

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

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<sup>7</sup> *Manuscript elements:* Figure 1 - Figure 6, Appendix A (including Figure A1 - Figure A15, Table  
<sup>8</sup> A1, and Supporting Methods).

<sup>9</sup> *Keywords:* climate change, plant-microbe symbiosis, herbarium, museum specimen, INLA,  
<sup>10</sup> spatially-varying coefficients model, *Epichloë*, *Poaceae*.<sup>1</sup>

<sup>11</sup> *Manuscript type:* Research Article.

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<sup>1</sup> *alphabetic order ?*

## Abstract

13 Species' distributions and abundances are shifting in response to ongoing global climate change.  
 14 Mutualistic microbial symbionts can provide hosts with protection from environmental stress that  
 15 may promote resilience under environmental change, however this change may also disrupt species  
 16 interactions and lead to declines in hosts and/or symbionts. Symbionts preserved within natural  
 17 history specimens offer a unique opportunity to quantify changes in microbial symbiosis across broad  
 18 temporal and spatial scales. We asked how the prevalence of seed-transmitted fungal symbionts  
 19 of grasses (*Epichloë* endophytes) has changed over time in response to climate change, and how  
 20 these changes vary across host species' distributions. Specifically, we examined 2,346 herbarium  
 21 specimens of three grass host species (*Agrostis hyemalis*, *Agrostis perennans*, *Elymus virginicus*)  
 22 collected over the past two centuries (1824 – 2019) for the presence or absence of *Epichloë* symbiosis.  
 23 Analysis of an approximate Bayesian spatially-varying coefficients model<sup>23</sup> revealed that endophytes  
 24 increased in prevalence over the last two centuries from ca. 25% to ca. 75% prevalence, on average,  
 25 across three host species. Changes in seasonal climate drivers were associated with increasing  
 26 endophyte prevalence. Notably, increasing precipitation during the peak growing season for *Agrostis*  
 27 species and decreasing precipitation for *E. virginicus* were associated with increasing endophyte  
 28 prevalence. Changes in the variability of precipitation and temperature during off-peak seasons were  
 29 also important predictors of increasing endophyte prevalence. Our analysis performed favorably in  
 30 an out-of-sample predictive test with contemporary survey data<sup>4</sup>, a rare extra step in collections-  
 31 based research. We identified greater local-scale variability in endophyte prevalence in contemporary  
 32 data compared to model predictions based on historic data, suggesting new directions that could

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<sup>2</sup> This is the first time INLA is mentioned. It seems to be a suggestion from the reviewer. However, I think most readers may not be familiar with it. I appreciate the precision regarding the type of Bayesian statistics (approximate), but I wonder if that might be too technical for the abstract. I would understand if it were included in the methods section.

<sup>3</sup> I agree. I cut ' implemented in INLA '

<sup>4</sup> Since you drop numbers for the herbarium samples I would drop some numbers here too.

<sup>33</sup> improve predictive accuracy. Our results provide novel evidence for a cryptic biological response  
<sup>34</sup> to climate change that may contribute to the resilience of host-microbe symbiosis through **fitness**  
<sup>35</sup> **benefits to symbiotic hosts.**

<sup>36</sup> Abstract : 300 words

## Introduction

38 Understanding how biotic interactions are altered by global change is a major goal of basic and  
 39 applied ecological research (Blois et al., 2013; Gilman et al., 2010). Documented responses to envi-  
 40 ronmental change, such as shifts in species' distributions (Aitken et al., 2008) and phenology (Piao  
 41 et al., 2019), are typically blind to concurrent changes in associated biotic interactions. Empirically  
 42 evaluating these biotic changes – whether interacting species shift in tandem with their partners  
 43 or not (HilleRisLambers et al., 2013) – is crucial to predicting the reorganization of Earth's biodi-  
 44 versity under global change. Such evaluations have been limited because few datasets on species  
 45 interactions extend over sufficiently long time scales of contemporary climate change (Poisot et al.,  
 46 2021).

47 Natural history specimens, which were originally collected to study and preserve taxonomic di-  
 48 versity, present a unique opportunity to explore long-term changes in ecological interactions across  
 49 broad spatial and temporal scales (Meineke et al., 2018). Natural history collections, built and  
 50 maintained by the efforts of thousands of scientists, are invaluable time machines, primarily com-  
 51 prised of physical specimens of organisms along with information about the time and place of their  
 52 collection. These specimens often preserve physical legacies of ecological processes and species' in-  
 53 teractions from dynamically changing environments across time and space. For example, previous  
 54 researchers have used plant collections (herbaria) to document shifts in phenology *by examining re-*  
 55 *productive structures*<sup>5</sup> (Berg et al., 2019; Park et al., 2019; Willis et al., 2017), pollination *through*  
 56 *examination of pollen removal* (Duan et al., 2019; Pauw and Hawkins, 2011), and herbivory *by*  
 57 *documenting leaf damage* (Meineke et al., 2019) related to anthropogenic climate change. However,  
 58 few previous studies have leveraged biological collections to examine climate change-related shifts  
 59 in a particularly common type of interaction: microbial symbiosis.

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<sup>5</sup> *I don't really understand this revision or what prompted it. Phenology studies do not examine reproductive structures, they examine timing. I also don't think the sentence works grammatical with each list element having a 'by' clause.*

60 Microbial symbionts are common to all macroscopic organisms and can have important effects  
61 on their hosts' survival, growth and reproduction (McFall-Ngai et al., 2013; Rodriguez et al., 2009).  
62 Many microbial symbionts act as mutualists, engaging in reciprocally beneficial interactions with  
63 their hosts that can ameliorate environmental stress. For example, bacterial symbionts of insects,  
64 such as *Wolbachia*, can improve their hosts' thermal tolerance (Reno et al., 2019; Truitt et al., 2019),  
65 and arbuscular mycorrhizal fungi, documented in 70-90% of families of land plants (Parniske, 2008),  
66 allow their hosts to persist through drought conditions by improving water and nutrient uptake  
67 (Cheng et al., 2021). On the other hand, changes in the mean and variance of environmental  
68 conditions may disrupt microbial mutualisms by changing the costs and benefits of the interaction  
69 for each partner in ways that can cause the interaction to deteriorate (Aslan et al., 2013; Fowler et al.,  
70 2024). Coral bleaching (the loss of symbiotic algae) due to temperature stress (Sully et al., 2019)  
71 is perhaps the best known example, but this phenomenon is not unique to corals. Lichens exposed  
72 to elevated temperatures experienced loss of photosynthetic function along with changes in the  
73 composition of their algal symbiont community (Meyer et al., 2022). How commonly and under what  
74 conditions microbial mutualisms deteriorate or strengthen under climate change remain unanswered  
75 questions (Frederickson, 2017). Previous work suggests that these alternative responses may depend  
76 on the intimacy and specialization of the interaction as well as the physiological tolerances of the  
77 mutualist partners (Rafferty et al., 2015; Toby Kiers et al., 2010; Warren and Bradford, 2014).

78 Understanding of how microbial symbioses are affected by climate change is additionally com-  
79 plicated by spatial heterogeneity in the direction and magnitude of environmental change (IPCC,  
80 2021). Beneficial symbionts are likely able to shield their hosts from environmental stress in loca-  
81 tions that experience a small degree of change, but symbionts in locations that experience changes  
82 of large magnitude may be pushed beyond their physiological limits (Webster et al., 2008). Ad-  
83 ditionally, symbionts are often unevenly distributed across their host's distribution. Facultative  
84 symbionts may be absent from portions of the host range (Afkhami et al., 2014), and hosts may en-  
85 gage with a diversity of partners (different symbiont species or locally-adapted strains) across their  
86 environments (Fowler et al., 2023; Fraude et al., 2008; Rolshausen et al., 2018). Identifying broader

87 spatial trends in symbiont prevalence is therefore an important step in developing predictions for  
88 where to expect changes in the symbiosis in future climates.

89 *Epichloë* fungal endophytes are specialized symbionts of cool-season grasses, which have been  
90 documented in ~ 30% of cool-season grass species (Leuchtmann, 1992). They are transmitted  
91 predominantly vertically<sup>6</sup> from maternal plants to offspring through seeds. Vertical transmission  
92 creates a feedback between the fitness of host and symbiont (Douglas, 1998; Fine, 1975; Rudgers  
93 et al., 2009). Over time, endophytes that act as mutualists should rise in prevalence within a  
94 host population, particularly under environmental conditions that elicit protective benefits (Donald  
95 et al., 2021). *Epichloë* are known to improve their hosts' drought tolerance (Decunta et al., 2021) and  
96 protect their hosts against herbivores (Crawford et al., 2010) and pathogens (Xia et al., 2018) likely  
97 through the production of a diverse suite of alkaloids and other secondary metabolites. The fitness  
98 feedback induced by vertical transmission leads to the prediction that endophyte prevalence should  
99 be high in populations where these fitness benefits are most important. Previous survey studies  
100 of contemporary populations have documented large-scale spatial patterns in endophyte prevalence  
101 structured by environmental gradients (Afkhami, 2012; Bazely et al., 2007; Granath et al., 2007;  
102 Sneck et al., 2017). We predicted that prevalence should track temporal changes in environmental  
103 drivers (i.e. drought) that elicit strong fitness benefits.

104 Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic diversity  
105 of host species that harbor these symbionts (White and Cole, 1985), establishing that endophyte  
106 symbiosis could be identified in plant tissue from as early as 1851. However, no subsequent stud-  
107 ies, to our knowledge, have used the vast resources of biological collections to quantitatively assess  
108 spatio-temporal trends in endophyte prevalence and their environmental correlates. Grasses are  
109 commonly collected and identified based on the presence of their reproductive structures, mean-  
110 ing that preserved specimens typically contain seeds, conveniently preserving the fungi along with  
111 their host plants on herbarium sheets. This creates the opportunity to leverage the unique spatio-  
112 temporal sampling of herbarium collections to examine the response of this symbiosis to historical

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<sup>6</sup> *Maybe two adverbs is awkward but I think we need to acknowledge the possibility of H transmission.*

113 climate change. However, the predictive ability derived from historical analyses is rarely tested  
114 against contemporary data (Lee et al., 2024). Critically evaluating whether insights from historical  
115 reconstruction are predictive of variation across contemporary populations is a crucial step for the  
116 field to move from reading signatures of the past to forecasting ecological dynamics into the future.

117 In this study, we assessed the long-term responses of *Epichloë* endophyte symbiosis to climate  
118 change through the use of herbarium specimens of three North American host grass species (*Agrostis*  
119 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*). We first addressed questions describing  
120 spatial and temporal trends in endophyte prevalence: (i) How has endophyte prevalence changed  
121 over the past two centuries? and (ii) How spatially variable are temporal trends in endophyte  
122 prevalence across eastern North America? We then addressed how climate change may be driving  
123 trends in endophyte prevalence by asking: (iii) What is the relationship between temporal trends  
124 in endophyte prevalence and associated changes in climate drivers? We predicted that overall  
125 endophyte prevalence would increase over time in tandem with climate warming, and that localized  
126 hotspots of endophyte change would correspond spatially to hotspots of climate change. Finally,  
127 we evaluated (iv) how our model, built on data from historic specimens, performed in an out-of-  
128 sample test using data on endophyte prevalence from contemporary surveys of host populations.  
129 To answer these questions we examined a total of 2,346 historic specimens collected across eastern  
130 North America between 1824 and 2019, and evaluated model performance against contemporary  
131 surveys comprising 1,442 individuals from 63 populations surveyed between 2013 and 2020.

132

## Methods

133

### *Focal species*

134 Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis perennans*,  
135 and *Elymus virginicus* that host *Epichloë* symbionts. These cool-season grass species are commonly  
136 represented in natural history collections with broad distributions covering much the eastern United  
137 States (Fig. 1). Cool-season grasses grow during the cooler temperatures of spring and autumn

138 due to their reliance on  $C_3$  photosynthesis. *A. hyemalis* is a small short-lived perennial species that  
139 germinates in winter<sup>7</sup> and typically flowers between March and July (most common collection month:  
140 May). *A. perennans* is of similar stature but is longer lived than *Agrostis hyemalis* and flowers in  
141 late summer and early autumn (most common collection month: September). *A. perennans* is more  
142 sparsely distributed, tending to be found in shadier and moister habitats, while *A. hyemalis* is  
143 commonly found in open and recently disturbed habitats. Both *Agrostis* species are recorded from  
144 throughout the Eastern US, but *A. perennans* has a slightly more northern distribution, whereas  
145 *A. hyemalis* is found rarely as far north as Canada and is listed as a rare plant in Minnesota.  
146 *E. virginicus* is a larger and relatively longer-lived species that is more broadly distributed than  
147 the *Agrostis* species. It begins flowering as early as March or April but continues throughout the  
148 summer (most common collection month: July).

149 Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001; Leuchtmann et al., 2014),  
150 while *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl, 2002). The fungal sym-  
151 bionts primarily reproduce asexually and are passed from mother to offspring by vertical transmis-  
152 sion through seeds. These traits contribute to highly specialized interactions between symbiont and  
153 host. Some host species have been shown to partner with multiple symbiont species or strains, and  
154 in some cases multiple symbiont lineages can co-exist within a host population (Mc Cargo et al.,  
155 2014). However, surveys have typically found limited *Epichloë* genotypic diversity within host popu-  
156 lations (Treindl et al., 2023). Across host populations, concentrations of biologically-active alkaloids  
157 and the genes associated with their production vary substantially (Schardl et al., 2012). In this anal-  
158 ysis, we focus on the presence/absence of *Epichloë* symbionts, and we discuss potential implications  
159 of symbiont genotypic diversity in the Discussion.

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<sup>7</sup> Winter, not spring. It's 'winter bentgrass'.

## Herbarium surveys

161 We visited nine herbaria between 2019 and 2022 (see [Table<sup>8</sup>](#) A1 for a summary of specimens included  
 162 from each collection). With permission from herbarium staff, we acquired seed samples from 1135  
 163 *A. hyemalis* specimens collected between 1824 and 2019, 357 *A. perennans* specimens collected  
 164 between 1863 and 2017, and 854 *E. virginicus* specimens collected between 1839 and 2019 (Fig. 1,  
 165 Fig. 2A, Fig. A1). We chose our focal species in part because they are commonly represented in  
 166 herbarium collections and produce many seeds, meaning that small samples would not diminish the  
 167 value of the specimens for future studies. We collected 5-10 seeds per specimen after examining the  
 168 herbarium sheet under a dissecting microscope to ensure that we collected mature seeds, not florets  
 169 or unfilled seeds, fit for our purpose of identifying fungal endophytes with microscopy. We excluded  
 170 specimens for which information about the collection location and date were unavailable.

171 Each specimen was assigned geographic coordinates based on collection information recorded  
 172 on the herbarium sheet using the geocoding functionality of the ggmap R package (Kahle et al.,  
 173 2019). Many specimens had digitized collection information readily available, but for those that did  
 174 not, we transcribed information printed on the herbarium sheet. Collections were geo-referenced  
 175 to the nearest county centroid, or nearest municipality when that information was available. For  
 176 fifteen of the oldest specimens, only information at the state level was available, and so we used the  
 177 state centroid. The median pairwise distance between georeferenced coordinate points was 841 km.  
 178 The median longitudinal width of the bounding boxes generated to geocode municipality, county, or  
 179 state centroids was 44.7 km. Among those specimens geo-referenced at the state level, the largest  
 180 bounding box, spanning the state of Texas, was 1233 km wide. The smallest bounding boxes were  
 181 less than 1 km across for small municipalities (while this suggests high precision, we note that some  
 182 specimens were collected in natural habitat nearby to small municipalities not encompassed by this  
 183 bounding boxes).

184 Our visits focused on herbaria with historic strengths in *Poaceae* collections (e.g. Texas A&M,

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<sup>8</sup> *In table, maybe replace - with 0.*

185 Missouri Botanic Garden) and other herbaria in the Southern Great Plains region of the United  
186 States. While these nine herbaria garnered specimens that span the focal species' ranges, our dataset  
187 unevenly samples across the study region<sup>9</sup>. Texas, Oklahoma, Louisiana, and Missouri are the most  
188 represented states. Uneven sampling was most pronounced for *A. perennans*, which has much of its  
189 range in the northeastern US. We explore the potential influence of spatial bias in sampling on our  
190 results through a simulation analysis (Appendix A - Supporting Methods).

191 After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hyphae  
192 in each specimen using microscopy. We first softened seeds with a 10% NaOH solution, then stained  
193 the seeds with aniline blue-lactic acid stain and squashed them under a microscope cover slip. We  
194 examined the squashed seeds for the presence of fungal hyphae at 200-400X magnification (Bacon  
195 and White, 2018). On average we scored 4.7 intact seeds per specimen of *A. hyemalis*, 4.2 seeds  
196 per specimen of *A. perennans*, and 3.8 seeds per specimen of *E. virginicus*; we scored 10,342 seeds  
197 in total. Due to imperfect vertical transmission, the production of symbiont-free offspring from  
198 symbiotic hosts (Afkhami and Rudgers, 2008), it is possible that symbiotic host-plants produce a  
199 mixture of symbiotic and non-symbiotic seeds. We therefore designated a specimen as endophyte-  
200 symbiotic if *Epichloë* hyphae were observed in one or more of its seeds, or non-symbiotic if *Epichloë*  
201 hyphae were observed in none of its seeds. To capture uncertainty in the endophyte identification  
202 process, we recorded both a "liberal" and a "conservative" endophyte score for each plant specimen.  
203 When we confidently identified endophytes within a specimen's seeds, we assigned matching liberal  
204 and conservative scores. When we identified potential endophytes with unusual morphology, low  
205 uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a positive  
206 identification for the liberal score and a negative identification for the conservative score. The  
207 liberal status assumed a potential endophyte identification was more likely to be endophyte-positive  
208 while the conservative status assumed that the potential endophyte identification was less likely to  
209 be endophyte-positive. <sup>10</sup> 89% of scored plants had matching liberal and conservative scores,

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<sup>9</sup> Reference Figure 1 here?

<sup>10</sup> I don't think this sentence is necessary.

reflecting high confidence in endophyte status. The following analyses used the liberal status, however repeating all analyses with the conservative status yielded qualitatively similar results (Fig. A8).

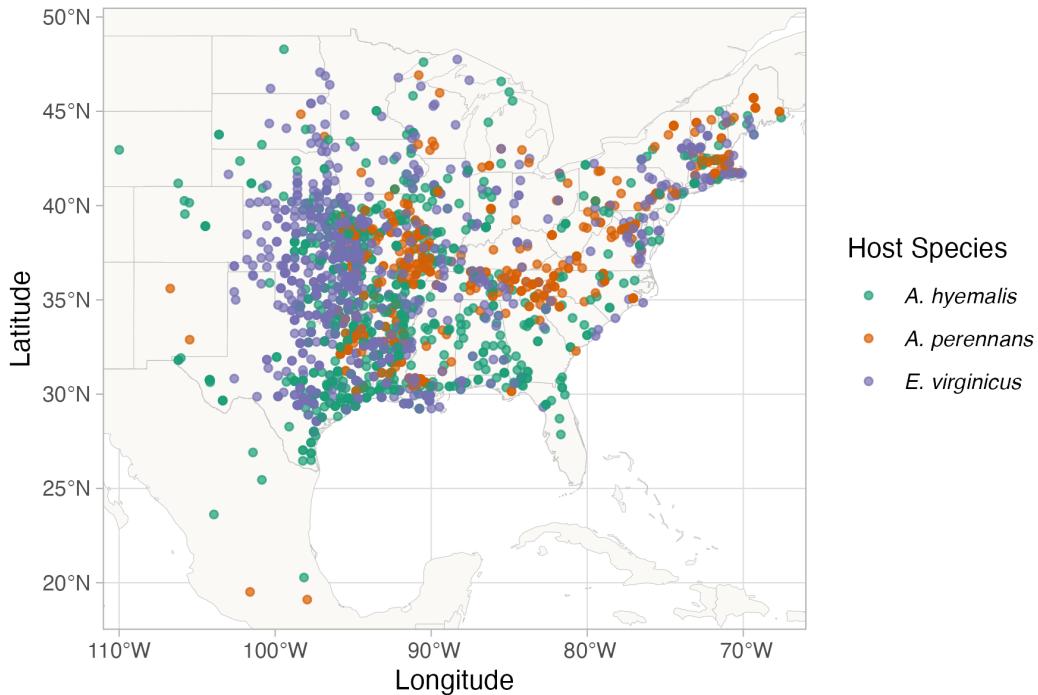


Figure 1: **Collection locations of herbarium specimens sampled for *Epichloë* endophytes.** Specimens span eastern North America from nine herbaria, and are colored by host species (*A. hyemalis*: green, *A. perennans*: orange, *E. virginicus*: purple). Map lines delineate study areas and do not necessarily depict accepted national boundaries.

### 213            *Modeling spatial and temporal changes in endophyte prevalence*

214    We assessed spatial and temporal changes in endophyte prevalence across each host distribution,  
215    quantifying the “global” temporal trends averaged across space, and then examining spatial hetero-  
216    geneity in the direction and magnitude of endophyte change (hotspots and coldspots) across the spa-  
217    tial extent of each host’s distribution. To account for the spatial non-independence of geo-referenced  
218    occurrences, we used an approximate Bayesian method, Integrated Nested Laplace Approximation

219 (INLA), to construct spatio-temporal models of endophyte prevalence. INLA provides a computa-  
 220 tionally efficient method of ascertaining parameter posterior distributions for certain models that  
 221 can be formulated as latent Gaussian Models (Rue et al., 2009). Many common statistical models,  
 222 including structured and unstructured mixed-effects models, can be represented as latent Gaussian  
 223 Models. We incorporated spatial heterogeneity into this analysis using spatially-structured intercept  
 224 and slope parameters implemented as stochastic partial differential equations (SPDE) to approxi-  
 225 mate a continuous spatial Gaussian process. This SPDE approach is a flexible method of smoothing  
 226 across space while explicitly accounting for spatial dependence between data-points (Bakka et al.,  
 227 2018; Lindgren et al., 2011). Fitting models with structured spatial effects is possible with MCMC  
 228 sampling but can require long computation times, making INLA an effective alternative. This ap-  
 229 proach has been used to model spatial patterns in flowering phenology (Willems et al., 2022), the  
 230 abundance of birds (Meehan et al., 2019) and butterflies (Crossley et al., 2022), the distribution of  
 231 temperate trees (Engel et al., 2022) as well as the population dynamics of endangered amphibians  
 232 (Knapp et al., 2016) and other ecological processes (Beguin et al., 2012).

233 We estimated global and spatially-varying trends in endophyte prevalence using a joint-likelihood  
 234 model. For each host species  $h$ , endophyte presence/absence of the  $i^{th}$  specimen ( $P_{h,i}$ ) was modeled  
 235 as a Bernoulli response variable with expected probability of endophyte occurrence  $\hat{P}_{h,i}$ . We modeled  
 236  $\hat{P}_{h,i}$  as a linear function of **intercept  $\mathbf{A}_h$**  and **slope  $\mathbf{T}_h$** <sup>11</sup> defining the global trend in endophyte  
 237 prevalence specific to each host species as well as with spatially-varying intercepts  $\alpha_{h,l_i}$  and slopes  
 238  $\tau_{h,l_i}$  associated with location ( $l_i$ , the unique latitude-longitude combination of the  $i$ th observation).  
 239 The joint-model structure allowed us to “borrow information” across species in the estimation of  
 240 shared variance terms for the spatially-dependent random effect  $\delta_{l_i}$ , intended to account for residual  
 241 spatial variation, and  $\chi_{c_i}$  and  $\omega_{s_i}$ , the i.i.d.-random effects indexed for each collector identity ( $c_i$ )  
 242 and scorer identity ( $s_i$ ) of the  $i^{th}$  specimen.

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<sup>11</sup> *Are these matrices? I am concerned that Eq 1 does not make mathematical sense if it's saying scalar = matrix + matrix\*scalar + scalar... etc.*

$$\text{logit}(\hat{P}_{h,i}) = \mathbf{A}_h + \mathbf{T}_h * \text{year}_i + \alpha_{h,l_i} + \tau_{h,l_i} * \text{year}_i + \delta_{l_i} + \chi_{c_i} + \omega_{s_i} \quad (1)$$

243 By including random effects for collectors and scorers, we accounted for “nuisance” variance that  
 244 may bias predictions for changes in endophyte prevalence. Previous work suggests that behavior of  
 245 historical botanists may introduce biases into ecological inferences made from historic collections  
 246 (Kozlov et al., 2020). Prolific collectors who contribute thousands of specimens may be more or  
 247 less likely to collect certain species, or specimens with certain traits (Daru et al., 2018). Similarly,  
 248 the process of scoring seeds for hyphae involved **multiple researchers (or "scorers")** who, even with  
 249 standardized training, may vary in their likelihood of positively identifying *Epichloë*.

250 We performed model fitting using the *inlabru* R package (Bachl et al., 2019). Global intercept  
 251 and slope parameters  $\mathbf{A}$ , and  $\mathbf{T}$ , were given vague priors. Collector and scorer random effects,  $\chi$   
 252 and  $\omega$  respectively, were centered at 0 with precision parameters were assigned **penalized complexity**  
 253 (**PC**) priors with parameter values  $U_{PC} = 1$  and  $a_{PC} = 0.01$  (Simpson et al., 2017). Each spatially-  
 254 structured parameter depended on a covariance matrix according to the proximity of each pair  
 255 of collection locations (Bakka et al., 2018; Lindgren et al., 2011). The covariance matrix was  
 256 approximated using a Matérn covariance function, with each data point assigned a location according  
 257 to the nodes of a mesh of non-overlapping triangles encompassing the study area (Fig. A2). We  
 258 assessed model fit with visual posterior predictive checks (A3) and measurements of AUC (Figs.  
 259 A4-A5) (Gelman and Hill, 2006). Priors for the Matérn covariance function, termed “range” and  
 260 “variance”, define how proximity effects decay with distance. Results presented in the main text  
 261 reflect a prior range of 342 kilometers (i.e. a 50% probability of estimating a range less than  
 262 342 kilometers). We tested a range of values (from 68 kilometers to 1714 kilometers) and meshes  
 263 (presented in the Supporting Methods), finding that while the magnitude and uncertainty of effects  
 264 varied, model results were qualitatively similar, i.e. the same direction of effects across space.  
 265 Through results and discussion that follow, we refer to the model described in this section as the  
 266 “endophyte prevalence model”.

267                   *Modeling distributions of host species*

268     The herbarium records did not encompass the entirety of each host species' range. Therefore, we  
269     used additional data sources to model the geographic distribution of each host species, with two  
270     goals: (1) generate realistic maps on which we could project the predictions of the INLA model,  
271     and (2) use the geographic distributions to test for relationships between climate change drivers and  
272     trends in endophyte prevalence. We followed the ODMAP (overview, data, model, assessment,  
273     prediction) protocol (Crossley et al., 2022) (see Supporting Methods). In short, we used presence-  
274     only observations of each host species from Global Biodiversity Information Facility (GBIF) between  
275     1990 to 2020.<sup>12</sup> We fit maximum entropy (MaxEnt) models using the maxent function in the R  
276     package dismo (Hijmans et al., 2017) using following seasonal climate predictors (1990-2020 climate  
277     normals): mean and standard deviation of spring, summer, and autumn temperature, and mean and  
278     standard deviation of spring, summer, and autumn cumulative precipitation. We generated 10,000  
279     pseudo-absences as background points, and split the occurrence data into 75% for model training and  
280     25% for model testing. The performance of models was evaluated with AUC (Jiménez-Valverde,  
281     2012). We found AUC values of 0.862, 0.838, 0.821 respectively for *Agrostis hyemalis*, *Agrostis*  
282     *perennans*, and *Elymus virginicus* indicating good model fit to data. To convert the continuous  
283     predicted probabilities into binary presence - absence maps on which we projected INLA predictions,  
284     we used the training sensitivity (true positive rate) and specificity threshold (true negative rate)  
285     (Liu et al., 2005).<sup>13</sup>

286                   *Assessing the role of climate drivers*

287     We assessed how the magnitude of climate change may have driven changes in endophyte prevalence  
288     by assessing correlations between changes in climate and changes in endophyte prevalence predicted  
289     from our spatial model at evenly spaced pixels across the study area. We first downloaded monthly

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<sup>12</sup> Maybe say how many records there were for each species?

<sup>13</sup> I don't really understand this sentence. How do the training sensitivity and specificity threshold lead to presence/absence maps?

temperature and precipitation rasters from the PRISM climate group (Daly and Bryant, 2013) covering the time period between 1895 and 2020 using the 'prism' R package (Hart and Bell, 2015). Prism provides reconstructions of historic climate variables across the United States by spatially interpolating weather station data (Di Luzio et al., 2008). We calculated 30-year climate normals for seasonal mean temperature and cumulative precipitation for the recent (1990 to 2020) and historic (1895 to 1925) periods. We used three four-month seasons within the year (Spring: January, February, March, April; Summer: May, June, July, August; Autumn: September, October, November, December). This division of seasons allowed us to quantify differences in climate associated with the two "cool" seasons, when we expected our focal species to be most active (*A. hyemalis* flowering phenology: spring; *E. virginicus*: spring and summer; *A. perennans*: autumn). In addition to mean climate conditions, environmental variability itself can influence population dynamics (Tuljapurkar, 1982) and changes in variability are a key prediction of climate change models (IPCC, 2021; Stocker et al., 2013). Therefore, we calculated the standard deviation for each annual and seasonal climate driver across each 30-year period. We then took the difference between recent and historic periods for the mean and standard deviation for each climate driver (Figs. A13-A15). All together, we assessed twelve potential climate drivers: the mean and standard deviation of spring, summer, and autumn temperature, as well as the mean and standard deviation of spring, summer, and autumn cumulative precipitation (the same climate covariates used in the MaxEnt models).

We then evaluated whether areas that have experienced the greatest changes in endophyte prevalence (hotspots of endophyte change) are associated with high degrees of change in climate (hotspots of climate change). To do so, we modeled the fitted, spatially-varying slopes of endophyte change through time ( $\tau_{[h]l}$ ) as a linear function of environmental covariates, with a Gaussian error distribution for a set of pixels across each host distribution. The continuous SPDE approach taken from our endophyte prevalence model allows us to generate predictions of temporal trends in prevalence at arbitrarily many pixels across each host distribution. Balancing computation time with resolution, we generated predicted trends for 546, 645, and 753 pixels across each host distribution for *A. perennans*, *A. hyemalis*, and *E. virginicus* respectively ( pixel dimensions: *A. perennans* = 65 km

317  $\times$  36 km; *A. hyemalis* = 61 km  $\times$  45 km; *E. virginicus* = 62 km  $\times$  40 km ). Fitting regressions to  
318 many pixels across the study region risks artificially inflating confidence in our results due to large  
319 sample sizes, and so we performed this analysis using only a random subsample of 250 pixels across  
320 the study region; other sizes of subsample yielded similar results. Data from each host species were  
321 analyzed separately. Throughout the results and discussion that follow, we refer to this analysis as  
322 the “*post hoc* climate regression analysis”.

323 *Validating model performance with in-sample and out-of-sample tests*

324 We evaluated the predictive ability of the endophyte prevalence model using both in-sample train-  
325 ing data from the herbarium surveys, and with out-of-sample test data, an important but rarely  
326 used strategy in ecological studies (Lee et al., 2024; Tredennick et al., 2021). We generated out-of-  
327 sample test data from contemporary surveys of endophyte prevalence in natural populations of *A.*  
328 *hyemalis* and *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted  
329 in 2013 as described in Sneck et al. (2017), and surveys of *A. hyemalis* took place between 2015 and  
330 2020. Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation in  
331 endophyte prevalence towards its range edge, while surveys of *E. virginicus* were designed to cover  
332 latitudinal variation. In total, we visited 43 populations of *A. hyemalis* and 20 populations of *E.*  
333 *virginicus* across the south-central US, with emphasis on Texas and neighboring states (Fig A12).  
334 During surveys, we collected seeds from up to 30 individuals per population (average number of  
335 plants sampled per population: 22.9); note that this sampling design provided greater local depth  
336 of information than the herbarium records, where only one plant was sampled at each locality. We  
337 quantified the endophyte status of each individual with microscopy as described for the herbarium  
338 surveys (with 5-10 seeds scored per individual), and calculated the prevalence of endophytes within  
339 the population (proportion of plants that were endophyte-symbiotic). For each population, we com-  
340 pared the observed fraction of endophyte-symbiotic hosts to the predicted probability of endophyte  
341 occurrence  $\hat{P}$  derived from the model for that location and year. The contemporary survey period  
342 (2013-2020) is at the most recent edge of the time period encompassed by the historical specimens

<sup>343</sup> used for model fitting.

<sup>344</sup>

## Results

<sup>345</sup> *How has endophyte prevalence changed over time?*

<sup>346</sup> Across more than 2300 herbarium specimens dating back to 1824, we found that prevalence of  
<sup>347</sup> *Epichloë* endophytes increased over the last two centuries for all three grass host species (Fig. 2).  
<sup>348</sup> On average, endophytes of *A. perennans* and *E. virginicus* increased from ~ 40 % to 70% prevalence  
<sup>349</sup> across the study region, and *A. hyemalis* increased from ~ 25% to over 50% prevalence. Our model  
<sup>350</sup> indicates high **confidence** that overall temporal trends are positive across species (99% probability  
<sup>351</sup> of a positive overall year slope in *A. hyemalis*, 92% probability of a positive overall year slope in *A.*  
<sup>352</sup> *perennans*, and 91% probability of a positive overall year slope in *E. virginicus*) (Fig. A6).

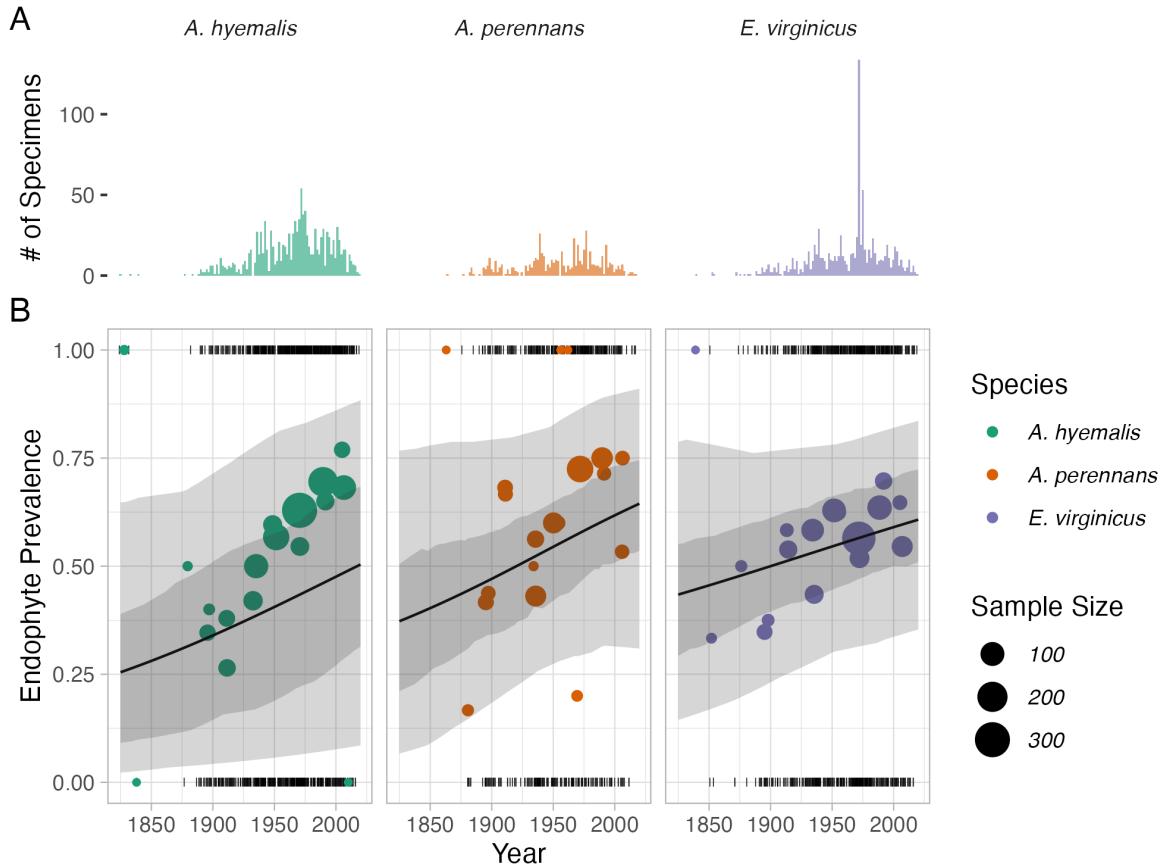


Figure 2: **Temporal trends in endophyte prevalence.** (A) Histograms show the frequency of scored specimens through time for each host species. (B) Lines show mean endophyte prevalence predicted by the endophyte prevalence model over the study period along with the 50% and 95% CI bands incorporating parameter uncertainty and variation associated with collector and scorer random effects. Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species (*A. hyemalis*: green, *A. perennans*: orange, *E. virginicus*: purple) and point size represents the number of specimens.

353 The model appears to under-predict the observed increase in endophyte prevalence relative to  
 354 the data, particularly for *A. hyemalis* (Fig. 2B), but the model is accounting for random effects  
 355 and spatial non-independence that are not readily seen in the figure. We found no evidence that  
 356 collector biases influenced our results. Collector random effects were consistently small (Fig. A9),

357 and models fit with and without this random effect provide qualitatively similar results. The identity  
358 of individual scorers, the researchers who identified endophyte status microscopically, did contribute  
359 to observed patterns in endophyte prevalence. For example, 3 of the 25 scorers were significantly  
360 more likely than average to assign positive endophyte status, as indicated by 95% credible intervals  
361 greater than zero (Fig. A10).<sup>14</sup>

362 *How spatially variable are temporal trends in endophyte prevalence?*

363 While there was an overall increase in endophyte prevalence, our model revealed hotspots and  
364 coldspots of change across the host species' ranges, which are mapped in Fig. 3 across geographic  
365 ranges predicted by MaxEnt species distribution models. In some regions, posterior mean esti-  
366 mates of spatially varying temporal trends indicate that *A. hyemalis* and *A. perennans* experienced  
367 increases in prevalence by as much as 2% per year over the study period, while *E. virginicus* ex-  
368 perienced increases up to around 1% per year. Both *Agrostis* species show areas of strong increase  
369 and areas of declining prevalence, while *E. virginicus* had an overall weaker and geographically  
370 more homogeneous increase in endophyte prevalence. Notably, endophytes are predicted to have  
371 increased most strongly towards the western range edge of *A. hyemalis* (Fig. 3A) and across the  
372 northeastern US for *A. perennans* (Fig. 3B). Broad increases in prevalence on average, along with  
373 increases towards range edges that had low historic prevalence result in range expansions of the  
374 symbiosis for both *Agrostis* species (Fig. 4). Increases in prevalence were strongest in regions with  
375 low historic prevalence for the *Agrostis* species (Fig. A11 A-B), but for *E. virginicus* trends did  
376 not differ according to historic prevalence (A11 C). Posterior estimates of uncertainty in spatially  
377 varying slopes indicate that these hotspots of change may have experienced increases of up to 5%  
378 per year while declines in prevalence may be as great as 4% per year<sup>15</sup> for *A. hyemalis* and *A.*  
379 *perennans*. For *E. virginicus*, uncertainty ranges between 3.5% increases and 2.5% decreases (Fig.  
380 A7).

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<sup>14</sup> And I count four that have significantly negative CIs.

<sup>15</sup> Should this be -4%?

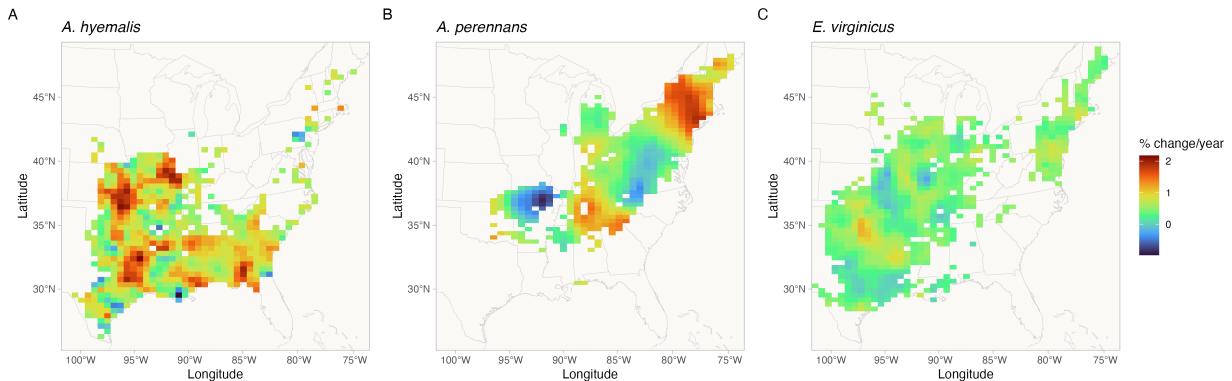


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species (A, *A. hyemalis*; B, *A. perennans*; C, *E. virginicus*). Spatially-varying trends are estimated from the endophyte prevalence model. Color indicates the relative change in predicted endophyte prevalence. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

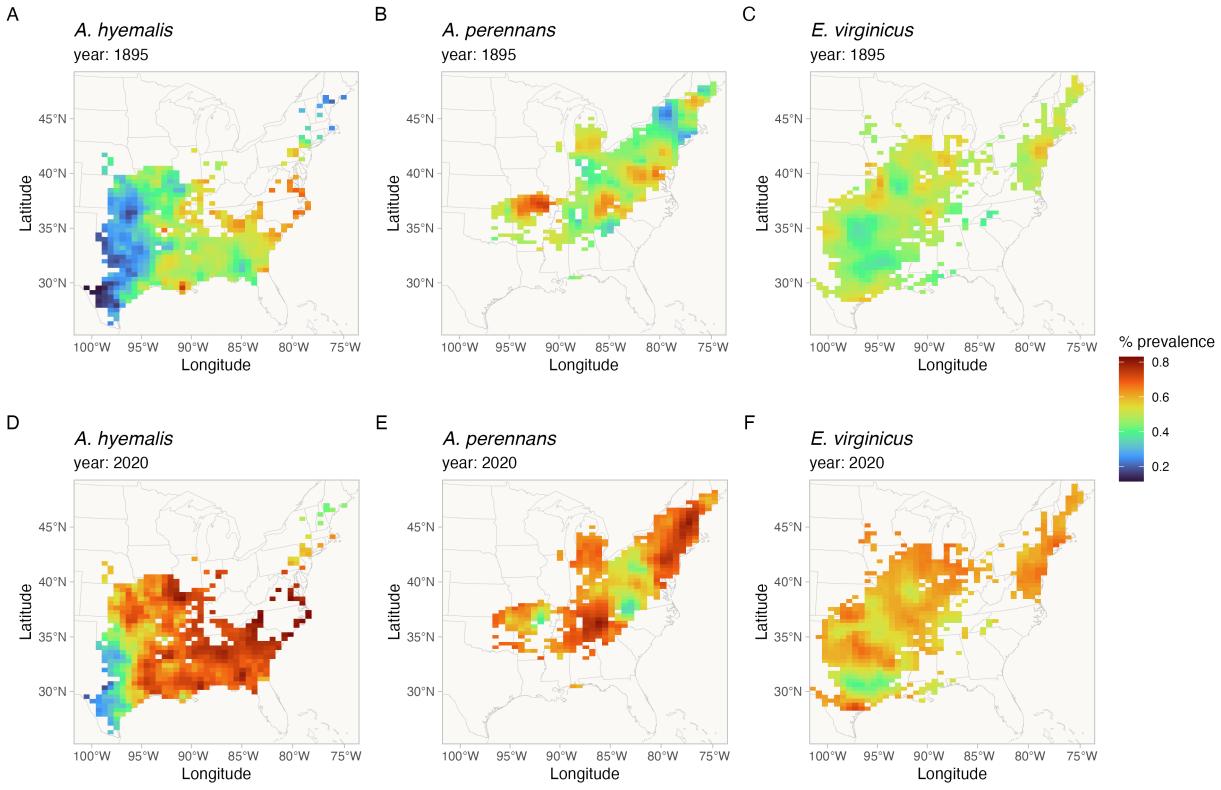


Figure 4: Predicted endophyte prevalence for each host species in 1895 and 2020. Predictions of prevalence come from the endophyte prevalence model. Color indicates the posterior mean endophyte prevalence for *A. hyemalis* (A,D), *A. perennans* (B,E), and *E. virginicus* (C,F). Map lines delineate study areas and do not necessarily depict accepted national boundaries.

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

383     We found that trends in endophyte prevalence were strongly associated with one or more seasonal  
 384        climate change drivers (Fig. 5). For the majority of the study region, the climate has become  
 385        wetter (an average increase in annual precipitation of 60 mm) with relatively little temperature  
 386        warming (an average increase in annual temperature of 0.02 °C) over the last century (Fig. A13-  
 387        A15), a consequence of regional variation in global climate change (IPCC, 2021). Within the

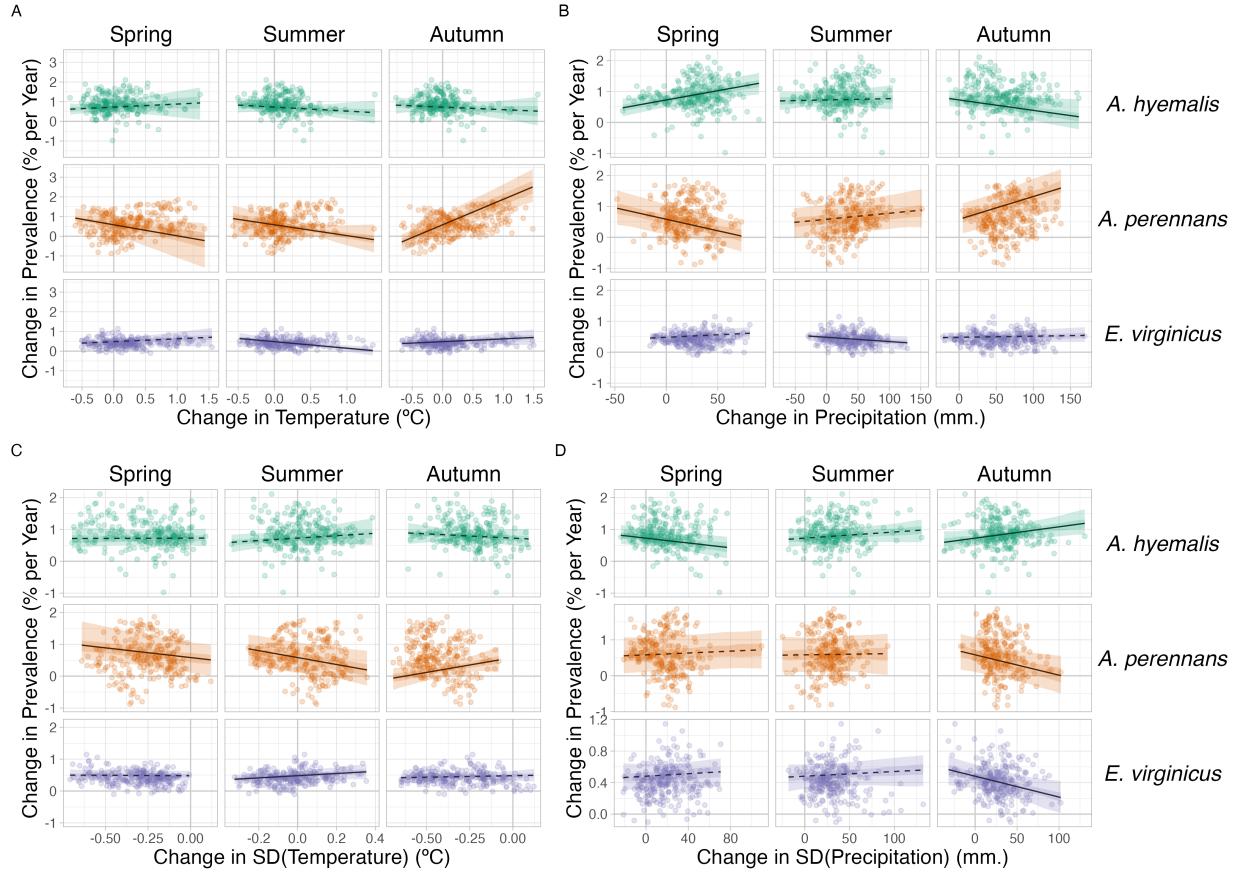
region, climate changes were spatially variable; certain locations experienced increases in annual precipitation as large as 375 mm or decreases up to 54 mm across the last century, while annual temperature changes ranged from warming as great as 1.4 °C to cooling by 0.46 °C. Spatially variable climate trends were predictive of trends in endophyte prevalence. For example, strong increases in endophyte prevalence for *A. perennans* were most strongly associated with increasing autumn precipitation and with increasing mean and variability in autumn temperature (greater than 97% posterior probabilities of positive slopes). For this species, a 1 °C increase in autumn temperature was associated with a 1.07 % increase per year in endophyte prevalence (Fig. 5A) and a 100 mm increase in precipitation was associated with a 0.8% increase per year in endophyte prevalence (Fig. 5B). This result aligns with the species' autumn active growing season, however other seasonal climate drivers were also associated with increasing endophyte prevalence in this host species. In particular, we found cooler and drier springs and cooler summers to be associated with increasing endophyte prevalence (greater than 99% posterior probabilities of negative slopes), though these slopes were generally of smaller magnitude than those for autumn climate drivers.

Changes in endophyte prevalence across the ranges of *A. hyemalis* and *E. virginicus* were less strongly driven by changes in climate. Like *A. perennans*, climate during peak growing season (spring for *A. perennans* and summer for *E. virginicus*) emerged most commonly as drivers of changes in endophyte prevalence. Increases in mean spring precipitation were the strongest predictor of increasing trends in endophyte prevalence for *A. hyemalis* (Fig. 5B) (greater than 99% posterior probability of a positive slope). For this species, an increase of 100 mm in spring precipitation led to an increase of 0.6% per year in endophyte prevalence. The next greatest slopes were those associated with variability in spring precipitation (greater than 96% posterior probability of a negative slope), as well as in the mean and variability of autumn climate (greater than 98% probability of negative and positive slopes, respectively). Changes in endophyte prevalence in *E. virginicus* were not strongly associated with changes in most climate drivers, but regions with reduced variability in autumn precipitation (Fig. 5B) and with cooler and more variable summer temperatures (Fig. 5A,C) experienced the largest increases in endophyte prevalence. Our analy-

415 sis indicated relatively high confidence that these climate drivers influence endophyte prevalence  
416 shifts in *E. virginicus* (greater than 99% posterior probability of either negative or positive slopes  
417 respectively), however they translate to less than 0.2% change in endophyte prevalence per year for  
418 a change of 100 mm change in precipitation over the century.<sup>16</sup> Repeating this analysis using all  
419 pixels across each species' distribution were qualitatively similar to these results.

---

<sup>16</sup> *I am not sure why you are highlighting the slope with respect to mm of precip, since the preceding sentences emphasize temperature and var(precip).*



**Figure 5: Relationships between predicted trends in endophyte prevalence and changes in seasonal climate drivers.** Lines show marginal predicted relationship between spatially-varying trends in endophyte prevalence and changes in mean and variability of climate ((A): mean temperature, (B): cumulative precipitation, (C): standard deviation in temperature, (D): standard deviation in precipitation) estimated from the *post hoc* climate regression analysis. Confidence bands represent the 50 and 95% CI, colored by host species (*A. hyemalis*: green, *A. perennans*: orange, *E. virginicus*: purple). Slopes with greater than 95% probability of being either positive or negative are represented as solid lines while those that have less than 95% probability are dashed. Points are the values of pre-computed SVC trends and climate drivers at 250 randomly sampled pixels across each host's distribution used in model fitting for the *post hoc* climate regression analysis.

420

## Evaluation of model performance on an out-of-sample test

421 Tests of models<sup>17</sup> predictive performance as quantified by AUC and by visual posterior predictive  
422 checks, indicated good predictive ability. Model performance was similar between historic herbarium  
423 specimens used as training data and the out-of-sample test data from contemporary surveys (AUC  
424 = 0.79 and 0.77 respectively; Fig. A5-A4). The model successfully captured broad regional trends in  
425 endophyte prevalence seen in the contemporary survey data, such as decline endophyte prevalence  
426 in *A. hyemalis* towards western longitudes (Fig. 6A) and northern latitudes<sup>18</sup> (Fig. 6B). It is  
427 notable that model predictions for endophyte prevalence exhibited relatively little local geographic  
428 variation, whereas the out-of-sample survey data were highly variable with populations spanning  
429 0% to 100% endophyte-symbiotic plants (Fig. 6C), indicating that...<sup>19</sup>.

<sup>17</sup> Which models are you referring to? Is this plural because each species had a different model?

<sup>18</sup> It's actually an increase toward N latitudes, not decline.

<sup>19</sup> I would finish this train of thought, even as you leave most of it for the discussion.

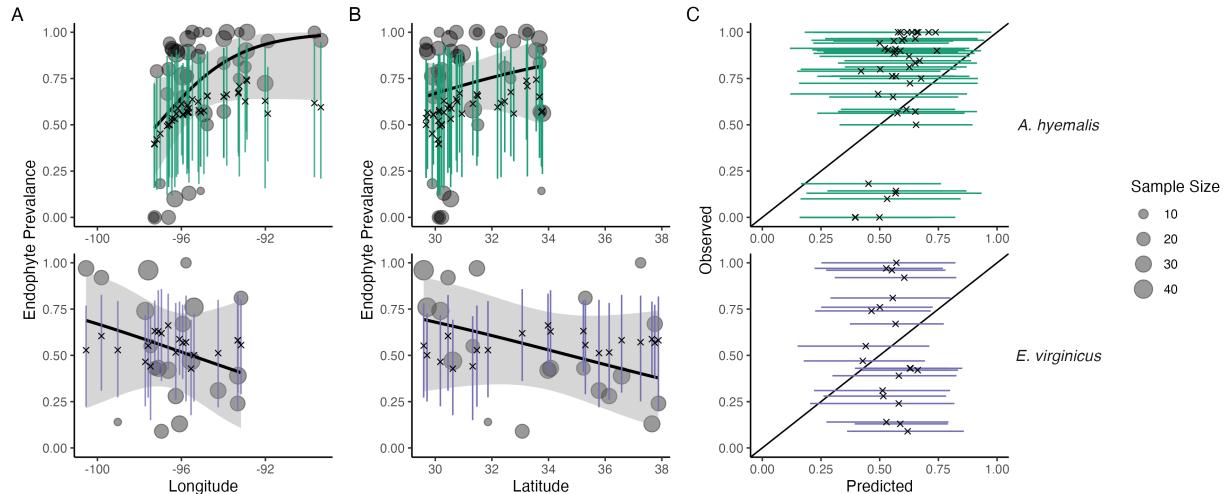


Figure 6: **Predictive performance for contemporary test data.** (A) The endophyte prevalence model, trained on historic herbarium collection data, performed modestly at predicting prevalence in contemporary population surveys. The model captured regional trends across (A) longitude and (B) latitude. Crosses indicate predicted mean prevalence along with the 95% CI (colored lines: *A. hyemalis*: green, orange, *E. virginicus*: purple) from the herbarium model. Contemporary prevalence is represented by grey points (point size reflects sample size) along with trend lines from generalized linear models (black line and shaded 95% confidence interval). (C) Comparison of observed vs. predicted endophyte prevalence shows that contemporary test data had more variance between populations than contemporary predictions.

## Discussion

430

431 Our examination of historic plant specimens revealed previously hidden shifts in microbial symbiosis  
 432 over the last two centuries. For the three grass host species we examined, there have been strong  
 433 increases in prevalence of *Epichloë* endophyte symbiosis. We interpret increases in prevalence of  
 434 *Epichloë*, which are vertically transmitted, as adaptive changes that improve the fitness of their  
 435 hosts under increasing environmental stress. This interpretation is in line with theory predicting  
 436 that positive fitness feedback caused by vertical transmission leads beneficial symbionts to rise in

437 prevalence within a population (Donald et al., 2021; Fine, 1975). We further found that trends  
438 in endophyte prevalence varied across the distribution of each species in association with changes  
439 in climate drivers, suggesting that the increases in endophyte prevalence are driven by context-  
440 dependent benefits to hosts that confer resilience under environmental change. Taken together, this  
441 suggests an overall strengthening of host-symbiont mutualism over the last two centuries.

442 *Responses of host-microbe symbioses to climate change*

443 Differences across host species underscore that while all of these  $C_3$  grasses share similar broad-scale  
444 distributions, each engages in unique biotic interactions and has unique responses to environmental  
445 drivers. We identified hotspots of change for *A. perennans*, which was the species that experienced  
446 the strongest absolute changes in endophyte prevalence (Fig. 3).<sup>20</sup> Predicted declines of 0.9% per  
447 year in the southern portion of its range and predicted increases of up to 2% per year in the north  
448 suggest a potential poleward range shift of endophyte-symbiotic plants; whether the overall host  
449 distribution is shifting in parallel is an exciting next question.

450 Based on previous work demonstrating that endophytes can shield their hosts from drought  
451 stress (reviewed in Decunta et al. (2021)), we generally predicted that drought conditions would be  
452 a driver of increasing endophyte prevalence. In contrast to this expectation, increasing prevalence  
453 for *A. perennans* was associated with increasing autumn temperature and precipitation (Fig. 5).  
454 To our knowledge, the response of the symbiosis in *A. perennans* to drought has not been examined  
455 experimentally, but in a greenhouse experiment, endophytes had a positive effect on host repro-  
456 duction under shaded, low-light conditions (Davitt et al., 2010). Our results also hint that it may  
457 be useful to investigate whether lagged climate effects are important predictors of host fitness in  
458 this system (Evers et al., 2021). Endophyte prevalence of the autumn-flowering *A. perennans* was  
459 strongly linked with decreasing spring precipitation, and that of the spring-flowering *A. hyemalis*  
460 was associated with decreasing autumn precipitation (Fig. 5B). For *A. hyemalis*, endophytes could  
461 be playing a role helping hosts weather autumn-season droughts, which is likely also an important

<sup>20</sup> Not sure what this is based on. In Fig 2 both *Agrostis* species look like they have similar increases.

time for the species' germination. Previous work demonstrated drought benefits in a greenhouse manipulation with this host-symbiont pair (Davitt et al., 2011), and early life stages may be particularly vulnerable to prolonged droughts. For *E. virginicus*, which experienced the weakest changes in endophyte prevalence overall (ranging between 1.1% increases and 0.2% decreases), we only found modest associations with changes in climate drivers. 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 contemporary endophyte prevalence in this species. The diverse relationships we detect between trends in endophyte prevalence and climate drivers suggest a more complicated picture than the simple explanation that drought alone, as measured through changes in annual precipitation, causes increasing endophyte prevalence through context-dependent fitness benefits.

While we show consistent increasing trends in prevalence between the three species, the mechanisms that explain these changes may be diverse and idiosyncratic. First, climate change responses may depend on genotype-specific responses that are not considered in our current analysis. While *Epichloë* symbioses are highly specialized, surveys have demonstrated genotypic and chemotypic diversity of the symbionts among and within populations (Treindl et al., 2023; von Cräutlein et al., 2021). Genotypic variation in *Epichloë* endophytes, particularly in genes responsible for alkaloid production, produces "chemotypes" with differing benefits for hosts against insect or mammalian herbivores mediated by environmental conditions (Saikkonen et al., 2013; Schardl et al., 2012). Genotypic variation of the hosts themselves can also influence interaction outcomes (Gundel et al., 2011; Parker et al., 2017). Whether hotspots of change in endophyte prevalence reflect selection for genotype-pairings with particularly strong fitness benefits is an unanswered question. Additionally, *Epichloë* endophytes have been connected to a suite of non-drought related fitness benefits including herbivory defense (Brem and Leuchtmann, 2001), salinity resistance (Wang et al., 2020), and mediation of pathogens (Vikuk et al., 2019) and the soil microbiome (Roberts and Ferraro, 2015). Broad changes in the distribution and abundance of natural enemies, along with stresses from anthropogenic changes in landcover and pollution (Sage, 2020) likely influence the benefits of

489 symbiosis (Rudgers et al., 2020). The historic trends that we observed result from the combination  
490 of these fitness benefits playing out across the heterogeneous map of a changing climate and other  
491 anthropogenic drivers.

492 Our results indicate that *Epichloë* symbiosis has likely improved host fitness in stressful envi-  
493 ronments leading to increasing prevalence. What is less clear is how this will influence future range  
494 shifts. Based on our analysis, it is likely that the symbiosis will facilitate range shifts for hosts by  
495 improving population growth at range edges. Previous population surveys (Rudgers and Swafford,  
496 2009; Semmarin et al., 2015; Sneck et al., 2017) attributed environment-dependent gradients in  
497 endophyte prevalence to symbiont-derived fitness benefit,s allowing hosts to persist in environments  
498 where they otherwise could not (Afkhami et al., 2014; Kazenel et al., 2015). However, symbiont-  
499 facilitated range shifts require that endophytes be present in the populations to be able to support  
500 population growth. The arid western range edge of *A. hyemalis* has had historically low endophyte  
501 prevalence (Fig. 4), and while prevalence has increased most quickly in the regions with historically  
502 low endophyte prevalence (Fig. A11), the complete absence of endophytes at range edges in this  
503 species would make it impossible for prevalence to increase without dispersal of symbiotic seeds  
504 (Fowler et al., 2023). These factors potentially contribute to the ability of the host species to track  
505 its environmental niche. Another interesting question is the degree to which symbiotic and non-  
506 symbiotic hosts, which occupy overlapping but distinct niches, are likely to experience distribution  
507 shifts in tandem or at different rates in future work. More extreme climate stresses, which are ex-  
508 pected more frequently in the future (Seneviratne et al., 2021), have the potential to alter the costs  
509 and benefits of the interaction. The past indicates a resilient symbiosis, but it will be crucial to  
510 understand whether more extreme future climate conditions could tip this interaction to deterioriate.

511 *Steps towards forecasts of host-microbe symbioses*

512 The combination of a spatially-explicit model and historic herbarium specimens allowed us to  
513 identify regions of both increasing and decreasing endophyte prevalence, however we see several  
514 next steps toward the goal of predicting host and symbiont niche-shifts in response to future cli-

515 mate change. While the model recreated the large-scale spatial trends observed in contemporary  
516 population surveys, test data contained more population-to-population variability in prevalence than  
517 could be explained by the model. We interpret this to mean that the model captures coarse-scale  
518 spatial and temporal trends reasonably well, but is not equipped to capture local-scale nuances that  
519 generate population-to-population differences. Validating our model predictions with this test, a  
520 rare extra step in collections-based studies, allows us to identify ways in which the model's out-of-  
521 sample predictive ability could be improved. Lack of information on local variability in symbiont  
522 prevalence may simply be a feature of data derived from herbarium specimens. Natural history col-  
523 lectors sample one or a few specimens from local populations, and these observations are aggregated  
524 by the model to derive broad-scale estimates. This suggests that increasing local replication should  
525 be a factor considered in future collection efforts of natural history specimens, balanced with the  
526 required time and effort and with limitations on storage space within collections. Poor predictive  
527 ability at local scales in this grass-endophyte system is not surprising, as previous studies have found  
528 that local variation, even to the scale of hundreds of meters<sup>21</sup> can structure endophyte-host niches  
529 (Kazenel et al., 2015). An important step would be integrating data from local and regional scales  
530 through modeling to constrain estimates of local and regional variation.

531 Predicting future niche-shifts of hosts and symbionts will require considering the coupled dynam-  
532 ics of host-symbiont dispersal in addition to fitness benefits. For example, transplanting symbiotic  
533 and non-symbiotic plants beyond the range edge of *A. hyemalis* could tell us whether low endophyte  
534 prevalence in that area (Fig. 4A) is a result of environmental conditions that lead the symbiosis  
535 to have negative fitness consequences, or is a result of some historical contingency or dispersal lim-  
536 itation that has thus far limited the presence of symbiotic hosts from a region where they would  
537 otherwise flourish and provide resilience. Incorporating available climatic and soil layers as covari-  
538 ates is another obvious step that could improve predictions. These steps will bridge gaps that often  
539 exist between large but broad bioclimatic and biodiversity data and small but high-resolution data

<sup>21</sup> Also see new paper in *Functional Ecology* by Pedro Gundel's group that has very local scale differences (I am a coauthor but did not do much.).

540 on biotic interactions, and move towards the goal of predicting the dynamics of microbial symbioses  
541 under climate change (Isaac et al., 2020; Miller et al., 2019).

542 *Herbaria for global change research*

543 Our analysis advances the use of herbarium specimens in global change biology in two ways.  
544 First and foremost, this is one of a growing number of studies to examine microbial symbiosis using  
545 specimens from natural history collections, and the first, to our knowledge, to link long-term changes  
546 in the symbioses to changes in climate. The responses of microbial symbioses are a rich target for  
547 future studies within historic specimens, particularly those that take advantage of advances in  
548 sequencing technology. While we used relatively coarse presence/absence data based on fungal  
549 morphology, other studies have examined historic plant microbiomes using molecular sequencing  
550 and sophisticated bioinformatics techniques, but these studies have so far been limited to relatively  
551 few specimens at limited spatial extents (Bieker et al., 2020; Bradshaw et al., 2021; Gross et al.,  
552 2021; Heberling and Burke, 2019; Yoshida et al., 2015). Continued advances in capturing historic  
553 DNA and in filtering out potential contamination during specimen storage (Bakker et al., 2020;  
554 Daru et al., 2019; Raxworthy and Smith, 2021) will be imperative in the effort to scale up these  
555 efforts. This scaling up will be essential to be able to quantify changes not just in the prevalence  
556 of symbionts, but also in symbionts' intraspecific variation and evolutionary responses to climate  
557 change, as well as in changes in the wider host microbiome. With improved molecular insights from  
558 historic specimens, we could ask whether the broad increases in endophytes that we have identified  
559 reflect selection for particular genetic strains or chemotypes and how this selection varies across  
560 space. Answering these questions as well as the unknown questions that future researchers may  
561 ask also reiterates the value in capturing meta-information during ongoing digitization efforts at  
562 herbaria around the world and during the accession of newly collected specimens (Edwards et al.;  
563 Lendemer et al., 2020).

564 The second major advance in this analysis is in accounting for several potential biases in the data  
565 observation process that may be common to many collections-based research questions by using a  
566 spatially-explicit random effects model. Potential biases introduced by the sampling habits of col-

567 lectors (Daru et al., 2018), and variation between contemporary researchers during the collection of  
568 trait data, if not corrected for could lead to over-confident inference about the strength and direction  
569 of historic change (Fig. 2). Previous studies that have quantified the effects of collector biases typ-  
570 ically find them to be small (Davis et al., 2015; Meineke et al., 2019), and we similarly did not find  
571 that collector has a strong effect on the results of our analysis, but that scorer identity did impact  
572 results. It is difficult to distinguish whether the impact of scorers was driven by true differences  
573 in scorers' biases or by unintended spatial or temporal clustering of the specimens examined by  
574 each scorer (Clayton et al., 1993; Urdangarin et al., 2023). By under-weighting endophyte-positive  
575 samples that are clustered spatially or by collector or observer, the endophyte prevalence model is  
576 appropriately accounting for nuisance variables and providing a conservative inference of endophyte  
577 change relative to the raw data. Spatial autocorrelation is another phenomenon likely common in  
578 data derived from herbarium specimens (Willems et al., 2022), which our spatially-explicit analysis  
579 models among samples. Beyond spatial autocorrelation of outcomes, systematic differences in sam-  
580 pling across space can result in spatial bias. One strength of herbaria as vehicles for global change  
581 research is the relative ease with which specimens from many distinct geographic locations can be  
582 examined. We visited just nine institutions in the central southern United States, and we were  
583 able to sample seeds from specimens across an area spanning over 300,000 sq. km, including speci-  
584 mens from Mexico and Canada. The specimens we examined are concentrated in the south-central  
585 United States, with fewer specimens in the rapidly warming northeastern United States. We provide  
586 a simulation analysis exploring the potential impact of spatially and temporally biased sampling  
587 (Appendix A - Supporting Methods). We found that the spatially-varying coefficient model had a  
588 strong ability to re-capitulate temporal trends across space in simulated data, and that this result  
589 was robust to relatively high levels of spatial bias (80% of data missing from region). Simulation  
590 analyses that extend this work to consider the myriad ways herbarium data may be biased (i.e. test-  
591 ing different spatial arrangements and scales of spatial bias, or testing different sample sizes) would  
592 be extremely valuable (Daru et al., 2018; Erickson and Smith, 2021; Gaul et al., 2020; Meineke and  
593 Daru, 2021).

594 *Conclusion*

595 Ultimately, a central goal of global change biology is to generate predictive insights into the  
596 future of natural systems on a rapidly changing planet. Beyond host-microbe symbioses, detect-  
597 ing ecological responses to anthropogenic global change and attributing their causes would inform  
598 public policy decision-makers and adaptive management strategies. This survey of historic endo-  
599 phyte prevalence is necessarily correlative, yet it serves as a foundation to develop better predictive  
600 models of the response of microbial symbioses to climate change. By comparing detected ecologi-  
601 cal responses with alternative mechanistic simulations of the past, we could attribute their cause,  
602 in a manner similar to methods from climate science and economics (Carleton and Hsiang, 2016;  
603 Stott et al., 2010; Trenberth et al., 2015). Combining the insights from this type of regional-scale  
604 survey with field experiments and physiological performance data could be invaluable to identify  
605 mechanisms driving shifts in host-symbiont dynamics. Evidence is strong that certain dimensions  
606 of climate change correlated with endophytes' temporal responses, however we do not know why  
607 trends in prevalence were weak in some areas or how endophytes would respond to more extreme  
608 changes in climate. The "time machine" of natural history collections revealed evidence of mutual-  
609 ism resilience for grass-endophyte symbioses in the face of environmental change, but more extreme  
610 changes could potentially push one or both partners beyond their physiological limits, leading to  
611 the collapse of the mutualism; more research is needed to understand what those limits might be.

612 **Acknowledgments**

613 We thank Dr. Jessica Budke for help in drafting our initial destructive sampling plan, and to the  
614 many staff members of herbaria who facilitated our research visits, as well as to the hundreds of  
615 collectors who contributed to the natural history collections. Several high school and undergraduate  
616 researchers contributed to data collection, including A. Appio-Riley, P. Bilderback, E. Chong, K.  
617 Dickens, L. Dufresne, B. Gutierrez, A. Johnson, S. Linder, E. Scales, B. Scherick, K. Schrader, E.  
618 Segal , G. Singla, and M. Tucker. This research was supported by funding from National Science  
619 Foundation (grants 1754468 and 2208857) and by funding from the Texas Ecolab Program. **Two**

620 anonymous reviewers greatly improved an earlier version of this manuscript.<sup>22</sup>

621 **Statement of Authorship**

622 J.C.F. contributed to research conception, data collection, data analysis, and led manuscript draft-  
623 ing. J.M. contributed to data analysis and manuscript revisions. T.E.X.M. contributed to research  
624 conception, data collection, data analysis, and manuscript revisions.

625 **Data and Code Availability**

626 Data from this publication can be found through a publicly available repository  
627 (<https://doi.org/10.5061/dryad.rn8pk0pn0>). Code for analyses can be found through a publicly  
628 available repository (<https://github.com/joshuacfowler/EndoHerbarium>) that will be permanently  
629 archived upon publication.

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<sup>22</sup> *Always thank the reviewers!*

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<sup>23</sup> I am not sure if it's because I messed with some of the font packages, but some of the citations are a little messy (some lowercase journal names, some uppercase). Latin names in titles should always be italicized. They will ask you to clean this up sooner or later, so I would do it sooner.

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949

## Appendix A

950

951      *Appendix to "Increasing Prevalence of plant-fungal symbiosis across two*  
952                   *centuries of environmental change"*

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957

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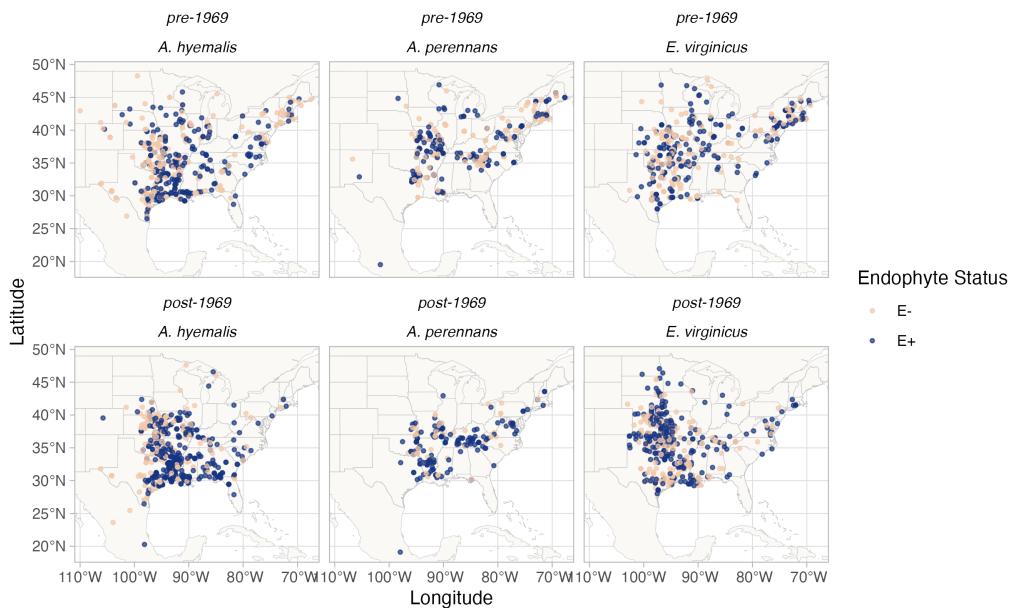
960      \* Corresponding author; e-mail: jcf221@miami.edu.

961      **Contents:**

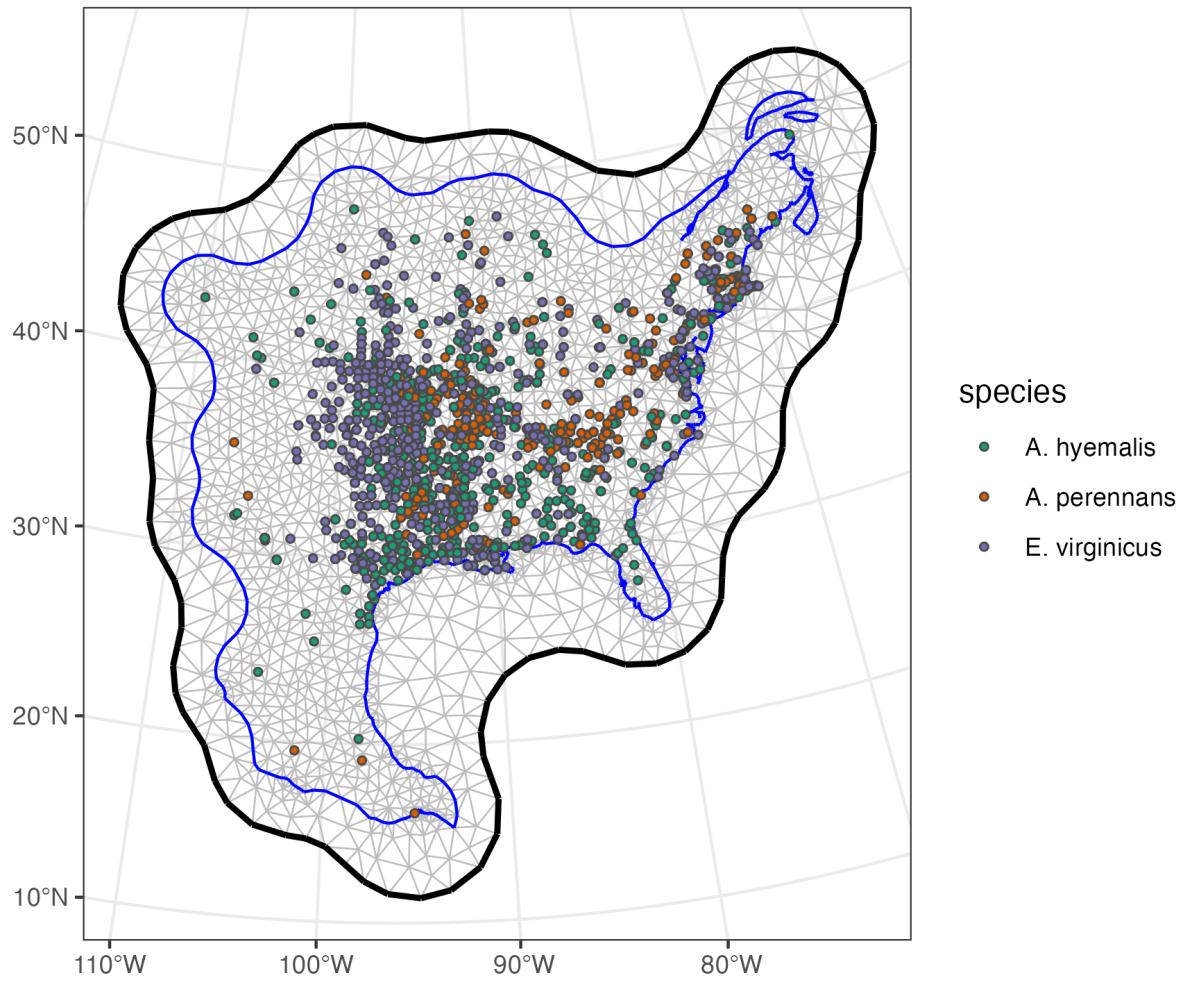
962      Appendix A includes: Figure A1 - Figure A15, Table A1, and Supporting Methods).

963

## Supplemental Figures



**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). To visualize temporal change, the data are faceted before and after the median year of collection. Map lines delineate study areas and do not necessarily depict accepted national boundaries.



**Figure A2: Triangulation mesh used to estimate spatial dependence between data points.**

Grey lines indicate edges of triangles used to define distances between observations. Colored points indicate locations of sampled herbarium specimens for each host species, and the blue line shows the convex hull and coastline used to define the edge of the mesh around the data points. The thick black line shows the convex hull defining a buffer space around the edge of the mesh to reduce the influence of edge effects on model estimates.

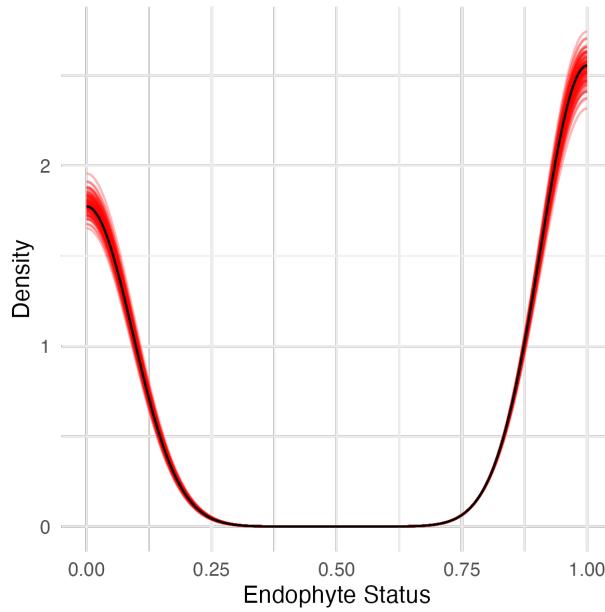


Figure A3: **Graphical posterior predictive check of the endophyte prevalence model fit.**

Consistency between **observed** data and **predicted** values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (black) along with 100 **predicted** datasets (red).

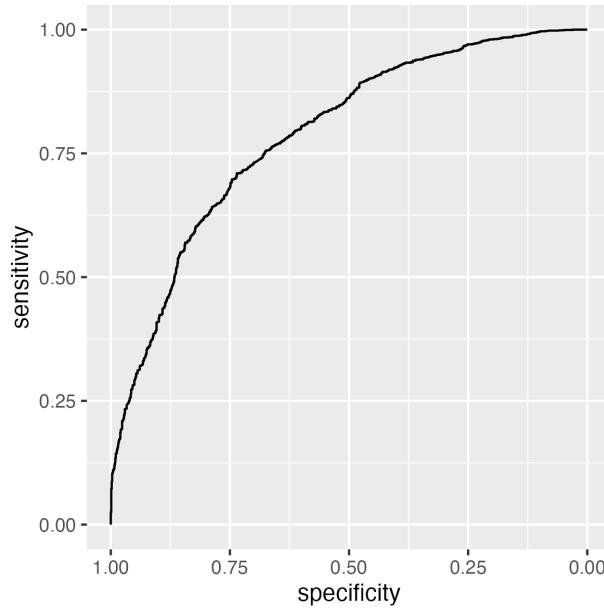


Figure A4: **ROC plot showing performance of the endophyte prevalence model in classifying observations according to endophyte status within the in-sample training data from herbarium collections.** The curves show adequate model performance for observed data. The AUC value is 0.79.

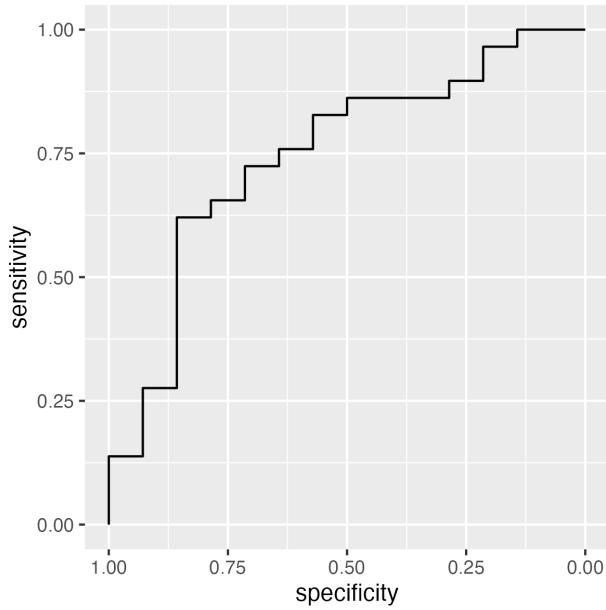
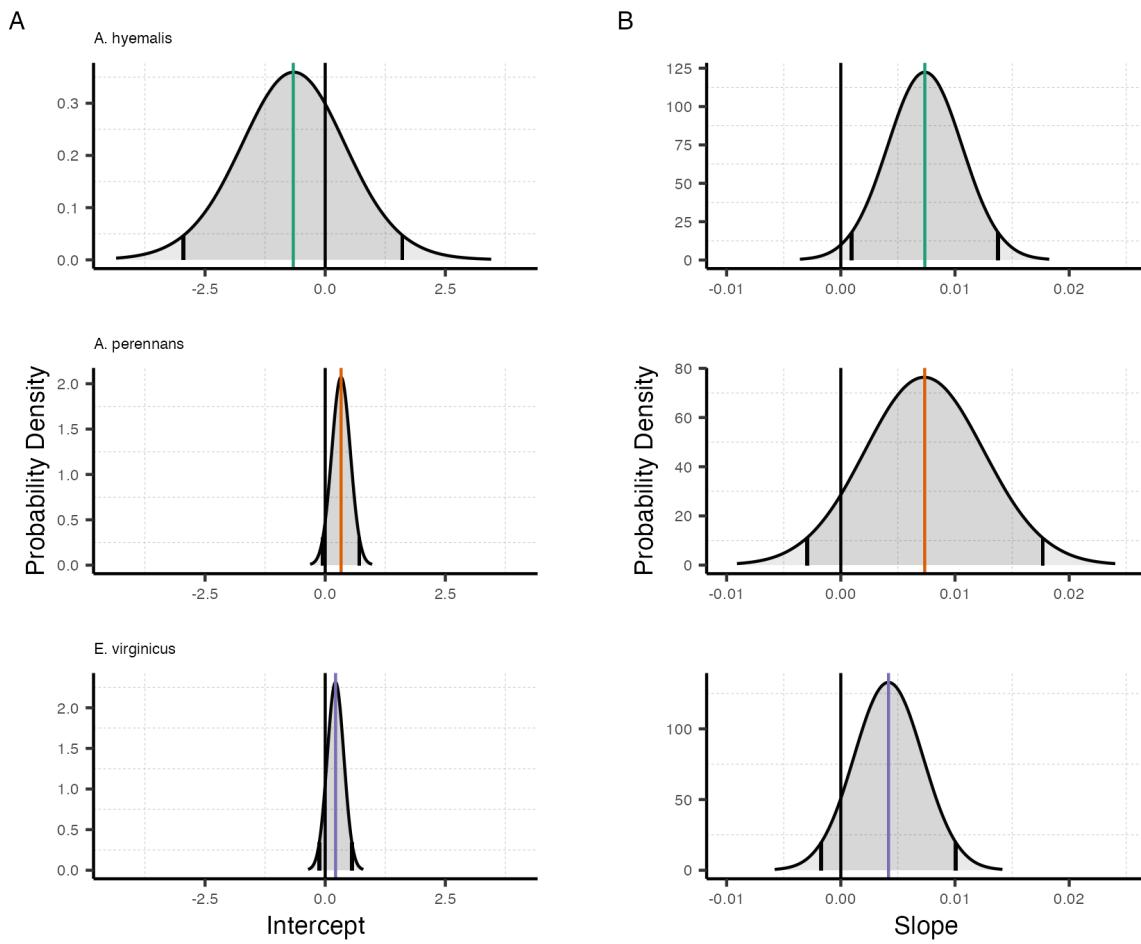
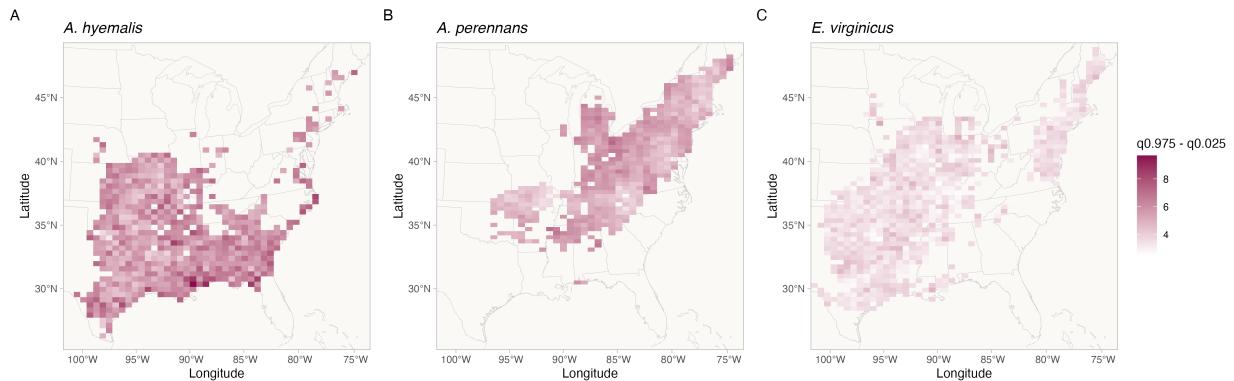


Figure A5: **ROC plot showing performance of the endophyte prevalence model in classifying observations according to endophyte status within the out-of-sample test data from contemporary surveys.** The curves show adequate model performance for test data. The AUC value is 0.77.



**Figure A6: Posterior estimates of parameters describing global intercept and temporal trends from the endophyte prevalence model.** Density curves show the probability density along with mean (colored line) and 95% CI (black lines) for the (A) intercept and (B) slope terms, **A** and **T** respectively from Eqn. 1. Colors represent each host species



**Figure A7: Credible interval width of temporal trends in endophyte prevalence across the distribution of each host species estimated from the endophyte prevalence model.**  
 Shading represents the range of the 95% posterior credible interval given in units of  $\%$  *change in prevalence/year* for spatially varying slopes,  $\tau$  from Eqn. 1. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

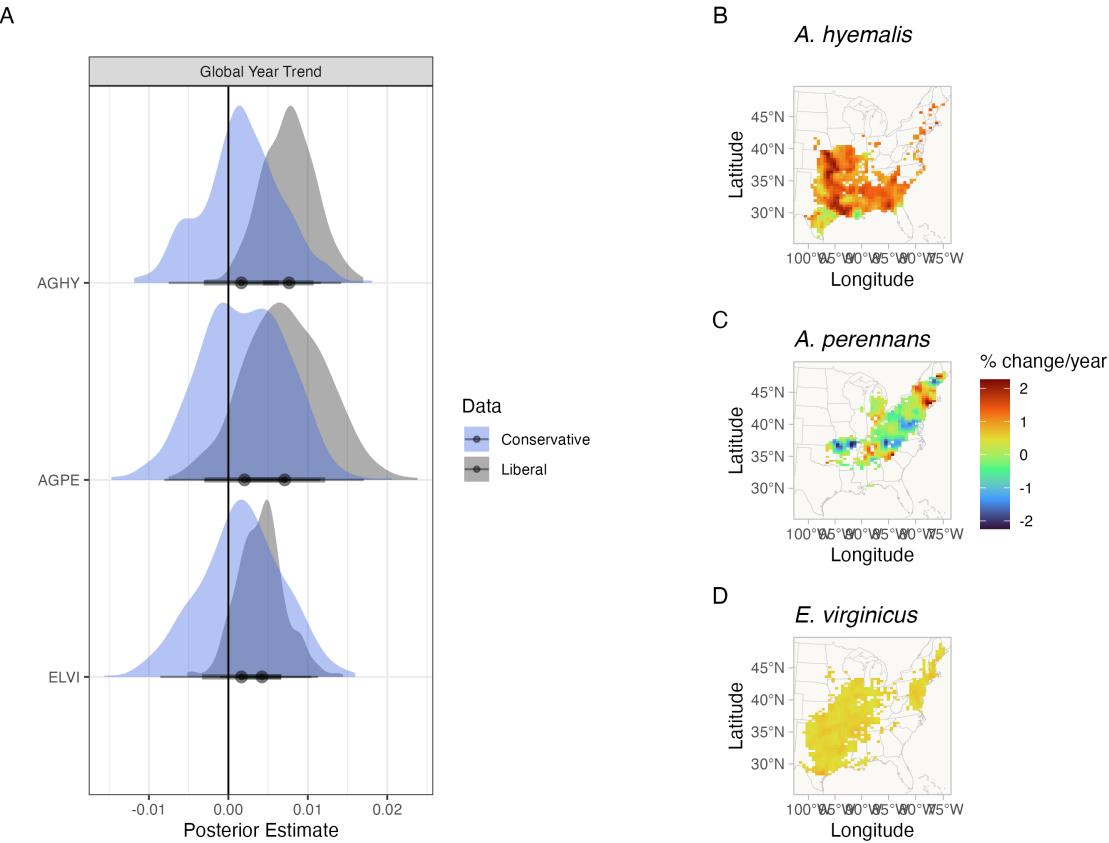


Figure A8: **Comparison of endophyte prevalence model estimates fit to data with liberal versus conservative endophyte scores.** Liberal and conservative scores document uncertainty in the endophyte identification process. Each specimen was given both a liberal and conservative scores. In cases of uncertain identification, the liberal status assumed a potential endophyte identification was more likely to be endophyte-positive while the conservative status assumed that the potential endophyte identification was less likely to be endophyte-positive. (A) Posterior estimates of global temporal trend ( $\mathbf{T}$  from Eqn. 1) for the endophyte prevalence model fit to liberal scores (grey) and to conservative scores (blue). Maps show the spatially varying temporal trend estimates ( $\tau$  from Eqn. 1) from the endophyte prevalence model fit to conservative scores for (B) *A. hyemalis*, (C) *A. perennans*, and (D) *E. virginicus*. Note that the color scale differs between this visualization and Fig. 3 that shows estimates fit using liberal endophyte scores.

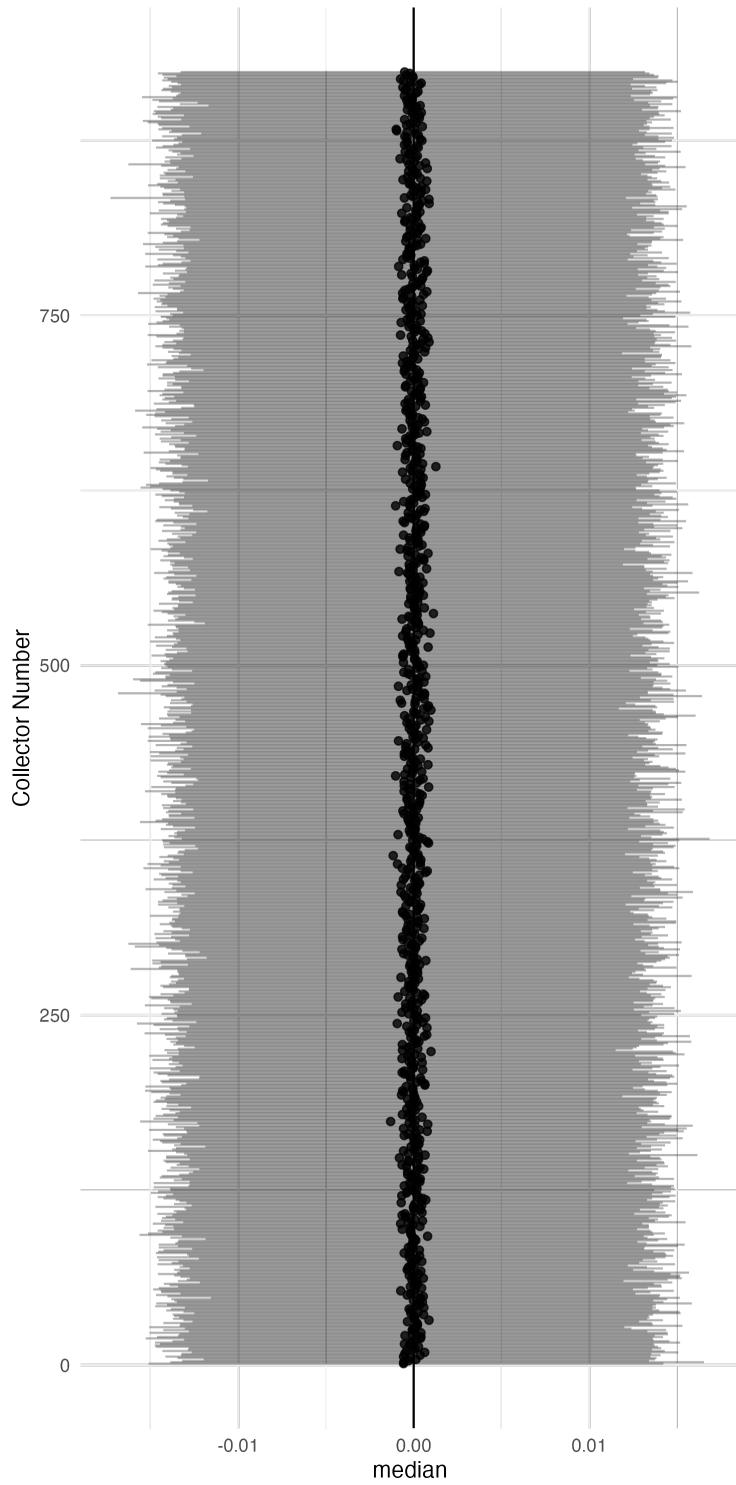


Figure A9: **Posterior estimates of collector random effects from endophyte prevalence model.** Collector random effects are denoted  $\chi$  in Eqn. 1 and represent variance associated with researchers who collected historic herbarium specimens. Points show posterior median along with 95% CI for each of 924 individual collectors.

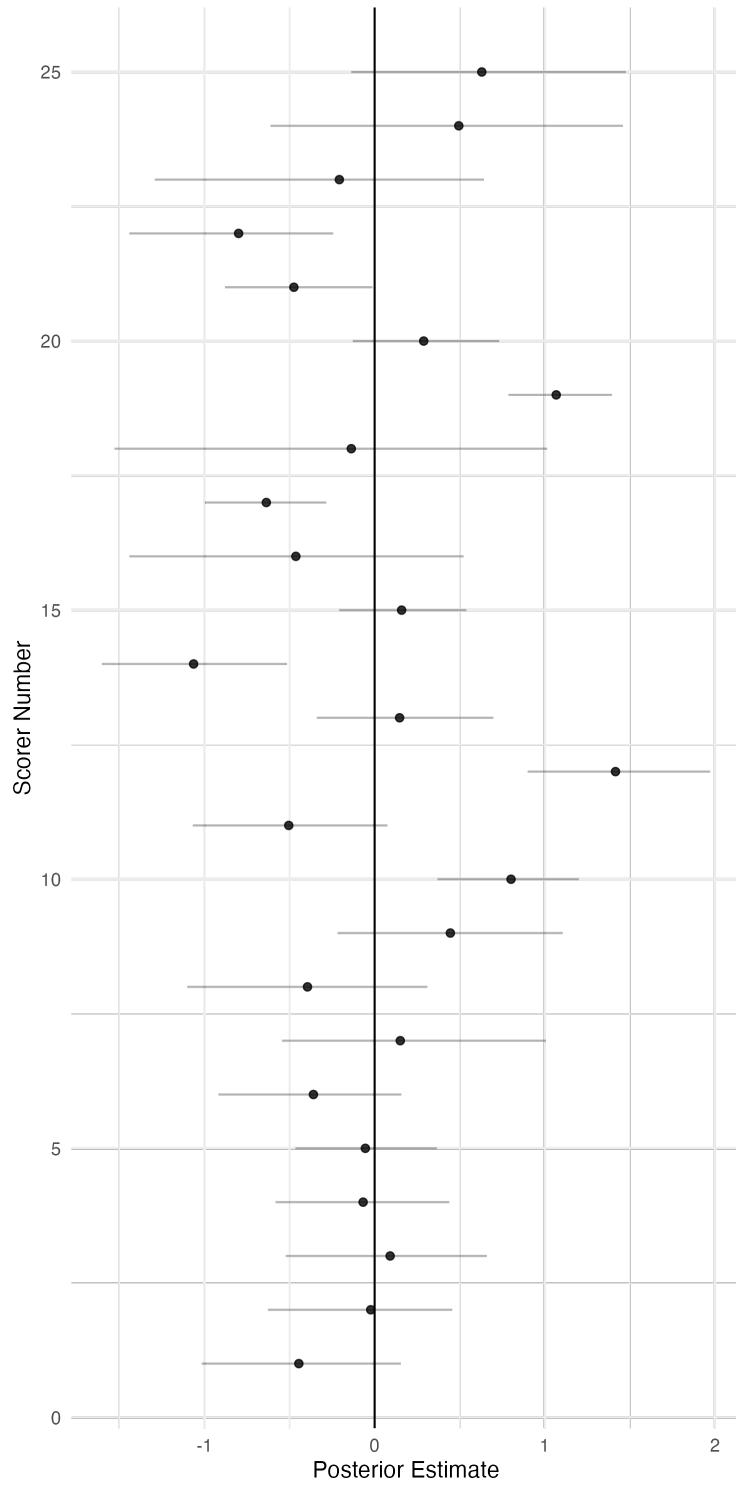
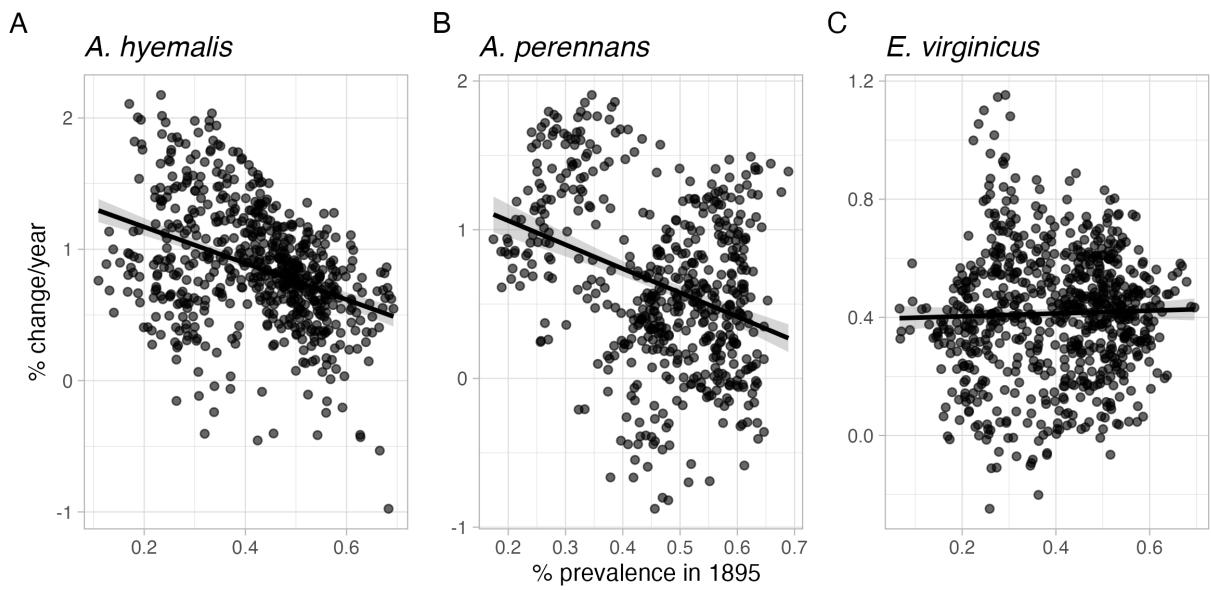


Figure A10: **Posterior estimates of scorer random effects from endophyte prevalence model.** Scorer random effects are denoted  $\omega$  in Eqn. 1 and represent variance associated with researchers who identified *Epichloë* endophytes within herbarium specimen tissue samples. Points show posterior median along with 95% CI for each of 25 individual scorers.



**Figure A11: Relationship between initial prevalence and temporal trends in prevalence estimated from the endophyte prevalence model.** Points show predicted posterior mean temporal trend for each species at pixels across each host distribution ((A) *A. hyemalis*, (B) *A. perennans*, and (C) *E. virginicus*). along with a linear regression and shaded ribbon showing 95% confidence interval.

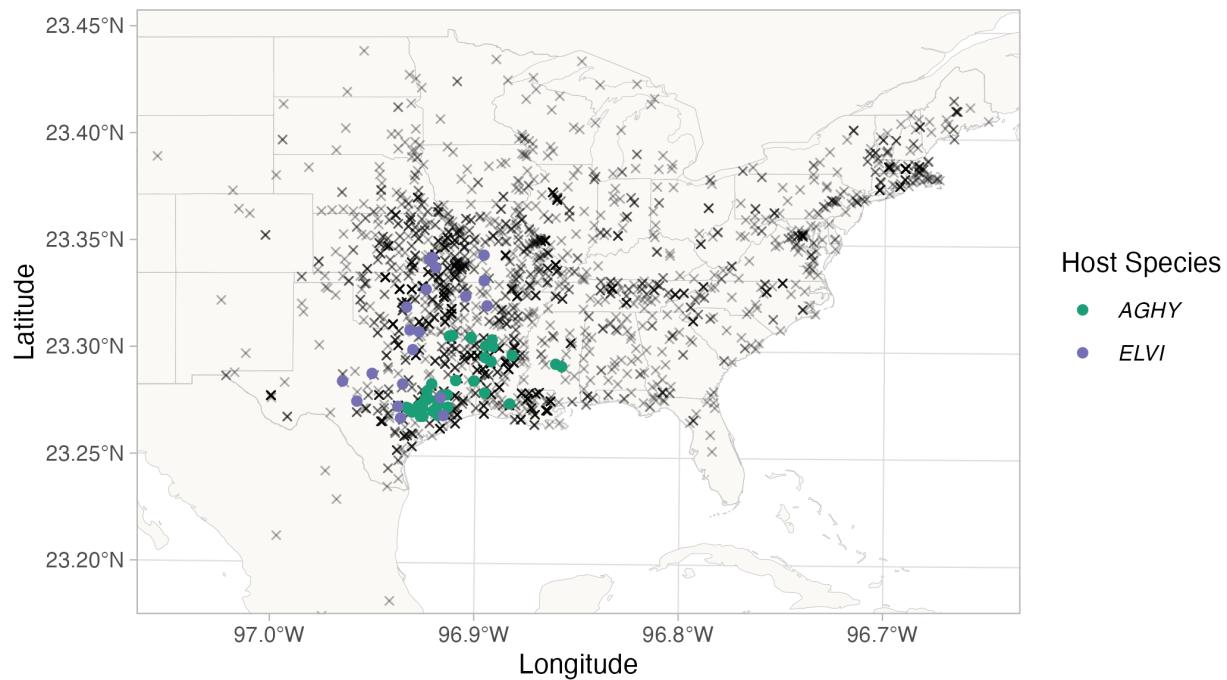


Figure A12: **Locations of contemporary surveys of endophytes used as "test" data to evaluate predictive ability of the endophyte prevalence model.** Points are locations of host populations surveyed between 2013 and 2019 for endophytes, colored by species (*A. hyemalis*: green, *E. virginicus*: purple). Black crosses show the historical herbarium collection locations used as "training" data for the endophyte prevalence model.

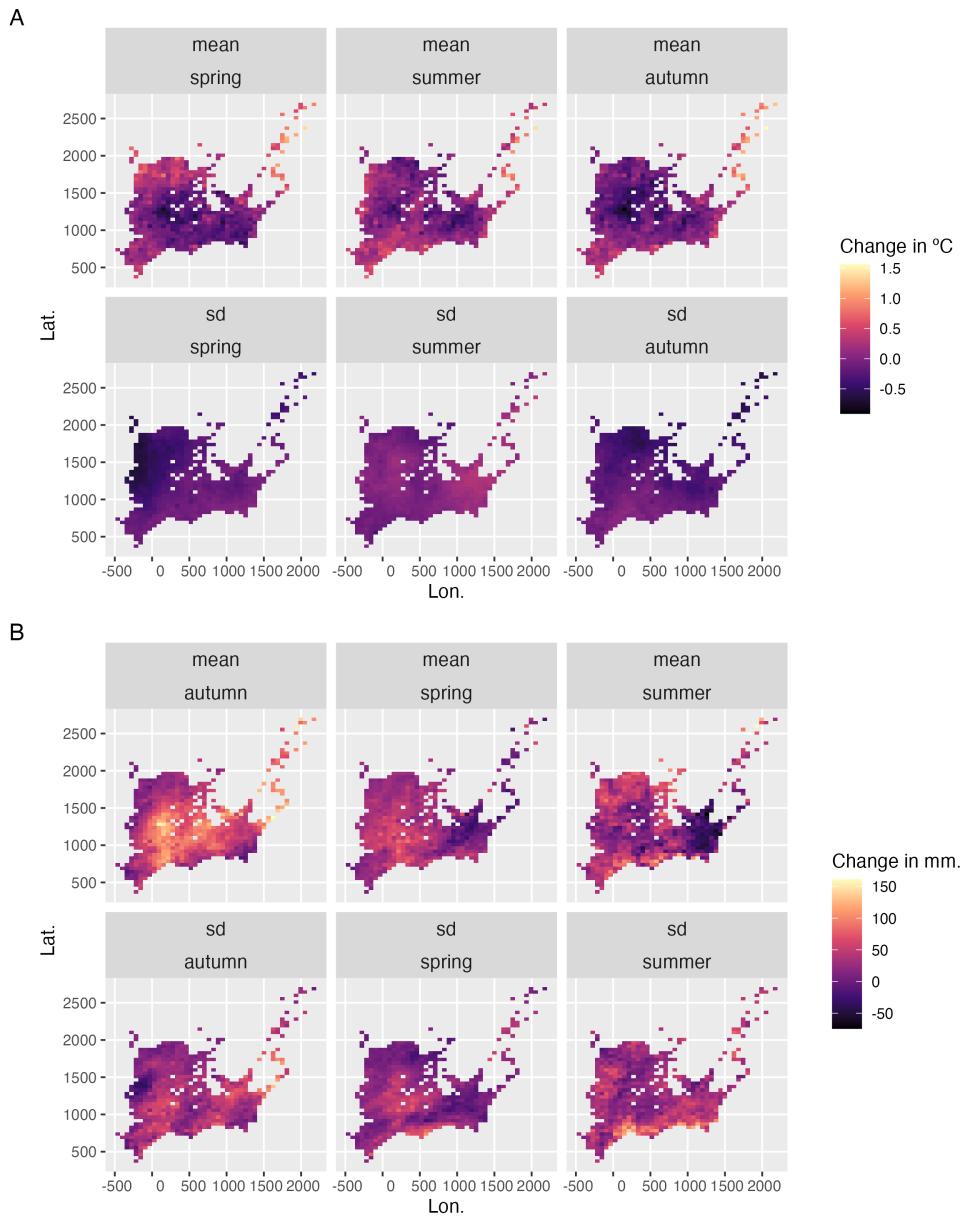


Figure A13: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *A. hyemalis*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution of *A. hyemalis* used in *post hoc* climate regression analysis.

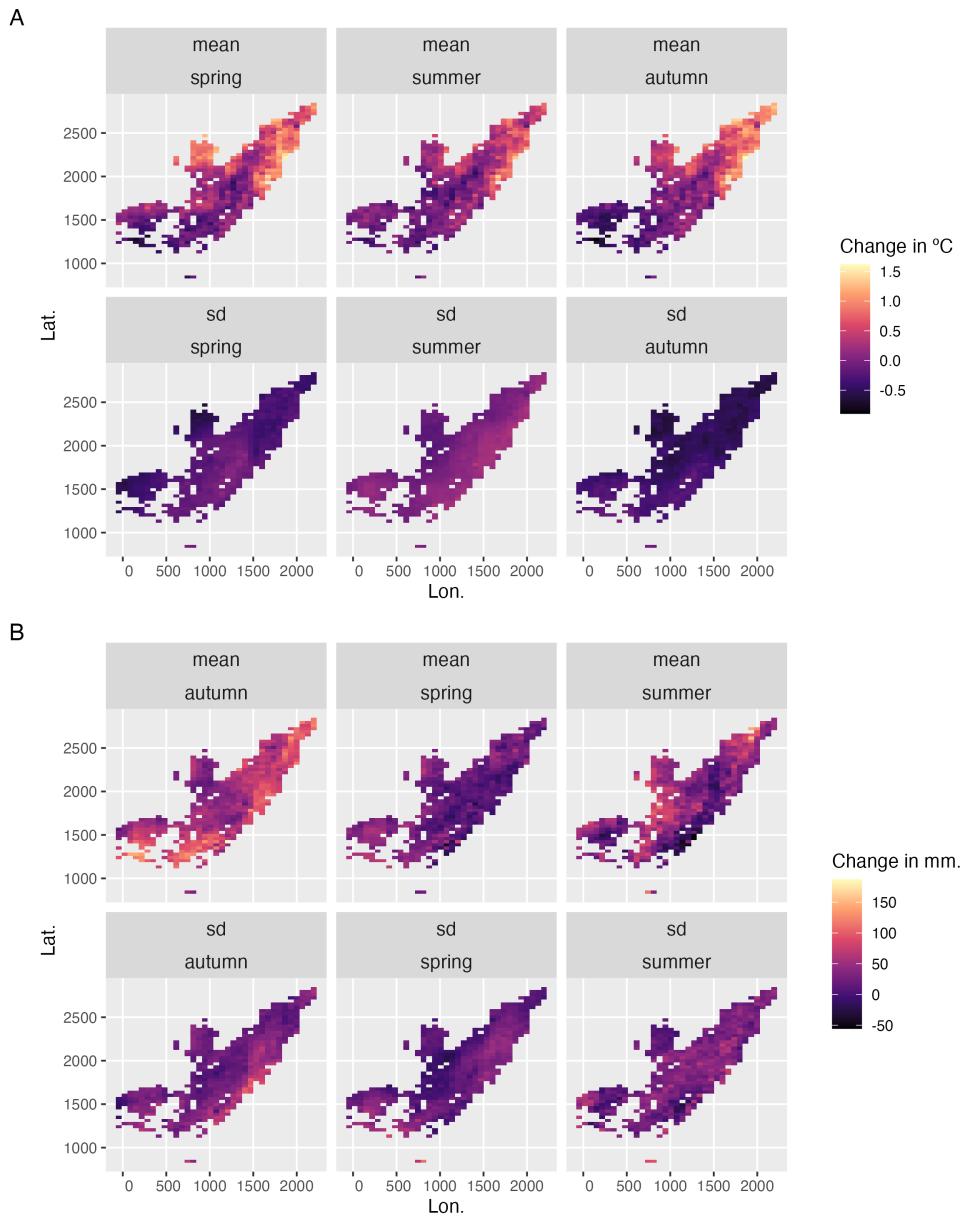


Figure A14: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *A. perennans*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution of *A. perennans* used in *post hoc* climate regression analysis.

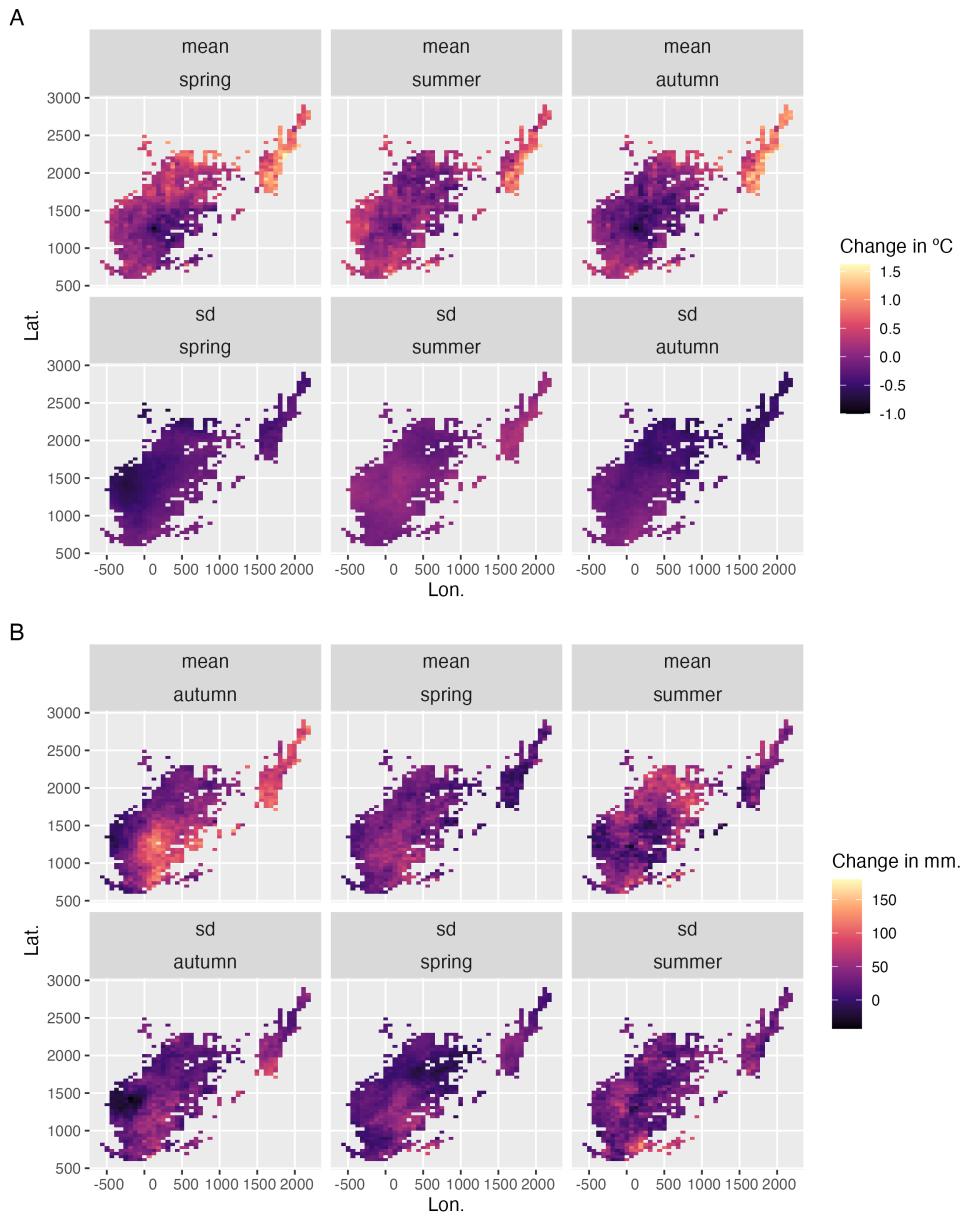


Figure A15: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *E. virginicus*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution of *E. virginicus* used in *post hoc* climate regression analysis.

Table A1: Summary of herbarium samples across collections (no. of specimens)

| Herbarium Collection                  | <i>A. hyemalis</i> | <i>A. perennans</i> | <i>E. virginicus</i> |
|---------------------------------------|--------------------|---------------------|----------------------|
| Botanical Research Institute of Texas | 350                | 190                 | 198                  |
| Louisiana State University            | 72                 | 38                  | 62                   |
| Mercer Botanic Garden                 | 3                  | –                   | 6                    |
| Missouri Botanic Garden               | 210                | 205                 | 122                  |
| Texas A&M                             | 100                | –                   | 72                   |
| University of Kansas                  | 134                | 34                  | 197                  |
| University of Oklahoma                | 85                 | 34                  | 95                   |
| University of Texas & Lundell         | 183                | 91                  | 102                  |
| Oklahoma State University             | 51                 | 10                  | 74                   |

965

## Supporting Methods

966

### ODMAP Protocol

967 [Overview](#)

968 **Model purpose:** Mapping current distribution of *Epichloë* host species.

969 **Target species:** *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*.

970 **Study area:** Eastern North America

971 **Spatial extent:** -125.0208, -66.47917, 24.0625, 49.9375 (xmin, xmax, ymin, ymax).

972 **Spatial resolution:** 0.04166667, 0.04166667 (x, y).

973 **Temporal extent:** 1990 to 2020.

974 **Boundary:** Natural.

975 [Data](#)

976 **Observation type:** Occurrence records from Global Biodiversity Information Facility and

977 herbarium collection across eastern North America. We used 713 occurrences records for *Agrostis*  
978 *hyemalis*, 656 occurrence records for *Agrostis perennans* and 2338 for *Elymus virginicus*.

979 **Response data type:** occurrence record, presence-only.

980 **Coordinate reference system:** WGS84 coordinate reference system (EPSG:4326 code)

981 **Climatic data:** raster data extracted from PRISM

982 [Model](#)

983 **Model assumption:** We assumed that the target species are at equilibrium with their environment.  
984

985 **Algorithms:** Maximum entropy (maxent)

986 **Workflow:** We described the workflow in the method section of the manuscript.

987 **Software:** All statistics were performed using Maxent 3.3.4 and R4.3.1 with packages terra, usdm,  
988 spThin and dismo.

989 **Code availability:** Available through this link: <https://github.com/joshuacfowler/EndoHerbarium>

990 **Data availability:** Will be available upon acceptance

991 [Assessment](#)

992 We used AUC to test model performance.

993 [Prediction](#)

994 We predicted the probability of presence of the host species as a binary maps (presence or absence)

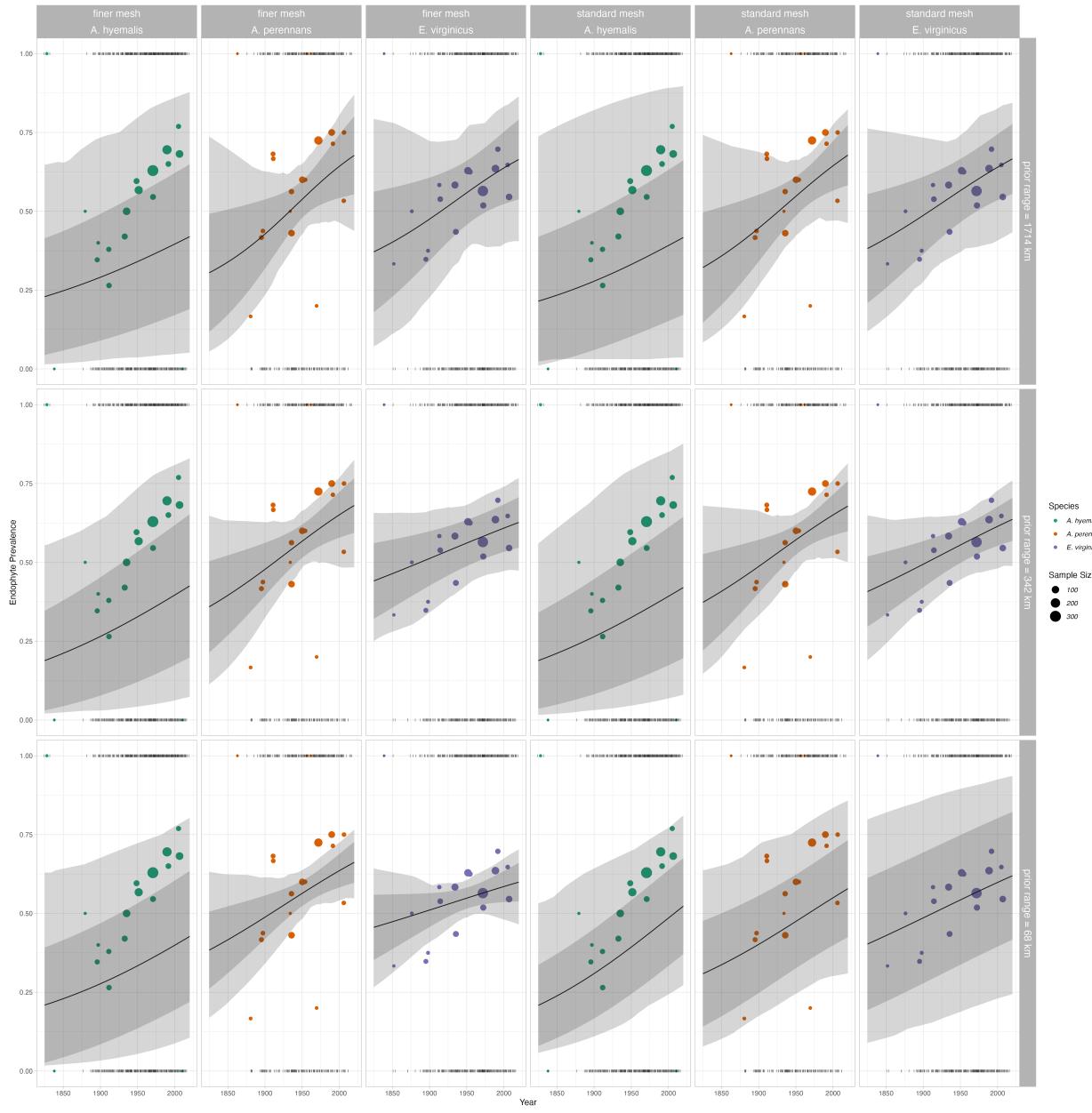
## 995 *Mesh and Prior Sensitivity Analysis*

996 To test the influence that the triangulation mesh and choice of priors has on results, we compared  
997 model results across a range of meshes and priors. We re-ran our model for the mesh used in main  
998 body of the text (Fig. A2), which we refer to as the "standard mesh", and with a mesh with smaller  
999 minimum vertices (finer mesh). Finer scale meshes increase computation time. For each of these  
1000 meshes, we ran the model with a range of priors defining the spatial range of our spatial random  
1001 effects: 342km (the prior used for presented results), as well as ranges five times smaller (68 km)  
1002 and five times larger (1714 km). We found generally that these choices did not alter the direction

1003 of model predictions, but did influence the associated uncertainty and magnitude of some effects.

1004 For overall temporal trends, we found that models with differing priors predicted consistently

1005 positive relationships over time (Fig. A16).



**Figure A16: Overall trend in endophyte prevalence evaluated for endophyte prevalence models with different range priors on spatially structured random effects, and for two different triangulation meshes.** Data used in model fitting is the same across all panels and as in the main text. Note that these plots, as compared to Fig. 2 in main text, show mean trends and do not incorporate variance associated with collector and scorer random effects.

1006 For spatially-varying temporal trends, we found that models with different priors predicted  
1007 consistent spatial patterns in temporal trends, although the range of this prediction varied depending  
1008 on the prior and mesh (Fig. A17 - A18). One noteworthy result of this analysis is that combinations  
1009 of prior choice and mesh can introduce instability in model fitting. This is evident in A17 panel B  
1010 and A18 panel B, where the prior range is smaller than the minimum vertex length of the mesh.  
1011 Model fitting takes an extended time period and the model struggles to identify variation across  
1012 space. Results with a set of prior ranges (Fig. A17 - A and C; Fig. A18 - A and C) result in  
1013 models that estimate trends across space of the same direction and order of magnitude, although  
1014 the “smoothness” of these predictions vary.

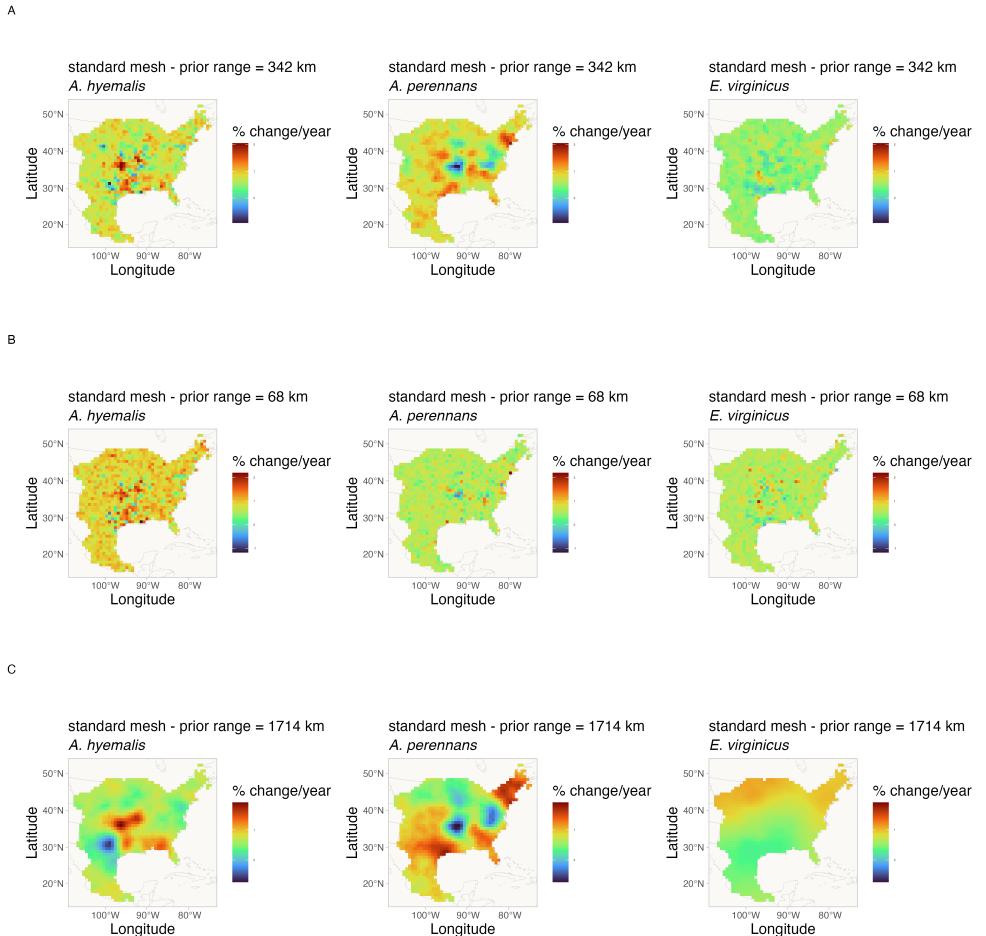


Figure A17: Spatially-varying trends in endophyte prevalence evaluated for the endophyte prevalence model with different range priors on spatially structured random effects, and for the "standard" mesh. Data used in model fitting is the same across all panels and as in the main text. Shading indicates the magnitude and direction of predicted trends for each of three host species for each of three prior ranges (rows A-C). Note that each plot has an individual scale bar.

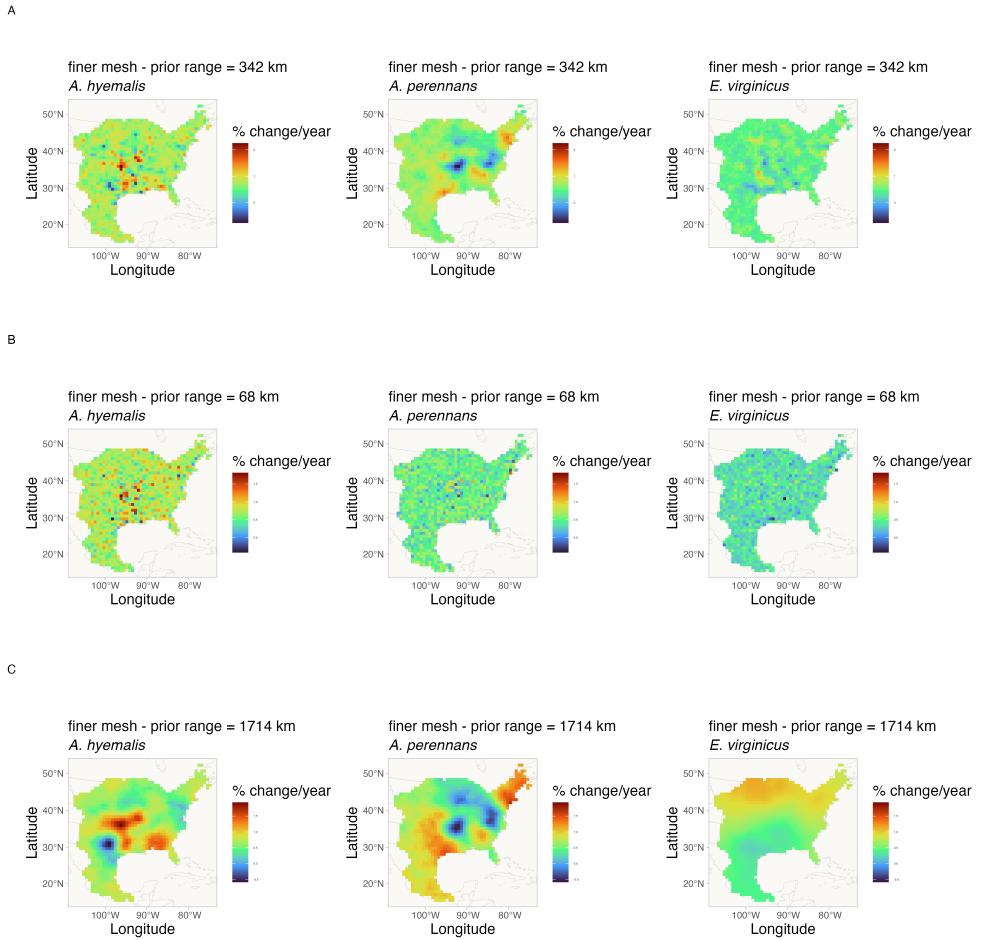


Figure A18: Spatially-varying trends in endophyte prevalence evaluated for the endophyte prevalence model with different range priors on spatially structured random effects, and for the "finer" mesh. Data used in model fitting is the same across all panels and as in the main text. Shading indicates the magnitude and direction of predicted trends for each of three host species for each of three prior ranges (rows A-C). Note that each plot has an individual scale bar.

1015

## *Spatially-biased Sample Size Simulation Analysis*

1016 To examine how data that is unevenly distributed across host distributions may influence interpreta-  
1017 tion of spatially-varying coefficients, we performed a simulation analysis. Our focal species, *Agrostis*  
1018 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*, are widely distributed grasses across the east-  
1019 ern United States that host *Epichloë* fungal endophytes. For logistical reasons, our sampling visits  
1020 to herbaria focused on herbaria in the central southern U.S., which resulted in unevenly distributed  
1021 data across each host species' range. This is particularly noteable for *Agrostis perennans* which has  
1022 the most northern distribution and relatively fewer total collected specimens compared to the other  
1023 focal species. Thus, a significant portion in the northeast of this species' range is relatively sparsely  
1024 sampled. Our analysis presented in the main text identified this region as having strong increase in  
1025 endophyte prevalence. Future visits to herbaria with regional focuses in the Northeastern US would  
1026 certainly garner new specimens that could provide valuable insights into shifting host and symbiont  
1027 distributions.

1028 *Simulation of spatially-biased symbiont occurrence data*

1029 We simulated datasets with varying levels of missing-ness to examine how this missing-ness influ-  
1030 enced the estimation of spatially-varying trend estimates. We first generated 300 data points for  
1031 each of three hypothetical species at random positions across an area approximating the scale of  
1032 our focal data. Each data point was randomly assigned a year of collection across 200 years. We  
1033 then simulated data from a Bernoulli process with trends alternating across nine regions (Fig. A19)  
1034 in a 3X3 grid pattern. This grid pattern was intended to create a complex spatial layout of trends,  
1035 where trends were either an increase of 1% per year or a decrease of 1% per year.

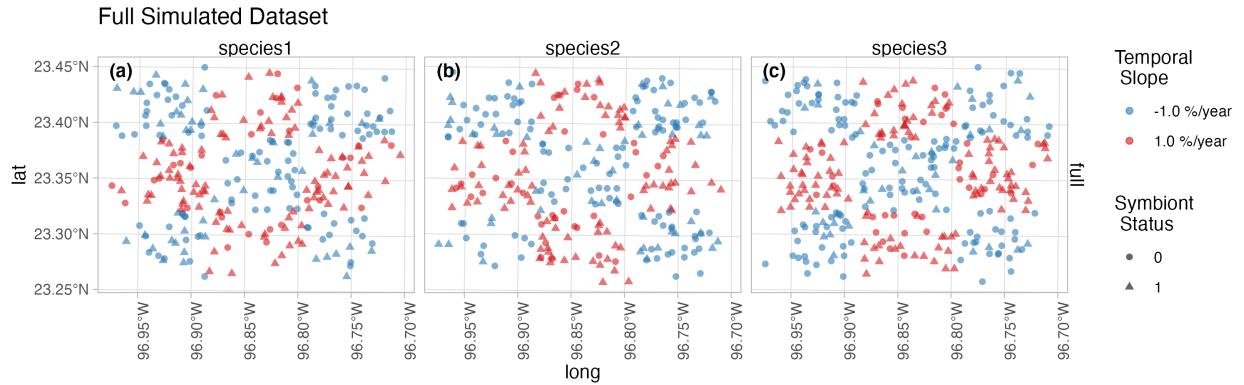
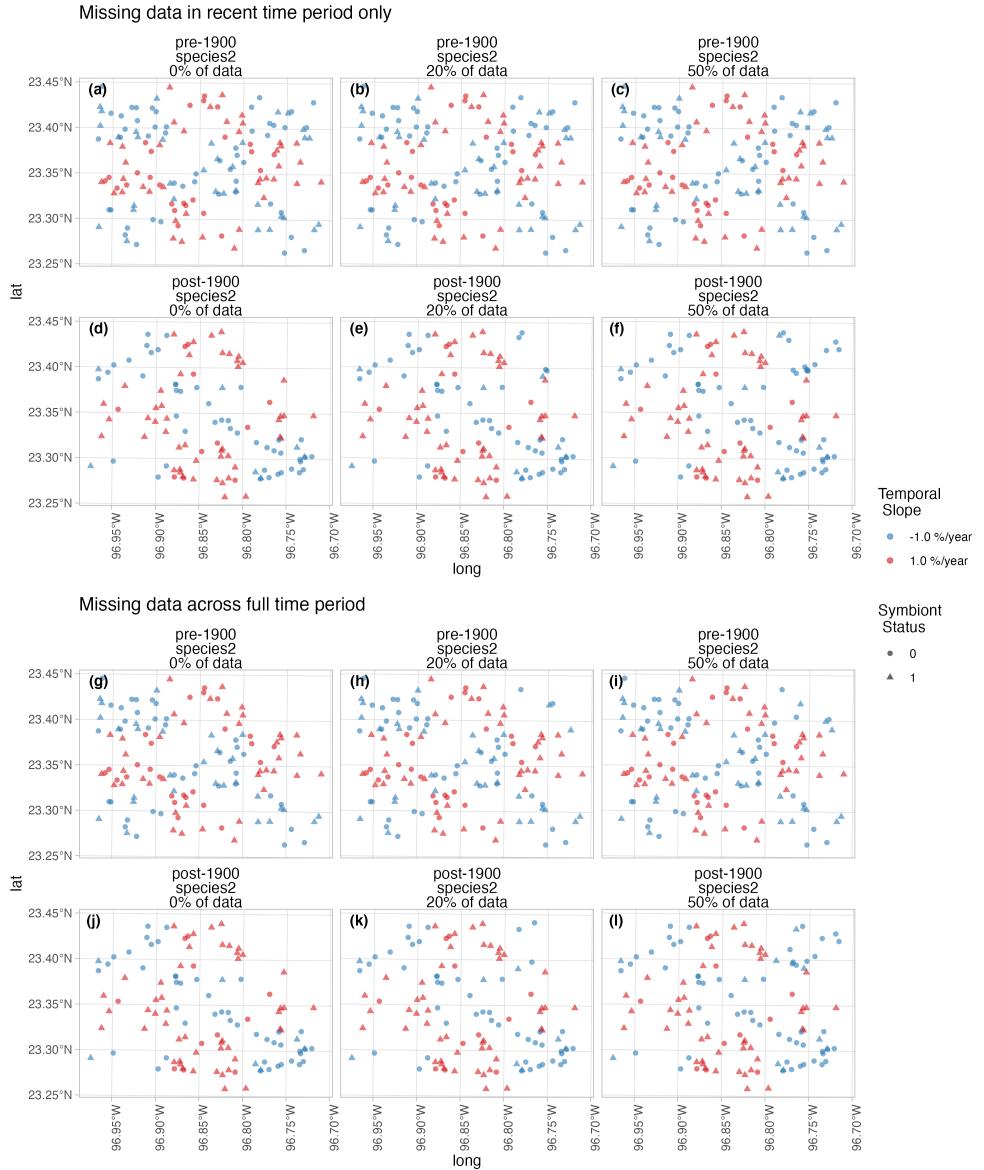


Figure A19: **Full simulated dataset of symbiotic association with spatially-varying temporal trends.** Color indicates the slope parameter used to simulate trends in endophyte status across nine "regions" for three species. Data are assigned collection years across a period of 200 years. Shape indicates the presence (1) or absence (0) of a symbiont.

1036 From this full data, we generated six additional datasets with missing-ness in the northeast  
 1037 region of the simulated data for hypothetical species 2. The data remained the same for Species 1  
 1038 and for species 3 across all datasets. For these six datasets, we removed data points at random in  
 1039 six ways: 0% of datapoints in northeast region, 0% of recent datapoints, only 20% of datapoints,  
 1040 only 20% of recent datapoints, only 50% of datapoints, and only 50% of recent datapoints (Fig.  
 1041 A20). We define the datapoints as part of the recent time period if they occur later than the median  
 1042 year. The result is 6 scenarios exploring degrees of spatial and temporal bias.



**Figure A20: Six simulated datasets representing scenarios of spatially-baised missingness for Species 2.** Missingness was imposed in the northeast region for six scenarios: 0% of recent datapoints available (a,d); only 20% of recent datapoints (b,e); only 50% of recent datapoints (c,f); 0% of datapoints across the full time period available (g,j); only 20% of datapoints across the full time period (h,k); and only 50% of datapoints across the full time period(i,l). Missingness was imposed only for hypothetical Species 2; Species 1 and 3 remain as in Figure A19. Color indicates the slope parameter used to simulate trends in endophyte status across 9 regions in a 3x3 grid. Shape indicates the presence (1) or absence (0) of a symbiont.

1043 *Statistical analysis*

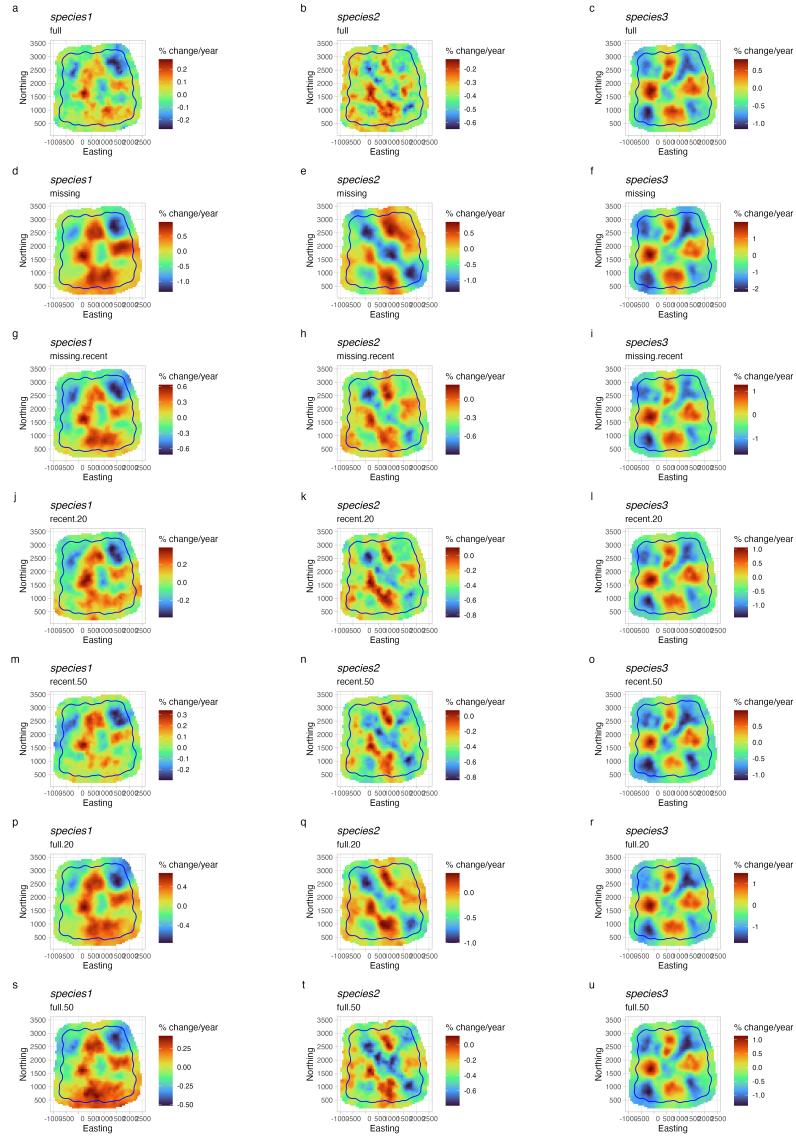
1044 We analyzed each dataset with a model given by Eqn. A1 similar in construction to that used in  
1045 our central analysis.

$$\text{logit}(\hat{P}_{h,i}) = \mathbf{A}_h + \mathbf{T}_h * \text{year}_i + \alpha_{h,l_i} + \tau_{h,l_i} * \text{year}_i + \delta_{l_i} \quad (\text{A1})$$

1046 Where symbiont presence/absence of the  $i^{th}$  specimen ( $P_{h,i}$ ) was modeled as a Bernoulli response  
1047 variable with expected probability of symbiont occurrence  $\hat{P}_{h,i}$  for each host species  $h$ . We modeled  
1048  $\hat{P}_{h,i}$  as a linear function of intercept  $\mathbf{A}_h$  and slope  $\mathbf{T}_h$  defining the global trend in endophyte  
1049 prevalence specific to each host species as well as with spatially-varying intercepts  $\alpha_{h,l_i}$  and slopes  
1050  $\tau_{h,l_i}$  associated with location ( $l_i$ , the unique latitude-longitude combination of the  $i$ th observation).  
1051 Similar to the SVC model of our central analysis (Eqn. 1), we estimated a shared variance term  
1052 with the spatially-dependent random effect  $\delta_{l_i}$ , intended to account for residual spatial variation.  
1053 However in this analysis we omit i.i.d.-random effects terms associated with collector and scorer  
1054 identity ( $\chi_{c_i}$  and  $\omega_{s_i}$  in Eqn. 1) for the sake of simplicity.

1055 *Influence of spatially-biased sampling on model interpretation*

1056 Our analysis of the full simulated data shows that our model is suitably flexible to capture complex  
1057 spatial patterns in temporal trends (Fig. A21 a-c). Beyond this, the model also qualitatively  
1058 captures the spatial patterns in temporal trends even with large amounts of data missingness (i.e  
1059 missing up to 80% of the datapoints (Fig. A21 p-r)).



**Figure A21: Mean predicted spatially-varying trend in symbiont prevalence across datasets with different levels of missingness.** Color indicates the estimated mean temporal trend within each pixel across the simulated data. Panels show estimates for models fit to different levels of missing data for species 2 in the northeast region ((a-c) the full dataset, (d-f) missing all datapoints across entire temporal period, (g-i) missing all datapoints only during the recent period, (j-l) missing 80% of the datapoints only during the recent period, (m-o) missing 50% of the datapoints only during the recent period, (p-r) missing 80% of the datapoints across the entire temporal period, (s-u) missing 50% of the datapoints across the entire temporal period). The mesh boundary that bounds the "full" simulated dataset is plotted in each panel.

1060 While this analysis is not an exhaustive examination of the influence of sampling bias on our  
1061 results for several reasons, including not examining how different strengths in temporal trends, dif-  
1062 ferent spatial arrangements of missing-ness influence model estimates, or different sample sizes, it  
1063 demonstrates that the spatially-varying modelling framework implemented in INLA we employ can  
1064 suitably recover regional trends even with significant spatially-bias within data collection, and fur-  
1065 ther the analysis is likely robust to temporally-structured bias (missing data within recent collection  
1066 period). Future work could more fully explore the scenarios that cause this ability to break down.  
1067 We expect this simulation reflects what may be a common scenario for research investigating global  
1068 change using natural history specimens. Collection effort by trained taxonomists and professional  
1069 collectors peaked in the past, and collections contain relatively fewer modern specimens in many  
1070 regions. Additionally, most global change research necessarily involves accessing many specimens  
1071 across collections. Research efforts such as ours will be unable to access every specimen from all  
1072 possible collections. Ongoing digitization efforts will make it possible to more clearly assess how  
1073 much data is missing from a particular study compared to the actual holdings of natural history  
1074 collections, but ultimately, the decision of what data and collections to include is a question of  
1075 sample size and study design.