

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.*

¹ *I think this is should be 2*

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. However, 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 2,346 herbarium specimens of three grass host species collected over the last two centuries (1824 – 2019) 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 seasonal climate drivers² corresponding to each host species' peak growing season. Our analysis performed favorably in an out-of-sample predictive test, however we identified XXX as suggesting the model fusion may be an important step moving forward. 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.³

²Describe "changes" – warming? drying?

³I 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

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

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

55 Microbial symbionts are common to all macroscopic organisms and can have important ef-
56 fects on their hosts' survival, growth and reproduction (McFall-Ngai et al., 2013; Rodriguez et al.,
57 2009). Many microbial symbionts act as mutualists, engaging in reciprocally beneficial interac-
58 tions with their hosts that can ameliorate environmental stress. For example, bacterial symbionts

59 of insects, such as *Wolbachia*, can improve their hosts' thermal tolerance (Renoz et al., 2019; Truitt
60 et al., 2019), and arbuscular mycorrhizal fungi, documented in 70-90% of families of land plants
61 (Parniske, 2008), allow their hosts to persist through drought conditions by improving water and
62 nutrient uptake (Cheng et al., 2021). On the other hand, changes in the mean and variance of
63 environmental conditions may disrupt microbial mutualisms by changing the costs and bene-
64 fits of the interaction for each partner, leading the interaction to deteriorate (Aslan et al., 2013;
65 Fowler et al., 2024). Coral bleaching (the loss of symbiotic algae) due to temperature stress (Sully
66 et al., 2019) is perhaps the best known example, but this phenomenon is not unique to corals.
67 Lichens exposed to elevated temperatures experienced loss of photosynthetic function along with
68 changes in the composition of their algal symbiont community (Meyer et al., 2022). How com-
69 monly and under what conditions microbial mutualisms deteriorate or strengthen under climate
70 change remain unanswered questions. Previous work suggests that these alternative responses
71 may depend on the intimacy and specialization of the interaction as well as the physiological
72 tolerances of the mutualist partners (Rafferty et al., 2015; Toby Kiers et al., 2010; Warren and
73 Bradford, 2014).

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

85 *Epichloë* fungal endophytes are specialized symbionts of cool-season grasses, which have been

86 documented in ~ 30% of cool-season grass species (Leuchtmann, 1992). They are transmitted ver-
87 tically from maternal plants to offspring through seeds. Vertical transmission creates a feedback
88 between the fitness of host and symbiont (Douglas, 1998; Fine, 1975; Rudgers et al., 2009). Over
89 time, endophytes that act as mutualists should rise in prevalence within a host population (Don-
90 ald et al., 2021). *Epichloë* are known to improve their hosts' drought tolerance (Decunta et al.,
91 2021) and protect their hosts against herbivores (Crawford et al., 2010) and pathogens (Xia et al.,
92 2018) likely through the production of a diverse suite of alkaloids and other secondary metabo-
93 lites. The fitness feedback induced by vertical transmission leads to the prediction that endophyte
94 prevalence should be high in populations where these fitness benefits are most important. Pre-
95 vious contemporary survey studies have documented large-scale spatial patterns in endophyte
96 prevalence structured by environmental gradients (Afkhami, 2012; Bazely et al., 2007; Granath
97 et al., 2007; Sneck et al., 2017). We predicted that prevalence should track temporal changes in
98 environmental drivers that elicit these fitness benefits.

99 Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic di-
100 versity of host species that harbor these symbionts (White and Cole, 1985), establishing that
101 endophyte symbiosis could be identified in plant tissue from as early as 1851.⁴ However, no
102 subsequent studies, to our knowledge, have used the vast resources of biological collections to
103 quantitatively assess spatio-temporal trends in endophyte prevalence and their environmental
104 correlates. Grasses are commonly collected and identified based on the presence of their re-
105 productive structures, meaning that preserved specimens typically contain seeds, conveniently
106 preserving the fungi along with their host plants on herbarium sheets. This creates the oppor-
107 tunity to leverage the unique spatio-temporal sampling of herbarium collections to examine the
108 response of the symbiosis to historical climate change. Research using historical collections has
109 clearly demonstrated other ecological signatures of a changing climate. However the predictive
110 ability of these historical analyses is rarely tested against contemporary data (Lee et al., 2024).
111 Identifying the ways in which these analyses fall short is a crucial step for the field move from

⁴Edited this a bit. This is the earliest year we have in the database that was part of JFWhites original paper.

112 reading signatures in the past to forecasting ecological dynamics into the future.⁵

113 In this study, we assessed the long-term responses of endophyte symbiosis to climate change
114 through the use of herbarium specimens of three North American host grass species (*Agrostis*
115 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*). We first address questions describing spatial
116 and temporal trends in endophyte prevalence: (i) How has endophyte prevalence changed over
117 the past two centuries? and (ii) How spatially heterogenous are temporal trends in endophyte
118 prevalence across eastern North America? We then address how climate change may be driv-
119 ing trends in endophyte prevalence by asking: (iii) What is the relationship between variation
120 in temporal trends in endophyte prevalence and changes in climate drivers? We predicted that
121 aggregate endophyte prevalence would increase over time in tandem with climate warming, and
122 that hotspots of endophyte change would correspond spatially to hotspots of climate change.
123 Finally, we evaluated the performance of models built on data from historic specimens with an
124 out-of-sample test, data on endophyte prevalence from contemporary surveys of host popula-
125 tions. To answer these questions we examined a total of 2,346 specimens collected across eastern
126 North America between 1824 and 2019.⁶

127 Methods

128 Focal species

129 Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis peren-*
130 *nans*, and *Elymus virginicus*. Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001;
131 Leuchtmann et al., 2014), while *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl,
132 2002). These C₃ grass species are commonly represented in natural history collections with broad
133 distributions covering much the eastern United States. *A. hyemalis* is a small short-lived perennial

5what do you think of this? trying to presage the out-of-sample test without over promising and without saying outright that our analysis sucks. Is this the right place for this? I had imagined some of this material will be really developed in the discussion.

6I 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.

¹³⁴ species that germinates in the spring and typically flowers between March and July (most com-
¹³⁵ mon collection month: May). *A. perennans* is of similar stature but is longer lived than *Agrostis*
¹³⁶ *hyemalis* and flowers in late summer and early autumn (most common collection month: Septem-
¹³⁷ ber). *A. perennans* is more sparsely distributed, tending to be found in shadier and more moist
¹³⁸ habitats, while *A. hyemalis* is commonly found in open and recently disturbed ground. Both
¹³⁹ *Agrostis* species are recorded from throughout the Eastern US, but *A. perennans* has a slightly
¹⁴⁰ more northern distribution, whereas *A. hyemalis* is found rarely as far north as Canada and is
¹⁴¹ listed as a rare plant in Minnesota. *E. virginicus* is a larger and relatively longer-lived species that
¹⁴² is more broadly distributed than the *Agrostis* species. It begins flowering as early as March or
¹⁴³ April but continues throughout the summer (most common collection month: July).

¹⁴⁴ *Herbarium surveys*

¹⁴⁵ We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens in-
¹⁴⁶ cluded from each collection). With permission from herbarium staff, we acquired seed samples
¹⁴⁷ from 1135 *A. hyemalis* specimens collected between 1824 and 2019, 357 *A. perennans* specimens
¹⁴⁸ collected between 1863 and 2017, and 854 *E. virginicus* specimens collected between 1839 and
¹⁴⁹ 2019 (Fig. 1, Fig. 2A, Fig. A1). We chose our focal species in part because they are commonly
¹⁵⁰ represented in herbarium collections, and produce high numbers of seeds, meaning that small
¹⁵¹ samples would not diminish the value of the specimens for future studies. We collected 5-10
¹⁵² seeds per specimen after examining the herbarium sheet under a dissecting microscope to en-
¹⁵³ sure that we collected mature seeds, not florets or unfilled seeds, fit for our purpose of identifying
¹⁵⁴ fungal endophytes with microscopy. We excluded specimens for which information about the
¹⁵⁵ collection location and date were unavailable. Each specimen was assigned geographic coor-
¹⁵⁶ dinates based on collection information recorded on the herbarium sheet using the geocoding
¹⁵⁷ functionality of the ggmap R package (Kahle et al., 2019). Many specimens had digitized collec-
¹⁵⁸ tion information readily available, but for those that did not, we transcribed information printed
¹⁵⁹ on the herbarium sheet. Collections were geo-referenced to the nearest county centroid, or near-

¹⁶⁰ est municipality when that information was available. For a few of the oldest specimens, only
¹⁶¹ information at the state level was available, and so we used the state centroid.

¹⁶² After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hy-
¹⁶³ phae, which grow intercellularly, in each specimen using microscopy. We first softened seeds
¹⁶⁴ with a 10% NaOH solution, then stained the seeds with aniline blue-lactic acid stain and squashed
¹⁶⁵ them under a microscope cover slip. We examined the squashed seeds for the presence of fungal
¹⁶⁶ hyphae at 200-400X magnification (Bacon and White, 2018). In some cases, the tissues examined
¹⁶⁷ during microscopy came from flowers or otherwise non-viable seeds, which were excluded for
¹⁶⁸ that specimen. On average we scored 4.7 seeds per specimen of *A. hyemalis*, 4.2 seeds per spec-
¹⁶⁹ imen of *A. perennans*, and 3.8 seeds per specimen of *E. virginicus*; we scored # seeds in total. .
¹⁷⁰ Due to imperfect vertical transmission (Afkhami and Rudgers, 2008), it is possible that symbiotic
¹⁷¹ host-plants produce a mixture of symbiotic and non-symbiotic seeds. We therefore designated a
¹⁷² specimen as endophyte-symbiotic if *Epichloë* hyphae were observed in one or more of its seeds,
¹⁷³ or non-symbiotic if hyphae were observed in none of its seeds. To capture uncertainty in the
¹⁷⁴ endophyte scoring process, we recorded both a "liberal" and a "conservative" endophyte status
¹⁷⁵ for each plant specimen. When we identified potential endophytes with unusual morphology,
¹⁷⁶ low uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a
¹⁷⁷ positive liberal status (more likely to be endophyte-positive) and a negative conservative status
¹⁷⁸ (less likely to be endophyte-positive). 89% of scored plants had matching liberal and conservative
¹⁷⁹ scores, reflecting high confidence in endophyte status. The following analyses in the main text
¹⁸⁰ used the liberal status, but we repeated all analyses with the conservative status which yielded
¹⁸¹ qualitatively similar results (Fig. A10)

¹⁸² *Modeling spatial and temporal changes in endophyte prevalence*

¹⁸³ We assessed spatial and temporal changes in endophyte prevalence across each host distribution,
¹⁸⁴ quantifying the "global" temporal trends, aggregating across space, and then examining spatial
¹⁸⁵ heterogeneity in the direction and magnitude of endophyte change (hotspots and coldspots)

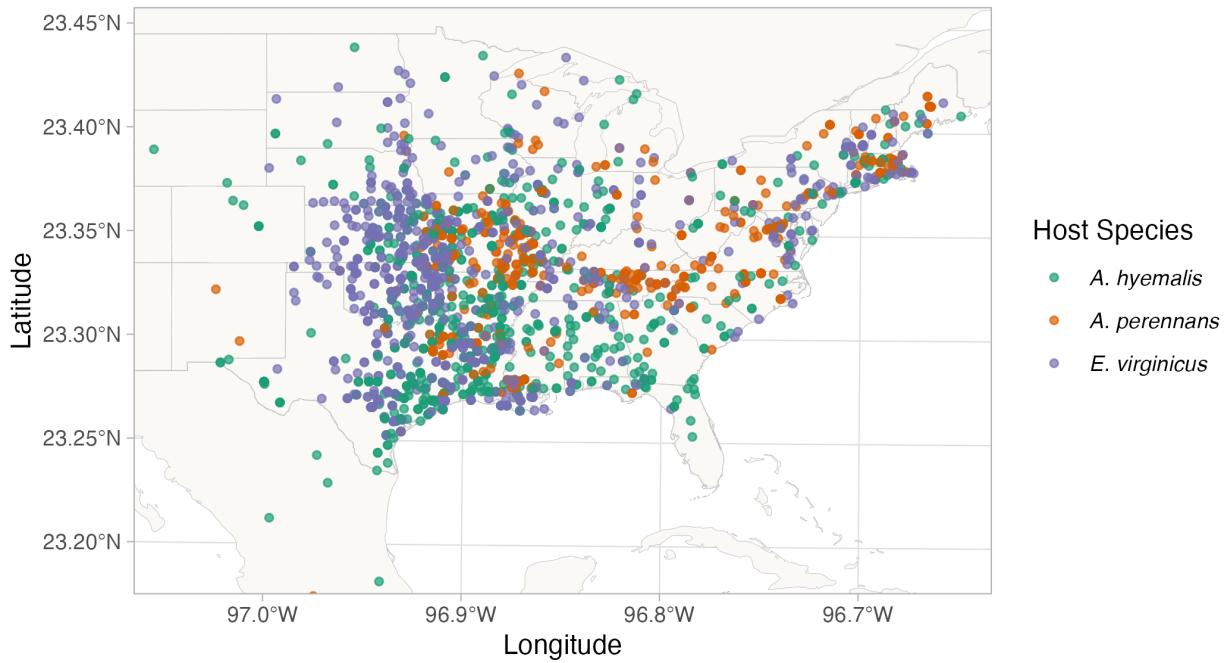


Figure 1: Collection locations of herbarium specimens of three grass host species across eastern North America that were sampled for *Epichloë* endophyte presence or absence.

across the spatial extent of each host's distribution. To appropriately account for the spatial non-independence of geo-referenced occurrences⁷, we used an approximate Bayesian method, Integrated Nested Laplace Approximation (INLA), to construct spatio-temporal models of endophyte prevalence. INLA provides a computationally efficient method of ascertaining parameter posterior distributions for certain models that can be formulated as latent Gaussian Models (Rue et al., 2009). Many common statistical models, including structured and unstructured mixed-effects models, can be represented as latent Gaussian Models. We incorporated spatial heterogeneity into this analysis using spatially-structured intercept and slope parameters implemented as stochastic partial differential equation (SPDE) approximations of a continuous spatial Gaussian process. This SPDE approach is a flexible method of smoothing across space while explicitly accounting for spatial dependence between data-points (Bakka et al., 2018; Lindgren

⁷ spelling?

¹⁹⁷ et al., 2011). Fitting models with structured spatial effects is possible with MCMC sampling but
¹⁹⁸ can require long computation times, making INLA an effective alternative, which has been used
¹⁹⁹ to model spatial patterns in flowering phenology (Willems et al., 2022), the abundance of bird
²⁰⁰ species (Meehan et al., 2019) and butterflies (Crossley et al., 2022), the distribution of temperate
²⁰¹ trees (Engel et al., 2022) as well as the population dynamics of endangered amphibians (Knapp
²⁰² et al., 2016) and other ecological processes (Beguin et al., 2012).

²⁰³ We estimated global and spatially-varying trends in endophyte prevalence using a joint-
²⁰⁴ likelihood model. For each host species h , endophyte presence/absence of the i^{th} specimen ($P_{[h]i}$)
²⁰⁵ was modeled as a Bernoulli response variable with expected probability of endophyte occurrence
²⁰⁶ $\hat{P}_{[h]i}$. We modeled $\hat{P}_{[h]i}$ as a linear function of intercept $A_{[h]i}$ and slope $T_{[h]}$ defining the global trend
²⁰⁷ in endophyte prevalence specific to each host species as well as with spatially-varying intercepts
²⁰⁸ $\alpha_{[h_1]l[i]}$ and slopes $\tau_{[h_1]l[i]}$ associated with location ($l[i]$, a unique latitude-longitude combination).
²⁰⁹ The joint-model structure allowed us to share variance terms across focal species to account for
²¹⁰ dependence associated with the collection of specimens and identification of endophytes. Shared
²¹¹ variance terms included the spatially-dependent random effect $\delta_{l[i]}$, intended to account for resid-
²¹² ual spatial variation, and $\chi_{c[i]}$ and $\omega_{s[i]}$ i.i.d.-random effects indexed for each collector identity
²¹³ ($c[i]$), and scorer identity ($s[i]$) of the i^{th} specimen.

$$\text{logit}(\hat{P}_{[h_1]i}) = A_{[h_1]i} + T_{[h_1]} * \text{year}_i + \alpha_{[h_1]l[i]} + \tau_{[h_1]l[i]} * \text{year}_i + \delta_{l[i]} + \chi_{c[i]} + \omega_{s[i]} \quad (1a)$$

$$\text{logit}(\hat{P}_{[h_2]i}) = A_{[h_2]i} + T_{[h_2]} * \text{year}_i + \alpha_{[h_2]l[i]} + \tau_{[h_2]l[i]} * \text{year}_i + \delta_{l[i]} + \chi_{c[i]} + \omega_{s[i]} \quad (1b)$$

$$\text{logit}(\hat{P}_{[h_3]i}) = A_{[h_3]i} + T_{[h_3]} * \text{year}_i + \alpha_{[h_3]l[i]} + \tau_{[h_3]l[i]} * \text{year}_i + \delta_{l[i]} + \chi_{c[i]} + \omega_{s[i]} \quad (1c)$$

$$(1d)$$

²¹⁴ Previous work suggests that behavior of historical botanists and uneven sampling may intro-
²¹⁵ duce biases into ecological inferences made from historic collections (Kozlov et al., 2020). Prolific
²¹⁶ collectors who contribute thousands of specimens may be more or less likely to collect certain
²¹⁷ species, or specimens with certain traits (Daru et al., 2018). Similarly, the process of scoring seeds

218 for hyphae involved several student researchers who, even with standardized training, may vary
219 in their likelihood of positively identifying *Epichloë* hyphae. By including a random effect for
220 collectors and for scorers, we attempted to account for variance across individual researchers
221 that may bias our predictions of changes in endophyte prevalence.

222 We performed model fitting using the inlabru R package (). Global parameters A, and T, and
223 i.i.d. random effects were given penalized complexity priors χ and ω . Each spatially-structured
224 parameter depended on a covariance matrix according to the proximity of each collection location
225 (Bakka et al., 2018; Lindgren et al., 2011). The covariance matrix was approximated using a
226 Matérn covariance function, with each data point assigned a location according to the nodes
227 of a mesh of non-overlapping triangles encompassing the study area (Fig. A2). Priors, termed
228 "range" and "variance", define the distance of spatial decay described by the Matérn covariance
229 function. Priors for results presented in the main text reflect a range of XX⁸ kilometers. We
230 found that model results were sensitive to this choice, and so tested a range of priors (from XX
231 kilometers to XX kilometers) and meshes (Supplemental Material), finding that model results
232 were qualitatively similar, i.e. the same direction of effects across space, but that the magnitude
233 and uncertainty varied.

234 *Validating the model with in-sample and out-of-sample tests*

235 In all cases, posterior modes were **stable**⁹ and equal to zero, indicating successful numeric con-
236 vergence. We assessed model fit with graphical posterior predictive checks (Fig. A3). **The model**
237 **performed adequately at classifying the historical data, comparing the accuracy of predictions**
238 **from the model with observed data (avg. AUC = 0.77; Fig. A4).**¹⁰

239 We evaluated the predictive ability of the model using contemporary endophyte surveys as
240 out-of-sample test data, **an important but rarely used strategy in ecological studies (Tredennick**

8

⁹ *Assessed how?*

¹⁰ *Maybe move this to validation section, and then have both in-sample and out-of-sample approaches.*

²⁴¹ et al., 2021).¹¹ ¹² We used data from contemporary surveys of endophyte prevalence in *A. hyemalis* and *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted ²⁴² in 2013 as described in Sneed et al. (2017), and surveys of *A. hyemalis* took place between 2015 and ²⁴³ 2020¹³. Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation ²⁴⁴ in endophyte prevalence towards its range edge, while surveys of *E. virginicus* were designed ²⁴⁵ to cover latitudinal variation along its range edge. In total, we visited 43 populations of *A. ²⁴⁶ hyemalis* and 20 populations of *E. virginicus* across the south-central US, with emphasis on Texas ²⁴⁷ and neighboring states (Fig A4¹⁴). During surveys, we collected seeds from up to 30 individuals ²⁴⁸ per location (average number of plants sampled: 22.9). We quantified the endophyte status ²⁴⁹ of each individual with staining microscopy as described for the herbarium surveys (with 5-10 ²⁵⁰ seeds scored per individual), and calculated the prevalence of endophytes within the population ²⁵¹ (proportion of symbiotic plants divided by the number of sampled plants). For each population, ²⁵² we compared the observed fraction of endophyte-symbiotic hosts to the predicted probability of ²⁵³ endophyte occurrence \hat{P} derived from the model based on location and year, with collector and ²⁵⁴ scorer random effects fixed at zero. The contemporary survey period (2013-2020) is at the most ²⁵⁵ recent edge of the time period encompassed by the historical observations used for model fitting. ²⁵⁶ We compared the model's prediction for these locations to the observed population prevalence.¹⁵

²⁵⁸ Assessing the role of climate drivers

²⁵⁹ We assessed how the magnitude of climate change may have driven changes in endophyte preva-
²⁶⁰ lence by assessing correlations between changes in climate and changes in endophyte prevalence
²⁶¹ predicted from our spatial model at evenly spaced pixels across the study area. We first down-

¹¹This 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.

¹²Add Benjamin lee paper, maybe? it's not just herbaria, but kind of related

¹³We 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.

¹⁴This is now A6. Good reminder to use the ref function.

¹⁵It is not clear if you are testing model 1 ("global trend") or model 2 ("spatially varying trends").

262 loaded monthly temperature and precipitation rasters from the PRISM climate group (Daly and
263 Bryant, 2013) covering the time period between 1895 and 2020 using the 'prism' R package (Hart
264 and Bell, 2015). Prism provides reconstructions of historic climate variables across the United
265 States by spatially-interpolating weather station data (Di Luzio et al., 2008). We calculated 30-
266 year climate normals for annual and seasonal mean temperature and cumulative precipitation
267 for the recent (1990 to 2020) and historic (1895 to 1925) periods. We used three four-month sea-
268 sons within the year (Spring: January, February, March, April; Summer: May, June, July, August;
269 Autumn: September, October, November, December). This division of seasons allowed us to
270 quantify differences in climate associated with the two "cool" seasons, when we expect our fo-
271 cal species to be most biologically active (*A. hyemalis* flowering phenology: Spring; *E. virginicus*:
272 Spring and Summer; *A. perennans*: Fall). In addition to mean climate conditions, environmental
273 variability itself can influence population dynamics (Tuljapurkar, 1982) and changes in variabil-
274 ity are a key prediction of climate change models (IPCC, 2021; Stocker et al., 2013). Therefore
275 we calculated the coefficient of variation (CV) during each period for each annual and seasonal
276 climate driver as the interannual standard deviation divided by the mean across each 30-year
277 period. We then took the difference between recent and historic periods for the mean and CV for
278 each climate driver (Fig. A5)¹⁶. Because initial analyses indicated a high degree of collinearity
279 between seasonal and annual changes in temperature, we used annual temperature only, along
280 with annual and seasonal precipitation, in the subsequent analysis. All together, this left us with
281 measurements of change in 10 potential climate drivers: the mean and coefficient of variation
282 of annual temperature, as well as the mean and coefficient of variation of cumulative annual
283 precipitation, cumulative spring precipitation, cumulative summer precipitation, and cumulative
284 autumn precipitation (Fig A8-A9)¹⁷.

285 To evaluate whether areas that have experienced the greatest changes in endophyte preva-
286 lence (hotspots of endophyte change) are associated with high degrees of change in climate

¹⁶This is Figure A7 – Can you make the color scale on these diverging at zero?

¹⁷The species names are not clear on Fig A9. I suggest increase the font siize

287 (hotspots of climate change), we modeled spatially varying slopes of endophyte change through
288 time (β_1) as a linear function of environmental covariates, with a Gaussian error distribution.¹⁸
289 Calculating correlations from many pixels across the study region risks artificially inflating confi-
290 dence in our results due to large sample sizes, and so we repeated this calculation using only a
291 random subsample of 100 pixels across the study region¹⁹.²⁰ ²¹

292 *Modeling distributions of host species*

293 We modeled the distribution of each host species to generate maps on which we predicted the
294 dynamics of *Epichloë* symbionts. We followed the ODMAP (overview, data, model, assessment,
295 prediction) protocol (Crossley et al., 2022), using presence-only observations of the host species
296 from GBIF between 1990 to 2020²². To reduce the potential influence of sampling bias and spa-
297 tial autocorrelation, we thinned the occurrences to the spatial scale ()²³ of our selected climatic
298 predictors. We selected climate variables that aligned with our analysis of climatic influences on
299 trends in endophyte prevalence described above. We calculated the mean and standard deviation
300 of seasonal temperature and precipitation across 1990 to 2020. Among this suite of variables,
301 we chose to include mean spring temperature, mean spring precipitation, and mean summer
302 temperature²⁴, which were uncorrelated (Variance Inflation Factor > 0.7) and allowed us to pre-
303 dict the occurrence probability of each host species in space and time. We fit maximum entropy

¹⁸I 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.

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

²⁰Are the methods above repeated for each species separately?

²¹I 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.

²²How many data points does this end up being?

²³I think this is 4km, but need to check

²⁴is this list accurate? my re-running my climate analysis means we ought to re-run the sdm's, and use SD instead of CV, so we can update this list based on whatever is appropriate

304 (MaxEnt) models using the maxent function in the package dismo (Hijmans et al., 2017). Max-
305 Ent is preferred because it has been shown to generate response curves with less unpredictable
306 behavior when applied to new climates (Hijmans and Graham, 2006).²⁵ We generated 10,000
307 pseudo-absences as background points, and split the occurrence data into 75% for model train-
308 ing and 25% for model testing. The performances of models were evaluated with AUC AUC²⁶
309 (Jiménez-Valverde, 2012). To convert the continuous predicted probabilities into binary presence
310 - absence maps, we used the training sensitivity (true positive rate) and specificity threshold
311 (true negative rate) (Liu et al., 2005). These binary maps serve as boundaries in presented maps
312 of change in endophyte prevalence, and outline the set of pixels used in our analysis of climate
313 correlates with trends in endophyte prevalence

314

Results

315

How has endophyte prevalence changed over time?

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

²⁵possibly could remove this sentence?

²⁶can we add these values?

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

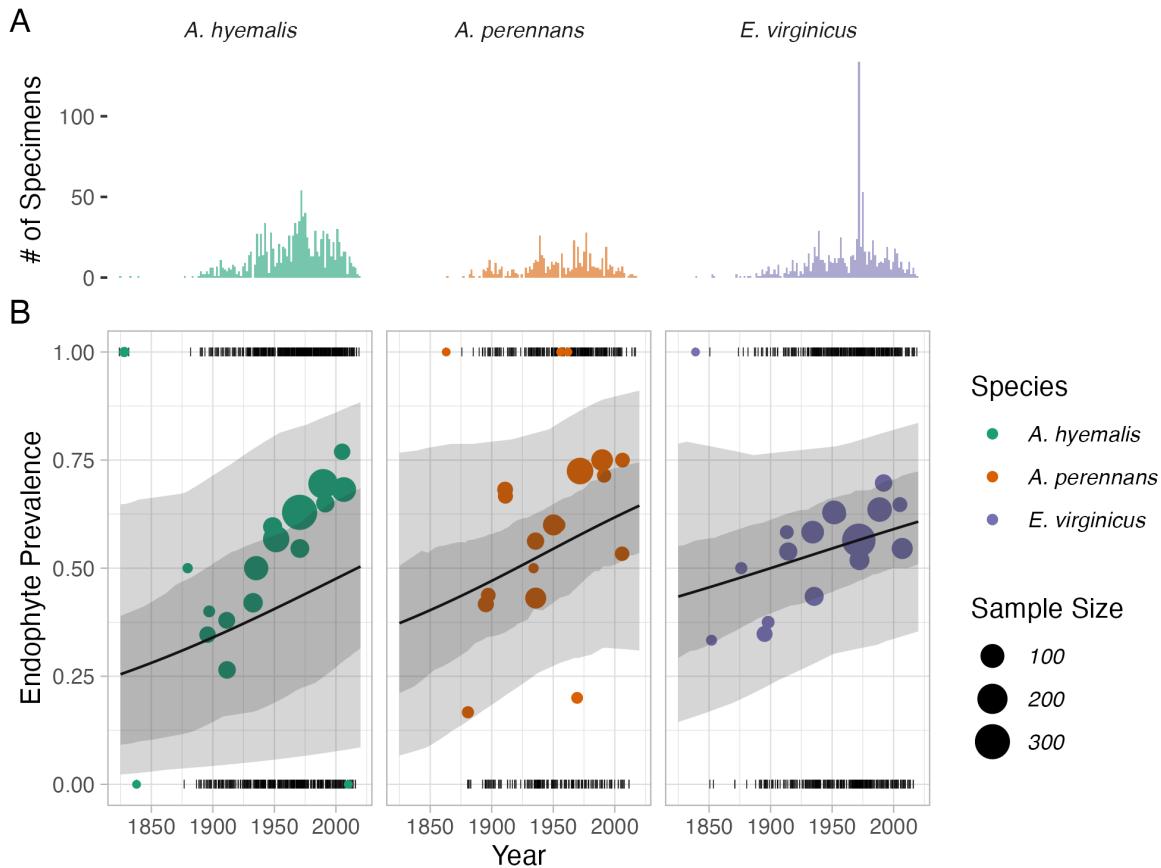


Figure 2: Temporal trends in endophyte prevalence. (A) Histograms show the frequency of collection through time for each host species. (B) Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species and point size is determined by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

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

324 Our model revealed hotspots of change in endophyte prevalence . While there was an overall
 325 increase in endophyte prevalence, these changes varied across the host species' ranges (Fig. 3).
 326 In some regions, posterior estimates of our spatially varying temporal trends, τ , indicate that *A.*

hyemalis and *A. perennans* experienced increases in percent prevalence by as much as 4% per year over the study period, while *E. virginicus* experienced increases up to around 1.5 % per year. In other regions, there were negligible changes. Notably, the symbionts of *E. virginicus* experienced only slight increases in prevalence, and were less spatially variable than the other two species. Regions that start with low endophyte prevalence, as in the southwestern portion of the range of *A. hyemalis*(Fig. A1), also experienced negligible change, suggesting that this may be driven more by the absence of the endophyte.²⁸ Predicted trends for *A. perennans* show certain areas of both large increase and of large decrease, however this species, for which we have the fewest samples, has the largest uncertainty. The posterior estimates of our spatially varying temporal trends, indicate relatively narrow certainty (need to compute²⁹).

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

29

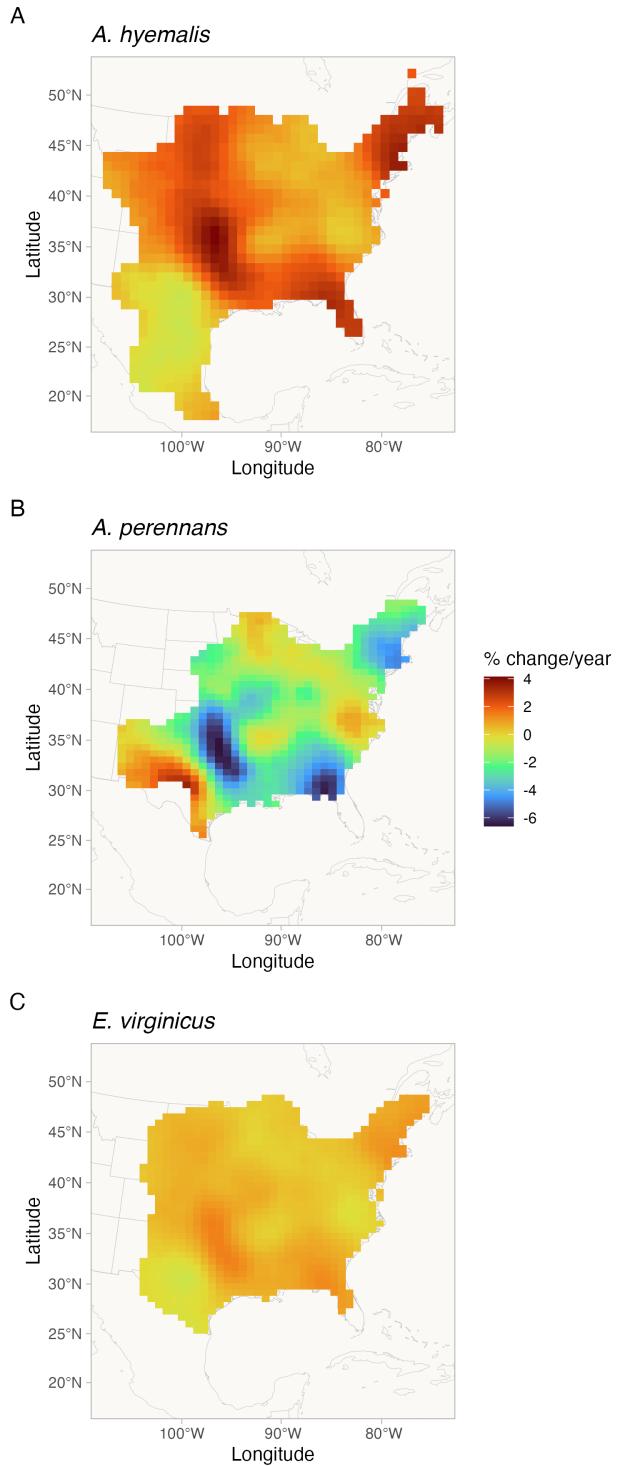


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species. Color indicates the relative change in predicted endophyte prevalence.

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

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

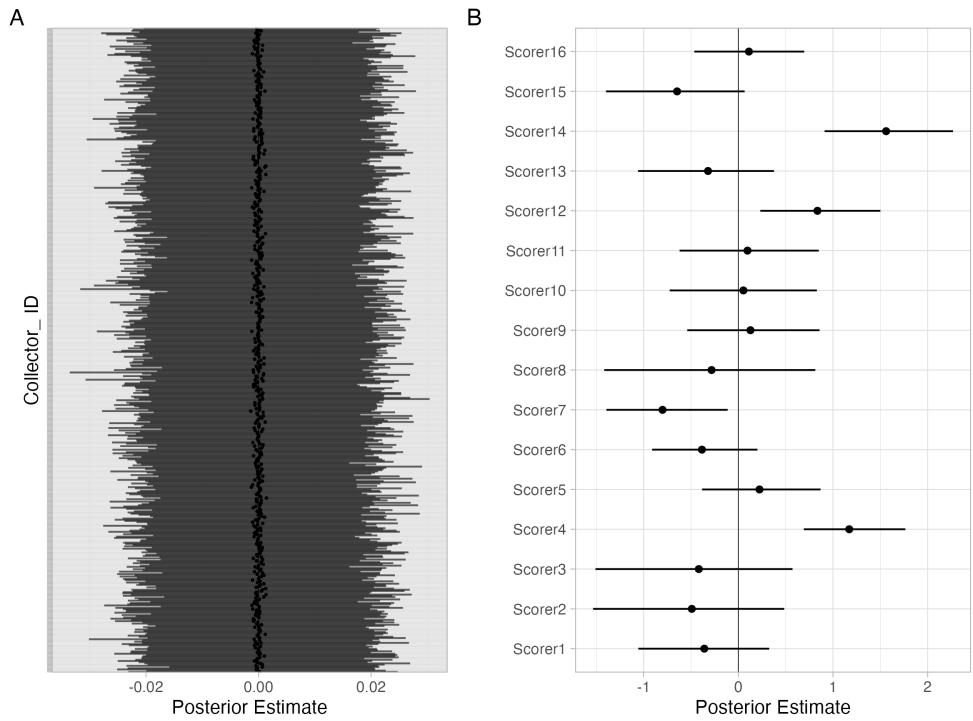


Figure 4: Posterior estimates of (A) collector and (B) scorer random effects. Points show the posterior mean along with 95% CI for random effects estimate from 532 collectors and 16 scorers.

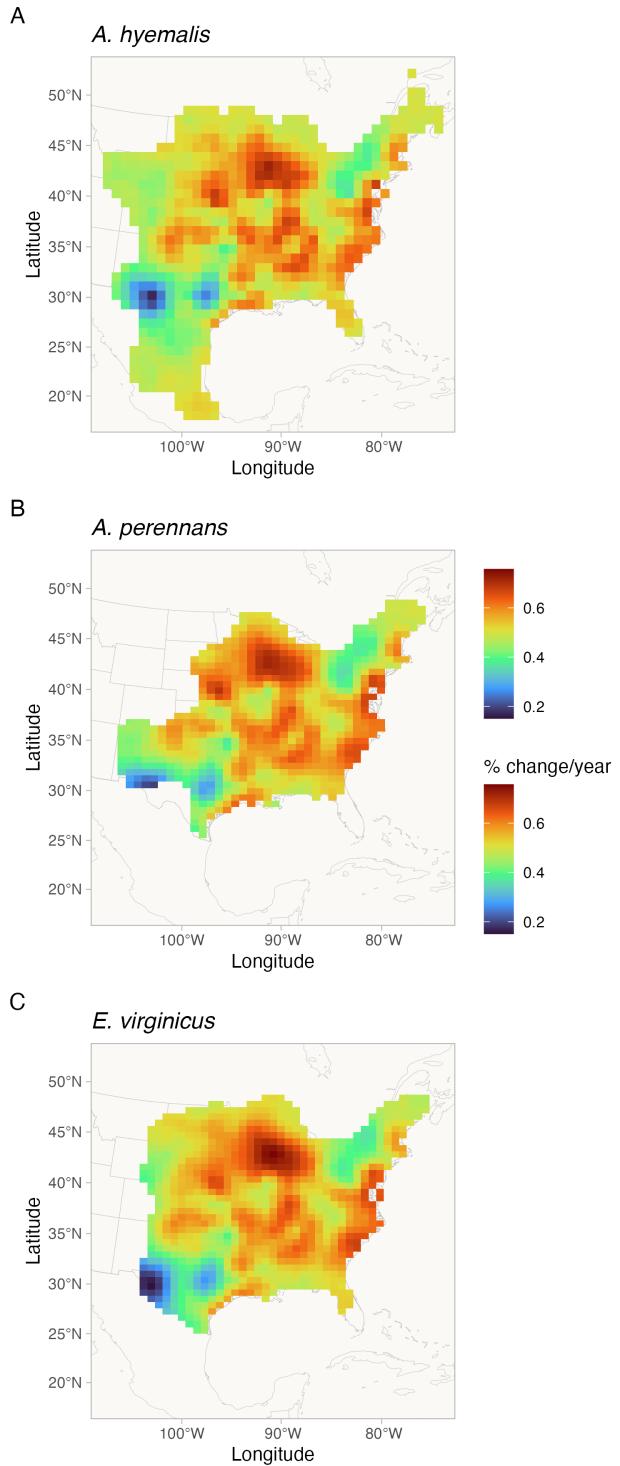


Figure 5: Mean predicted endophyte prevalence for each host species (columns) in 1925 (top row) and 2020 (bottom row). Color indicates mean predicted rate of endophyte prevalence across the predicted distribution of each species.

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

351 We found that trends in endophyte prevalence were strongly associated with seasonal climate
352 change drivers (Fig. 6). For the majority of the study region, the climate has become wetter and
353 cooler over the last century (Fig. A7-A8), a consequence of regional variation in global climate
354 change (IPCC, 2021). Within the study region, spatially heterogeneous environmental changes
355 were predictive of changes in endophyte prevalence. For example, strong increases in prevalence
356 within *E. virginicus* were most associated with declines in Summer precipitation (a negative cor-
357 relation in Fig. 7) as well as with increases in the year-to-year variability of annual temperature
358 (a positive correlation in Fig. 7). Changes were also associated with reductions in average an-
359 nual temperatures, and increases in year-to-year temperature variability. *A. perennans* endophyte
360 prevalence increased most strongly in regions that experienced reduced spring precipitation and
361 reduced variability in annual temperature. Although these correlations were weaker, changes
362 in *A. perennans* endophyte prevalence were also associated with increased in increases in annual
363 precipitation and increasing autumn precipitation. For *A. hyemalis*, endophyte prevalence in-
364 creased most strongly in regions that experienced reductions in autumn precipitation variability.
365 Correlations using only a subsampling of pixels were qualitatively similar to these results (Fig.
366 A11), suggesting that the patterns we find are not spurious associations.

367 30

³⁰Only have plotted results for AGHY right now.

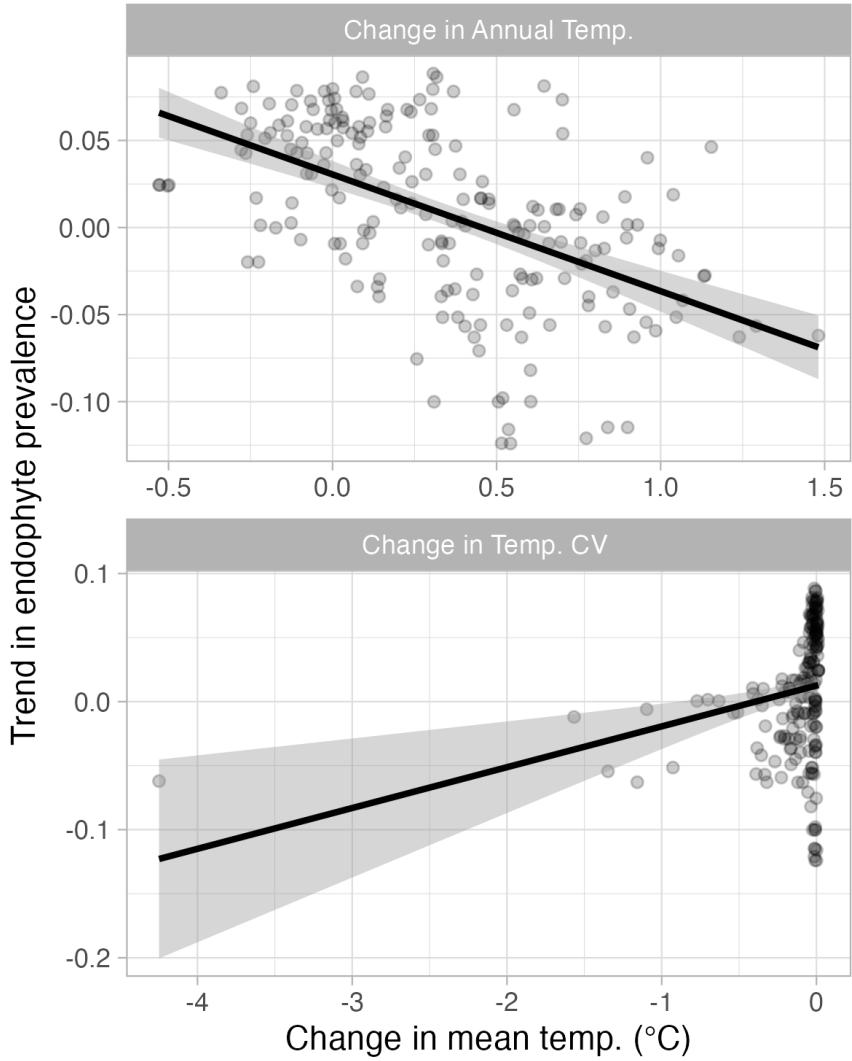


Figure 6: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation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

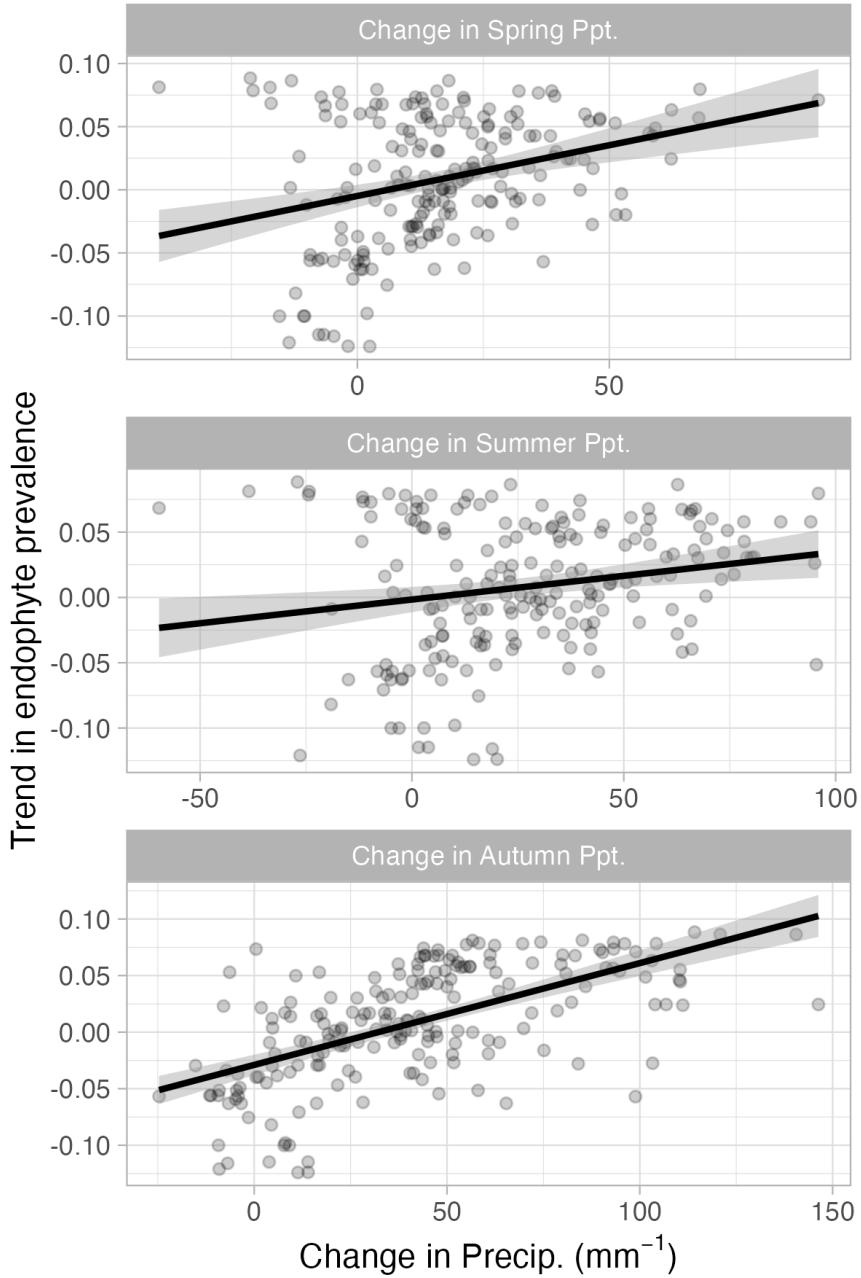


Figure 7: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation (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 .
 Asterisks denote correlation coefficients $> .3$ or $< -.3$.
 24

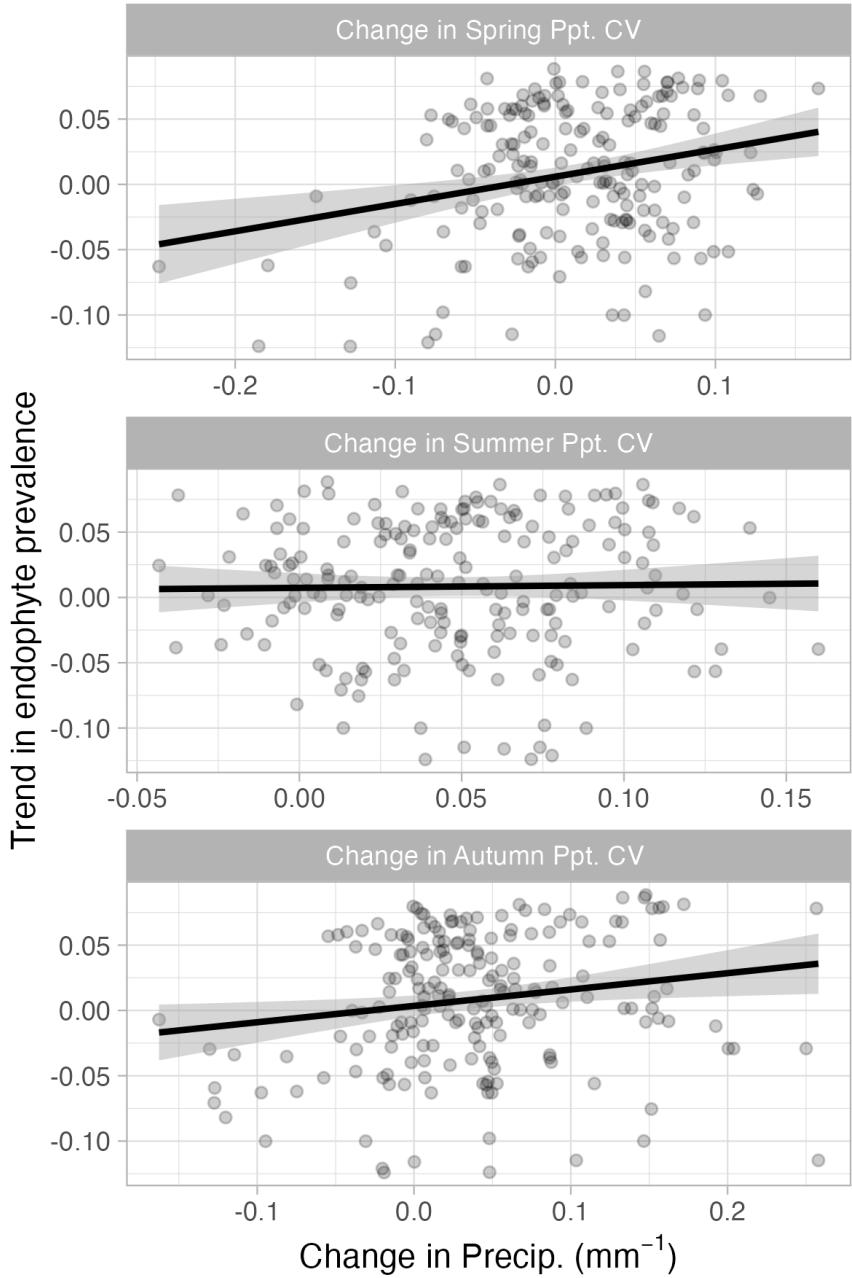


Figure 8: Correlations between changes in climate drivers and changes in endophyte prevalence. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ($^{\circ}\text{C}$) and total annual and seasonal precipitation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

368 *Performance on test data*

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

374 We discuss potential model improvements in the Discussion.

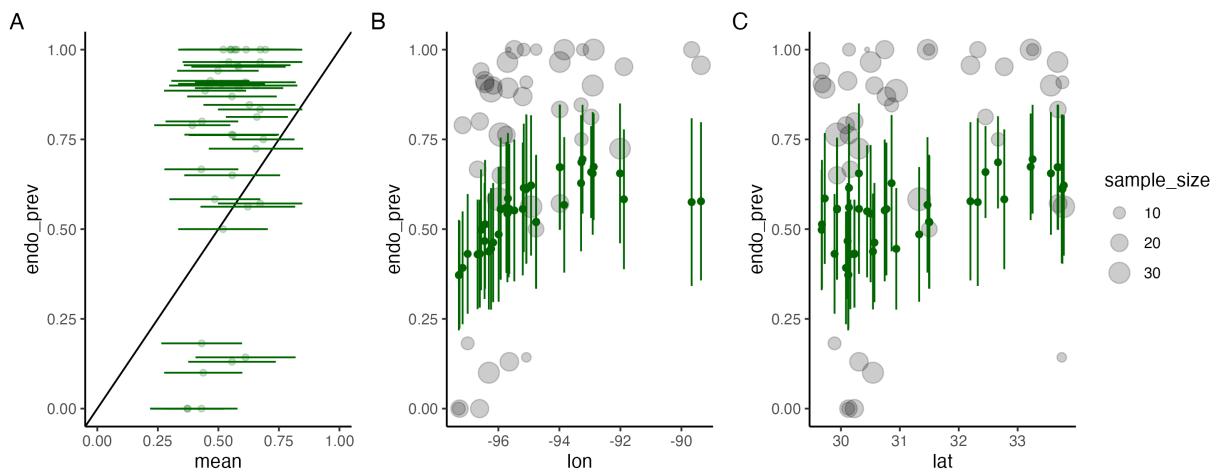


Figure 9: **Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporaneous endophyte prevalence in *A. hyemalis*, as indicated by some overlap of predicted 95% CI with the 1:1 line,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

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

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

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

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

428 Using test data to validate our model predictions allows us to evaluate places to improve the
429 model's ability to perform well at out-of-sample prediction, which will be particularly important
430 for predicting host and symbiont niche-shifts under future climate change. Lack of information
431 on local variability may simply be a feature of data derived from herbarium specimens. Even
432 though they are samples from local populations, they are single specimens that are aggregated
433 over in broad-scale model estimates. Poor predictive ability at local scales in this grass-endophyte
434 system is not surprising, as previous studies have found that local variation, even to the scale of
435 hundreds of meters can structure endophyte-host niches (Kazenel et al., 2015). Sneck et al. (2017)
436 also identified host genotype as an important predictor of endophyte prevalence in *E. virginicus*.
437 Other studies have found factors including land-use history (Vikuk et al., 2019) and the biotic
438 environment, including herbivory (Rudgers et al., 2016), to be important predictors of endophyte
439 ecology. Incorporating available climatic and soil layers as covariates is an obvious first step
440 that could improve predictions. Towards the goal of predicting the dynamics of microbial sym-
441 bioses under climate change, models that integrate data from local and regional scales would be
442 an important step to bridge the gap that often exists between large but broad bioclimatic and
443 biodiversity data and small but local data on biotic interactions (Isaac et al., 2020; Miller et al.,
444 2019).

445 Our analysis advances the use of herbarium specimens in global change biology in two ways.
446 First and foremost, this is the first study to link long-term changes in microbial symbioses to
447 changes in climate using specimens from natural history collections. The responses of micro-
448 bial symbioses are a rich target for future studies within museum specimens, particularly those
449 that take advantage of advances in sequencing technology. While we used relatively coarse
450 presence/absence data based on fungal morphology, other studies have examined historic plant
451 microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these
452 studies have so far been limited to relatively few specimens at limited spatial extents (Bieker
453 et al., 2020; Bradshaw et al., 2021; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al.,
454 2015). Continued advances in capturing historic DNA and in filtering out potential contami-

455 nation during specimen storage (Bakker et al., 2020; Daru et al., 2019; Raxworthy and Smith,
456 2021) will be imperative in the effort to scale up these efforts. This scaling up will be essential
457 to be able to quantify changes not just in the prevalence of symbionts, but also in symbionts'
458 intraspecific variation and evolutionary responses to climate change, as well as in changes in the
459 wider microbial community. Answering these questions as well as the unknown questions that
460 future researchers may ask also reiterates the value in capturing meta-information during ongo-
461 ing digitization efforts at herbaria around the world and during the accession of newly collected
462 specimens (Lendemer et al., 2020). Second, we accounted for several potential biases in the data
463 observation process that may be common to many collections-based research questions by using
464 a spatially-explicit random effects model. Spatial autocorrelation (Willems et al., 2022), potential
465 biases introduced by the sampling habits of collectors (Daru et al., 2018), and variation between
466 contemporary researchers during the collection of trait data, if not corrected for could lead to
467 over-confident inference about the strength and direction of historic change. Previous studies
468 that have quantified the effects of collector biases typically find them to be small (Davis et al.,
469 2015; Meineke et al., 2019), and we similarly did not find that collector has a strong effect on the
470 results of our analysis. Fitting this model in a Bayesian framework allows for full propagation of
471 uncertainty.

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

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

492 Acknowledgments

493 We thank Jessica Budke for help in drafting our initial destructive sampling plan, and to the
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495 of collectors who contributed to the natural history collections. Several high schooler and un-
496 dergraduate researchers contributed to data collection, including A. Appio-Riley, P. Bilderback,
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499 National Science Foundation (grants 1754468 and 2208857) and by funding from the Texas Ecolab
500 Program.

501 Statement of Authorship

502 Data and Code Availability

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

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

507

Appendix A

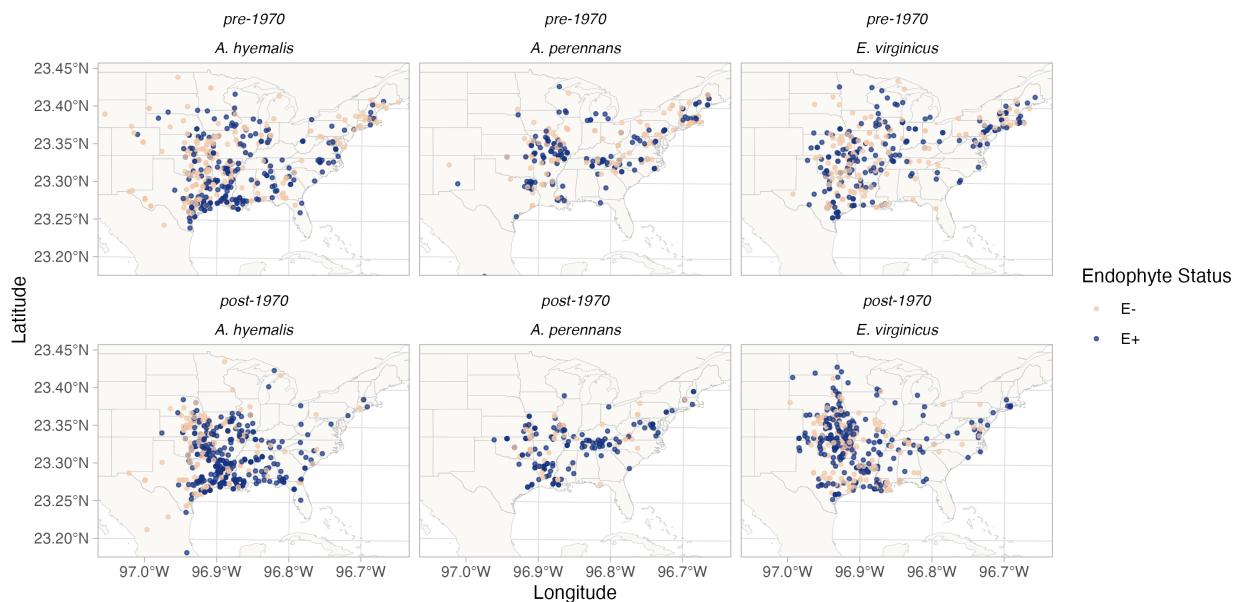


Figure A1: **Endophyte presence/absence in specimens of each host species.** Points show collection locations colored according to whether the specimen contained endophytes (E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.

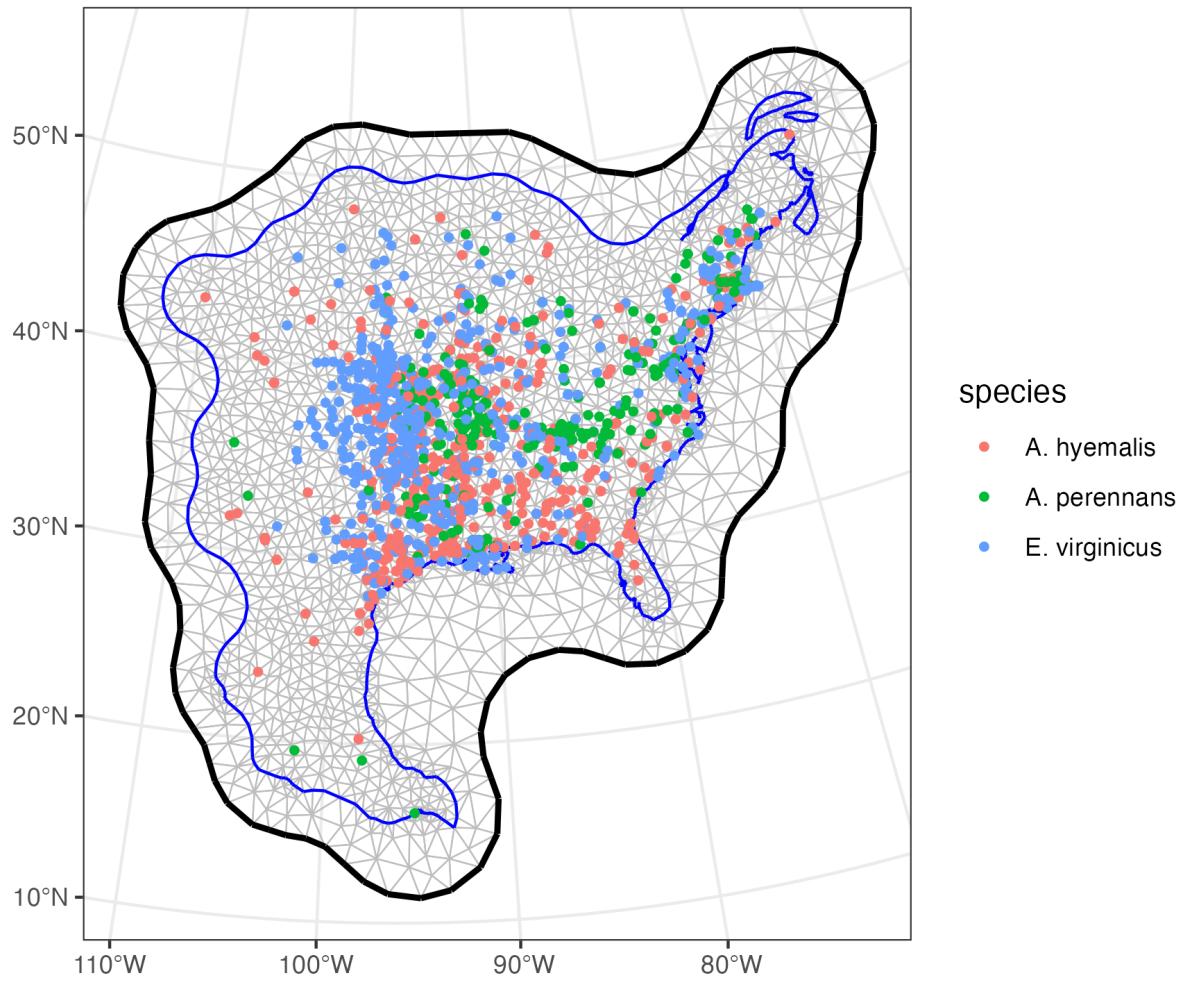


Figure A2: **Delauney triangulation mesh used to estimate spatial dependence between data points.** Grey lines indicate edges of triangles used to define distances between observations. Red points indicate locations of sampled herbarium specimens, and the blue outlines show the international borders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

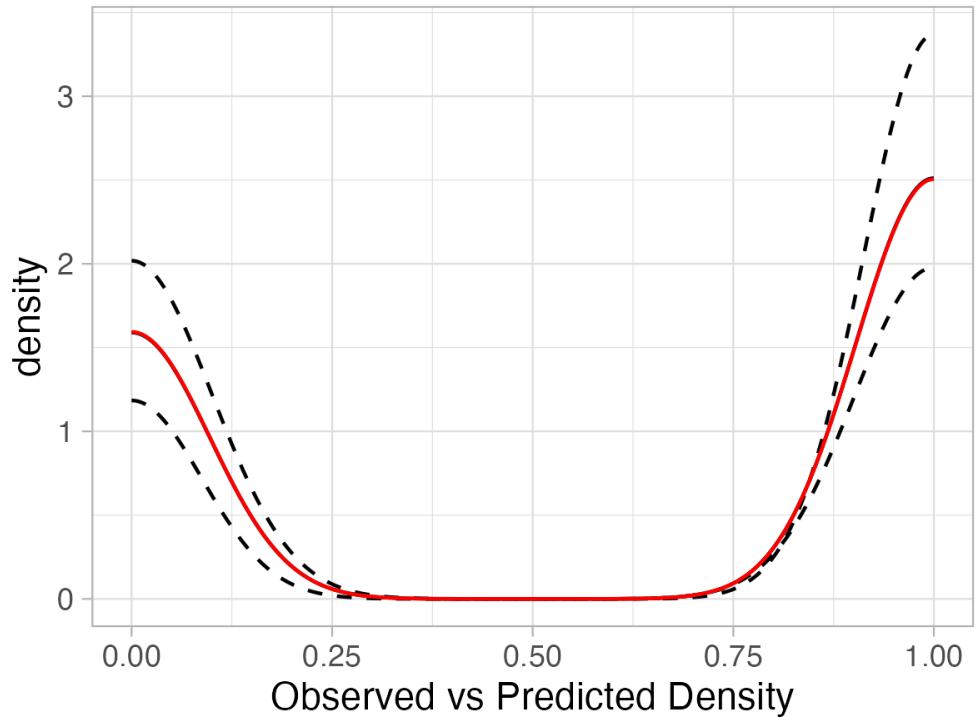


Figure A3: Consistency between real data and simulated values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (red) along with the mean(solid) and 95% CI (dashed) of simulated values (black).

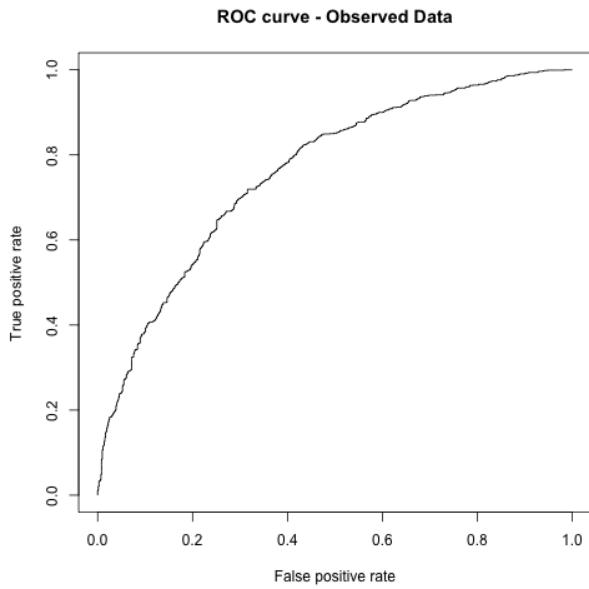


Figure A4: ROC plot showing model performance classifying observations according to endophyte status. The curves show adequate model performance for observed (top) and test (bottom) data. The AUC for each is 0.77.

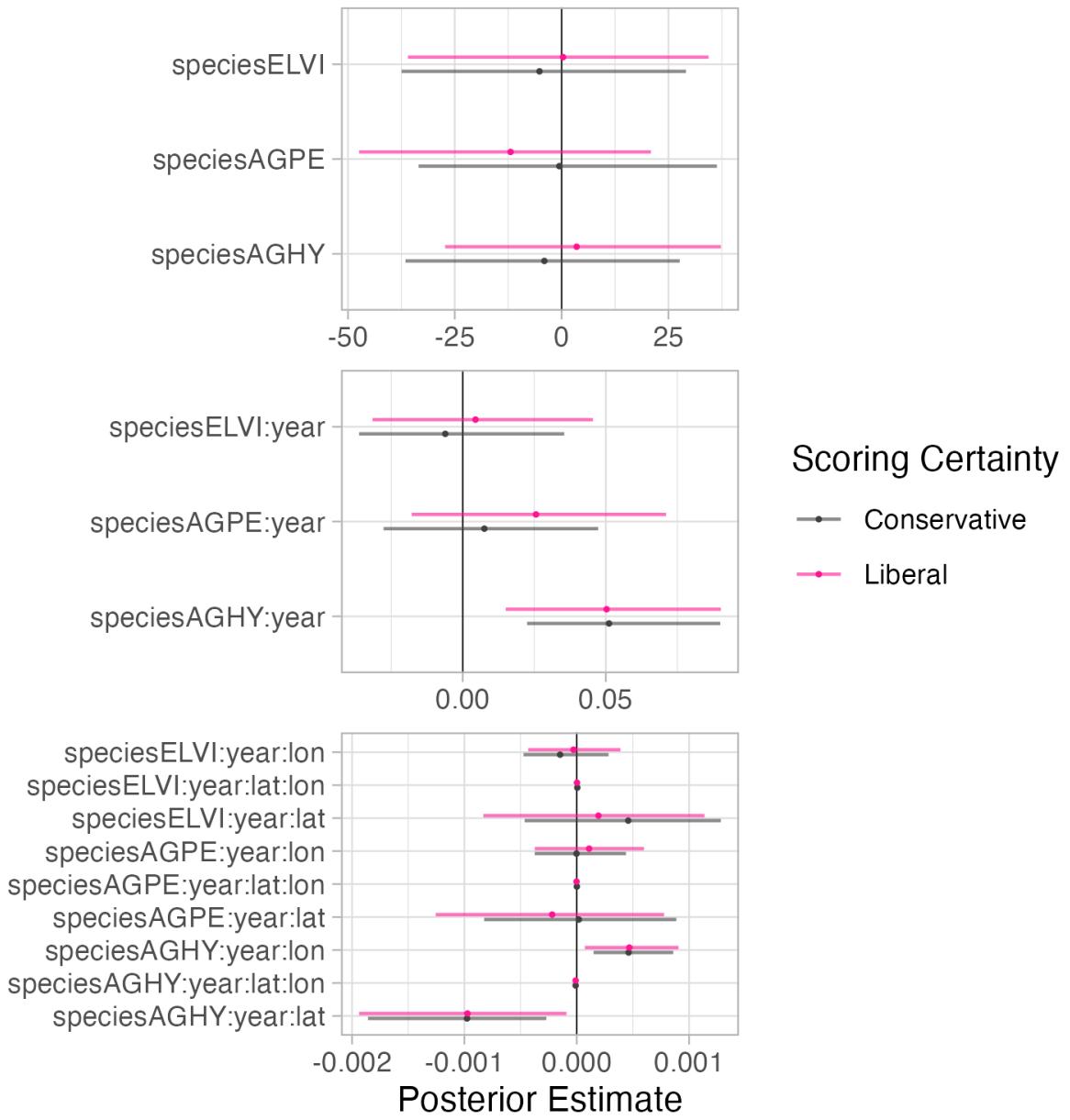


Figure A5: Comparison of posterior estimates of fixed effects when using Liberal or Conservative endophyte scores.

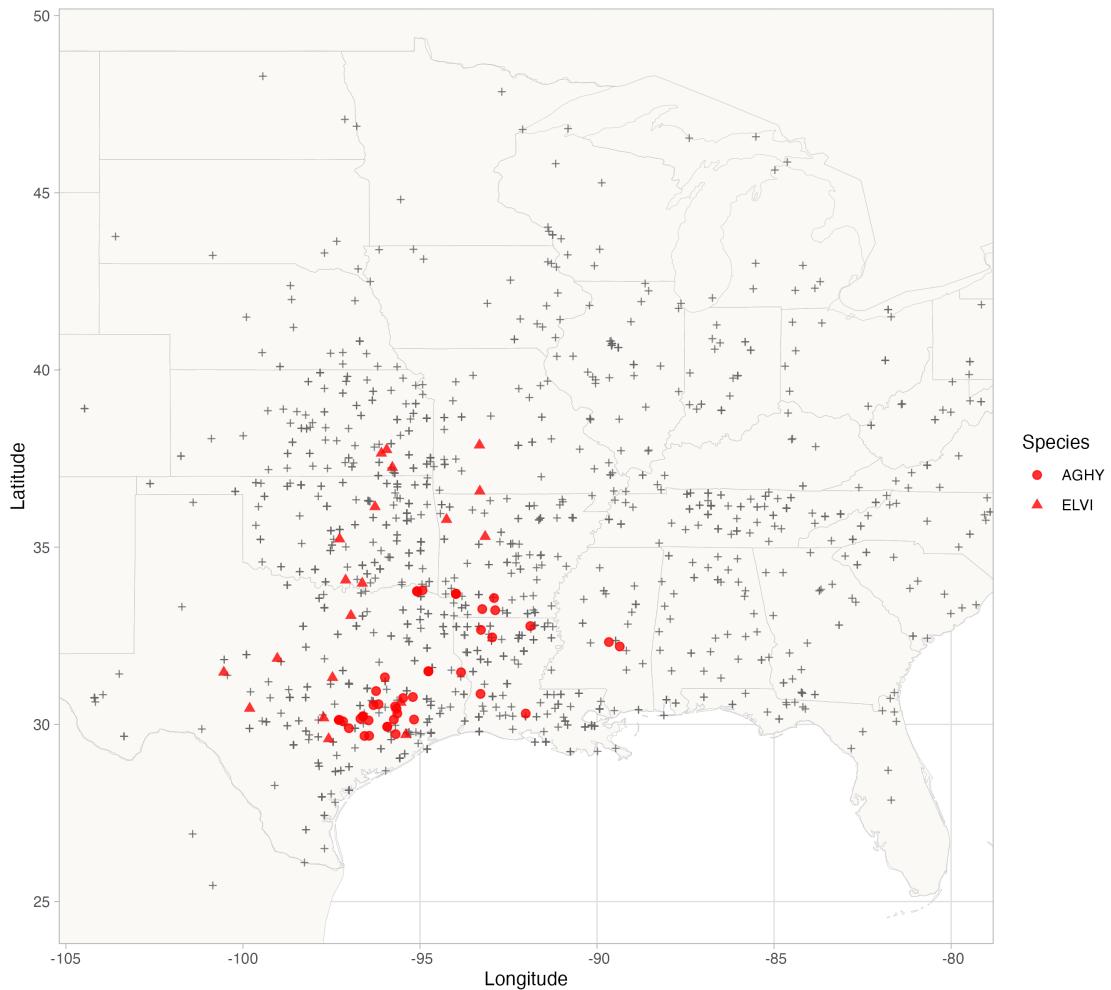


Figure A6: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).

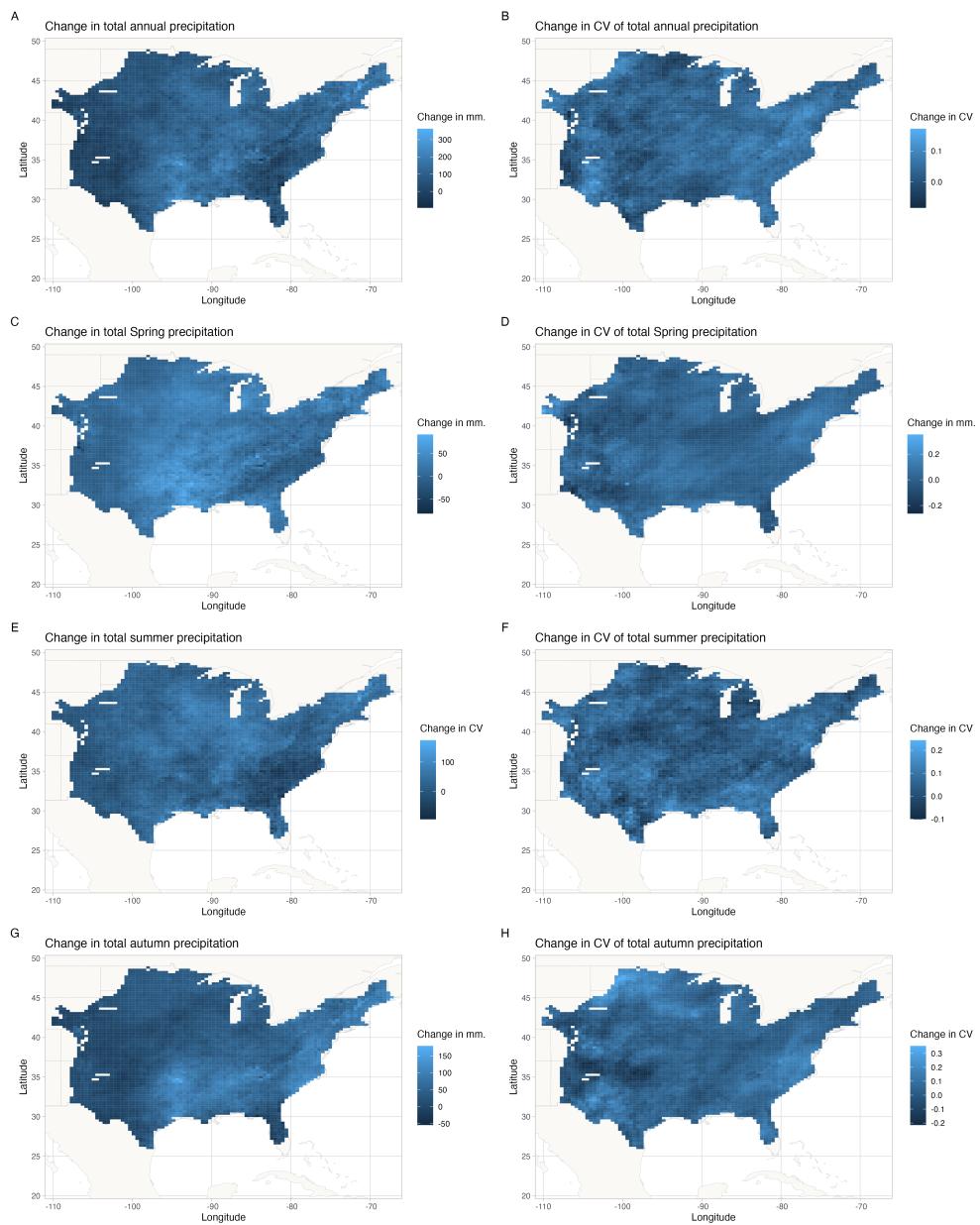


Figure A7: Change in precipitation between the periods 1895-1925 and 1990-2020. Color represents change in annual or seasonal total precipitation (A,C,E,G) and in the coefficient of variation of annual or seasonal total precipitation (B,D,F,H). Maps show the study area of *A. hyemalis*. Map pixels used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

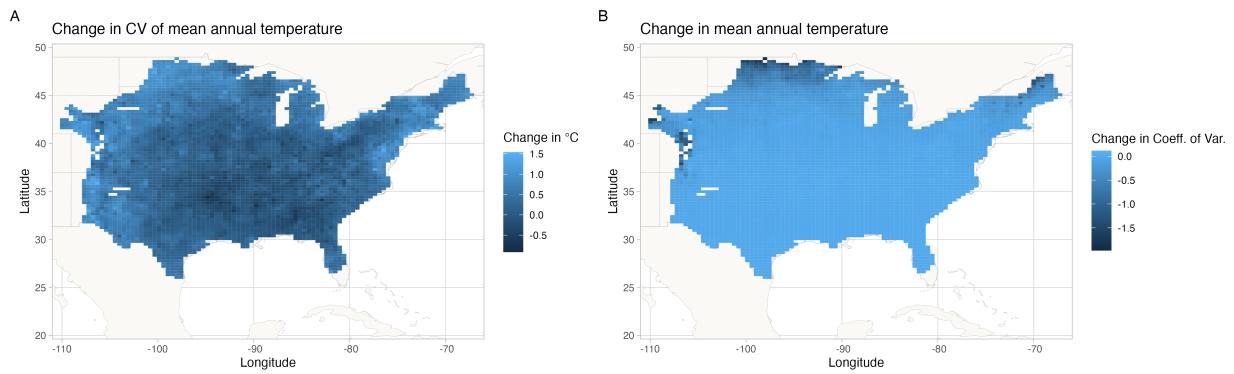


Figure A8: Change in temperature between the periods 1895-1925 and 1990-2020. Color represents change in annual mean temperature (A) and in the coefficient of variation of annual mean temperature (B). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change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

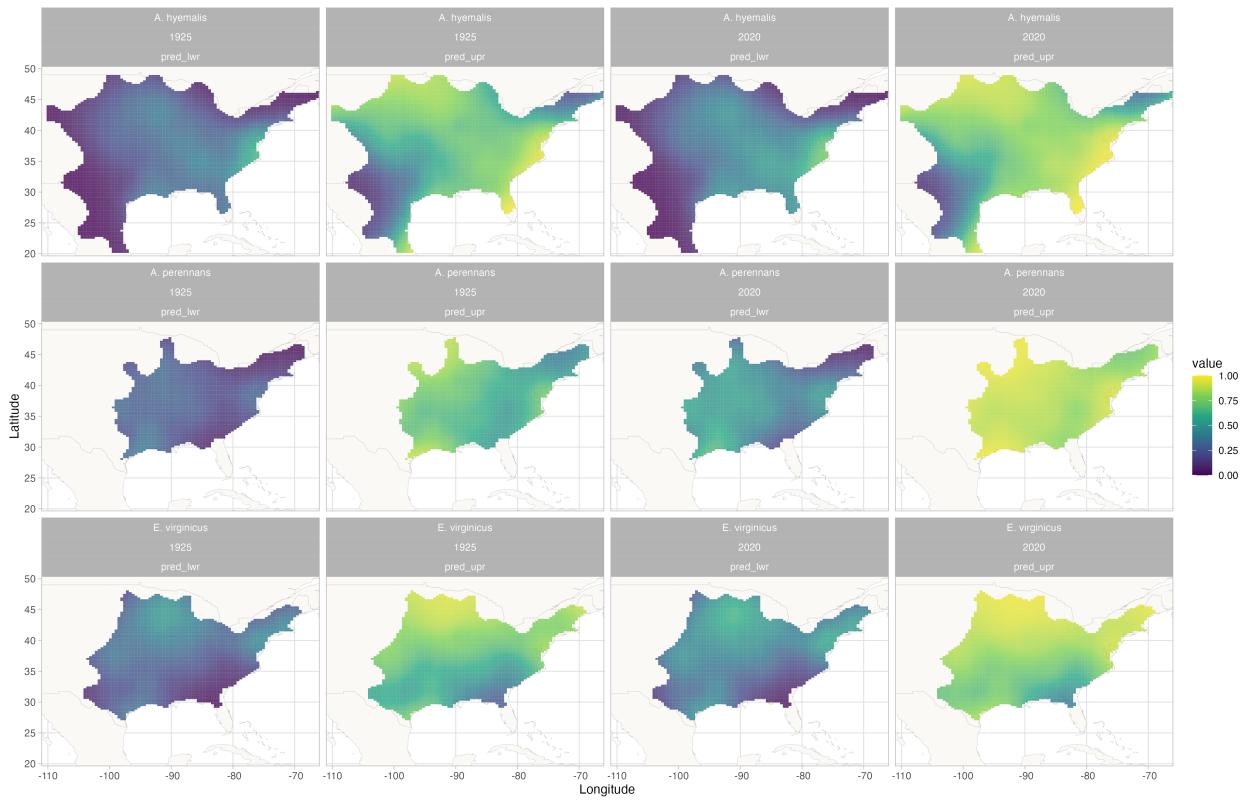


Figure A9: Uncertainty associated with spatial trends in endophyte prevalence. Color represents change in predicted endophyte prevalence. Panels show upper and lower 95% posterior probability for each host species between 1925 and 2020.

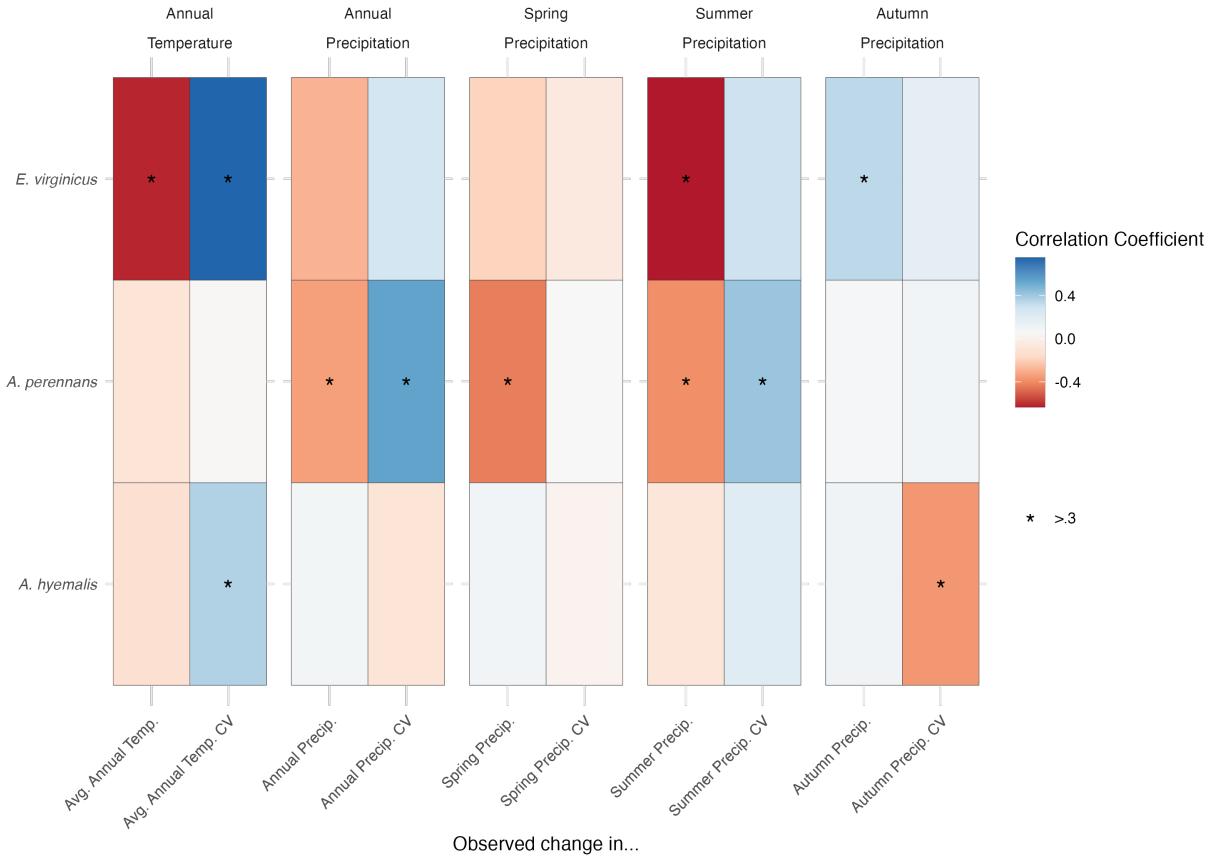


Figure A10: Correlations between changes in climate drivers and changes in endophyte prevalence from a random sample of 100 pixels across the study region. Color denotes the Spearman correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature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

508

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