

<sup>1</sup> Increasing prevalence of plant-fungal symbiosis across two  
<sup>2</sup> centuries of environmental change

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<sup>1</sup> *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. However, 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 2,346 herbarium specimens of three grass host species collected over the last two centuries (1824  
23 – 2019) 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  
26 three host species. We also found that changes in prevalence were associated with observed  
27 changes in seasonal climate drivers<sup>2</sup> corresponding to each host species' peak growing season.  
28 Our analysis performed favorably in an out-of-sample predictive test, however we identified  
29 XXX as suggesting the model fusion may be an important step moving forward. Our results  
30 provide novel evidence for a cryptic biological response to climate change that may contribute to  
31 the resilience of host-microbe symbiosis through context-dependent benefits that confer a fitness  
32 advantage to symbiotic hosts under environmental change.<sup>3</sup>

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<sup>2</sup>Describe "changes" – warming? drying?

<sup>3</sup>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

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.<sup>4</sup> 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

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<sup>4</sup>Edited 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.<sup>5</sup>

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.<sup>6</sup>

## 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 C<sub>3</sub> 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.

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

<sup>144</sup> *Herbarium surveys*

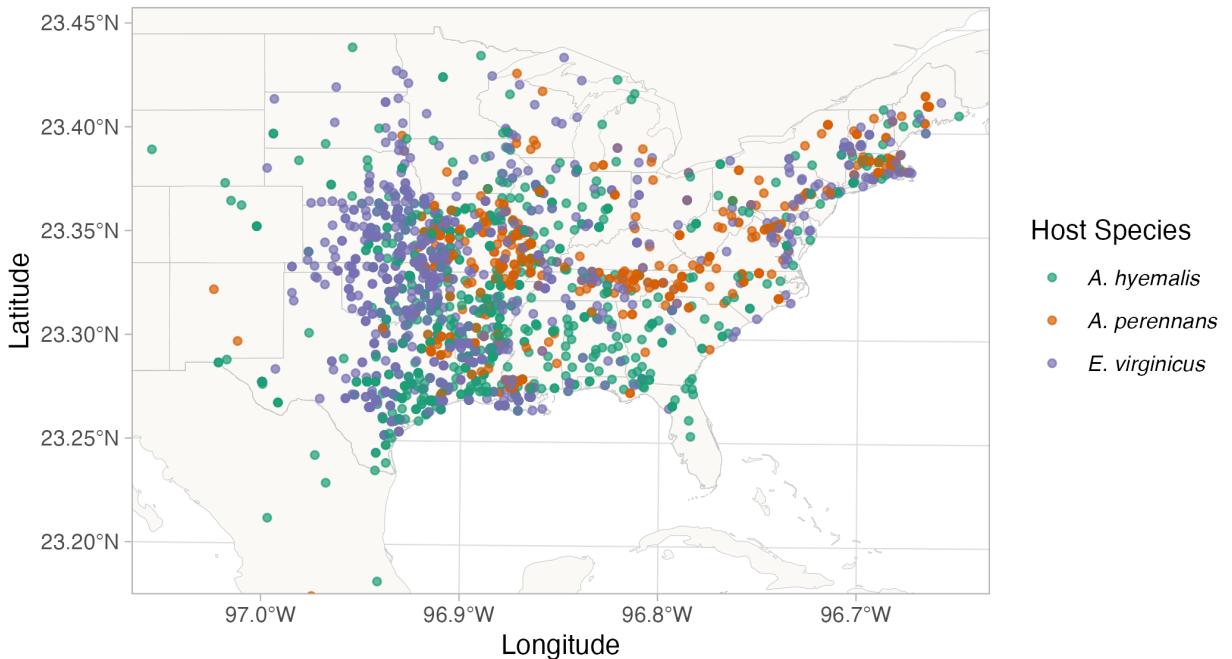
<sup>145</sup> We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens in-  
<sup>146</sup> cluded from each collection). With permission from herbarium staff, we acquired seed samples  
<sup>147</sup> from 1135 *A. hyemalis* specimens collected between 1824 and 2019,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

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

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

<sup>182</sup> *Modeling spatial and temporal changes in endophyte prevalence*

<sup>183</sup> We assessed spatial and temporal changes in endophyte prevalence across each host distribu-  
<sup>184</sup> tion, first quantifying the "global" trends, aggregating across space, and then examining spatial  
<sup>185</sup> 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 occurrences, 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 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. We  
 211 included the spatial random effect  $\delta_{l[i]}$ , intended to account for residual spatial variation, and  
 212  $\chi_{c[i]}$  and  $\omega_{s[i]}$  i.i.d.-random effects indexed for each collector identity ( $c[i]$ ), and scorer identity  
 213 ( $s[i]$ ) 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 Each spatially-structured parameter depended on a covariance matrix according to the prox-  
 215 imity of each collection location (Bakka et al., 2018; Lindgren et al., 2011). The covariance matrix  
 216 was approximated using a Matérn covariance function, with each data point assigned a location  
 217 according to the nodes of a mesh of non-overlapping triangles across our study area (Fig. A2).

218 Previous work suggests that behavior of historical botanists and uneven sampling may intro-

219 duce biases into ecological inferences made from historic collections (Kozlov et al., 2020). Prolific  
220 collectors who contribute thousands of specimens may be more or less likely to collect certain  
221 species, or specimens with certain traits (Daru et al., 2018). Similarly, the process of scoring seeds  
222 for hyphae involved several student researchers who, even with standardized training, may vary  
223 in their likelihood of positively identifying *Epichloë* hyphae. By including a random effect for  
224 collectors and for scorers, we attempted to account for variance across individual researchers  
225 that may bias our predictions of changes in endophyte prevalence. **Models for each host species**  
226 **were fit separately.**<sup>7</sup> <sup>8</sup>

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

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

227 The spatially-varying year slope  $\beta_l$  allowed us to flexibly estimate variation in the temporal  
228 trajectory of endophyte change at locations across the study region.

229 For both models, spatially-structured random intercepts ( $\alpha_l$ ) and slopes ( $\beta_l$ ) were constructed  
230 using stochastic partial differential equations (SPDE) that depend on a covariance matrix accord-  
231 ing to the proximity of each collection location (Bakka et al., 2018; Lindgren et al., 2011). The  
232 covariance matrix was approximated using a Matérn covariance function, with each data point  
233 assigned a location according to the nodes of a mesh of non-overlapping triangles across our  
234 study area (Fig A2).<sup>9</sup>

235 We performed model fitting using the inlabru R package (), with vague priors<sup>10</sup>, and com-  
236 pared models with different sizes of mesh, which had little effect on the resulting model esti-

<sup>7</sup>It 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.

<sup>8</sup>I 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  $\alpha$ ,  $\chi$ , and  $\omega$ . I presume the latter two are Gaussian but I don't know how to represent the distribution of  $\alpha$ .

<sup>9</sup>This paragraph would be a place to describe the variance terms for the other random effects.

<sup>10</sup>I thought you needed informative priors on the spatial decay parameters

237 mates<sup>11</sup>. Each mesh<sup>12</sup> was bounded by the predicted host distribution, described below. Posterior  
238 modes were stable<sup>13</sup> indicating that numeric convergence was successful. We assessed model fit  
239 with graphical posterior predictive checks (Fig. A3). The model performed adequately at clas-  
240 sifying the historical data, comparing the accuracy of predictions from the model with observed  
241 data (avg. AUC = 0.77; Fig. A4).<sup>14</sup>

242 *Modeling distributions of host species*

243 We modeled epicloë host species distribution to predict their occurrences in space and time as  
244 continuous and binary maps of potential presences. These maps were used as a backbone to  
245 predict endophyte prevalence on epicloë host species. The species distribution models were built  
246 following the ODMAP (overview, data, model, assessment, prediction) protocol (Crossley et al.,  
247 2022). We used the observed presence of the host species collected from GBIF from 1990 to 2020.  
248 These occurrences were corrected for spatial autocorrelation due to sampling bias by thinning the  
249 occurrences to the spatial scale of the climatic variables. The climatic variables were temperature  
250 of the spring, precipitation of the spring and precipitation of the summer. We preferred these  
251 variables because they were not correlated (Variance Inflation Factor > 0.7) and also allowed  
252 the model to account for the influence of seasonal climate variation on species presence. The  
253 occurrence data was split into 75% for model training and 25% for model testing. We fitted  
254 the model using maximum entropy (MaxEnt) using the maxent function in the package dismo  
255 (Hijmans et al., 2017). MaxEnt was preferred because it does not generate response curves that  
256 may cause unpredictable behavior when applied to new climates (Hijmans and Graham, 2006).  
257 We used 10000 pseudo-absences as background points. To convert the continuous predicted  
258 probabilities into binary presence - absence maps, we used the training sensitivity (true positive  
259 rate) and specificity threshold (true negative rate) (Liu et al., 2005). The performances of the

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<sup>11</sup>That'sa good but you still need to state what mesh size you used and what that means, biologically.

<sup>12</sup>You have not defined what you mean by "mesh".

<sup>13</sup>Assessed how?

<sup>14</sup>Maybe move this to validation section, and then have both in-sample and out-of-sample approaches.

260 model were evaluated using the AUC (Jiménez-Valverde, 2012).

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

262 We evaluated the predictive ability of the model using contemporary endophyte surveys as out-  
263 of-sample test data, an important but rarely used strategy in ecological studies (Tredennick et al.,  
264 2021).<sup>15</sup> We used data from contemporary surveys of endophyte prevalence in *A. hyemalis* and  
265 *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted in 2013 as  
266 described in Sneck et al. (2017), and surveys of *A. hyemalis* took place between 2015 and 2020<sup>16</sup>.  
267 Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation in endo-  
268 phyte prevalence towards its range edge, while surveys of *E. virginicus* were designed to cover  
269 latitudinal variation along its range edge. In total, we visited 43 populations of *A. hyemalis* and 20  
270 populations of *E. virginicus* across the south-central US, with emphasis on Texas and neighboring  
271 states (Fig A4<sup>17</sup>). During surveys, we collected seeds from up to 30 individuals per location (av-  
272 erage number of plants sampled: 22.9). We quantified the endophyte status of each individual  
273 with staining microscopy as described for the herbarium surveys (with 5-10 seeds scored per  
274 individual), and calculated the prevalence of endophytes within the population (proportion of  
275 symbiotic plants divided by the number of sampled plants). For each population, we compared  
276 the observed fraction of endophyte-symbiotic hosts to the predicted probability of endophyte oc-  
277 currence  $\hat{P}$  derived from the model based on location and year, with collector and scorer random  
278 effects fixed at zero. The contemporary survey period (2013-2020) is at the most recent edge of  
279 the time period encompassed by the historical observations used for model fitting. We compared  
280 the model's prediction for these locations to the observed population prevalence.<sup>18</sup>

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<sup>15</sup>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.

<sup>16</sup>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.

<sup>17</sup>This is now A6. Good reminder to use the ref function.

<sup>18</sup>It is not clear if you are testing model 1 ("global trend") or model 2 ("spatially varying trends").

281

## Assessing the role of climate drivers

282 We assessed how the magnitude of climate change may have driven changes in endophyte preva-  
283 lence by assessing correlations between changes in climate and changes in endophyte prevalence  
284 predicted from our spatial model at evenly spaced pixels across the study area. We first down-  
285 loaded monthly temperature and precipitation rasters from the PRISM climate group (Daly and  
286 Bryant, 2013) covering the time period between 1895 and 2020 using the 'prism' R package (Hart  
287 and Bell, 2015). Prism provides reconstructions of historic climate variables across the United  
288 States by spatially-interpolating weather station data (Di Luzio et al., 2008). We calculated 30-  
289 year climate normals for annual and seasonal mean temperature and cumulative precipitation  
290 for the recent (1990 to 2020) and historic (1895 to 1925) periods. We used three four-month sea-  
291 sons within the year (Spring: January, February, March, April; Summer: May, June, July, August;  
292 Autumn: September, October, November, December). This division of seasons allowed us to  
293 quantify differences in climate associated with the two "cool" seasons, when we expect our fo-  
294 cal species to be most biologically active (*A. hyemalis* flowering phenology: Spring; *E. virginicus*:  
295 Spring and Summer; *A. perennans*: Fall). In addition to mean climate conditions, environmental  
296 variability itself can influence population dynamics (Tuljapurkar, 1982) and changes in variabil-  
297 ity are a key prediction of climate change models (IPCC, 2021; Stocker et al., 2013). Therefore  
298 we calculated the coefficient of variation (CV) during each period for each annual and seasonal  
299 climate driver as the interannual standard deviation divided by the mean across each 30-year  
300 period. We then took the difference between recent and historic periods for the mean and CV for  
301 each climate driver (Fig. A5)<sup>19</sup>. Because initial analyses indicated a high degree of collinearity  
302 between seasonal and annual changes in temperature, we used annual temperature only, along  
303 with annual and seasonal precipitation, in the subsequent analysis. All together, this left us with  
304 measurements of change in 10 potential climate drivers: the mean and coefficient of variation  
305 of annual temperature, as well as the mean and coefficient of variation of cumulative annual

<sup>19</sup>This is Figure A7 – Can you make the color scale on these diverging at zero?

306 precipitation, cumulative spring precipitation, cumulative summer precipitation, and cumulative  
307 autumn precipitation (Fig A8-A9)<sup>20</sup>.

308 To evaluate whether areas that have experienced the greatest changes in endophyte preva-  
309 lence (hotspots of endophyte change) are associated with high degrees of change in climate  
310 (hotspots of climate change), we modeled spatially varying slopes of endophyte change through  
311 time ( $\beta_l$ ) as a linear function of environmental covariates, with a Gaussian error distribution.<sup>21</sup>  
312 Calculating correlations from many pixels across the study region risks artificially inflating confi-  
313 dence in our results due to large sample sizes, and so we repeated this calculation using only a  
314 random subsample of 100 pixels across the study region<sup>22</sup>.<sup>23</sup> <sup>24</sup>

315 **Results**

316 *How has endophyte prevalence changed over time?*

317 We found that endophyte prevalence increased within the examined specimens over the last  
318 two centuries for all three host species (Fig. 4). On average, *A. hyemalis* and *E. virginicus* both  
319 increased from 30 % to over 70% prevalence across the study region, and *A. perennans* increased  
320 from 15% to over 70% prevalence. Our model indicates a higher certainty that overall temporal  
321 trends are positive for *A. hyemalis* and *A. perennans* than for *E. virginicus* (99% probability of a  
322 positive overall year slope in *A. hyemalis*, 89% probability of a positive overall year slope in *A.*  
323 *perennans*, and 58% probability of a positive overall year slope in *E. virginicus*).<sup>25</sup>

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<sup>20</sup> The species names are not clear on Fig A9. I suggest increase the font size

<sup>21</sup> 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.

<sup>22</sup> 100 seems like a low number to me. What if we did this for all of the herbarium collection locations?

<sup>23</sup> Are the methods above repeated for each species separately?

<sup>24</sup> 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.

<sup>25</sup> These numbers are currently outdated. I am making some adjustments to models, and will update with final model

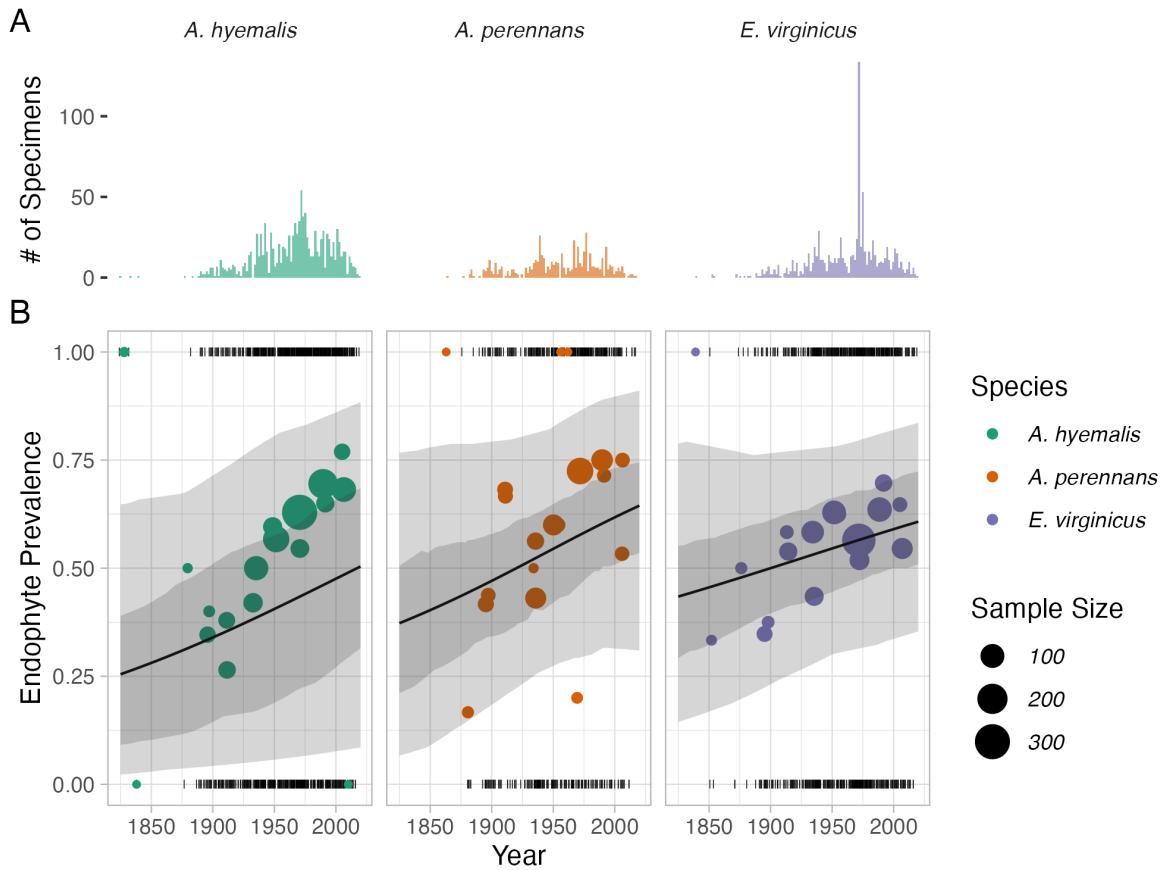


Figure 2: Temporal trends in endophyte prevalence. (A) Histograms show the frequency of collection through time for each host species. (B) Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species and point size is determined by 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.

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

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

328 *hyemalis* and *A. perennans* experienced increases in percent prevalence by as much as 4% per year  
329 over the study period, while *E. virginicus* experienced increases up to around 1.5 % per year. In  
330 other regions, there were negligible changes. Notably, the symbionts of *E. virginicus* experienced  
331 only slight increases in prevalence, and were less spatially variable than the other two species.  
332 Regions that start with low endophyte prevalence, as in the southwestern portion of the range  
333 of *A. hyemalis*(Fig. A1), also experienced negligible change, suggesting that this may be driven  
334 more by the absence of the endophyte.<sup>26</sup> Predicted trends for *A. perennans* show certain areas  
335 of both large increase and of large decrease, however this species, for which we have the fewest  
336 samples, has the largest uncertainty. The posterior estimates of our spatially varying temporal  
337 trends, indicate relatively narrow certainty (need to compute<sup>27</sup>).

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<sup>26</sup>more discussion material, but putting it here for now.

27

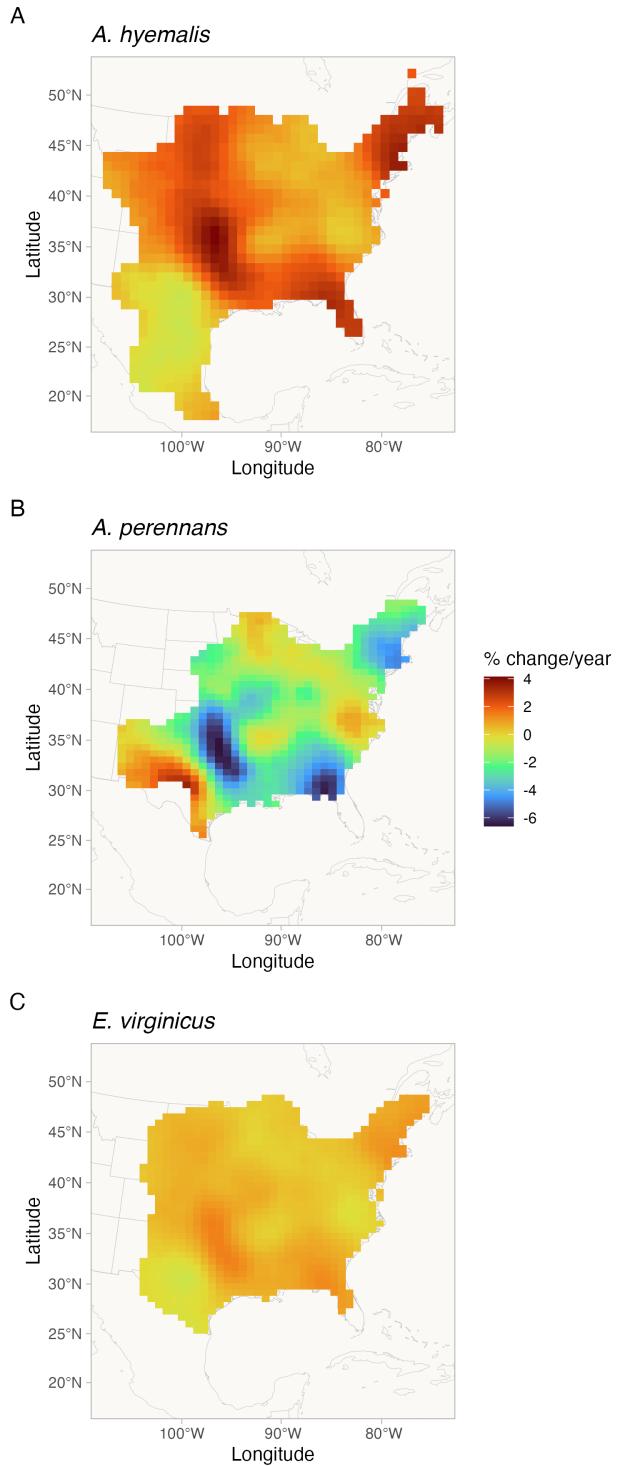
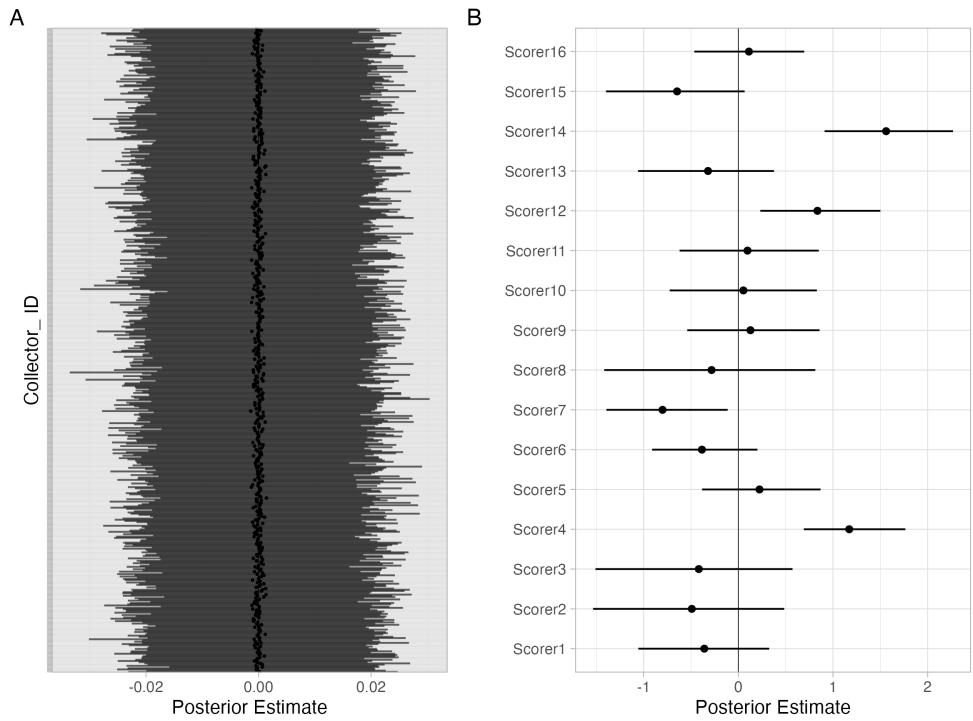


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species. Color indicates the relative change in predicted endophyte prevalence.

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

339    We quantified temporal and spatial trends in endophyte prevalence while accounting for poten-  
340    tial biases introduced by collectors and by individuals who quantified endophyte presence/absence  
341    with the use of random effects. We found no evidence that collector biases influenced our re-  
342    sults. Collector random effects were consistently small; Fig 4A, and models fit with and without  
343    this random effect provide qualitatively similar results. The identity of individual scorers did  
344    contribute to observed patterns in endophyte prevalence. For example, 3 of the 16 scorers were  
345    more likely than average to assign positive endophyte status, as indicated by 95% credible in-  
346    tervals that do not overlap 0) (Fig 4B). However, this may have been driven by differences in  
347    scorers biases during the seed scoring process, or by unintended spatial clustering of the speci-  
348    mens scored by each scorer. Interpreting our models with the inclusion of the scorer effect thus  
349    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.

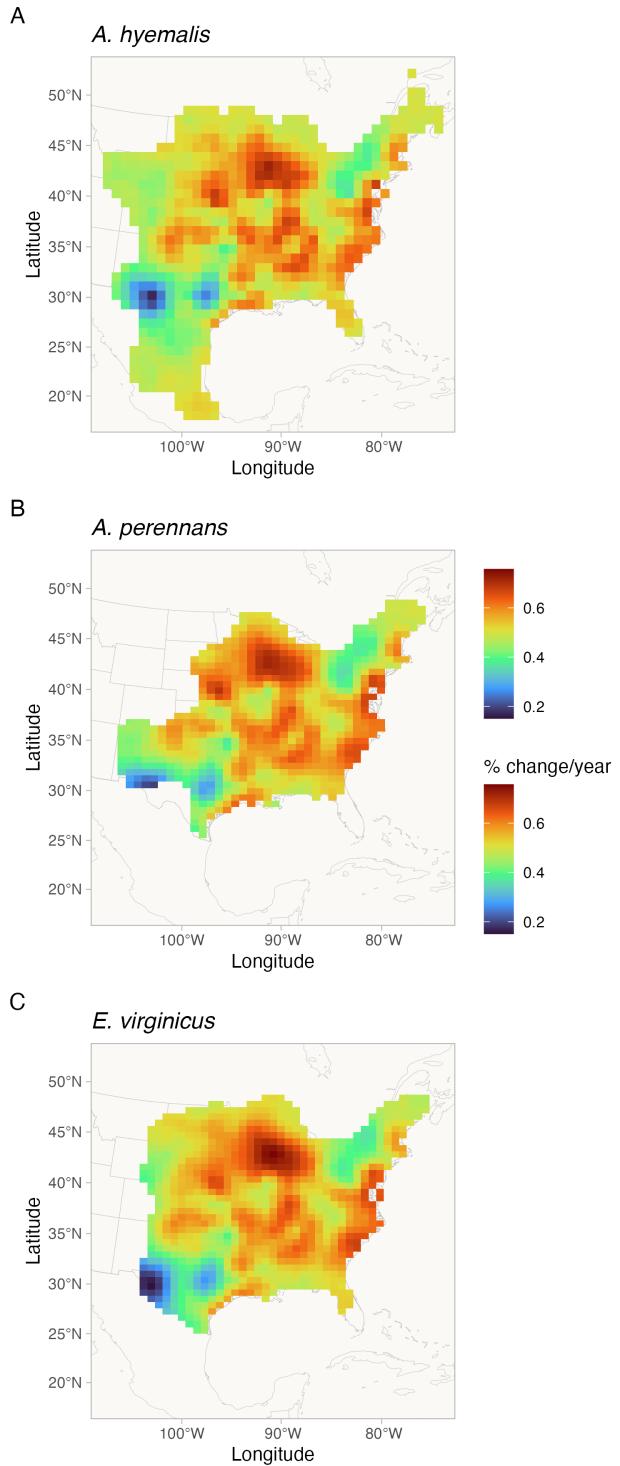


Figure 5: Mean predicted endophyte prevalence for each host species (columns) in 1925 (top row) and 2020 (bottom row). Color indicates mean predicted rate of endophyte prevalence across the predicted distribution of each species.

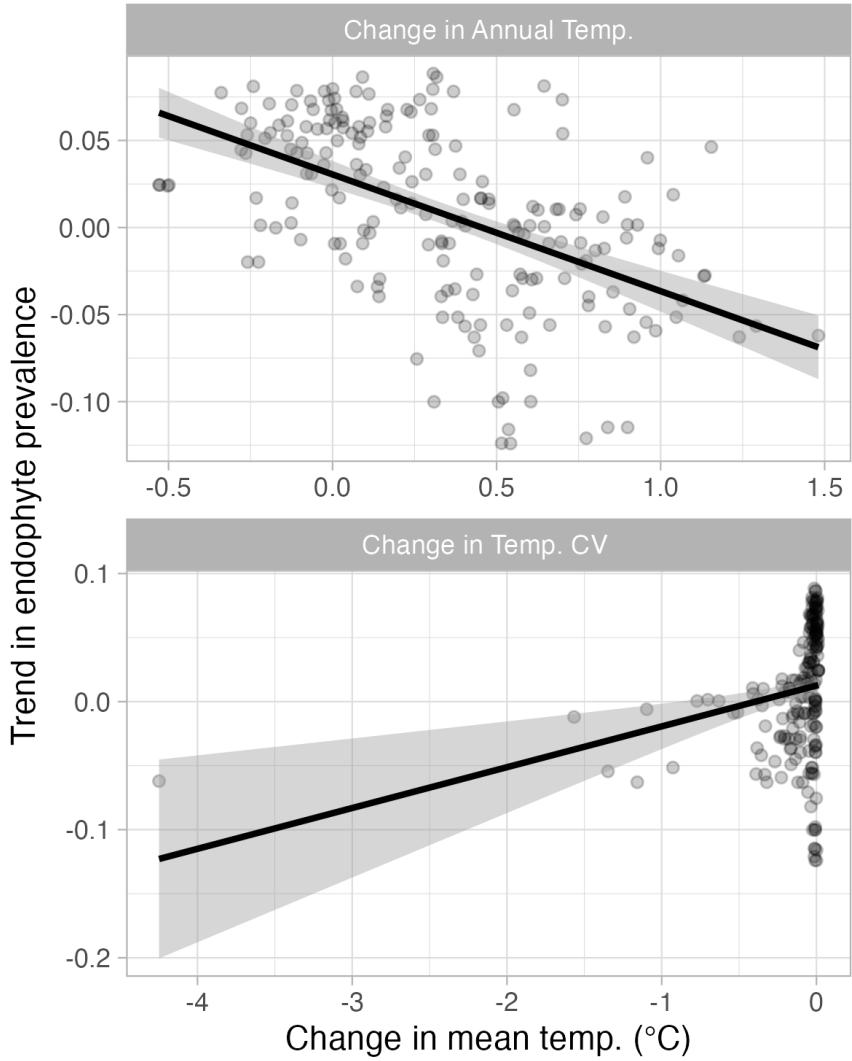
350     What is the relationship between variation in temporal trends in endophyte  
351            prevalence and changes in climate drivers?

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

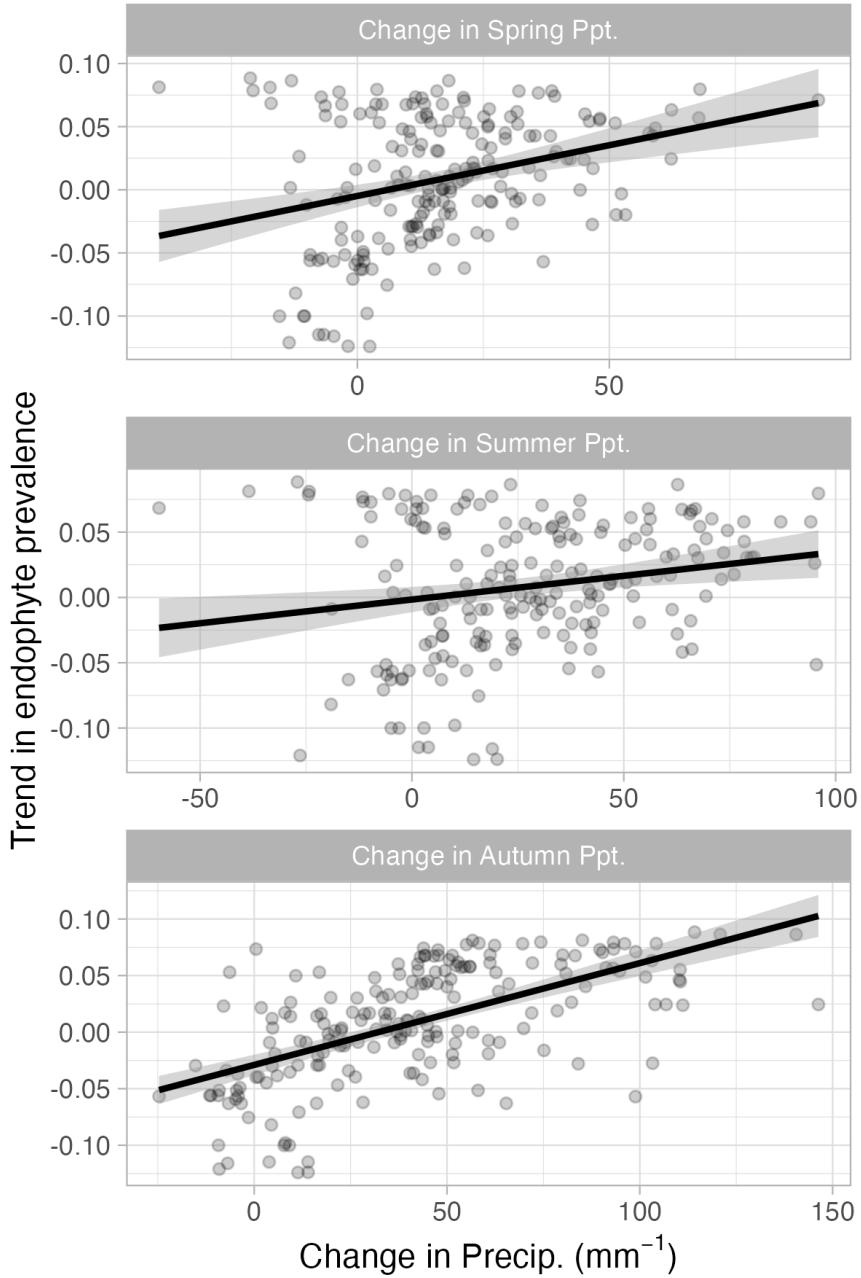
368            28

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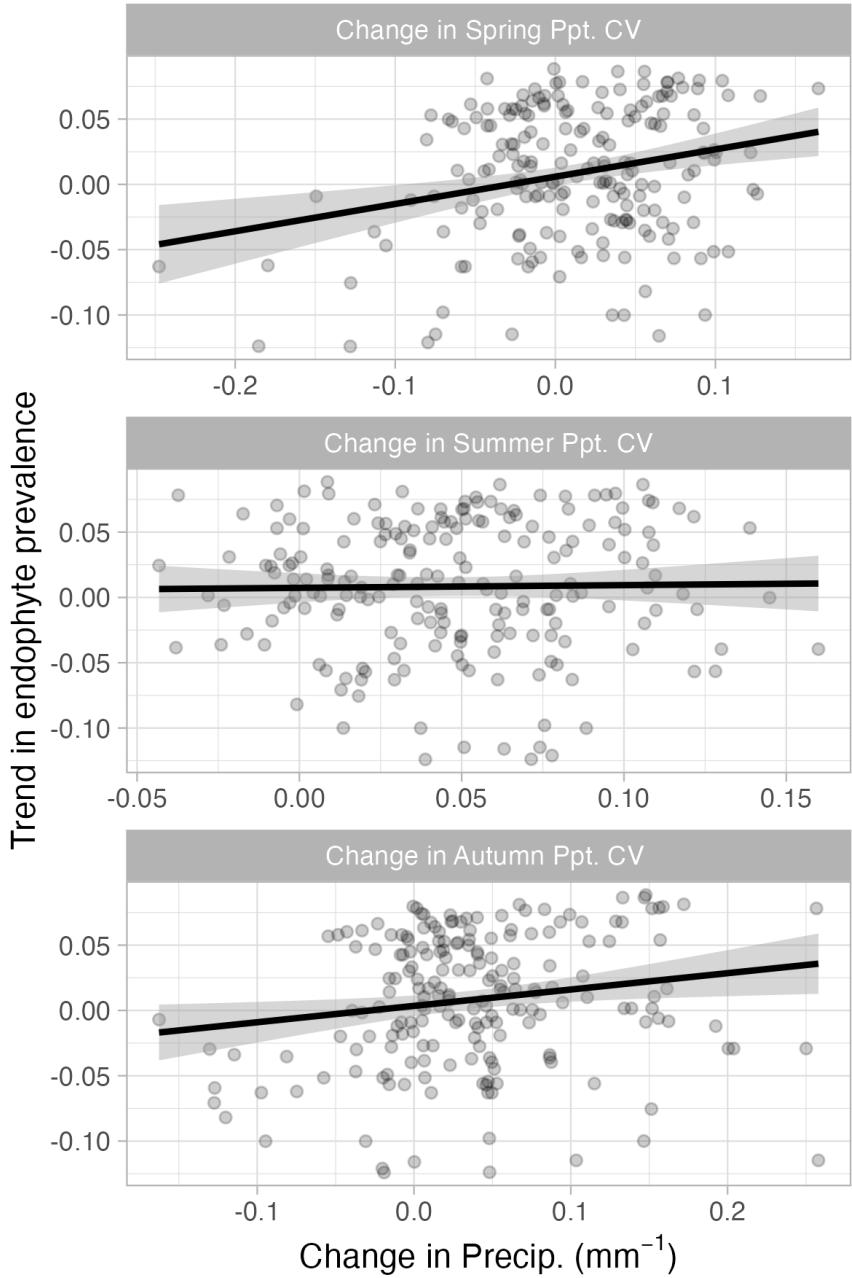
28 Only have plotted results for AGHY right now.



**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 .  
25 Asterisks denote correlation coefficients  $> .3$  or  $< -.3$ .

369 *Performance on test data*

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

375 We discuss potential model improvements in the Discussion.

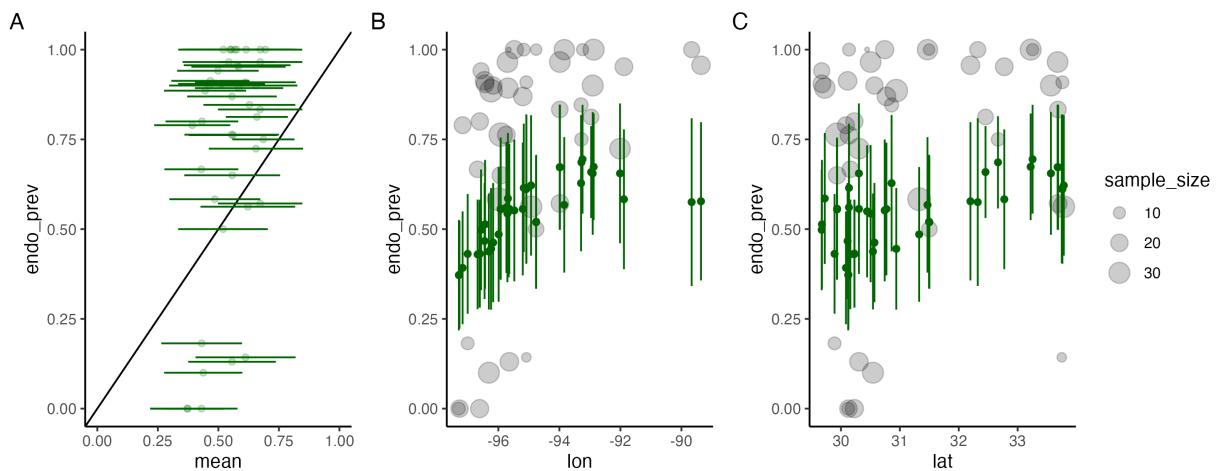


Figure 9: **Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporaneous endophyte prevalence in *A. hyemalis*, as indicated by some overlap of predicted 95% CI with the 1:1 line,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

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

387 Differences between the responses of each host species underscore that while all of these  
388 C<sub>3</sub> grasses share similar broad-scale distributions, each engages in unique biotic interactions  
389 and has unique niche requirements. We identified hotspots of change for *A. perennans*, which  
390 experienced the strongest absolute changes in endophyte prevalence (Fig. 5). Declines in the  
391 southern portion of its range and increases in the north suggest a potential poleward range shift  
392 of endophytic plants. Based on previous work demonstrating that endophytes can shield their  
393 hosts from drought stress (Decunta et al., 2021), we generally predicted that drought conditions  
394 could be a driver of increasing endophyte prevalence. In line with this expectation, increas-  
395 ing prevalence for this species was associated with decreasing precipitation, most strongly with  
396 autumn-season declines (Fig. 7). *A. perennans* typically blooms in the autumn. Endophytes could  
397 be playing a role helping hosts weather autumn-season droughts while the species is dormant.  
398 It may be useful to investigate whether lagged climate effects are important predictors of host  
399 fitness in this system (Evers et al., 2021). To our knowledge, the response of the symbiosis in  
400 *A. perennans* to drought has not been examined experimentally, but in a greenhouse experiment,  
401 endophytes had a positive effect on host reproduction under shaded, low-light conditions (Davitt

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

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

429 Using test data to validate our model predictions allows us to evaluate places to improve the  
430 model's ability to perform well at out-of-sample prediction, which will be particularly important  
431 for predicting host and symbiont niche-shifts under future climate change. Lack of information  
432 on local variability may simply be a feature of data derived from herbarium specimens. Even  
433 though they are samples from local populations, they are single specimens that are aggregated  
434 over in broad-scale model estimates. Poor predictive ability at local scales in this grass-endophyte  
435 system is not surprising, as previous studies have found that local variation, even to the scale of  
436 hundreds of meters can structure endophyte-host niches (Kazenel et al., 2015). Sneck et al. (2017)  
437 also identified host genotype as an important predictor of endophyte prevalence in *E. virginicus*.  
438 Other studies have found factors including land-use history (Vikuk et al., 2019) and the biotic  
439 environment, including herbivory (Rudgers et al., 2016), to be important predictors of endophyte  
440 ecology. Incorporating available climatic and soil layers as covariates is an obvious first step  
441 that could improve predictions. Towards the goal of predicting the dynamics of microbial sym-  
442 bioses under climate change, models that integrate data from local and regional scales would be  
443 an important step to bridge the gap that often exists between large but broad bioclimatic and  
444 biodiversity data and small but local data on biotic interactions (Isaac et al., 2020; Miller et al.,  
445 2019).

446 Our analysis advances the use of herbarium specimens in global change biology in two ways.  
447 First and foremost, this is the first study to link long-term changes in microbial symbioses to  
448 changes in climate using specimens from natural history collections. The responses of micro-  
449 bial symbioses are a rich target for future studies within museum specimens, particularly those  
450 that take advantage of advances in sequencing technology. While we used relatively coarse  
451 presence/absence data based on fungal morphology, other studies have examined historic plant  
452 microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these  
453 studies have so far been limited to relatively few specimens at limited spatial extents (Bieker  
454 et al., 2020; Bradshaw et al., 2021; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al.,  
455 2015). Continued advances in capturing historic DNA and in filtering out potential contami-

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

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

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

## 493 Acknowledgments

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

## 502 Statement of Authorship

## 503 Data and Code Availability

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

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

508

## Appendix A

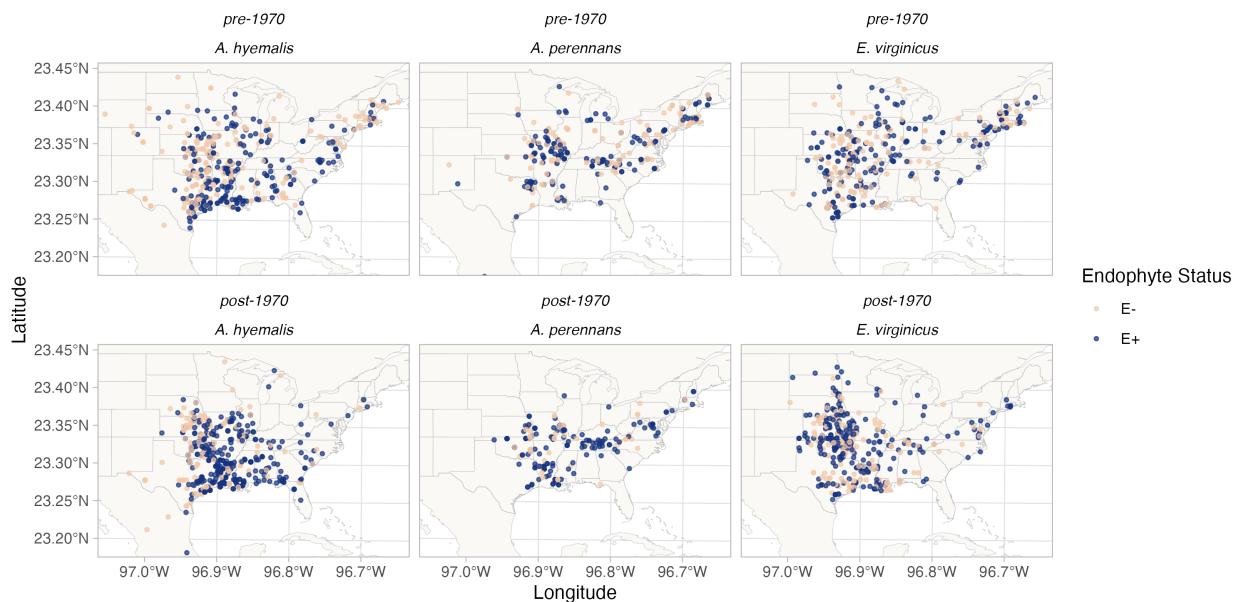


Figure A1: **Endophyte presence/absence in specimens of each host species.** Points show collection locations colored according to whether the specimen contained endophytes ( E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.

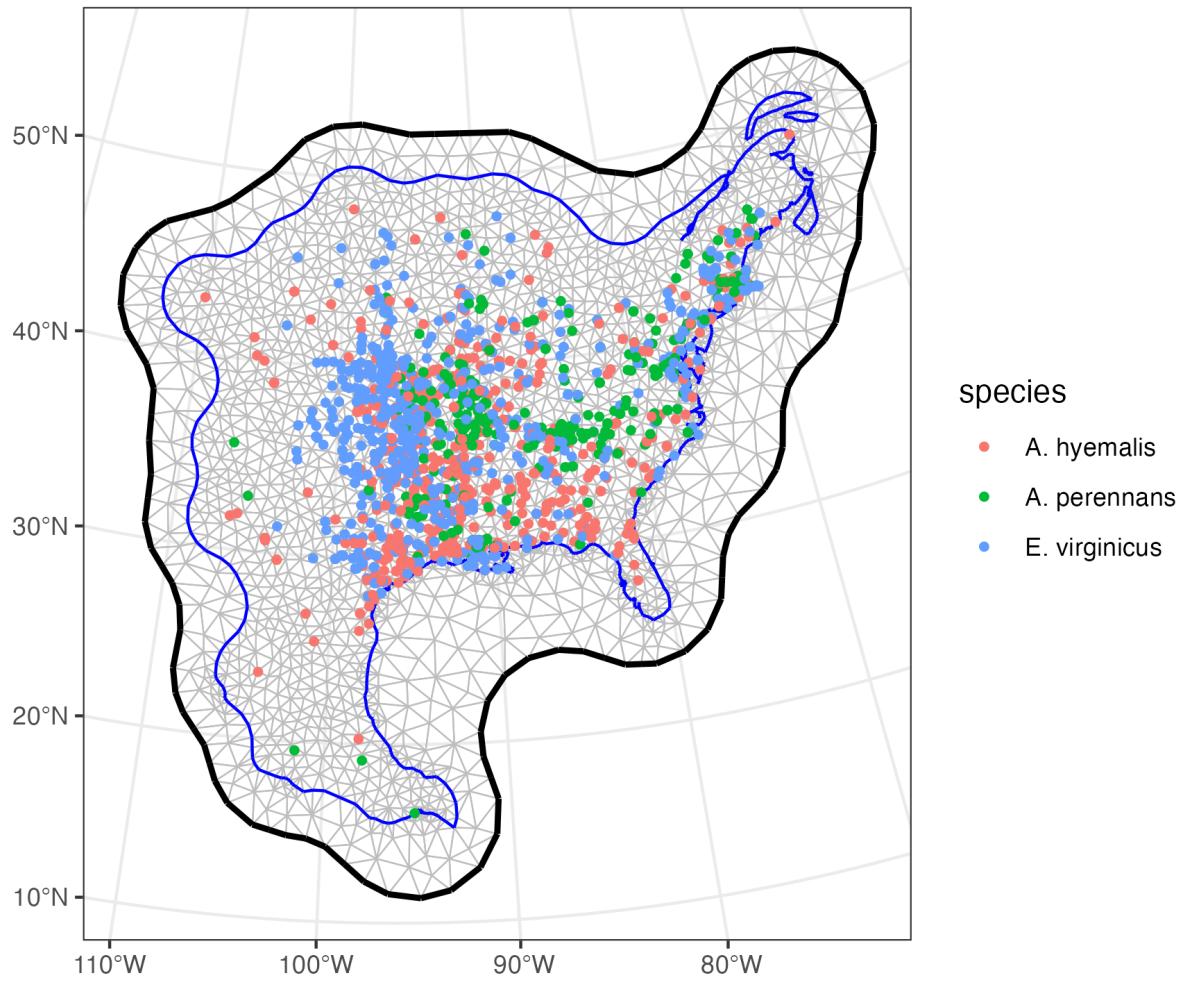
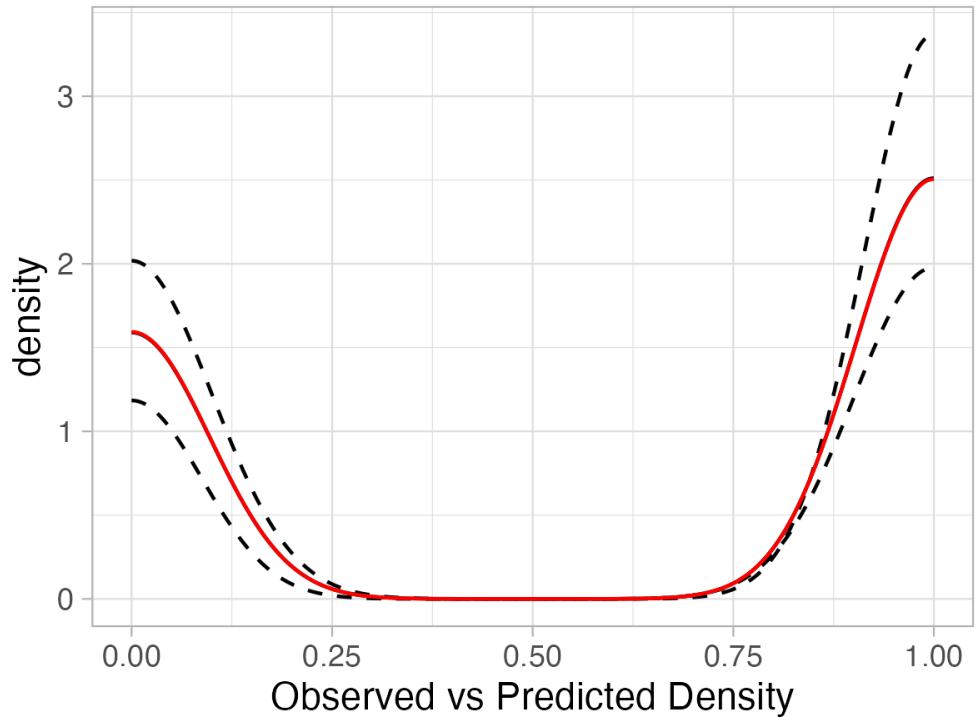
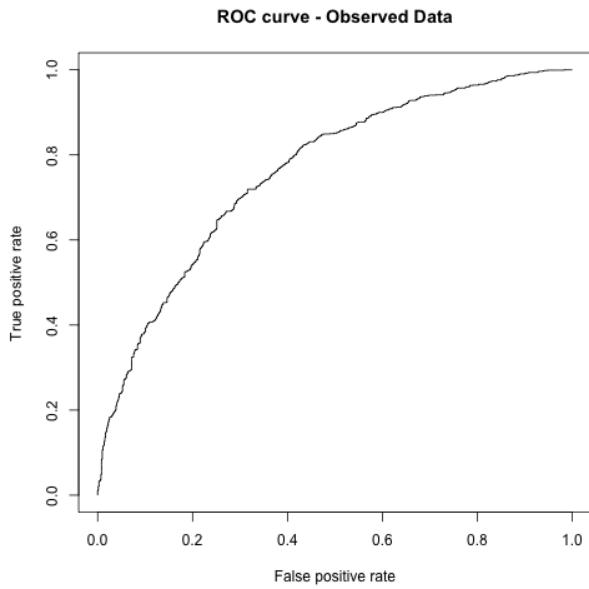


Figure A2: **Delauney triangulation mesh used to estimate spatial dependence between data points.** Grey lines indicate edges of triangles used to define distances between observations. Red points indicate locations of sampled herbarium specimens, and the blue outlines show the international borders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

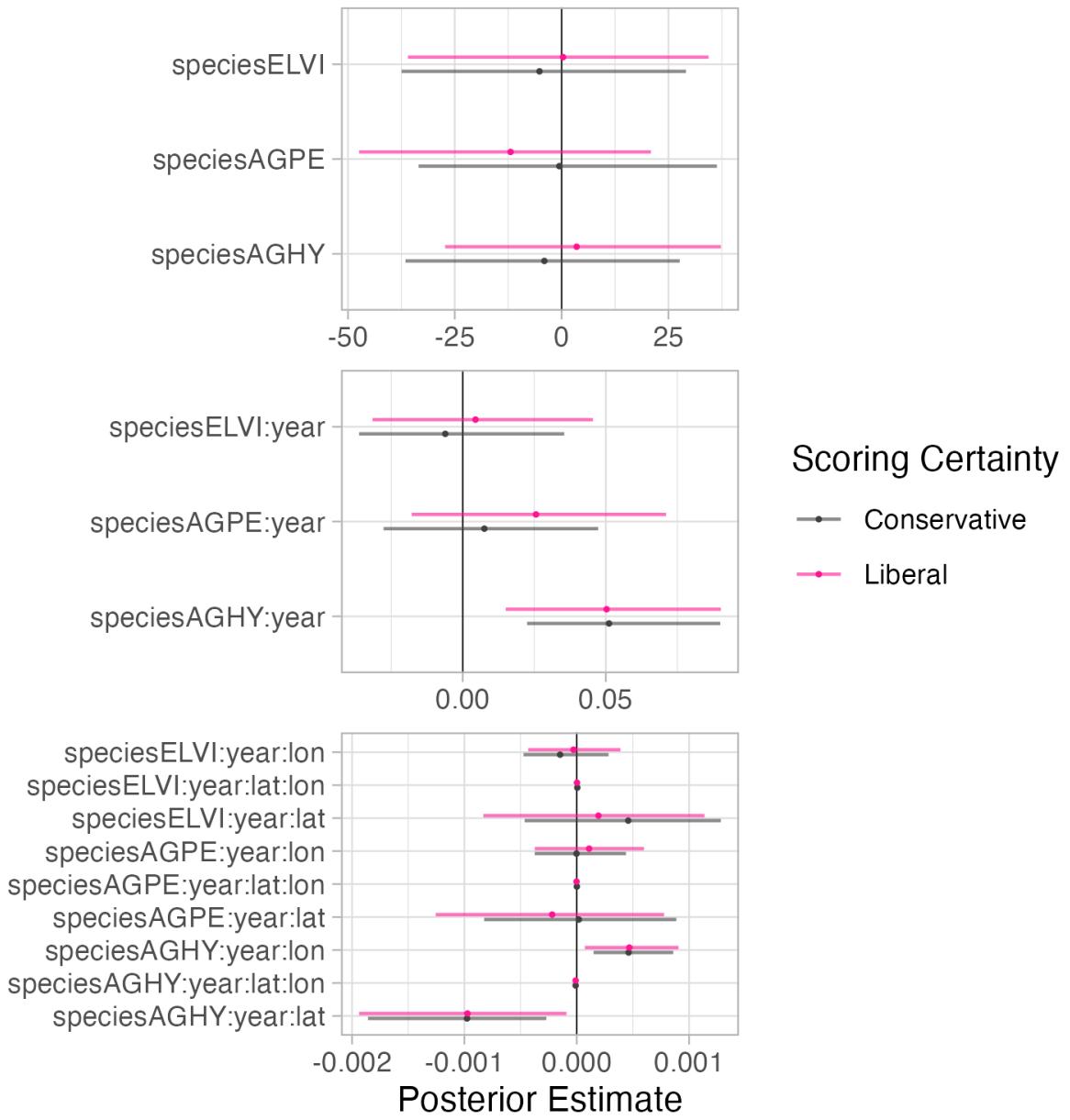


Figure A5: Comparison of posterior estimates of fixed effects when using Liberal or Conservative endophyte scores.

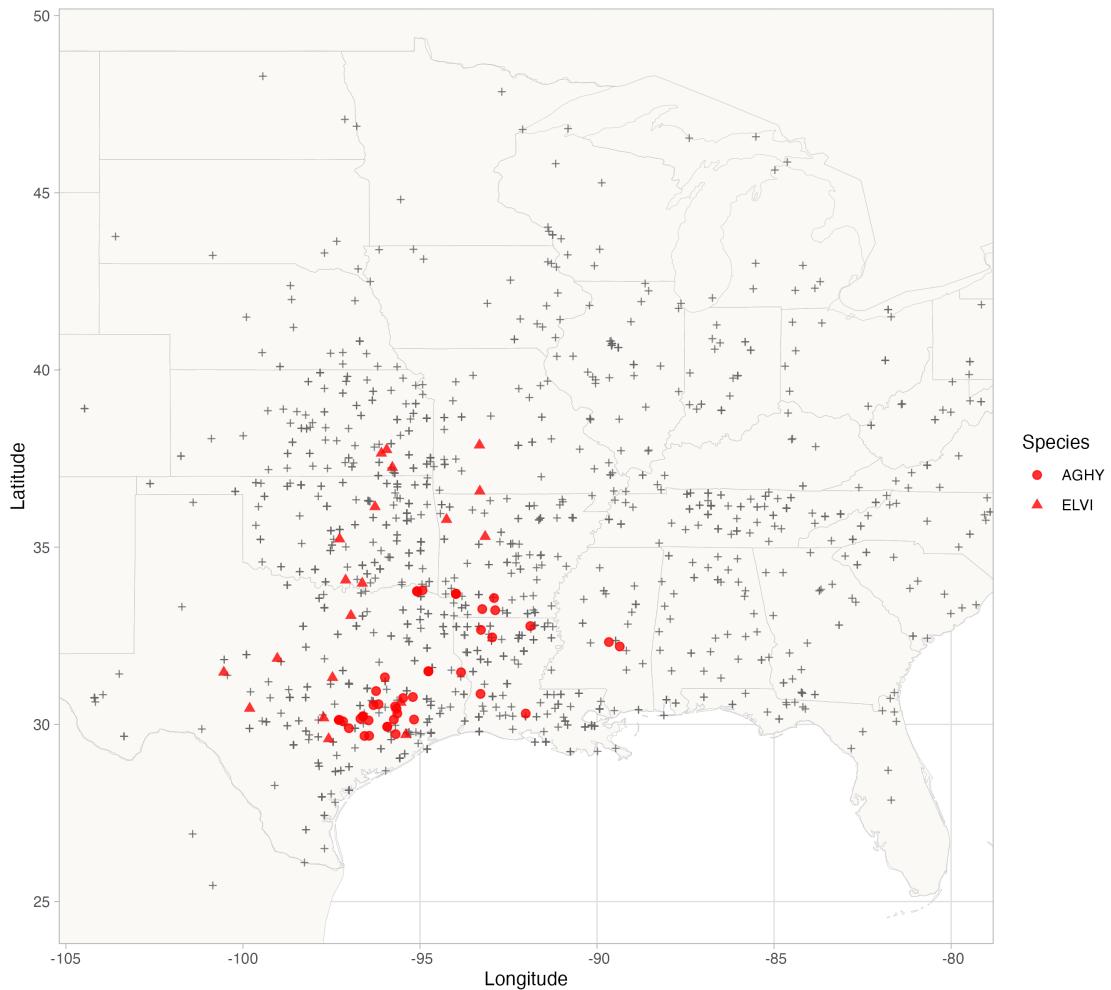
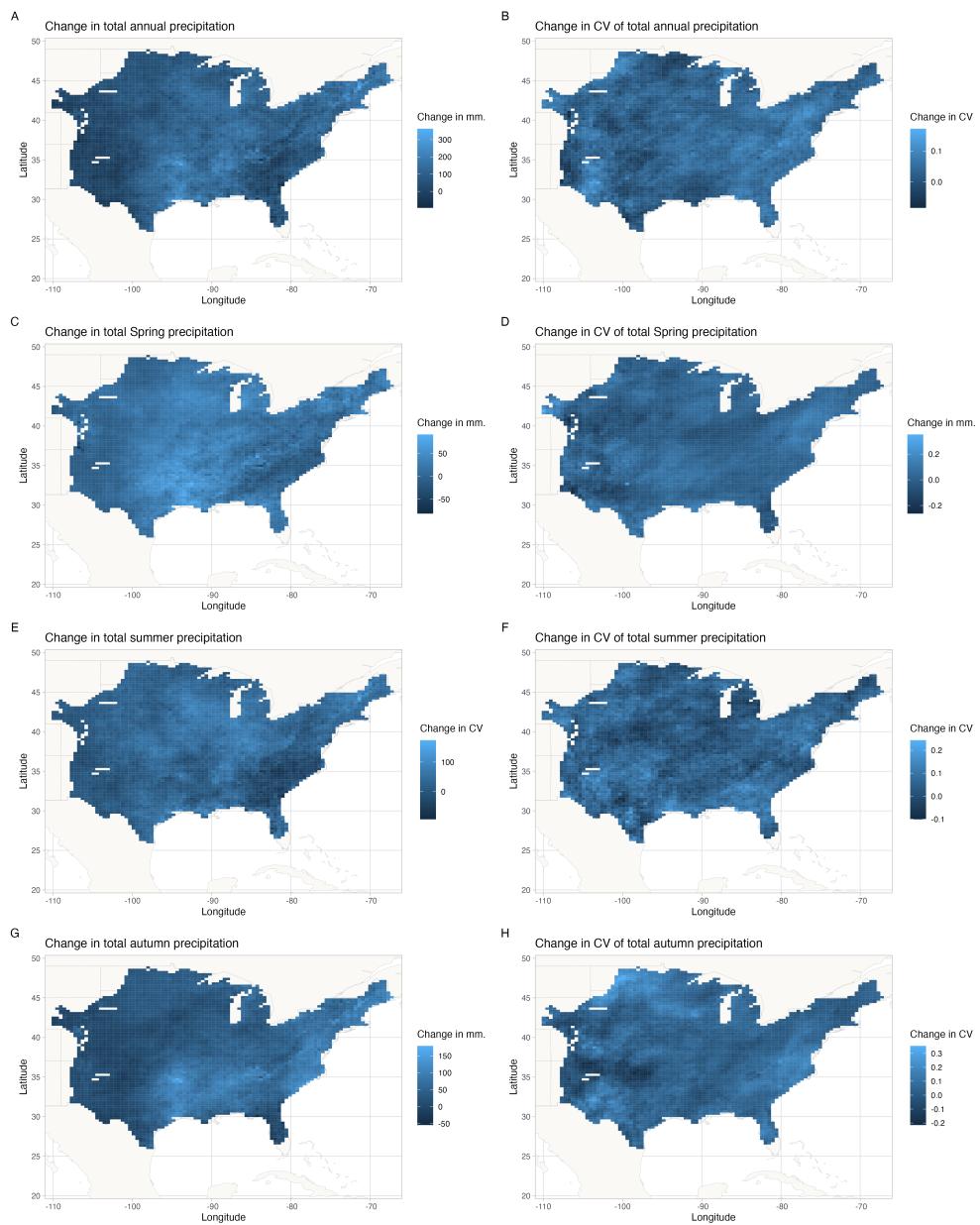
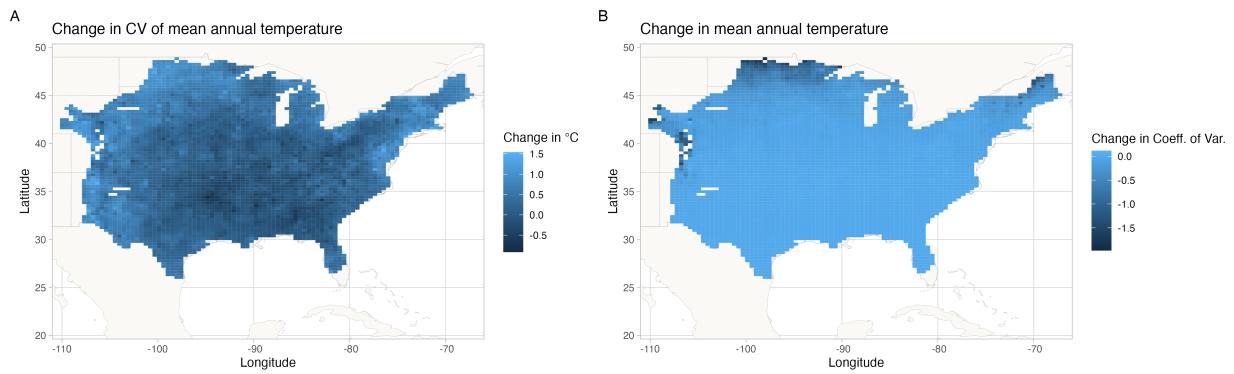


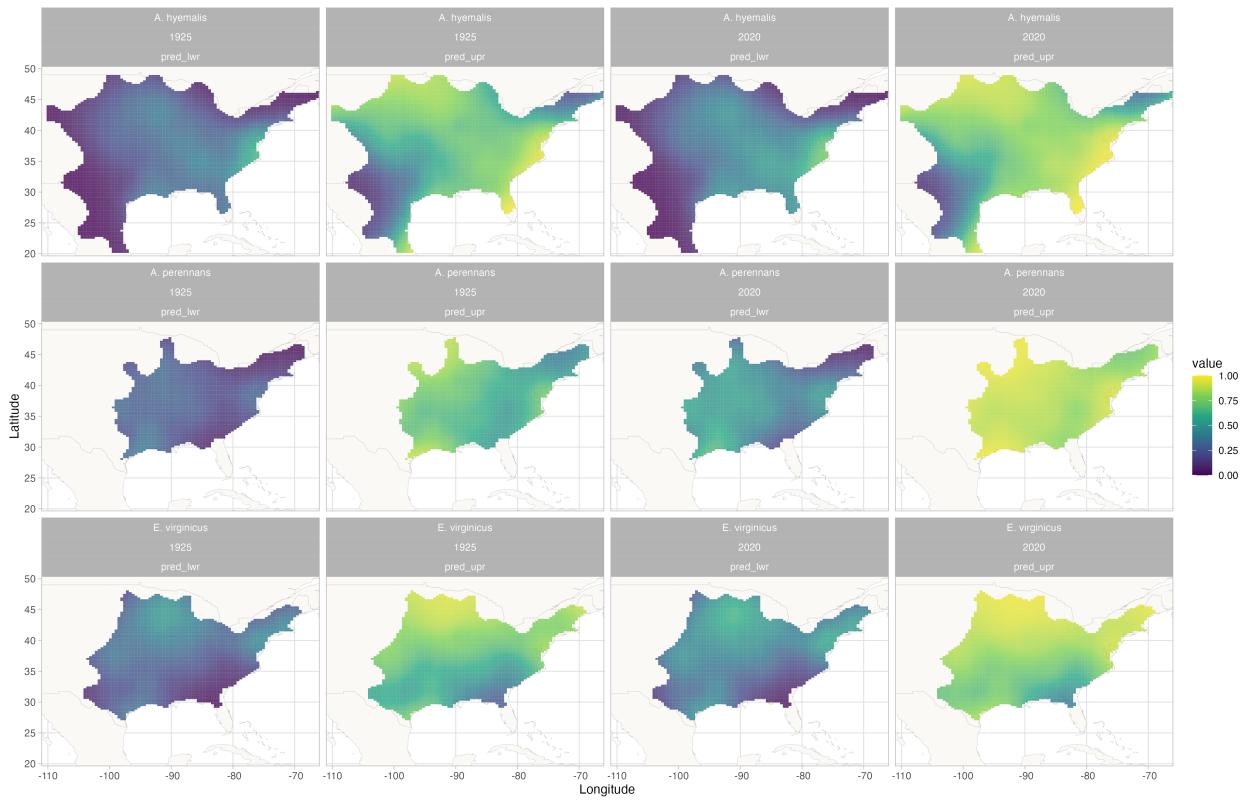
Figure A6: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).



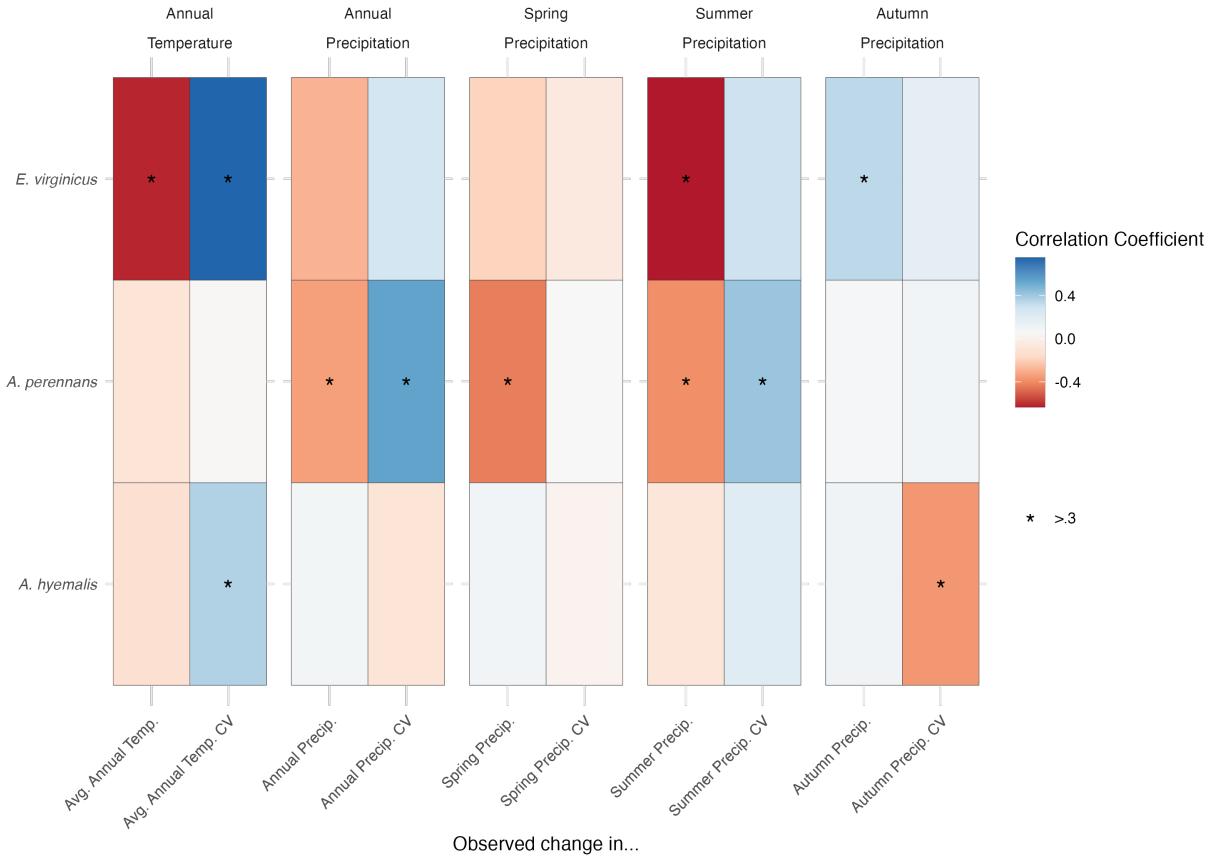
**Figure A7: Change in precipitation between the periods 1895-1925 and 1990-2020.** Color represents change in annual or seasonal total precipitation (A,C,E,G) and in the coefficient of variation of annual or seasonal total precipitation (B,D,F,H). Maps show the study area of *A. hyemalis*. Map pixels 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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