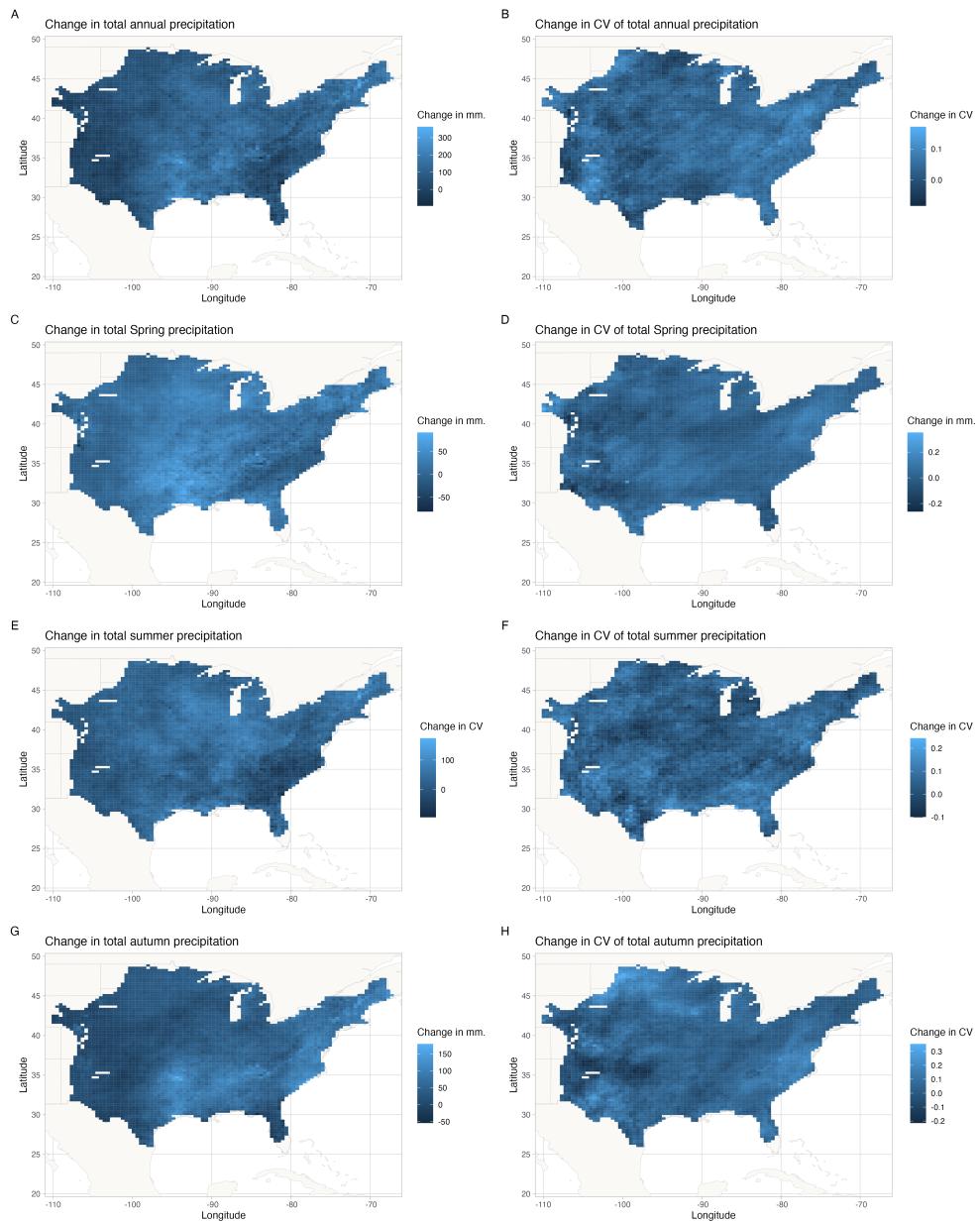


Figure A7: Change in precipitation between the periods 1895-1925 and 1990-2020. Color represents change in annual or seasonal total precipitation (A,C,E,G) and in the coefficient of variation of annual or seasonal total precipitation (B,D,F,H). Maps show the study area of *A. hyemalis*. Map pixels used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

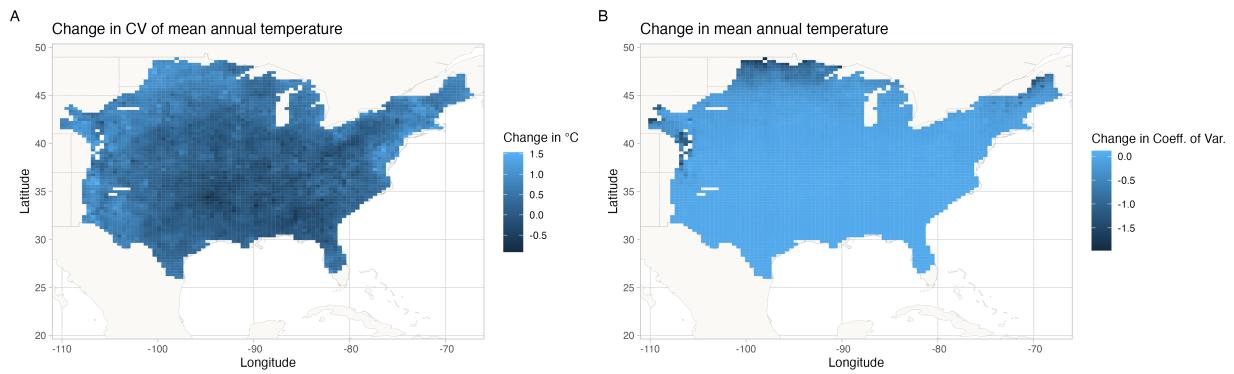


Figure A8: Change in temperature between the periods 1895-1925 and 1990-2020. Color represents change in annual mean temperature (A) and in the coefficient of variation of annual mean temperature (B). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change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

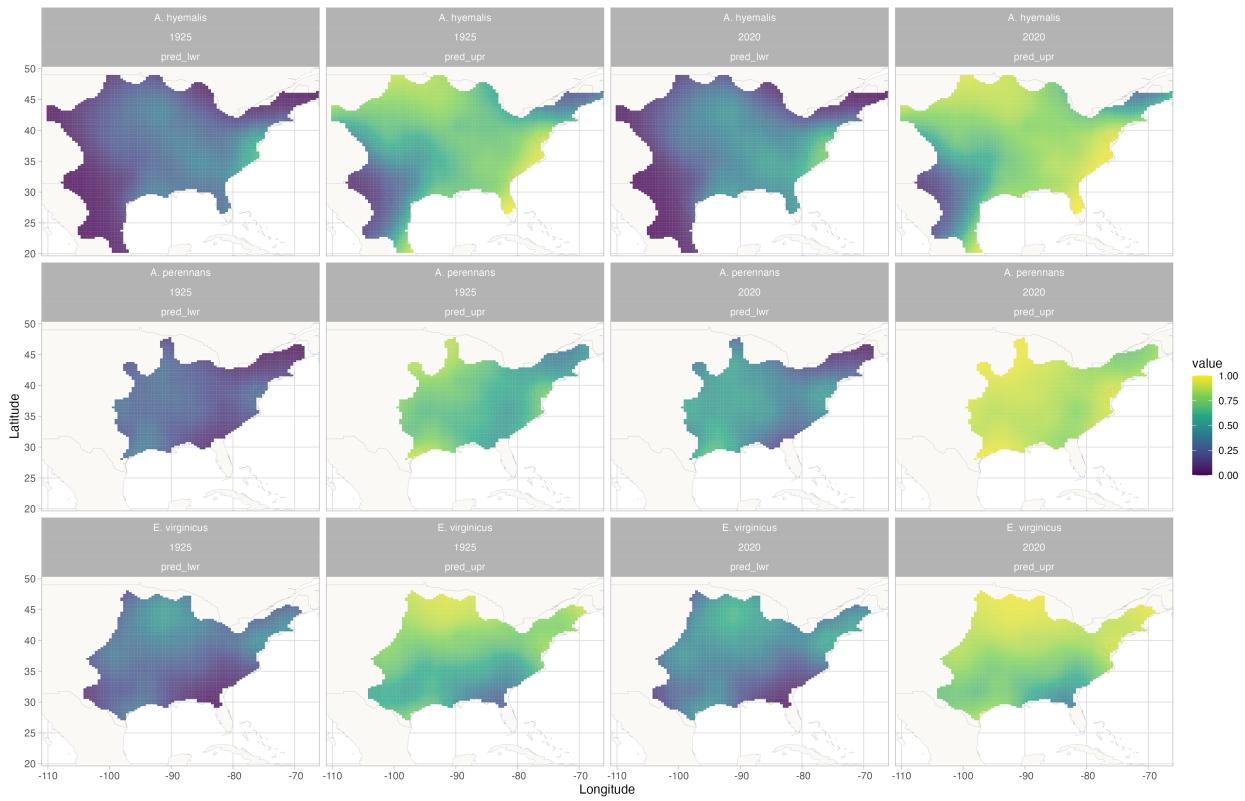


Figure A9: Uncertainty associated with spatial trends in endophyte prevalence. Color represents change in predicted endophyte prevalence. Panels show upper and lower 95% posterior probability for each host species between 1925 and 2020.

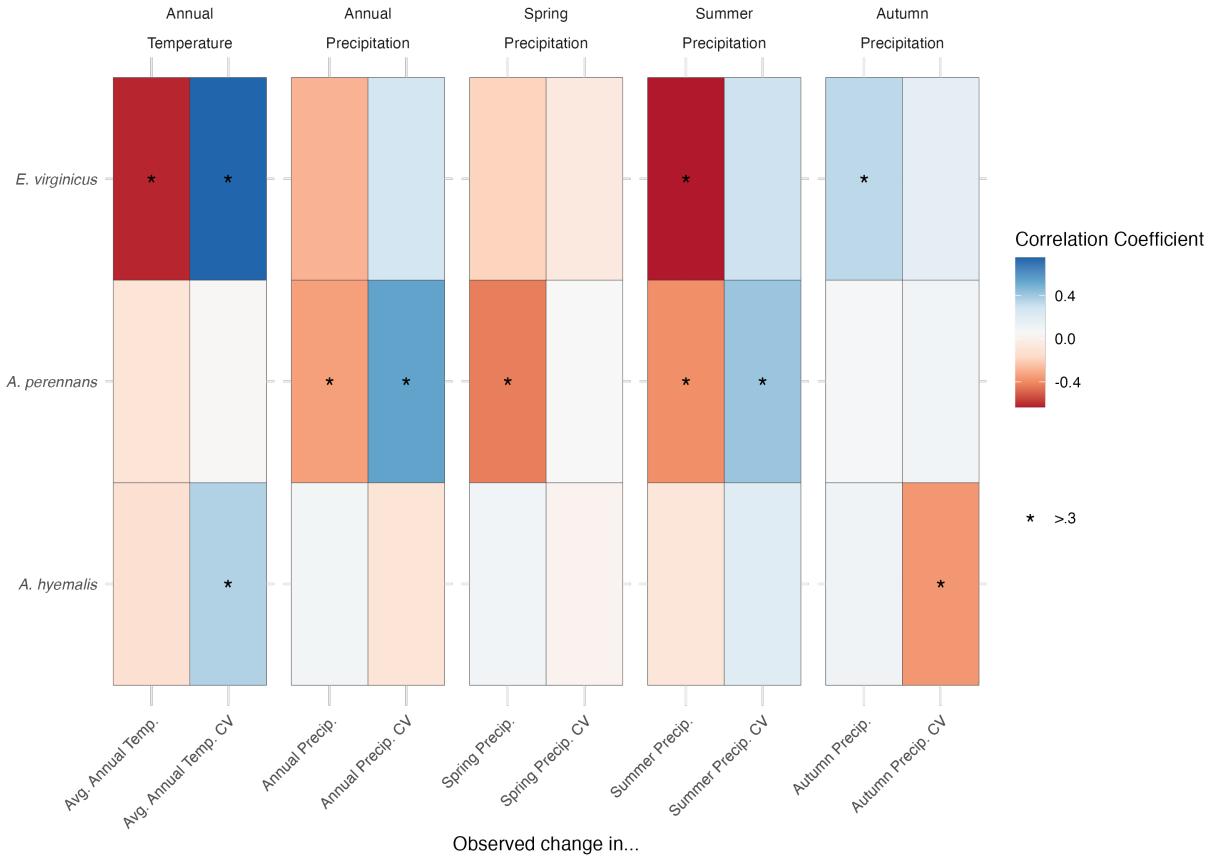


Figure A10: Correlations between changes in climate drivers and changes in endophyte prevalence from a random sample of 100 pixels across the study region. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) 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 |

469

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