

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

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⁹ *Keywords:* .

¹⁰ *Manuscript type:* Article.

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

¹ *I think this is should be 2*

Abstract

13 Species' distributions and abundances are shifting in response to climate change. Most species
14 harbor microbial symbionts that have the potential to influence these responses. Mutualistic
15 microbial symbionts may provide resilience to environmental change by protecting their hosts
16 from increasing stress. Alternatively, environmental change that disrupts these interactions may
17 lead to declines in hosts or symbionts. Microbes preserved within herbarium specimens offer a
18 unique opportunity to quantify changes in microbial symbiosis across broad temporal and spatial
19 scales. We asked how the prevalence of seed-transmitted fungal symbionts of grasses (*Epichloë*
20 endophytes), which can protect hosts from abiotic stress, have changed over time in response to
21 climate change, and how these changes vary across host species' ranges. Specifically, we analyzed
22 #² herbarium specimens of three grass host species collected over the last two centuries (18## –
23 20##) for the presence or absence of endophyte symbiosis, and evaluated spatial and temporal
24 trends in endophyte prevalence. We found that endophytes have increased in prevalence over
25 the last two centuries from ca. 25% prevalence to ca. 75% prevalence, on average, across the three
26 host species. We also found that changes in prevalence were associated with observed **changes in**
27 **annual and seasonal climate drivers**³ corresponding to each host species' peak growing season.
28 Our results provide novel evidence for a cryptic biological response to climate change that may
29 contribute to the resilience of host-microbe symbiosis through context-dependent benefits that
30 confer a fitness advantage to symbiotic hosts under environmental change.⁴

²*Give numbers*

³*Describe "changes" – warming? drying?*

⁴*I like this and the abstract in general. I agree with Tom and I think we have some space to add these details. Abstract : 300*

Introduction

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

40 Natural history specimens, 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 and temporal scales (?). Natural history collections, built and maintained
43 by the efforts of thousands of scientists, are invaluable time machines, primarily comprised of
44 physical specimens of organisms along with information about the time and place of their col-
45 lection. These specimens often preserve physical legacies of ecological processes and species'
46 interactions from dynamically changing environments across time and space. For example, pre-
47 vious researchers have used plant collections (herbaria) to document shifts in phenology (???),
48 pollination (??), and herbivory (?) related to anthropogenic climate change. However, few pre-
49 vious studies have leveraged biological collections to examine climate change-related shifts in a
50 particularly common type of interaction: microbial symbiosis.

51 Microbial symbionts are common to all macroscopic organisms and can have important ef-
52 fects on their hosts' survival, growth and reproduction (??). Many microbial symbionts act as
53 mutualists, engaging in reciprocally beneficial interactions with their hosts that can ameliorate
54 environmental stress. For example, bacterial symbionts of insects, such as *Wolbachia*, can improve
55 their hosts' thermal tolerance (?), and arbuscular mycorrhizal fungi, documented in 70-90% of
56 families of land plants (?), allow their hosts to persist through drought conditions by improving

57 water and nutrient uptake (?). On the other hand, changes in the mean and variance of envi-
58 ronmental conditions may disrupt microbial mutualisms by changing the costs and benefits of
59 the interaction for each partner, leading the interaction to deteriorate (?). Coral bleaching (the
60 loss of symbiotic algae) due to temperature stress (?) is perhaps the best known example, but
61 this phenomenon is not unique to corals. Lichens exposed to elevated temperatures experienced
62 loss of photosynthetic function along with changes in the composition of their algal symbiont
63 community (?). How commonly and under what conditions microbial mutualisms deteriorate
64 or strengthen under climate change remain unanswered questions. Previous work suggests that
65 these alternative responses may depend on the intimacy and specialization of the interaction as
66 well as the physiological tolerances of the mutualist partners (???).

67 Understanding of how microbial symbioses are affected by climate change is additionally
68 complicated by spatial heterogeneity in the direction and magnitude of environmental change
69 (?). Beneficial symbionts are likely able to shield their hosts from environmental stress in loca-
70 tions that experience a small degree of change, but symbionts in locations that experience changes
71 of large magnitude may be pushed beyond their physiological limits (?). Additionally, symbionts
72 are often unevenly distributed across their hosts' distribution. Facultative symbionts may be
73 absent from portions of the host range (?), and hosts may engage with a diversity of partners
74 (different symbiont species or locally-adapted strains) across their environments (??). Identify-
75 ing broader spatial trends in symbiont prevalence is therefore an important step in developing
76 predictions for where to expect changes in the symbiosis in future climates.

77 *Epichloë* fungal endophytes are specialized symbionts of cool-season grasses, which have been
78 documented in ~ 30% of cool-season grass species (?). They are transmitted vertically from
79 maternal plants to offspring through seeds. Vertical transmission creates a feedback between
80 the fitness of host and symbiont (???). Over time, endophytes that act as mutualists should rise
81 in prevalence within a host population (?). *Epichloë* are known to improve their hosts' drought
82 tolerance (?) and protect their hosts against herbivores (?) and pathogens (?) likely through the
83 production of a diverse suite of alkaloids and other secondary metabolites. The fitness feedback

84 induced by vertical transmission leads to the prediction that endophyte prevalence should be
85 high in populations where these fitness benefits are most important. Previous survey studies have
86 documented large-scale spatial patterns in endophyte prevalence structured by environmental
87 gradients (????). We predicted that prevalence should track temporal changes in environmental
88 drivers that elicit these fitness benefits.

89 Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic diver-
90 sity of host species that harbor these symbionts (?), establishing that signatures of endophyte
91 symbiosis can be recovered from long-preserved plant tissue.⁵ However, no subsequent studies,
92 to our knowledge, have used the vast resources of biological collections to quantitatively assess
93 spatio-temporal trends in endophyte prevalence and their environmental correlates. Grasses are
94 commonly collected and identified based on the presence of their reproductive structures, mean-
95 ing that preserved specimens typically contain seeds, conveniently preserving the fungi along
96 with their host plants on herbarium sheets. This creates the opportunity to leverage the unique
97 spatio-temporal sampling of herbarium collections to examine the response of the symbiosis to
98 historical climate change.

99 In this study, we assessed the long-term responses of endophyte symbiosis to climate change
100 through the use of herbarium specimens of three North American host grass species (*Agrostis*
101 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*). We first address questions describing spatial
102 and temporal trends in endophyte prevalence: (i) How has endophyte prevalence changed over
103 the past two centuries? and (ii) How spatially heterogeneous are temporal trends in endophyte
104 prevalence across eastern North America? We then address how climate change may be driving
105 trends in endophyte prevalence by asking: (iii) What is the relationship between variation in
106 temporal trends in endophyte prevalence and changes in climate drivers? We predicted that
107 aggregate endophyte prevalence would increase over time in tandem with climate warming, and
108 that hotspots of endophyte change would correspond spatially to hotspots of climate change. To
109 answer these questions we examined a total of 2,346 specimens collected across eastern North

⁵Not sure about this but I am trying to pre-empt skepticism that fungi are less detectable in older samples.

¹¹⁰ America between 1824 and 2019.⁶

¹¹¹ Methods

¹¹² Focal species

¹¹³ Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis peren-*
¹¹⁴ *nans*, and *Elymus virginicus*. Both *Agrostis* species host *Epichloë amarillans* (?), while *Elymus*
¹¹⁵ *virginicus* typically hosts *Epichloë elymi* (?). These C₃ grass species are commonly represented in
¹¹⁶ natural history collections with broad distributions covering much the eastern United States. *A.*
¹¹⁷ *hyemalis* is a small short-lived perennial species that germinates in the spring and typically flow-
¹¹⁸ ers between March and July (most common collection month: May). *A. perennans* is of similar
¹¹⁹ stature but is longer lived than *Agrostis hyemalis* and flowers in late Summer⁷ and early Autumn
¹²⁰ (most common collection month: September). *A. perennans* is more sparsely distributed, tending
¹²¹ to be found in shadier and more moist habitats, while *A. hyemalis* is commonly found in open
¹²² and recently disturbed ground. Both *Agrostis* species are recorded from throughout the Eastern
¹²³ US, but *A. perennans* has a slightly more northern distribution, whereas *A. hyemalis* is found rarely
¹²⁴ as far north as Canada and is listed as a rare plant in Minnesota. *E. virginicus* is a larger and
¹²⁵ relatively longer-lived species that is more broadly distributed than the *Agrostis* species. It be-
¹²⁶ gins flowering as early as March or April but continues throughout the Summer (most common
¹²⁷ 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). We permission from herbarium staff, we acquired seed samples

⁶*I 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.*

⁷*Are seasons capitalized?*

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

146 After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hy-
147 phae, which grow intercellularly, in each specimen using microscopy. We first softened seeds
148 with a 10% NaOH solution, then stained the seeds with aniline blue⁸ dye and squashed them
149 under a microscope cover slip. We examined the squashed seeds for the presence of fungal hy-
150 phae at 200-400X magnification (?). In some cases, the tissues examined during microscopy came
151 from flowers or otherwise non-viable seeds, which were excluded for that specimen. On average
152 we scored 4.7 seeds per specimen of *A. hyemalis*, 4.2 seeds per specimen of *A. perennans*, and
153 3.8 seeds per specimen of *E. virginicus*; we scored # seeds in total. . Due to imperfect vertical
154 transmission (?), it is possible that symbiotic host-plants produce a mixture of symbiotic and non-
155 symbiotic seeds. We therefore designated a specimen as endophyte-symbiotic if *Epichloë* hyphae
156 were observed in one or more of its seeds, or non-symbiotic if hyphae were observed in none of

⁸Most of our stains in the lab also have lactic acid.

157 its seeds. To capture uncertainty in the endophyte scoring process, we recorded both a "liberal"
 158 and a "conservative" endophyte status for each plant⁹. When we identified potential endophytes
 159 with unusual morphology, low uptake of stain, or a small amount of fungal hyphae across the
 160 scored seeds, we recorded a positive liberal status (more likely to be endophyte-positive) and
 161 a negative conservative status (less likely to be endophyte-positive). 89% of scored plants had
 162 matching liberal and conservative scores, reflecting high confidence in endophyte status. The
 163 following analyses in the main text used the liberal status, but we repeated all analyses with the
 164 conservative status which yielded qualitatively similar results (Figure A5)¹⁰.

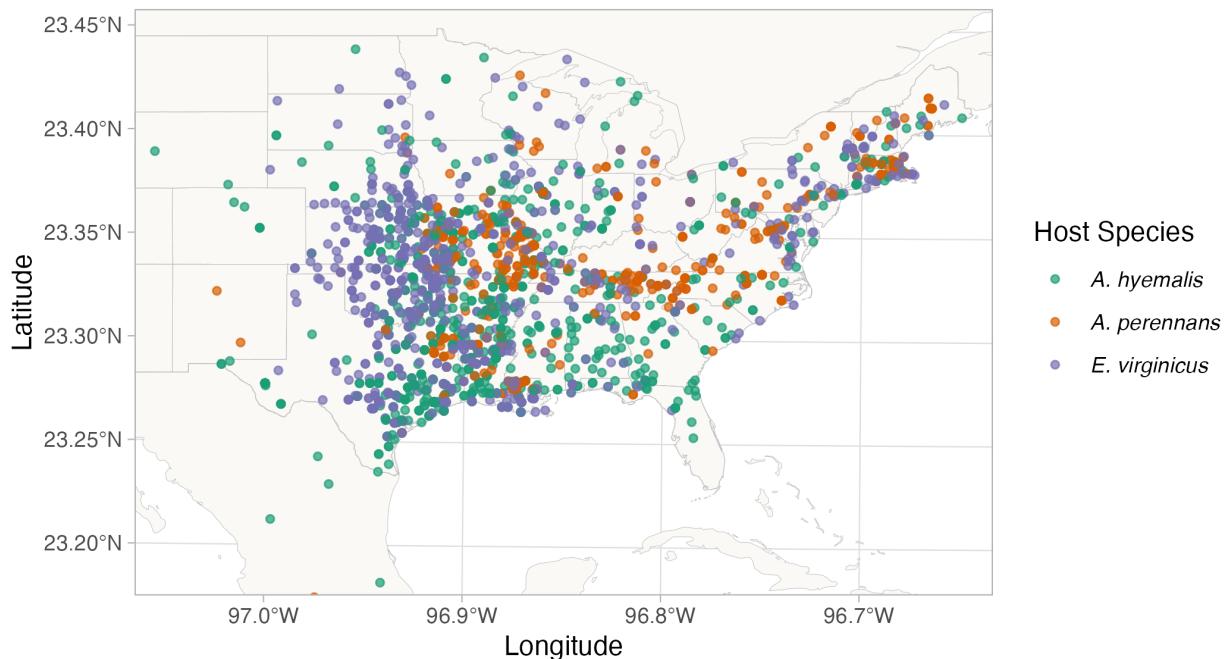


Figure 1: Collection locations of herbarium specimens of three grass host species across eastern North America that were sampled for *Epichloë* endophyte presence or absence.

⁹Plant or seed?

¹⁰Use ?? for citing figures, and stay consistent between Figure and Fig.

165 *Modeling spatial and temporal changes in endophyte prevalence*

166 We assessed spatial and temporal changes in endophyte prevalence across each host distribu-
167 tion, first quantifying the “global” trends, aggregating across space, and then examining spatial
168 heterogeneity in the direction and magnitude of endophyte change (hotspots and coldspots)
169 across the spatial extent of each host’s distribution. To appropriately account for the spatial
170 non-independence of geo-referenced occurrences we used an approximate Bayesian method,
171 Integrated Nested Laplace Approximation (INLA), to construct spatio-temporal models of en-
172 dophyte prevalence. INLA provides a computationally more efficient method of ascertaining
173 parameter posterior distributions for certain models that can be formulated as latent Gaussian
174 Models (?). Many common statistical models, including structured and unstructured mixed-
175 effects models, can be represented as latent Gaussian Models. Fitting models with structured
176 spatial effects is possible with MCMC sampling but can require long computation times, mak-
177 ing INLA an effective alternative, which has been used to model spatial patterns in flowering
178 phenology (?), the abundance of bird species (?) and butterflies citecrossley2022opposing, the
179 distribution of temperate trees (?) as well as the population dynamics of endangered amphibians
180 (?) and other ecological processes (?).¹¹

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

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

186 Spatially-indexed random intercepts $\alpha_{l[i]}$ account for potential spatial autocorrelation between

¹¹I 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.

187 data points, and year slope β describes the overall temporal trend in endophyte prevalence. We
188 accounted for potential biases introduced during the process of collecting specimens as well as
189 in scoring ability by including random effects specific to each collector χ and scorer ω . Pre-
190 vious work suggests that behavior of historical botanists and uneven sampling may introduce
191 biases into ecological inferences made from historic collections (?). Prolific collectors who con-
192 tribute thousands of specimens may be more or less likely to collect certain species, or specimens
193 with certain traits (?). Similarly, the process of scoring seeds for hyphae involved many student
194 researchers who, even with standardized training, may vary in their likelihood of positively iden-
195 tifying *Epichloë* hyphae. By including a random effect for collectors and for scorers, we accounted
196 for variance across individual researchers that may bias our predictions of changes in endophyte
197 prevalence. Models for each host species were fit separately.^{12 13}

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

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

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

202 For both models, spatially-structured random intercepts (α_l) and slopes (β_l) were constructed
203 using stochastic partial differential equations (SPDE) that depend on a covariance matrix accord-
204 ing to the proximity of each collection location (??). The covariance matrix was approximated
205 using a Matérn covariance function, with each data point assigned a location according to the
206 nodes of a mesh of non-overlapping triangles across our study area (Fig A2).¹⁴

¹²It 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.

¹³I 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 .

¹⁴This paragraph would be a place to describe the variance terms for the other random effects.

207 We performed model fitting using the inlabru R package (), with vague priors¹⁵, and com-
208 pared models with different sizes of mesh, which had little effect on the resulting model esti-
209 mates¹⁶. Each mesh¹⁷ was bounded by the predicted host distribution, described below. Posterior
210 modes were stable¹⁸ indicating that numeric convergence was successful. We assessed model fit
211 with graphical posterior predictive checks (Fig. A3). The model performed adequately at clas-
212 sifying the historical data, comparing the accuracy of predictions from the model with observed
213 data (avg. AUC = 0.77; Fig. A4).¹⁹

214 *Modeling distributions of host species*

215 We modeled epicloe host species distribution to predict their occurrences in space and time as
216 continuous and binary maps of potential presences. These maps were used as a backbone to
217 predict endophyte prevalence on epicloe host species. The species distribution models were built
218 following the ODMAP (overview, data, model, assessment, prediction) protocol (?). We used the
219 observed presence of the host species collected from GBIF from 1990 to 2020. These occurrences
220 were corrected for spatial autocorrelation due to sampling bias by thinning the occurrences to
221 the spatial scale of the climatic variables. The climatic variables were temperature of the spring,
222 precipitation of the spring and precipitation of the summer. We preferred these variables because
223 they were not correlated (Variance Inflation Factor 0.7) and also allowed the model to account for
224 the influence of seasonal climate variation on species presence. The occurrence data was split into
225 75% for model training and 25% for model testing. We fitted the model using maximum entropy
226 (MaxEnt) using the maxent function in the package dismo (?). MaxEnt was preferred because it
227 does not generate response curves that may cause unpredictable behavior when applied to new
228 climates (?). We used 10000 pseudo-absences as background points. To convert the continuous

¹⁵I thought you needed informative priors on the spatial decay parameters

¹⁶That'sa good but you still need to state what mesh size you used and what that means, biologically.

¹⁷You have not defined what you mean by "mesh".

¹⁸Assessed how?

¹⁹Maybe move this to validation section, and then have both in-sample and out-of-sample approaches.

²²⁹ predicted probabilities into binary presence - absence maps, we used the training sensitivity (true
²³⁰ positive rate) and specificity threshold (true negative rate) (?). The performances of the model
²³¹ were evaluated using the AUC (?).

²³² *Validating the model with an out-of-sample test*

²³³ We evaluated the predictive ability of the model using contemporary endophyte surveys as out-
²³⁴ of-sample test data, **an important but rarely used strategy in ecological studies (?)**.²⁰ We used
²³⁵ data from contemporary surveys of endophyte prevalence in *A. hyemalis* and *E. virginicus* in
²³⁶ Texas and the southern US. Surveys of *E. virginicus* were conducted in 2013 as described in
²³⁷ ?, and **surveys of *A. hyemalis* took place between 2015 and 2020**²¹. Population surveys of *A.*
²³⁸ *hyemalis* were initially designed to cover longitudinal variation in endophyte prevalence towards
²³⁹ its range edge, while surveys of *E. virginicus* were designed to cover latitudinal variation along its
²⁴⁰ range edge. In total, we visited 43 populations of *A. hyemalis* and 20 populations of *E. virginicus*
²⁴¹ across the south-central US, with emphasis on Texas and neighboring states (Fig A4²²). During
²⁴² surveys, we collected seeds from up to 30 individuals per location (average number of plants
²⁴³ sampled: 22.9). We quantified the endophyte status of each individual with staining microscopy
²⁴⁴ as described for the herbarium surveys (with 5-10 seeds scored per individual), and calculated
²⁴⁵ the prevalence of endophytes within the population (proportion of symbiotic plants divided
²⁴⁶ by the number of sampled plants). For each population, we compared the observed fraction
²⁴⁷ of endophyte-symbiotic hosts to the predicted probability of endophyte occurrence \hat{P} derived
²⁴⁸ from the model based on location and year, with collector and scorer random effects fixed at
²⁴⁹ zero. **The contemporary survey period (2013-2020) is at the most recent edge of the time period**
²⁵⁰ **encompassed by the historical observations used for model fitting. We compared the model's**

²⁰*This 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.*

²¹*We 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.*

²²*This is now A6. Good reminder to use the ref function.*

251 prediction for these locations to the observed population prevalence.²³

252 *Assessing the role of climate drivers*

253 We assessed how the magnitude of climate change may have driven changes in endophyte preva-
254 lence by assessing correlations between changes in climate and changes in endophyte prevalence
255 predicted from our spatial model at evenly spaced pixels across the study area. We first down-
256 loaded monthly temperature and precipitation rasters from the PRISM climate group (?) cov-
257 ering the time period between 1895 and 2020 using the 'prism' R package (?). Prism provides
258 reconstructions of historic climate variables across the United States by spatially-interpolating
259 weather station data (?). We calculated 30-year climate normals for annual and seasonal mean
260 temperature and cumulative precipitation for the recent (1990 to 2020) and historic (1895 to 1925)
261 periods. We used three four-month seasons within the year (Spring: January, February, March,
262 April; Summer: May, June, July, August; Autumn: September, October, November, December).
263 This division of seasons allowed us to quantify differences in climate associated with the two
264 "cool" seasons, when we expect our focal species to be most biologically active (*A. hyemalis* flow-
265 ering phenology: Spring; *E. virginicus*: Spring and Summer; *A. perennans*: Fall). In addition
266 to mean climate conditions, environmental variability itself can influence population dynamics
267 (?) and changes in variability are a key prediction of climate change models (??). Therefore
268 we calculated the coefficient of variation (CV) during each period for each annual and seasonal
269 climate driver as the interannual standard deviation divided by the mean across each 30-year
270 period. We then took the difference between recent and historic periods for the mean and CV for
271 each climate driver (Fig. A5)²⁴. Because initial analyses indicated a high degree of collinearity
272 between seasonal and annual changes in temperature, we used annual temperature only, along
273 with annual and seasonal precipitation, in the subsequent analysis. All together, this left us with
274 measurements of change in 10 potential climate drivers: the mean and coefficient of variation

²³*It is not clear if you are testing model 1 ("global trend") or model 2 ("spatially varying trends").*

²⁴*This is Figure A7 – Can you make the color scale on these diverging at zero?*

275 of annual temperature, as well as the mean and coefficient of variation of cumulative annual
276 precipitation, cumulative spring precipitation, cumulative summer precipitation, and cumulative
277 autumn precipitation (Fig A8-A9)²⁵.

278 To evaluate whether areas that have experienced the greatest changes in endophyte preva-
279 lence (hotspots of endophyte change) are associated with high degrees of change in climate
280 (hotspots of climate change), we modeled spatially varying slopes of endophyte change through
281 time (β_t) as a linear function of environmental covariates, with a Gaussian error distribution.²⁶
282 Calculating correlations from many pixels across the study region risks artificially inflating confi-
283 dence in our results due to large sample sizes, and so we repeated this calculation using only a
284 random subsample of 100 pixels across the study region²⁷.²⁸²⁹

285

Results

286

How has endophyte prevalence changed over time?

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

²⁵ The species names are not clear on Fig A9. I suggest increase the font size

²⁶ I 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.

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

²⁸ Are the methods above repeated for each species separately?

²⁹ I 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.

293 *perennans*, and 58% probability of a positive overall year slope in *E. virginicus*) .³⁰

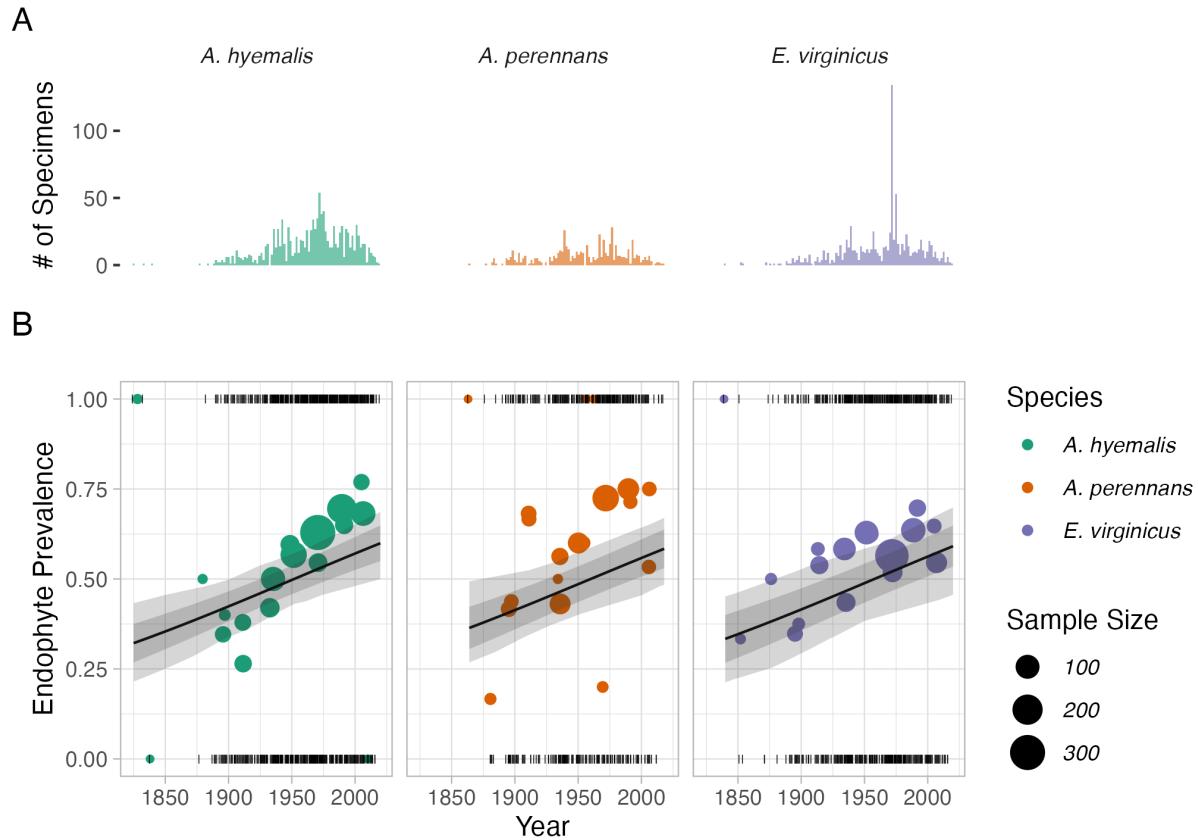


Figure 2: Temporal trends in endophyte prevalence. (A) Histograms show the frequency of collection through time for each host species. (B) Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species and point size is determined by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

294 *How spatially heterogeneous are temporal trends in endophyte prevalence?*

295 Our model revealed hotspots of change in endophyte prevalence . While there was an overall
296 increase in endophyte prevalence, these changes varied across the host species' ranges (Fig. 3).

³⁰These numbers are currently outdated. I am making some adjustments to models, and will update with final model

297 In some regions, posterior estimates of our spatially varying temporal trends, τ , indicate that *A.*
298 *hyemalis* and *A. perennans* experienced increases in percent prevalence by as much as 4% per year
299 over the study period, while *E. virginicus* experienced increases up to around 1.5 % per year. In
300 other regions, there were negligible changes. Notably, the symbionts of *E. virginicus* experienced
301 only slight increases in prevalence, and were less spatially variable than the other two species.
302 Regions that start with low endophyte prevalence, as in the southwestern portion of the range
303 of *A. hyemalis*(Fig. A1), also experienced negligible change, suggesting that this may be driven
304 more by the absence of the endophyte.³¹ Predicted trends for *A. perennans* show certain areas
305 of both large increase and of large decrease, however this species, for which we have the fewest
306 samples, has the largest uncertainty. The posterior estimates of our spatially varying temporal
307 trends, indicate relatively narrow certainty (need to compute³²).

³¹more discussion material, but putting it here for now.

32

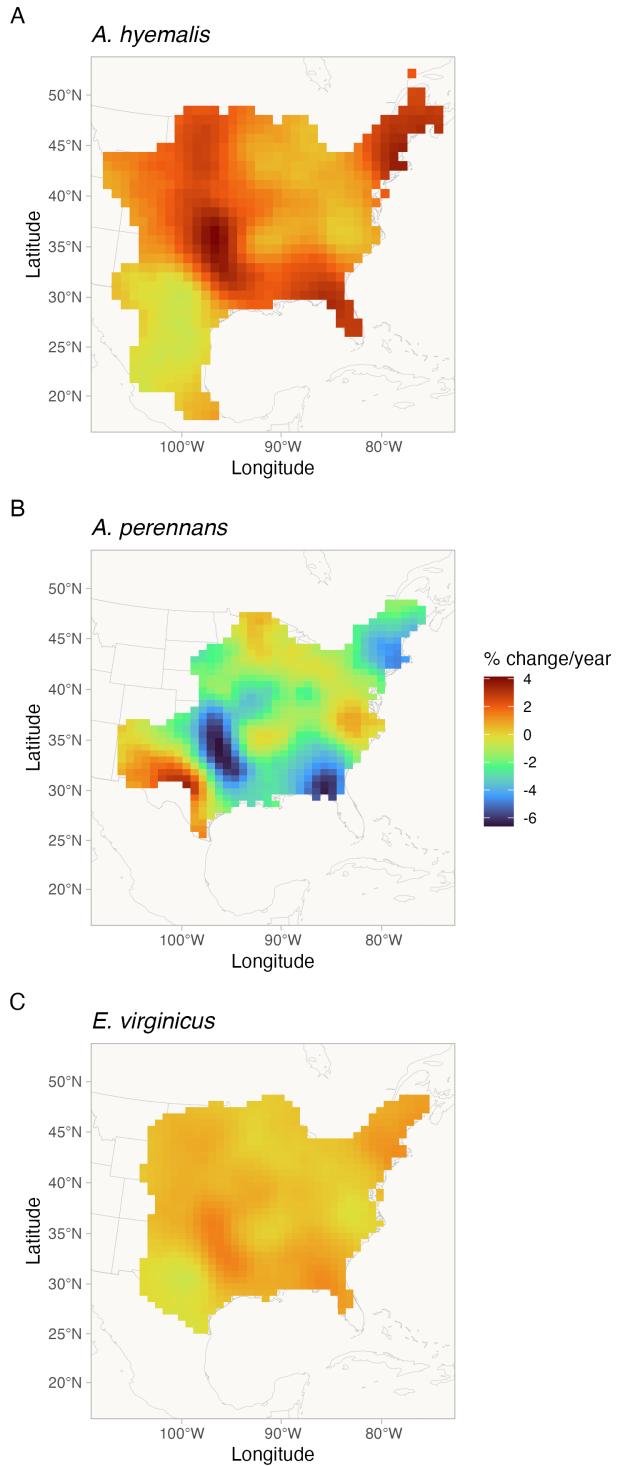


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species. Color indicates the relative change in predicted endophyte prevalence.

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

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

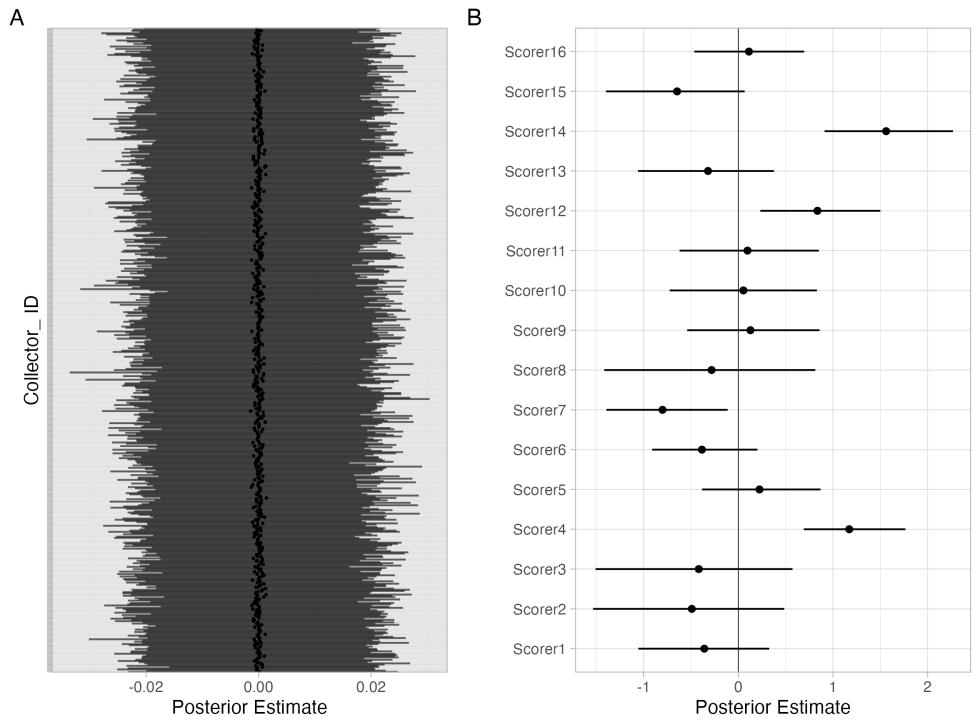


Figure 4: Posterior estimates of (A) collector and (B) scorer random effects. Points show the posterior mean along with 95% CI for random effects estimate from 532 collectors and 16 scorers.

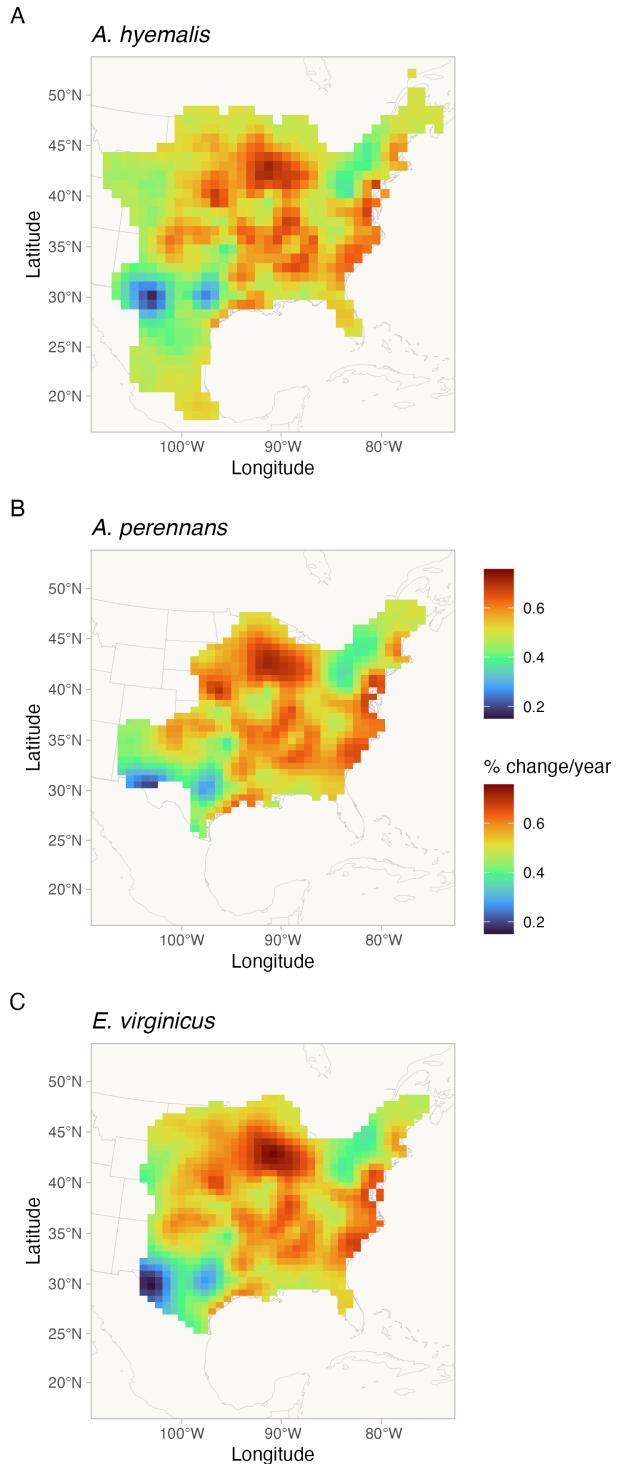


Figure 5: Mean predicted endophyte prevalence for each host species (columns) in 1925 (top row) and 2020 (bottom row). Color indicates mean predicted rate of endophyte prevalence across the predicted distribution of each species.

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

322 We found that trends in endophyte prevalence were strongly associated with seasonal climate
323 change drivers (Fig. 6). For the majority of the study region, the climate has become wetter and
324 cooler over the last century (Fig. A7-A8), a consequence of regional variation in global climate
325 change (?). Within the study region, spatially heterogeneous environmental changes were pre-
326 dictive of changes in endophyte prevalence. For example, strong increases in prevalence within
327 *E. virginicus* were most associated with declines in Summer precipitation (a negative correla-
328 tion in Fig. 7) as well as with increases in the year-to-year variability of annual temperature
329 (a positive correlation in Fig. 7). Changes were also associated with reductions in average an-
330 nual temperatures, and increases in year-to-year temperature variability. *A. perennans* endophyte
331 prevalence increased most strongly in regions that experienced reduced spring precipitation and
332 reduced variability in annual temperature. Although these correlations were weaker, changes
333 in *A. perennans* endophyte prevalence were also associated with increased in increases in annual
334 precipitation and increasing autumn precipitation. For *A. hyemalis*, endophyte prevalence in-
335 creased most strongly in regions that experienced reductions in autumn precipitation variability.
336 Correlations using only a subsampling of pixels were qualitatively similar to these results (Fig.
337 A11), suggesting that the patterns we find are not spurious associations.

338 33

³³ Only have plotted results for AGHY right now.

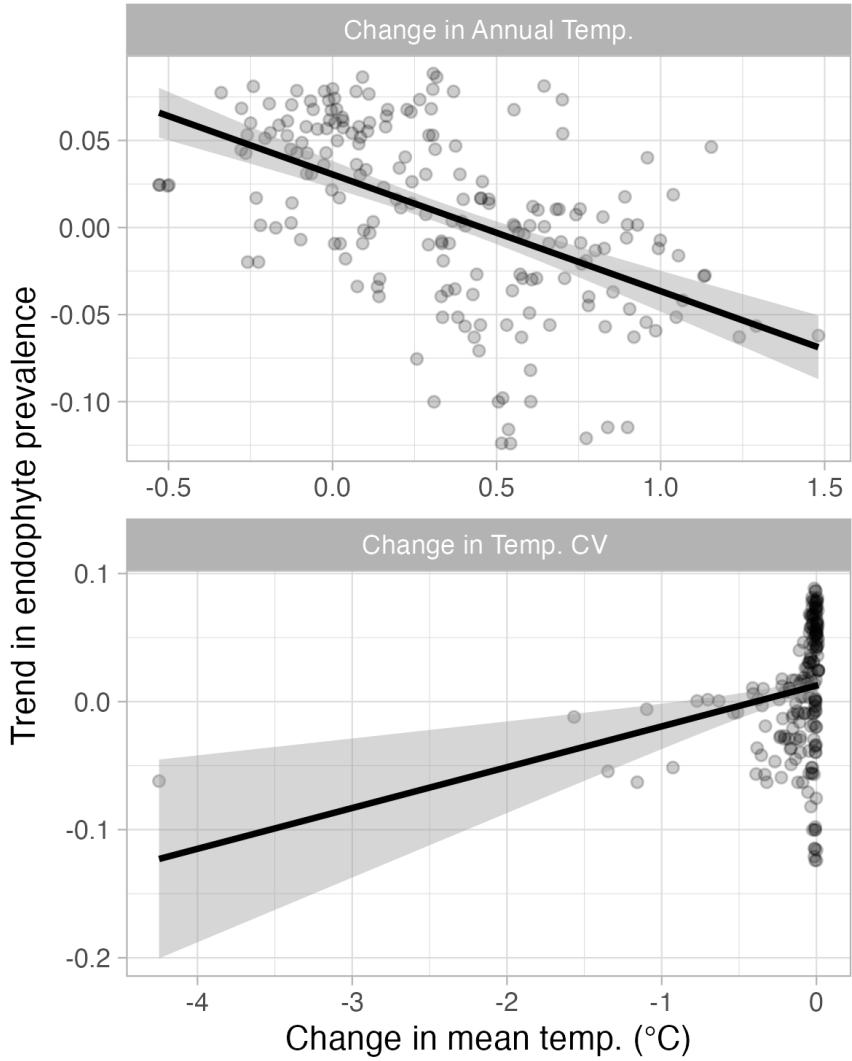


Figure 6: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation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

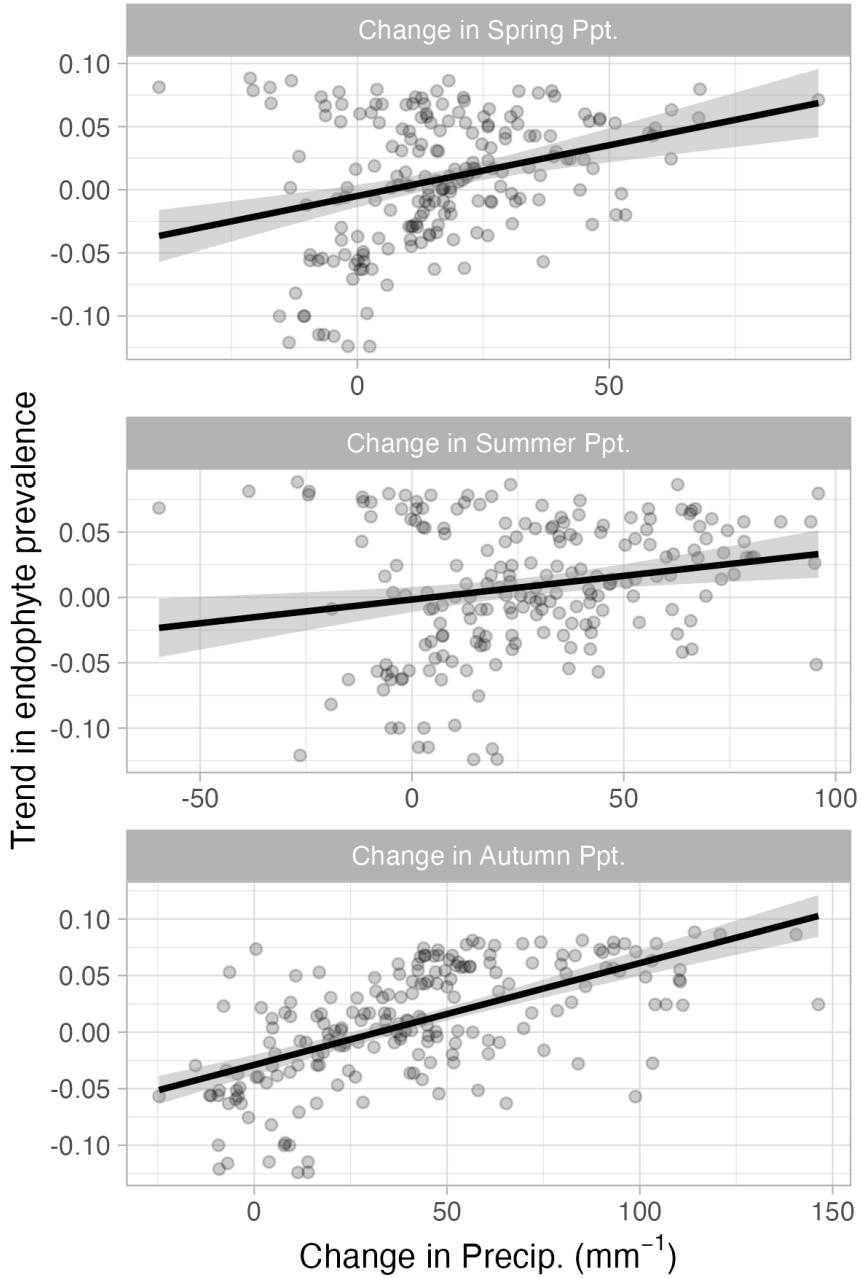


Figure 7: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation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

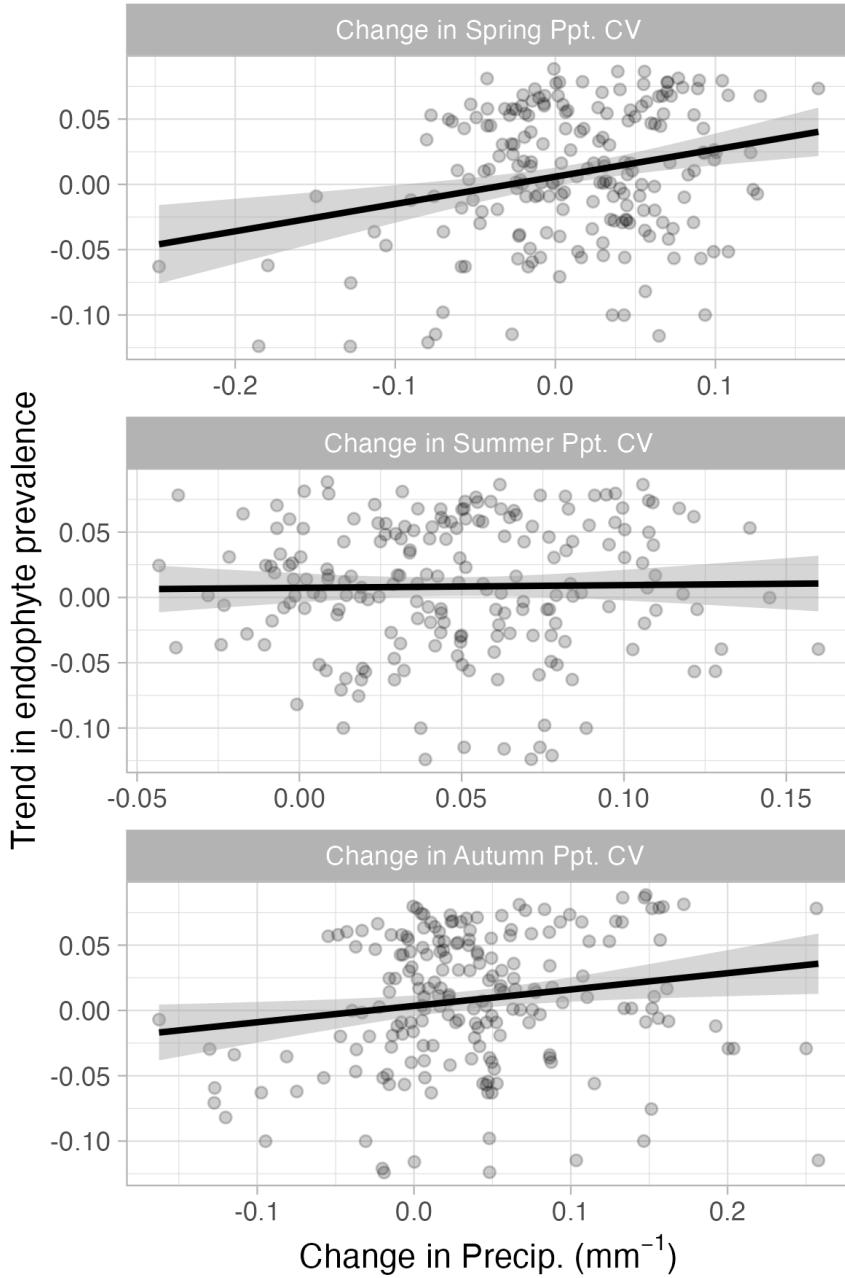


Figure 8: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation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

339 *Performance on test data*

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

345 We discuss potential model improvements in the Discussion.

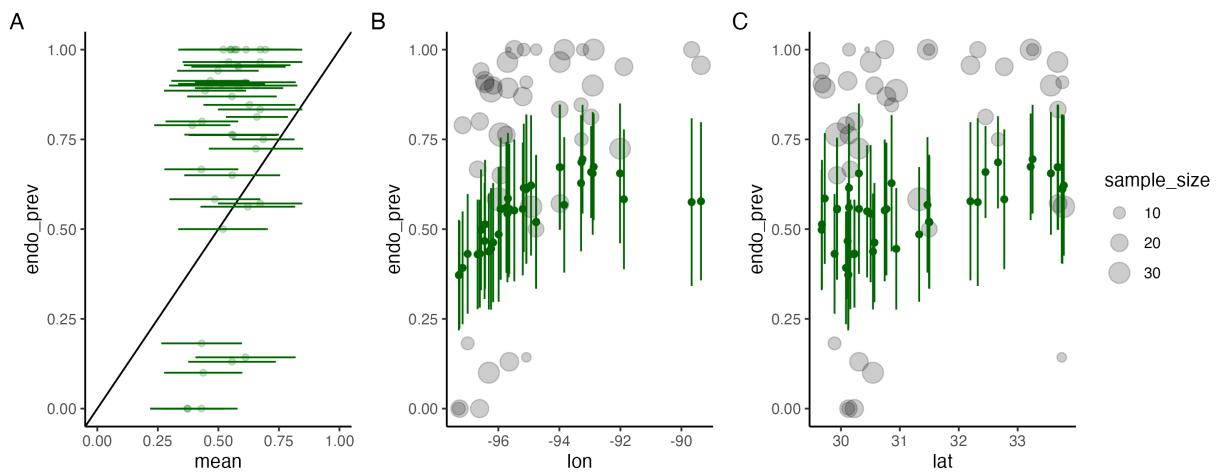


Figure 9: **Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporaneous endophyte prevalence in *A. hyemalis*, as indicated by some overlap of predicted 95% CI with the 1:1 line,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

347 Our examination of historic plant specimens revealed a cryptic biotic reponse to climate change.
348 For the three host species we examined, there have been clear increases in fungal endophyte
349 prevalence over the last two centuries. Increases in prevalence of *Epichloë*, which are vertically
350 transmitted, can potentially be interpreted as adaptive changes that improve the fitness of their
351 hosts under stressful conditions. This interpretation is in line with theory predicting that the
352 positive fitness feedback caused by vertical transmission leads beneficial symbionts to rise in
353 prevalence within a population (?). We found that trends in endophyte prevalence varied across
354 the distribution of each species in association with observed changes in climate drivers, suggesting
355 that the endophytes have contributed to host resilience under environmental change. Taken
356 together, this suggests a strengthening of the mutualism over the last two centuries.

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

372 been connected to a suite of non-drought related fitness benefits including herbivore protection
373 (?), salinity resistance (?), and mediation of the soil microbiome (?) These effects are potentially
374 mediated by the diverse bioactive alkaloids and other signaling compounds they produce (?). The
375 strong increase in symbiotic *A. perennans* could be explained, at least in part, by these diverse
376 benefits. *A. hyemalis* experienced more consistently positive increases in endophyte prevalence
377 related to changes in spring temperature and precipitation. This result is in line with previous
378 work demonstrating drought benefits in a greenhouse manipulation with this species (?) that
379 led us to expect that endophyte prevalence should similarly increase at a greater rate in regions
380 that have experienced increasing drought. For *E. virginicus*, which experienced the most modest
381 changes in endophte prevalence overall, we found a strong relationship between temporal trends
382 and changes in the mean and variability of temperature, as well as with decreases in summer
383 precipitation. Surveys by ?, used as part of the test data in this study, identified a drought index
384 (SPEI) that integrates precipitation with estimated evapotranspiration as an important predictor
385 of endophyte prevalence. While we show consistent increasing trends in prevalence between the
386 three species, the mechanisms that explain these changes may be diverse and idiosyncratic.

387 Our spatially-explicit model predicted regions of both high and low endophyte prevalence,
388 suggesting that symbiotic and non-symbiotic host plants have overlapping, but non-identical
389 niche requirements. Endophytes fitness benefits potentially explain the spatial distribution of
390 prevalence by allowing their hosts to persist in environments where they otherwise could not
391 (??). For example, fitness benefits of the symbiosis could explain high predicted prevalence in *E.*
392 *virginicus* towards the north or in *A. hyemalis* towards its range center coinciding with strong envi-
393 ronmental gradients. Previous population surveys for endophytes, which were used as test data
394 for our model, found similar latitudinal trends in prevalence in these species (??), but at smaller
395 scales. While the model recreated these large-scale spatial trends, test data was more variable.
396 Using test data to validate our model predictions allows us to evaluate places to improve the
397 model's ability to perform well at out-of-sample prediction, which will be particularly important
398 for predicting host and symbiont niche-shifts under future climate change. Lack of information

399 on local variability may simply be a feature of data derived from herbarium specimens. Even
400 though they are samples from local populations, they are single specimens that are aggregated
401 over in broad-scale model estimates. Poor predictive ability at local scales in this grass-endophyte
402 system is not surprising, as previous studies have found that local variation, even to the scale
403 of hundreds of meters can structure endophyte-host niches (?). ? also identified host genotype
404 as an important predictor of endophyte prevalence in *E. virginicus*. Other studies have found
405 factors including land-use history (?) and the biotic environment, including herbivory (?), to be
406 important predictors of endophyte ecology. Incorporating available climatic and soil layers as
407 covariates is an obvious first step that could improve predictions. Towards the goal of predicting
408 the dynamics of microbial symbioses under climate change, models that integrate data from local
409 and regional scales would be an important step to bridge the gap that often exists between large
410 but broad bioclimatic and biodiversity data and small but local data on biotic interactions. (??)

411 Our analysis advances the use of herbarium specimens in global change biology in two ways.
412 First and foremost, this is the first study to link long-term changes in microbial symbioses to
413 changes in climate using specimens from natural history collections. The responses of micro-
414 bial symbioses are a rich target for future studies within museum specimens, particularly those
415 that take advantage of advances in sequencing technology. While we used relatively coarse
416 presence/absence data based on fungal morphology, other studies have examined historic plant
417 microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these
418 studies have so far been limited to relatively few specimens at limited spatial extents (?????). Con-
419 tinued advances in capturing historic DNA and in filtering out potential contamination during
420 specimen storage (???) will be imperative in the effort to scale up these efforts. This scaling up
421 will be essential to be able to quantify changes not just in the prevalence of symbionts, but also
422 in symbionts' intraspecific variation and evolutionary responses to climate change, as well as in
423 changes in the wider microbial community. Answering these questions as well as the unknown
424 questions that future researchers may ask also reiterates the value in capturing meta-information
425 during ongoing digitization efforts at herbaria around the world and during the accession of

426 newly collected specimens (?). Second, we accounted for several potential biases in the data
427 observation process that may be common to many collections-based research questions by using
428 a spatially-explicit random effects model. Spatial autocorrelation (?), potential biases introduced
429 by the sampling habits of collectors (?), and variation between contemporary researchers during
430 the collection of trait data, if not corrected for could lead to over-confident inference about the
431 strength and direction of historic change. Previous studies that have quantified the effects of
432 collector biases typically find them to be small (??), and we similarly did not find that collector
433 has a strong effect on the results of our analysis. Fitting this model in a Bayesian framework
434 allows for full propagation of uncertainty.

435 Ultimately, a central goal of global change biology is to generate predictive insights into the
436 future of natural systems. While this survey of historic endophyte prevalence is necessarily cor-
437 relative, it serves as a foundation to develop better predictive models of the response of microbial
438 symbioses to climate change. Combining the insights from this type of regional-scale survey with
439 field experiments and physiological data could be invaluable. While we found that climate is
440 strongly correlated with endophytes' temporal responses, we do not know why trends in preva-
441 lence were weak in some areas or how endophytes would respond to more extreme changes in
442 climate. For example, transplanting symbiotic and non-symbiotic plants beyond the range edge
443 of *A. hyemalis* could tell us whether persistent low endophyte prevalence in that area is a result of
444 environmental conditions that lead the symbiosis to negative fitness consequences, or is a result
445 of some historical contingency or dispersal limitation that has thus far limited the presence of
446 symbiotic hosts from a region where they would otherwise flourish and provide resilience. While
447 we observed evidence of mutualism resilience, more extreme environmental changes than those
448 observed in our study could potentially push one or both partners beyond their physiological
449 limit, leading to the collapse of the mutualism. Our analysis thus far is agnostic to changes in the
450 distributions of hosts. Mechanistic models could connect the responses of both host and sym-
451 bionts to abiotic climate drivers integrating dispersal processes. Beyond host-microbe symbioses,
452 building these types of models would work towards quantitatively attributing biotic responses

⁴⁵³ to anthropogenically driven climate change, similar to methods in climate science and economics
⁴⁵⁴ (??).

⁴⁵⁵ **Acknowledgments**

⁴⁵⁶ We thank Jessica Budke for help in drafting our initial destructive sampling plan, and to the
⁴⁵⁷ many members of herbarium staff who facilitated our research visits, as well as to the hundreds
⁴⁵⁸ of collectors who contributed to the natural history collections. Several high schooler and un-
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⁴⁶³ Program.

⁴⁶⁴ **Statement of Authorship**

⁴⁶⁵ **Data and Code Availability**

⁴⁶⁶ On initial submission, you may use this section to provide a URL for editors and reviewers that
⁴⁶⁷ is ‘private for peer review’. After acceptance, this section must be updated with correct, working
⁴⁶⁸ DOIs for data deposits (typically on the Dryad Digital Repository,) and code deposits (such as
⁴⁶⁹ in Zenodo).

Appendix A

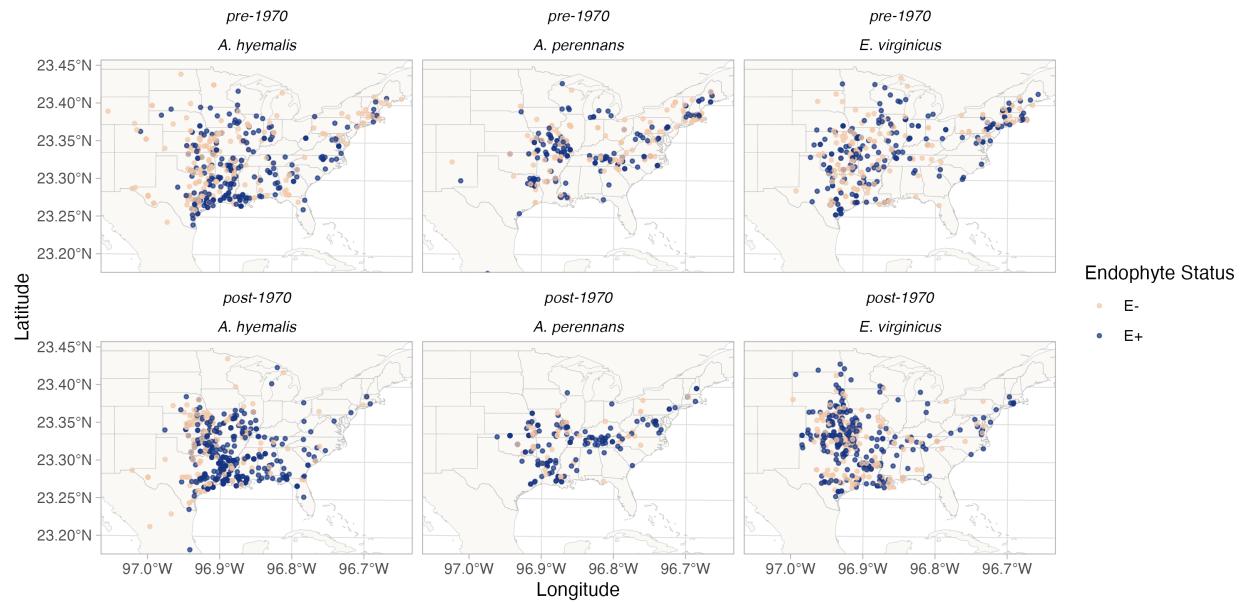


Figure A1: Endophyte presence/absence in specimens of each host species. Points show collection locations colored according to whether the specimen contained endophytes (E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.

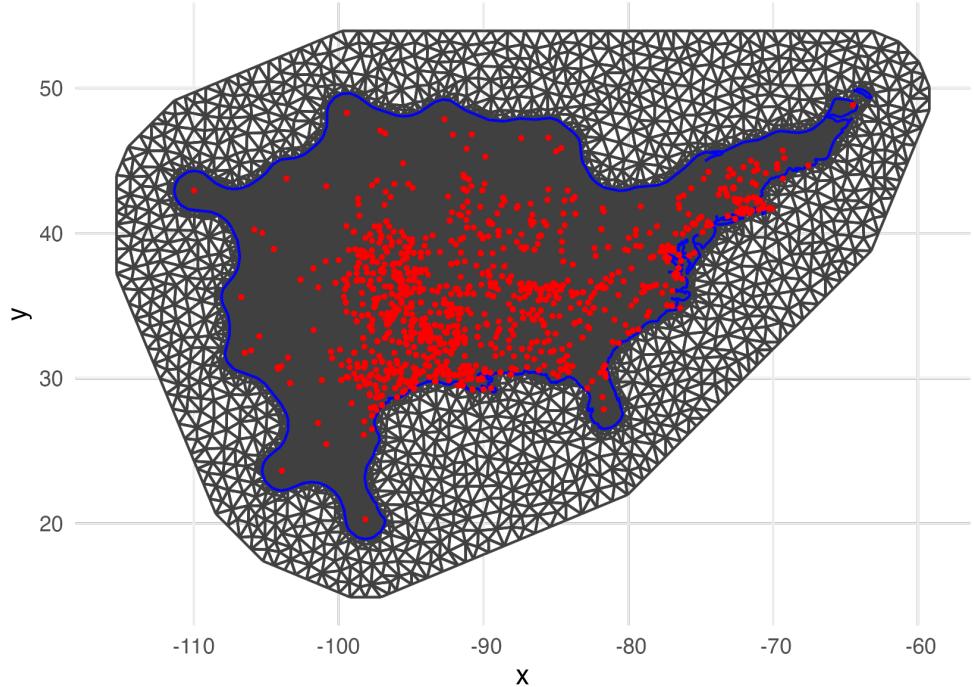


Figure A2: Delauney triangulation mesh used to estimate spatial dependence between data points. Grey lines indicate edges of triangles used to define distances between observations. Red points indicate locations of sampled herbarium specimens, and the blue outlines show the international borders used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

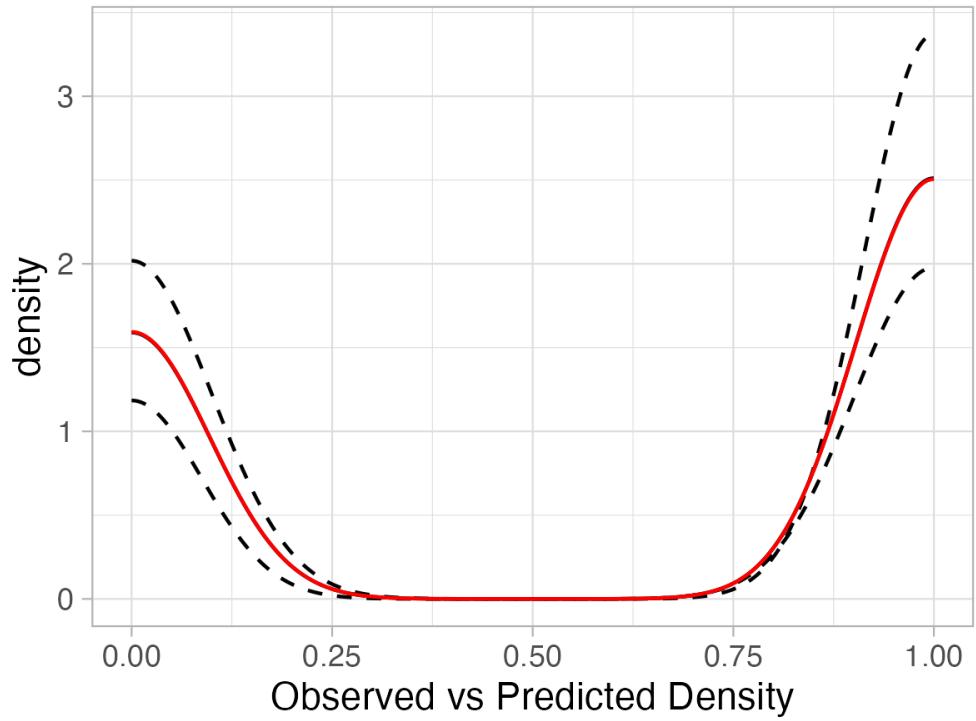


Figure A3: Consistency between real data and simulated values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (red) along with the mean(solid) and 95% CI (dashed) of simulated values (black).

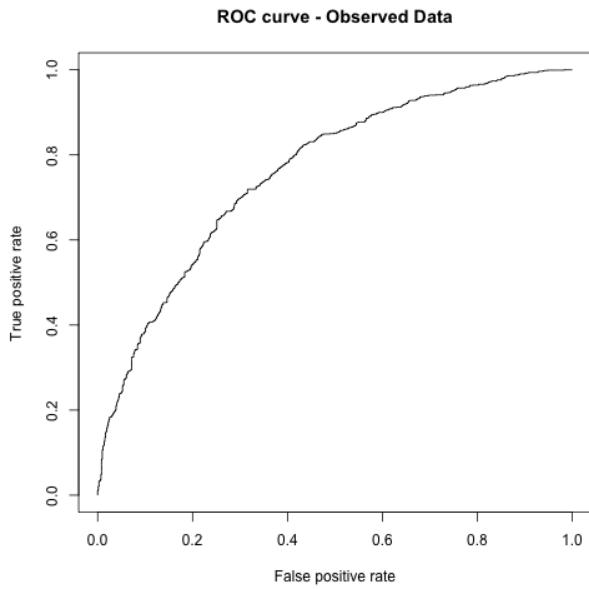


Figure A4: ROC plot showing model performance classifying observations according to endophyte status. The curves show adequate model performance for observed (top) and test (bottom) data. The AUC for each is 0.77.

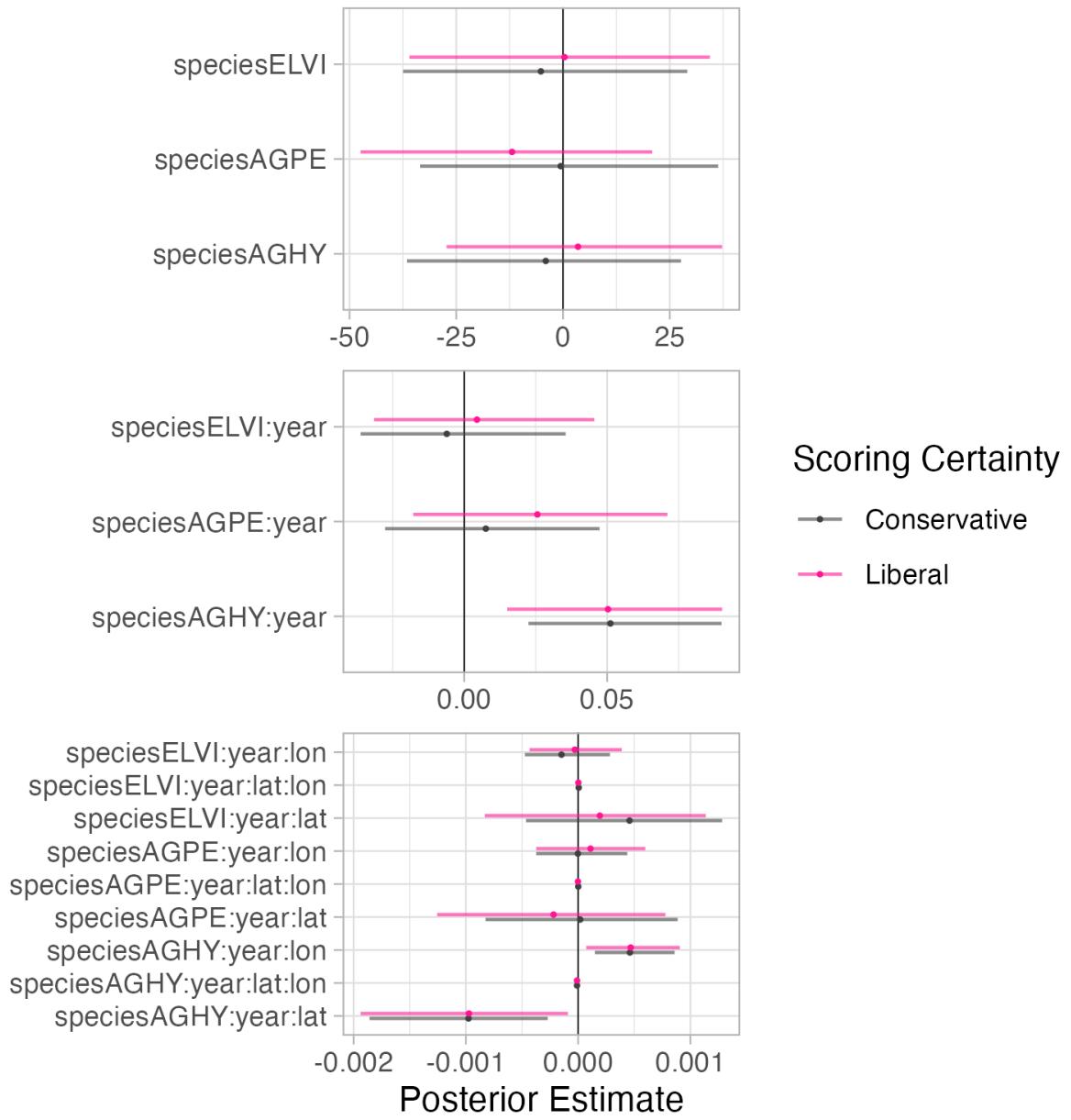


Figure A5: Comparison of posterior estimates of fixed effects when using Liberal or Conservative endophyte scores.

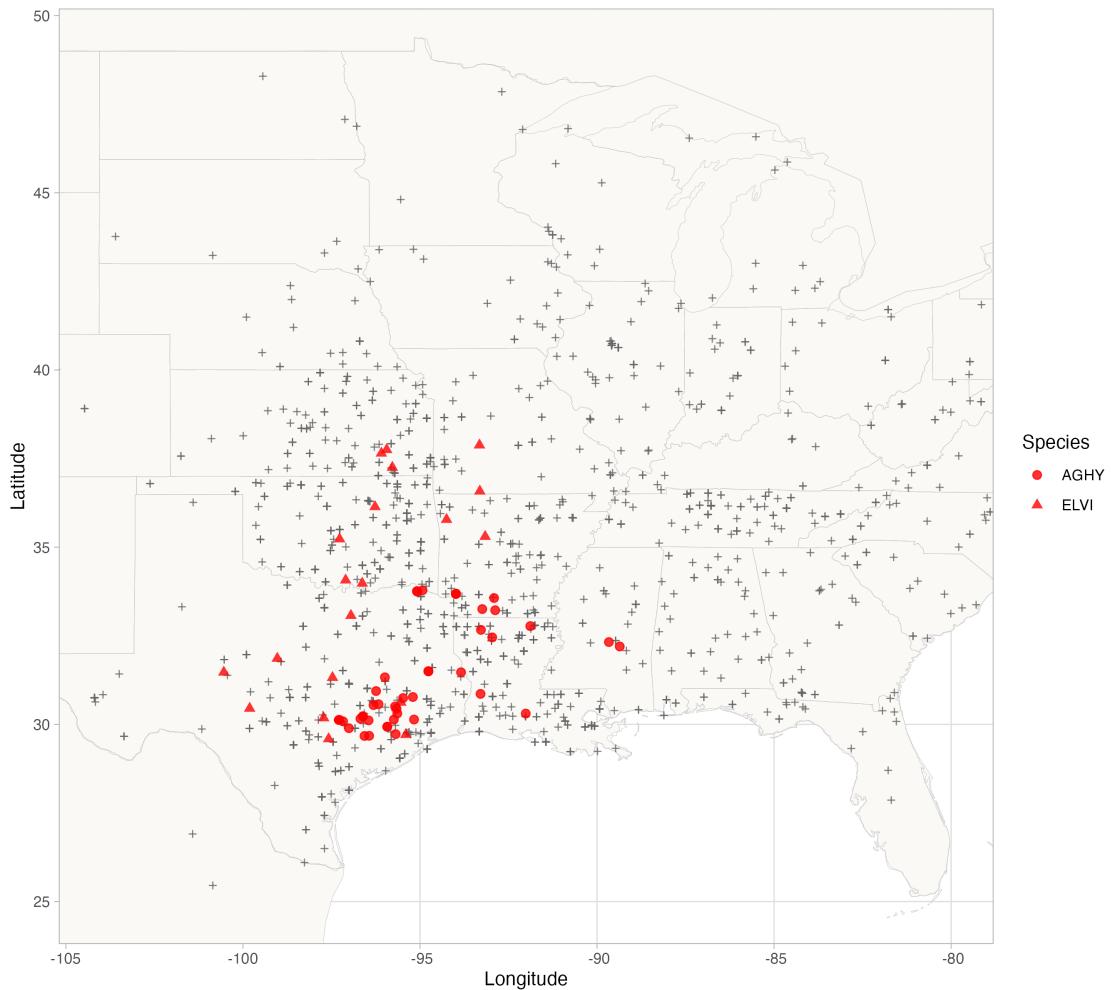


Figure A6: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).

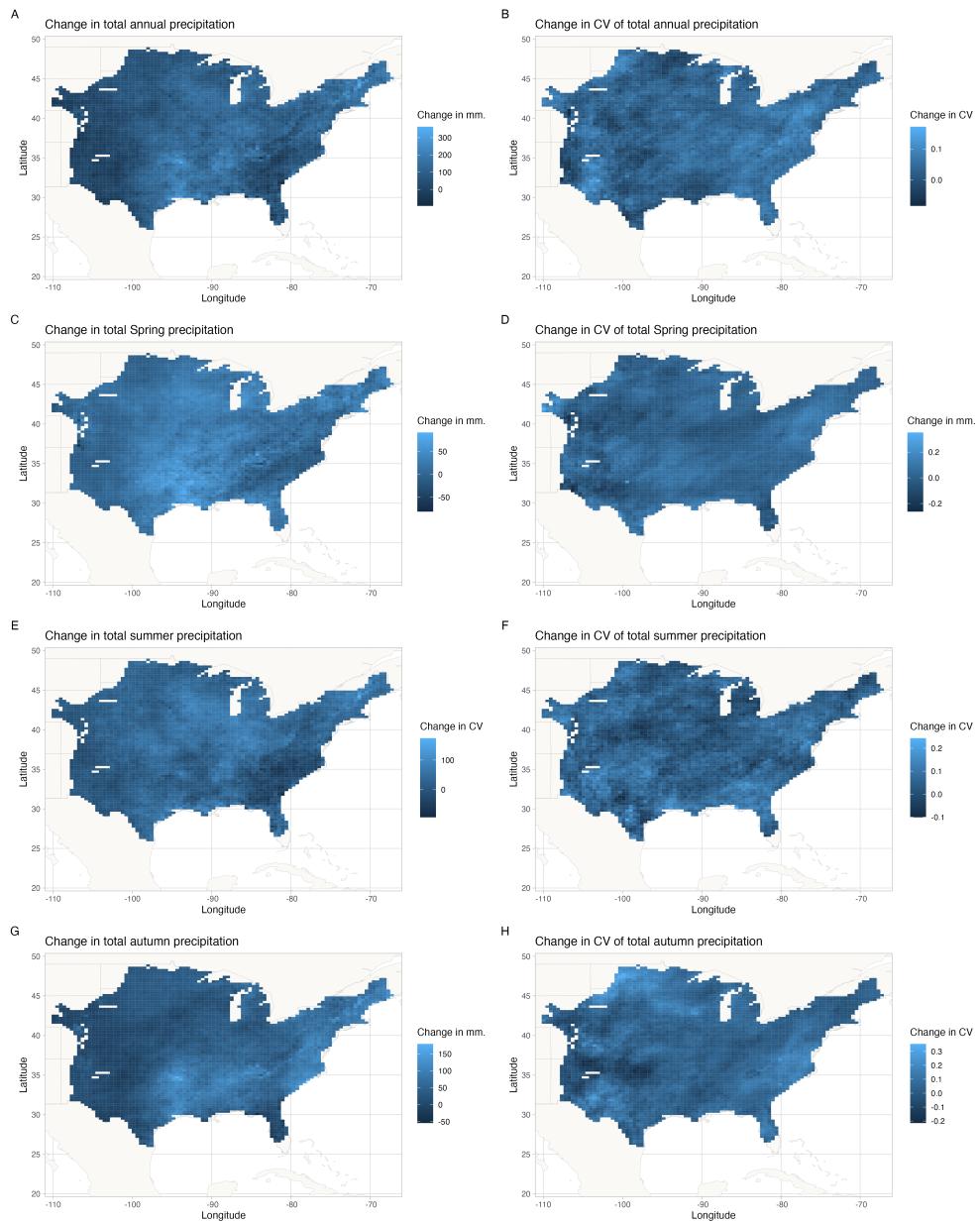


Figure A7: Change in precipitation between the periods 1895-1925 and 1990-2020. Color represents change in annual or seasonal total precipitation (A,C,E,G) and in the coefficient of variation of annual or seasonal total precipitation (B,D,F,H). Maps show the study area of *A. hyemalis*. Map pixels used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

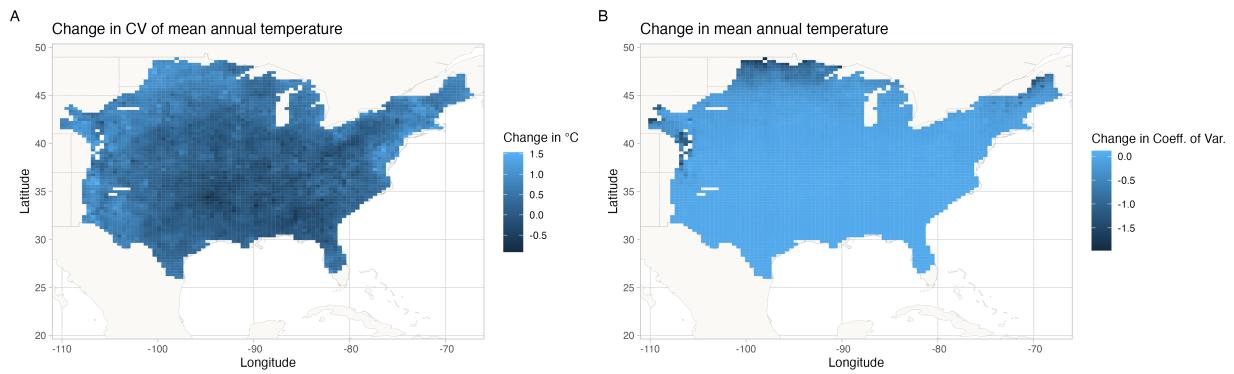


Figure A8: Change in temperature between the periods 1895-1925 and 1990-2020. Color represents change in annual mean temperature (A) and in the coefficient of variation of annual mean temperature (B). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change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

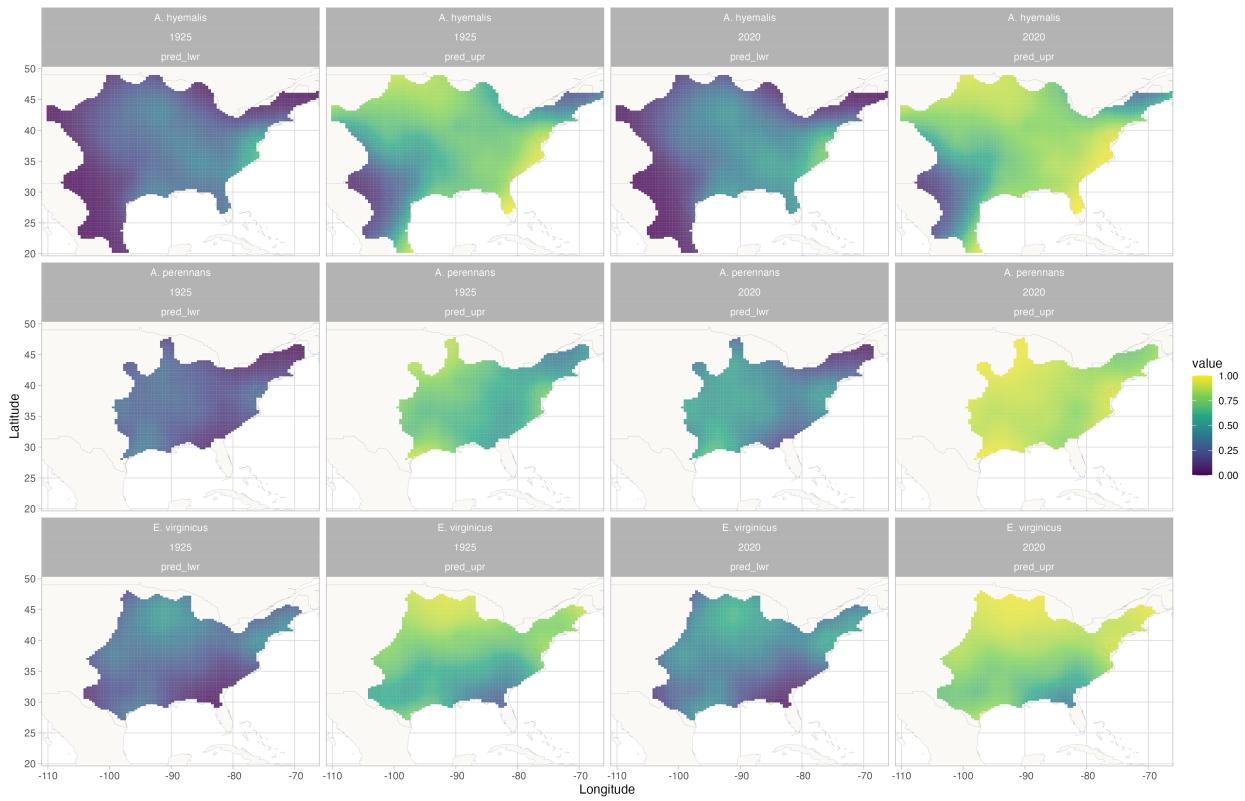


Figure A9: Uncertainty associated with spatial trends in endophyte prevalence. Color represents change in predicted endophyte prevalence. Panels show upper and lower 95% posterior probability for each host species between 1925 and 2020.

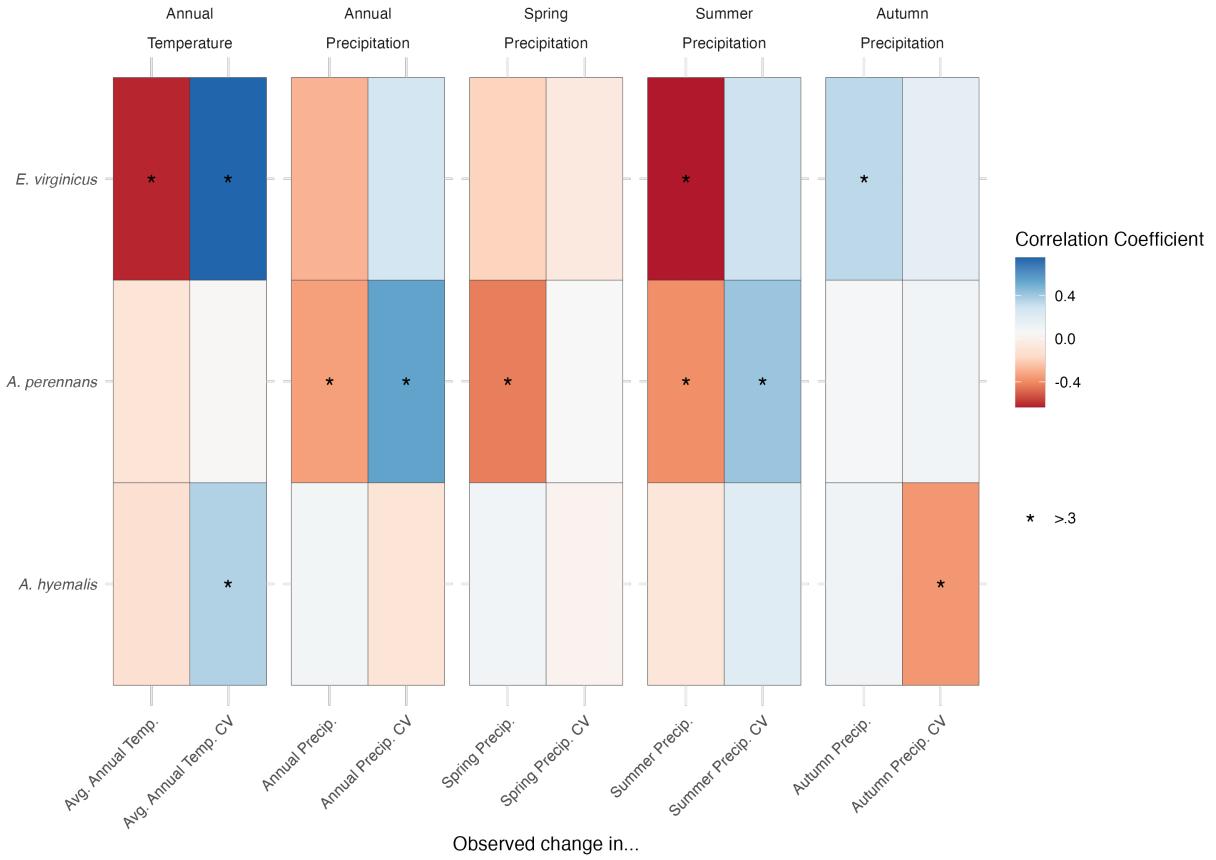


Figure A10: Correlations between changes in climate drivers and changes in endophyte prevalence from a random sample of 100 pixels across the study region. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature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

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