

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

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## Abstract

Species' distributions and abundances are shifting in response to ongoing global climate change. Mutualistic microbial symbionts can provide hosts with protection from environmental stress that may promote resilience under environmental change, however this change may also disrupt species interactions and lead to declines in hosts and/or symbionts. Symbionts preserved within natural history specimens offer a unique opportunity to quantify changes in microbial symbiosis across broad temporal and spatial scales. We asked how the prevalence of seed-transmitted fungal symbionts of grasses (*Epichloë* endophytes) has changed over time in response to climate change, and how these changes vary across host species' distributions. Specifically, we examined 2,346 herbarium specimens of three grass host species (*Agrostis hyemalis*, *Agrostis perennans*, *Elymus virginicus*) collected over the past two centuries (1824 – 2019) for the presence or absence of *Epichloë* symbiosis. Analysis of an approximate Bayesian spatially-varying coefficients model revealed that endophytes increased in prevalence over the last two centuries from ca. 25% to ca. 75% prevalence, on average, across three host species. Changes in seasonal climate drivers were associated with increasing endophyte prevalence. Notably, increasing precipitation during the peak growing season for *Agrostis* species and decreasing precipitation for *E. virginicus* were associated with increasing endophyte prevalence. Changes in the variability of precipitation and temperature during off-peak seasons were also important predictors of increasing endophyte prevalence. Our model performed favorably in an out-of-sample predictive test with contemporary survey data from across 63 populations, a rare extra step in collections-based research. However, there was greater local-scale variability in endophyte prevalence in contemporary data compared to model predictions, suggesting new directions that could improve predictive accuracy. Our results provide novel evidence for a cryptic biological response to climate change that may contribute to the resilience of host-microbe symbiosis through fitness benefits to symbiotic hosts.

## 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 document and preserve taxonomic  
48 diversity, present a unique opportunity to explore long-term changes in biodiversity and ecological  
49 interactions across broad spatial and temporal scales (Davis, 2023; Meineke et al., 2018). Natural  
50 history collections, built and maintained by the efforts of thousands of scientists, are invaluable  
51 time machines, primarily comprised of physical specimens of organisms along with information  
52 about the time and place of their collection. These specimens often preserve physical legacies  
53 of ecological processes and species' interactions from dynamically changing environments across  
54 time and space (Lendemer et al., 2020). For example, previous researchers have examined the  
55 flowers, pollen grains, and leaves of specimens within plant collections (herbaria) to document shifts  
56 in reproductive phenology (Berg et al., 2019; Park et al., 2019; Willis et al., 2017), pollination  
57 (Duan et al., 2019; Pauw and Hawkins, 2011), and herbivory (Meineke et al., 2019) related to  
58 anthropogenic climate change. Herbarium specimens have also been used to identify the origins  
59 and population genomics of plant diseases such as *Phytophthora*, the Irish potato famine pathogen  
60 (Ristaino et al., 2001; Ristaino, 2002; Yoshida et al., 2013), and have been proposed as vehicles  
61 to track other emerging plant pathogens (Bradshaw et al., 2021; Ristaino, 2020). However, few  
62 previous studies have leveraged biological collections to examine climate change-related shifts in a

63 particularly common type of interaction: mutualistic microbial symbiosis.

64 Microbial symbionts are common to all macroscopic organisms and can have important effects  
65 on their hosts' survival, growth and reproduction (McFall-Ngai et al., 2013; Rodriguez et al., 2009).  
66 Many microbial symbionts act as mutualists, engaging in reciprocally beneficial interactions with  
67 their hosts in ways that can ameliorate environmental stress. For example, bacterial symbionts of  
68 insects, such as *Wolbachia*, can improve their hosts' thermal tolerance (Renoz et al., 2019; Truitt  
69 et al., 2019), and arbuscular mycorrhizal fungi, documented in 70-90% of families of land plants  
70 (Parniske, 2008), allow their hosts to persist through drought conditions by improving water and  
71 nutrient uptake (Cheng et al., 2021). On the other hand, changes in the mean and variance of  
72 environmental conditions may disrupt microbial mutualisms by changing the costs and benefits  
73 of the interaction for each partner in ways that can cause the interaction to deteriorate (Aslan  
74 et al., 2013; Fowler et al., 2024). Coral bleaching (the loss of symbiotic algae) due to temperature  
75 stress (Sully et al., 2019) is perhaps the best known example, but this phenomenon is not unique  
76 to corals. Lichens exposed to elevated temperatures experienced loss of photosynthetic function  
77 along with changes in the composition of their algal symbiont community (Meyer et al., 2022).  
78 How commonly and under what conditions microbial mutualisms deteriorate or strengthen under  
79 climate change remain unanswered questions (Frederickson, 2017). Previous work suggests that  
80 these alternative responses may depend on the intimacy and specialization of the interaction as well  
81 as the physiological tolerances of the mutualist partners (Rafferty et al., 2015; Toby Kiers et al.,  
82 2010; Warren and Bradford, 2014).

83 Understanding how microbial symbioses are affected by climate change is additionally compli-  
84 cated by spatial heterogeneity in the direction and magnitude of environmental change (IPCC, 2021).  
85 Beneficial symbionts are likely able to shield their hosts from environmental stress in locations that  
86 experience a small degree of change, but symbionts in locations that experience changes of large  
87 magnitude may be pushed beyond their physiological limits (Webster et al., 2008). Additionally,  
88 symbionts are often unevenly distributed across their host's distribution. Facultative symbionts  
89 may be absent from portions of the host range (Afkhami et al., 2014), and hosts may engage with

90 a diversity of partners (different symbiont species or locally-adapted strains) across environments  
91 (Fowler et al., 2023; Frade et al., 2008; Rolshausen et al., 2018). Identifying broader spatial trends  
92 in symbiont prevalence is therefore an important step in developing predictions for where to expect  
93 changes in the symbiosis in future climates.

94 *Epichloë* fungal endophytes are specialized symbionts of cool-season grasses, estimated from  
95 surveys to associate with ~ 20 – 30% of species in the diverse Poaceae family (Leuchtmann, 1992).  
96 Surveys within the cool-season grass subfamily (Pooideae) have found that associations occur for  
97 between 17% to 40% of sampled species (Card et al., 2014; Iannone et al., 2011). <sup>1</sup> They are  
98 predominantly transmitted vertically from maternal plants to offspring through seeds. Vertical  
99 transmission creates a feedback between the fitness of host and symbiont (Douglas, 1998; Fine,  
100 1975; Rudgers et al., 2009). Over time, endophytes that act as mutualists should rise in prevalence  
101 within a host population, particularly under environmental conditions that elicit protective benefits  
102 (Donald et al., 2021). *Epichloë* are known to improve their hosts' drought tolerance (Decunta et al.,  
103 2021) and protect their hosts against herbivores (Ambrose et al., 2014; Crawford et al., 2010) and  
104 pathogens (Tian et al., 2017; Xia et al., 2018) likely through the production of a suite of biologically  
105 active molecules, including diverse alkaloids, proteins, and other secondary metabolites. The fitness  
106 feedback induced by vertical transmission leads to the prediction that endophyte prevalence should  
107 be high in populations where these fitness benefits are most important. Previous survey studies  
108 of contemporary populations have documented large-scale spatial patterns in endophyte prevalence  
109 structured by environmental gradients (Afkhami, 2012; Bazely et al., 2007; Granath et al., 2007;  
110 Sneck et al., 2017). We predicted that endophyte prevalence should track temporal changes in  
111 environmental drivers (i.e. drought) that elicit strong fitness benefits.

112 Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic diversity  
113 of grass host species that harbor these symbionts (White and Cole, 1985), establishing that endo-  
114 phyte symbiosis could be identified in plant tissue from as early as 1851. However, no subsequent

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<sup>1</sup> *I have not made any edits here but I find it a little confusing to provide two sets of estimates for endophyte prevalence, one from Poaceae and one from Pooideae. Maybe that's just a me thing and not worth changing.*

115 studies, to our knowledge, have used the vast resources of biological collections to quantitatively  
116 assess spatio-temporal trends in endophyte prevalence and their environmental correlates. Grasses  
117 are commonly collected and identified based on the presence of their reproductive structures, mean-  
118 ing that preserved specimens typically contain seeds, conveniently preserving the seed-transmitted  
119 fungi along with their host plants on herbarium sheets. This creates the opportunity to leverage  
120 the unique spatio-temporal sampling of herbarium collections to examine the response of this sym-  
121 biosis to historical climate change. However, the predictive ability derived from historical analyses  
122 is rarely tested against contemporary data (Lee et al., 2024). Critically evaluating whether insights  
123 from historical reconstruction are predictive of variation across contemporary populations is a cru-  
124 cial step for the field to move from reading signatures of the past to forecasting ecological dynamics  
125 into the future.

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

141

## Methods

142

### *Focal species*

143 Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis perennans*,  
144 and *Elymus virginicus* that host *Epichloë* symbionts. These cool-season grass species have broad  
145 distributions covering much the eastern United States (Fig. 1) and are commonly represented in  
146 natural history collections. Cool-season grasses grow during the cooler temperatures of spring and  
147 autumn due to their reliance on *C*<sub>3</sub> photosynthesis. *A. hyemalis* is a small short-lived perennial  
148 species that germinates in autumn to late winter winter and typically flowers between March and  
149 July (most common collection month: May). *A. perennans* is of similar stature but is longer lived  
150 than *Agrostis hyemalis* and flowers in late summer and early autumn (most common collection  
151 month: September). *A. perennans* is more sparsely distributed, tending to be found in shadier and  
152 moister habitats, while *A. hyemalis* is commonly found in open and recently disturbed habitats.  
153 Both *Agrostis* species are recorded from throughout the Eastern US, but *A. perennans* has a slightly  
154 more northern distribution, whereas *A. hyemalis* is found rarely as far north as Canada and is listed  
155 as a rare plant in Minnesota. *E. virginicus* is a larger and longer-lived species that is more broadly  
156 distributed than the *Agrostis* species. It begins flowering as early as March or April but continues  
157 throughout the summer (most common collection month: July).

158 Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001; Leuchtmann et al., 2014),  
159 and *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl, 2002). The fungal sym-  
160 bionts primarily reproduce asexually and are passed from maternal plant to offspring by vertical  
161 transmission through seeds. These symbionts are also capable of horizontal transmission between  
162 hosts via the production of external reproductive structures, including sexual spore-bearing stromata  
163 that grow over host inflorescences (known as 'choke disease') and epiphyllous conidia on leaf surfaces  
164 that produce asexual spores (Tadych et al., 2014). Evidence suggests that production of horizontal  
165 transmission structures by *Epichloë* occurs at low levels, and may be influenced by environmental

166 and genotypic factors (Brem and Leuchtmann, 1999; Meijer and Leuchtmann, 2000; Tintjer et al.,  
167 2008). In line with this, monitoring of long-term plots of *A. perennans* and *E. virginicus* showed  
168 no production of stromata on *A. perennans* and on only less than 1% of *E. virginicus* plants over  
169 14 years (Fowler et al., 2024). Some host species have shown the capacity to partner with multiple  
170 symbiont species or strains, and in some cases multiple symbiont lineages can co-exist within a  
171 host population (Mc Cargo et al., 2014). However, surveys have typically found limited *Epichloë*  
172 genotypic diversity within host populations (Treindl et al., 2023). Across host populations, con-  
173 centrations of biologically-active biomolecules and the genes associated with their production vary  
174 substantially (Schardl et al., 2012). In this analysis, we focus on the presence/absence of *Epichloë*  
175 symbionts, and we discuss potential implications of symbiont genotypic diversity in the Discussion.

176

177

### *Herbarium surveys*

178 We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens included  
179 from each collection). With permission from herbarium staff, we acquired seed samples from 1135  
180 *A. hyemalis* specimens collected between 1824 and 2019, 357 *A. perennans* specimens collected  
181 between 1863 and 2017, and 854 *E. virginicus* specimens collected between 1839 and 2019 (Fig. 1,  
182 Fig. 2A, Fig. A1). We chose our focal species in part because they are commonly represented in  
183 herbarium collections and produce many seeds, meaning that small samples would not diminish the  
184 value of specimens for future studies. We collected 5-10 seeds per specimen after examining the  
185 herbarium sheet under a dissecting microscope to ensure that we collected mature seeds, not florets  
186 or unfilled seeds, fit for our purpose of identifying fungal endophytes with microscopy. We excluded  
187 specimens for which information about the collection location and date were unavailable.

188 Each specimen was assigned geographic coordinates based on collection information recorded on  
189 the herbarium sheet using the geocoding functionality of the *ggmap* R package (Kahle and Wickham,  
190 2019). Many specimens had digitized collection information readily available, but for those that  
191 did not, we transcribed information printed on the herbarium sheet. The identity of each specimen

192 collector was gathered as part of the sample's metadata. Collections were geo-referenced to the  
193 nearest county centroid, or nearest municipality when that information was available. For fifteen  
194 of the oldest specimens, only information at the state level was available, and so we used the state  
195 centroid. The median pairwise distance between georeferenced coordinate points was 841 km. The  
196 median longitudinal width of the bounding boxes generated to geocode municipality, county, or  
197 state centroids was 44.7 km. Among those specimens geo-referenced at the state level, the largest  
198 bounding box, spanning the state of Texas, was 1233 km wide. The smallest bounding boxes were  
199 less than 1 km across for small municipalities (while this suggests high precision, we note that some  
200 specimens were collected in natural habitat nearby to small municipalities not encompassed by these  
201 bounding boxes).

202 Our visits focused on herbaria with historic strengths in grass collections (e.g. Texas A&M,  
203 Missouri Botanic Garden) and other herbaria in the Southern Great Plains region of the United  
204 States. While these nine herbaria garnered specimens that span the focal species' ranges, our dataset  
205 unevenly samples across the study region (Fig. 1). Texas, Oklahoma, Louisiana, and Missouri are  
206 the most represented states. Uneven sampling was most pronounced for *A. perennans*, which has  
207 much of its range in the northeastern US. We explore the potential influence of spatial bias in  
208 sampling on our results through a simulation analysis (Appendix A - Supporting Methods).

209 After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hyphae  
210 in each specimen using microscopy. We first softened seeds with a 10% NaOH solution, then stained  
211 the seeds with aniline blue-lactic acid stain and squashed them under a microscope cover slip. We  
212 examined the squashed seeds for the presence of fungal hyphae at 200-400X magnification (Bacon  
213 and White, 2018). On average we scored 4.7 intact seeds per specimen of *A. hyemalis*, 4.2 seeds  
214 per specimen of *A. perennans*, and 3.8 seeds per specimen of *E. virginicus*; we scored 10,342 seeds  
215 in total. Due to imperfect vertical transmission, the production of symbiont-free offspring from  
216 symbiotic hosts (Afkhami and Rudgers, 2008), it is possible that symbiotic host-plants produce a  
217 mixture of symbiotic and non-symbiotic seeds. We therefore designated a specimen as endophyte-  
218 symbiotic if *Epichloë* hyphae were observed in one or more of its seeds, or non-symbiotic if *Epichloë*

219 hyphae were observed in none of its seeds. To capture uncertainty in the endophyte identification  
220 process, we recorded both a "liberal" and a "conservative" endophyte score for each plant specimen.  
221 When we confidently identified endophytes within a specimen's seeds, we assigned matching liberal  
222 and conservative scores. When we identified potential endophytes with unusual morphology, low  
223 uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a positive  
224 identification for the liberal score and a negative identification for the conservative score. We  
225 recorded the identity of each scorer as part of the data collection process. 89% of scored plants  
226 had matching liberal and conservative scores, reflecting high confidence in endophyte status. The  
227 following analyses used the liberal status, however repeating all analyses with the conservative status  
228 yielded qualitatively similar results (Fig. A8).

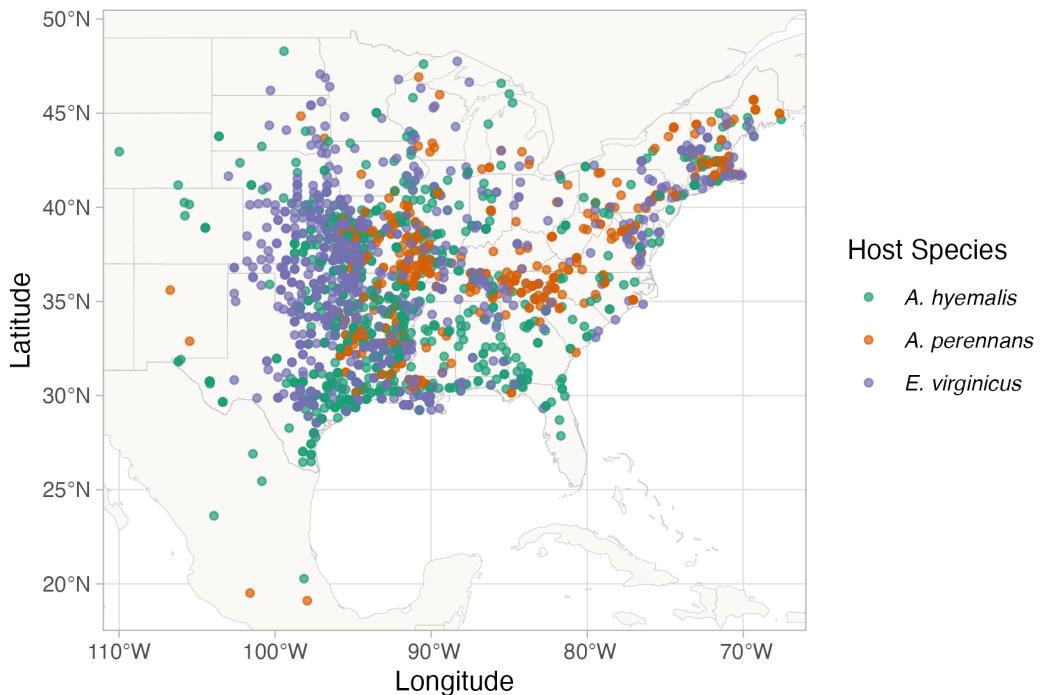


Figure 1: **Collection locations of herbarium specimens sampled for *Epichloë* endophytes.** Specimens span eastern North America from nine herbaria, and are colored by host species (*A. hyemalis*: green, *A. perennans*: orange, *E. virginicus*: purple). Map lines delineate study areas and do not necessarily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

229        *Modeling spatial and temporal changes in endophyte prevalence*

230        We assessed spatial and temporal changes in endophyte prevalence across each host distribution,  
231        quantifying the “global” temporal trends averaged across space, and then examining spatial hetero-  
232        geneity in the direction and magnitude of endophyte change (hotspots and coldspots) across the spa-  
233        tial extent of each host’s distribution. To account for the spatial non-independence of geo-referenced  
234        occurrences, we used an approximate Bayesian method, Integrated Nested Laplace Approximation  
235        (INLA), to construct spatio-temporal models of endophyte prevalence. INLA provides a computa-  
236        tionally efficient method of ascertaining parameter posterior distributions for certain models that  
237        can be formulated as latent Gaussian Models (Rue et al., 2009). Many common statistical models,  
238        including structured and unstructured mixed-effects models, can be represented as latent Gaussian  
239        Models. We incorporated spatial heterogeneity into this analysis using spatially-structured intercept  
240        and slope parameters implemented as stochastic partial differential equations (SPDE) to approxi-  
241        mate a continuous spatial Gaussian process. This SPDE approach is a flexible method of smoothing  
242        across space while explicitly accounting for spatial dependence between data-points (Bakka et al.,  
243        2018; Lindgren et al., 2011). Fitting models with structured spatial effects is possible with MCMC  
244        sampling but can require long computation times, making INLA an effective alternative. This ap-  
245        proach has been used to model spatial patterns in flowering phenology (Willems et al., 2022), the  
246        abundance of birds (Meehan et al., 2019) and butterflies (Crossley et al., 2022), the distribution of  
247        temperate trees (Engel et al., 2022) as well as the population dynamics of endangered amphibians  
248        (Knapp et al., 2016) and other ecological processes (Beguin et al., 2012).

249        We estimated global and spatially-varying trends in endophyte prevalence using a joint-likelihood  
250        model. For each host species  $h$ , endophyte presence/absence of the  $i^{th}$  specimen ( $P_{h,i}$ ) was modeled  
251        as a Bernoulli response variable with expected probability of endophyte occurrence  $\hat{P}_{h,i}$ . We modeled  
252         $\hat{P}_{h,i}$  as a linear function of collection year, with intercept  $A_h$  and slope  $T_h$  defining the global  
253        temporal trend in endophyte prevalence specific to each host species as well as with spatially-  
254        varying intercepts  $\alpha_{h,l_i}$  and slopes  $\tau_{h,l_i}$  associated with location ( $l_i$ , the unique latitude-longitude

combination of the  $i$ th observation). The joint-model structure allowed us to “borrow information” across species in the estimation of shared variance terms for the spatially-dependent random effect  $\delta_{l_i}$ , intended to account for residual spatial variation, and  $\chi_{c_i}$  and  $\omega_{s_i}$ , the i.i.d.-random effects indexed for each collector identity ( $c_i$ ) and scorer identity ( $s_i$ ) of the  $i^{th}$  specimen.

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

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

We performed model fitting using the *inlabru* R package (Bachl et al., 2019). Global intercept and slope parameters,  $A$  and  $T$ , were given vague priors. Collector and scorer random effects,  $\chi$  and  $\omega$  respectively, were centered at 0 with precision parameters assigned penalized complexity (PC) priors with parameter values  $U_{PC} = 1$  and  $a_{PC} = 0.01$  (Simpson et al., 2017). Each spatially-structured parameter depended on a covariance matrix according to the proximity of each pair of collection locations (Bakka et al., 2018; Lindgren et al., 2011). The covariance matrix was approximated using a Matérn covariance function, with each data point assigned a location according to the nodes of a mesh of non-overlapping triangles encompassing the study area (Fig. A2). Matérn covariance functions are widely used in spatially explicit statistical modeling because of their mathematical tractability and flexibility. This covariance structure relies on the assumption that the underlying process is stationary and isotropic, such that spatial autocorrelation between data points depends only on their relative positions (Bakka et al., 2018).

Implementing spatially-structured parameters in INLA with this SPDE approach is useful particularly because space is treated as a continuous variable, allowing the model to make efficient use

280 of the data and generate predictions across the entire study region. The SPDE approach is flexible  
281 enough that it can capture smooth trends across space that are informed by the data rather than  
282 by spatial regions chosen *a priori* by researchers. However this flexibility also invites the risk of  
283 overfitting, as with other non-linear modeling approaches (Lapeyrolerie and Boettiger, 2023; Ra-  
284 mampiandra et al., 2023; Ward et al., 2014). Priors for the Matérn covariance function, termed  
285 “range” and “variance”, define how proximity effects decay with distance. The choice of priors for  
286 these types of spatial models is an area of active research (Bakka et al., 2018; Simpson et al., 2017),  
287 but another advantage of the INLA approach is that its computational efficiency allows for prior  
288 sensitivity analyses. Results presented in the main text reflect a prior range of 342 kilometers (i.e.  
289 a 50% probability of estimating a range less than 342 kilometers). We tested a range of values  
290 (from 68 kilometers to 1714 kilometers) and meshes (presented in the Supporting Methods – *Mesh*  
291 and *Prior Sensitivity Analysis*), finding that while the magnitude and uncertainty of effects varied,  
292 model results were qualitatively similar, i.e. the same direction of effects across space. We assessed  
293 model fit with visual posterior predictive checks (Fig. A3) and measurements of AUC (Figs. A4-  
294 A5) (Gelman and Hill, 2006). Through results and discussion that follow, we refer to the model  
295 described in this section as the “endophyte prevalence model”.

## 296 *Modeling distributions of host species*

297 The herbarium records did not encompass the entirety of each host species’ range. Therefore, we  
298 used additional data sources to model the geographic distribution of each host species, with two  
299 goals: (1) generate realistic maps on which we could project the predictions of the INLA model,  
300 and (2) use the geographic distributions to test for relationships between climate change drivers and  
301 trends in endophyte prevalence. We followed the ODMAP (overview, data, model, assessment,  
302 prediction) protocol (Crossley et al., 2022) (see Supporting Methods). In short, we used presence-  
303 only observations of each host species from Global Biodiversity Information Facility (GBIF) between  
304 1990 to 2020 (713 occurrence records for *A. hyemalis* (GBIF.Org, 2025a), 656 occurrence records  
305 for *A. perennans* (GBIF.Org, 2025b), and 2338 occurrence records for *E. virginicus* (GBIF.Org,

306 2025c)). We fit maximum entropy (MaxEnt) models using the maxent function in the R package  
307 *dismo* (Hijmans et al., 2017) using the following seasonal climate predictors (1990-2020 climate  
308 normals): mean and standard deviation of spring, summer, and autumn temperature, and mean  
309 and standard deviation of spring, summer, and autumn cumulative precipitation.

310 We generated 10,000 pseudo-absences as background points, and split the occurrence data into  
311 75% for model training and 25% for model testing. The performance of models was evaluated with  
312 AUC (Jiménez-Valverde, 2012). We found AUC values of 0.862, 0.838, 0.821 respectively for *Agrostis*  
313 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus* indicating good model fit to data. We used  
314 the training sensitivity (true positive rate) and specificity (true negative rate) to set a threshold for  
315 transforming the continuous predicted probabilities into binary presence - absence host distribution  
316 maps on which we projected INLA predictions of endophyte prevalence (Liu et al., 2005).

317 *Assessing the role of climate drivers*

318 We assessed how the magnitude of climate change may have driven changes in endophyte prevalence  
319 by assessing correlations between changes in climate and changes in endophyte prevalence predicted  
320 from our spatial model at evenly spaced pixels across the study area.

321 We first downloaded monthly temperature and precipitation rasters from the PRISM climate  
322 group (Daly and Bryant, 2013) covering the time period between 1895 and 2020 using the *prism*  
323 R package (Hart and Bell, 2015). PRISM provides reconstructions of historic climate variables  
324 across the United States by spatially interpolating weather station data (Di Luzio et al., 2008).  
325 Because the magnitude of observed climate change differs across seasons, and because different  
326 growing seasons is a key feature of the biology of our focal host species, we calculated 30-year  
327 climate normals for seasonal mean temperature and cumulative precipitation for the recent (1990  
328 to 2020) and historic (1895 to 1925) periods. We used three four-month seasons within the year  
329 (Spring: January, February, March, April; Summer: May, June, July, August; Autumn: September,  
330 October, November, December). This division of seasons allowed us to quantify differences in  
331 the primary climate change drivers, temperature and precipitation, associated with the two “cool”

332 seasons, when we expected our focal species to be most active (*A. hyemalis* flowering phenology:  
333 spring; *E. virginicus*: spring and summer; *A. perennans*: autumn). In addition to mean climate  
334 conditions, environmental variability itself can influence population dynamics (Tuljapurkar, 1982)  
335 and changes in variability are a key prediction of climate change models (IPCC, 2021; Stocker et al.,  
336 2013). Therefore, we calculated the standard deviation for each annual and seasonal climate driver  
337 across each 30-year period. We then took the difference between recent and historic periods for the  
338 mean and standard deviation for each climate driver (Figs. A13-A15). All together, we assessed  
339 twelve potential climate drivers: the mean and standard deviation of spring, summer, and autumn  
340 temperature, as well as the mean and standard deviation of spring, summer, and autumn cumulative  
341 precipitation (the same climate covariates used in the MaxEnt models).

342 We then evaluated whether areas that have experienced the greatest changes in endophyte preva-  
343 lence (hotspots of endophyte change) are associated with high degrees of change in climate (hotspots  
344 of climate change). To do so, we modeled the fitted, spatially-varying slopes of endophyte change  
345 through time ( $\tau_{h,l}$ ) as a linear function of environmental covariates, with a Gaussian error distribu-  
346 tion for a set of pixels across each host distribution. The continuous SPDE approach taken from  
347 our endophyte prevalence model allows us to generate predictions of temporal trends in prevalence  
348 at arbitrarily many pixels across each host distribution. Balancing computation time with resolu-  
349 tion, we generated predicted trends for 546, 645, and 753 pixels across each host distribution for *A.*  
350 *perennans*, *A. hyemalis*, and *E. virginicus* respectively (pixel dimensions: *A. perennans* = 65 km  
351 x 36 km; *A. hyemalis* = 61km x 45 km; *E. virginicus* = 62 km x 40 km ). Fitting regressions to  
352 many pixels across the study region risks artificially inflating confidence in our results due to large  
353 sample sizes, and so we performed this analysis using only a random subsample of 250 pixels across  
354 the study region; other sizes of subsample yielded similar results. Data from each host species were  
355 analyzed separately. Throughout the results and discussion that follow, we refer to this analysis as  
356 the “*post hoc* climate regression analysis”.

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

358        We evaluated the predictive ability of the endophyte prevalence model using both in-sample training  
359        data from the herbarium surveys, and with out-of-sample test data, an important but rarely used  
360        strategy in ecological studies (Lee et al., 2024; Tredennick et al., 2021). We generated out-of-  
361        sample test data from contemporary surveys of endophyte prevalence in natural populations of *A.*  
362        *hyemalis* and *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted  
363        in 2013 as described in Sneck et al. (2017), and surveys of *A. hyemalis* took place between 2015  
364        and 2020. Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation  
365        in endophyte prevalence towards its range edge, while surveys of *E. virginicus* were designed to  
366        cover latitudinal variation. In total, we visited 43 populations of *A. hyemalis* and 20 populations  
367        of *E. virginicus* across the south-central US, with emphasis on Texas and neighboring states (Fig  
368        A12). number of plants sampled per population: 22.9); note that this sampling design provided  
369        greater local depth of information than the herbarium records, where only one plant was sampled at  
370        each locality. We quantified the endophyte status of each individual with microscopy as described  
371        for the herbarium surveys (with 5-10 seeds scored per individual), and calculated the prevalence  
372        of endophytes within the population (proportion of plants that were endophyte-symbiotic). For  
373        each population, we compared the observed fraction of endophyte-symbiotic hosts to the predicted  
374        probability of endophyte occurrence  $\hat{P}$  derived from the model for that location and year. The  
375        contemporary survey period (2013-2020) is at the most recent edge of the time period encompassed  
376        by the historical specimens used for model fitting.

377        **Results**

378        *How has endophyte prevalence changed over time?*

379        Across more than 2300 herbarium specimens dating back to 1824, we found that prevalence of  
380        *Epichloë* endophytes increased over the last two centuries for all three grass host species (Fig. 2).

<sup>381</sup> On average, endophytes of *A. perennans* and *E. virginicus* increased from ~ 40 % to 70% prevalence  
<sup>382</sup> across the study region, and *A. hyemalis* increased from ~ 25% to over 50% prevalence. Our model  
<sup>383</sup> indicates high confidence that overall temporal trends are positive across species (99% probability  
<sup>384</sup> of a positive overall year slope in *A. hyemalis*, 92% probability of a positive overall year slope in *A.*  
<sup>385</sup> *perennans*, and 91% probability of a positive overall year slope in *E. virginicus*) (Fig. A6).

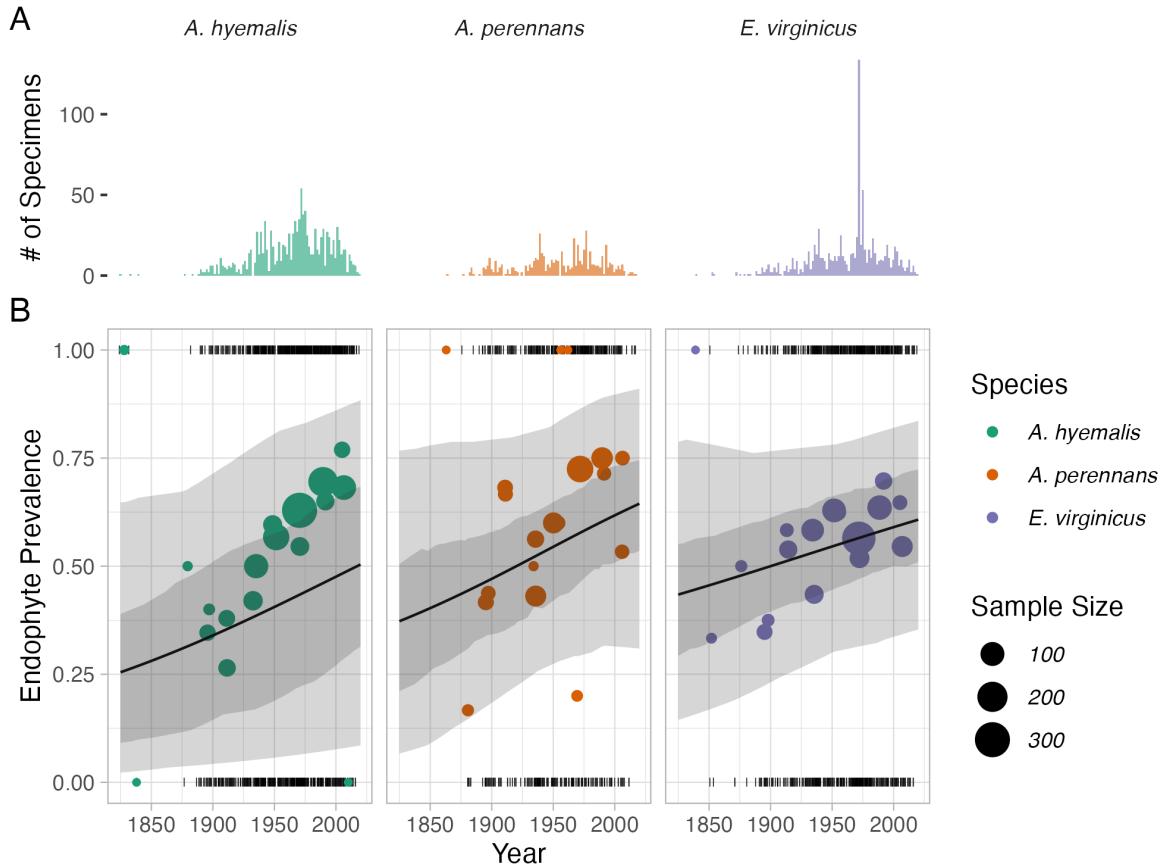


Figure 2: **Temporal trends in endophyte prevalence.** (A) Histograms show the frequency of scored specimens through time for each host species. (B) Lines show mean endophyte prevalence predicted by the endophyte prevalence model over the study period along with the 50% and 95% CI bands incorporating 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.

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

390 and models fit with and without this random effect provide qualitatively similar results. The identity  
391 of individual scorers, the researchers who identified endophyte status microscopically, did contribute  
392 to observed patterns in endophyte prevalence. For example, 3 of the 25 scorers were significantly  
393 more likely than average to assign positive endophyte status, as indicated by 95% credible intervals  
394 greater than zero, while 4 of the 25 had 95% credible intervals below zero (Fig. A10).

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

396 While there was an overall increase in endophyte prevalence, our model revealed hotspots and  
397 coldspots of change across the host species' ranges, which are mapped in Fig. 3 across geographic  
398 ranges predicted by MaxEnt species distribution models. In some regions, posterior mean estimates  
399 of spatially varying temporal trends indicate that *A. hyemalis* and *A. perennans* experienced in-  
400 creases in prevalence by as much as 2% per year over the study period. Posterior estimates of  
401 uncertainty in spatially varying slopes indicate that these hotspots of change may have experienced  
402 increases of up to 5% per year while declines in prevalence may be as great as -4% per year for  
403 the *Agrostis* species. (Fig. A7) In contrast, *E. virginicus* experienced increases up to around 1%  
404 per year, with uncertainty ranging between 3.5% increases and 2.5% decreases (Fig. A7) Taken  
405 together, both *Agrostis* species show areas of both strong increasing and declining prevalence, while  
406 *E. virginicus* had an overall weaker and geographically more homogeneous increase in endophyte  
407 prevalence. Notably, endophytes are predicted to have increased most strongly towards the western  
408 range edge of *A. hyemalis* (Fig. 3A) and across the northeastern US for *A. perennans* (Fig. 3B).  
409 Broad increases in prevalence on average, along with increases towards range edges that had low  
410 historic prevalence result in range expansions of the symbiosis for both *Agrostis* species (Fig. 4).  
411 Increases in prevalence were strongest in regions with low historic prevalence for the *Agrostis* species  
412 (Fig. A11 A-B), but for *E. virginicus* trends did not differ according to historic prevalence (A11  
413 C).

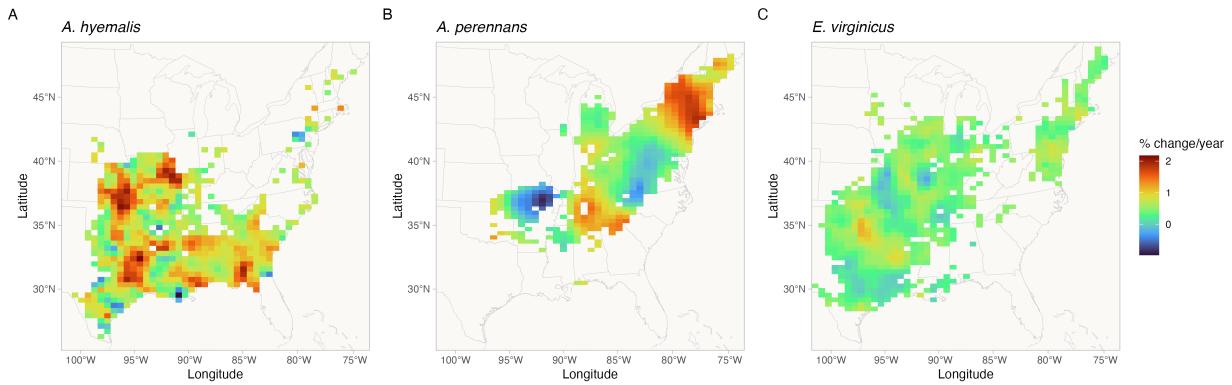


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species ((A)*A. hyemalis*; (B)*A. perennans*; (C)*E. virginicus*). Spatially-varying trends are estimated from the endophyte prevalence model. Color indicates the relative change in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

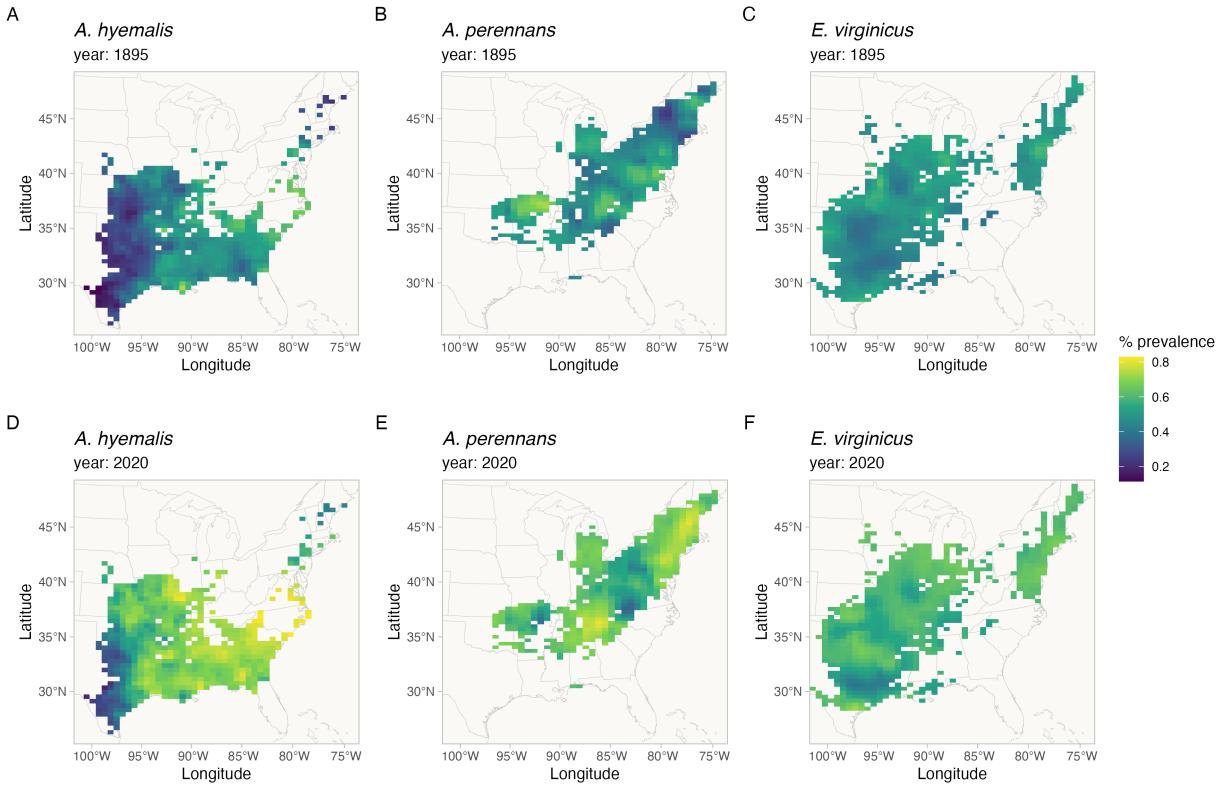


Figure 4: Predicted endophyte prevalence for each host species in 1895 and 2020. Predictions of prevalence come from the endophyte prevalence model. Color indicates the posterior mean endophyte prevalence for (A, D) *A. hyemalis*, (B, E) *A. perennans*, and (C, F) *E. virginicus*. Map lines delineate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

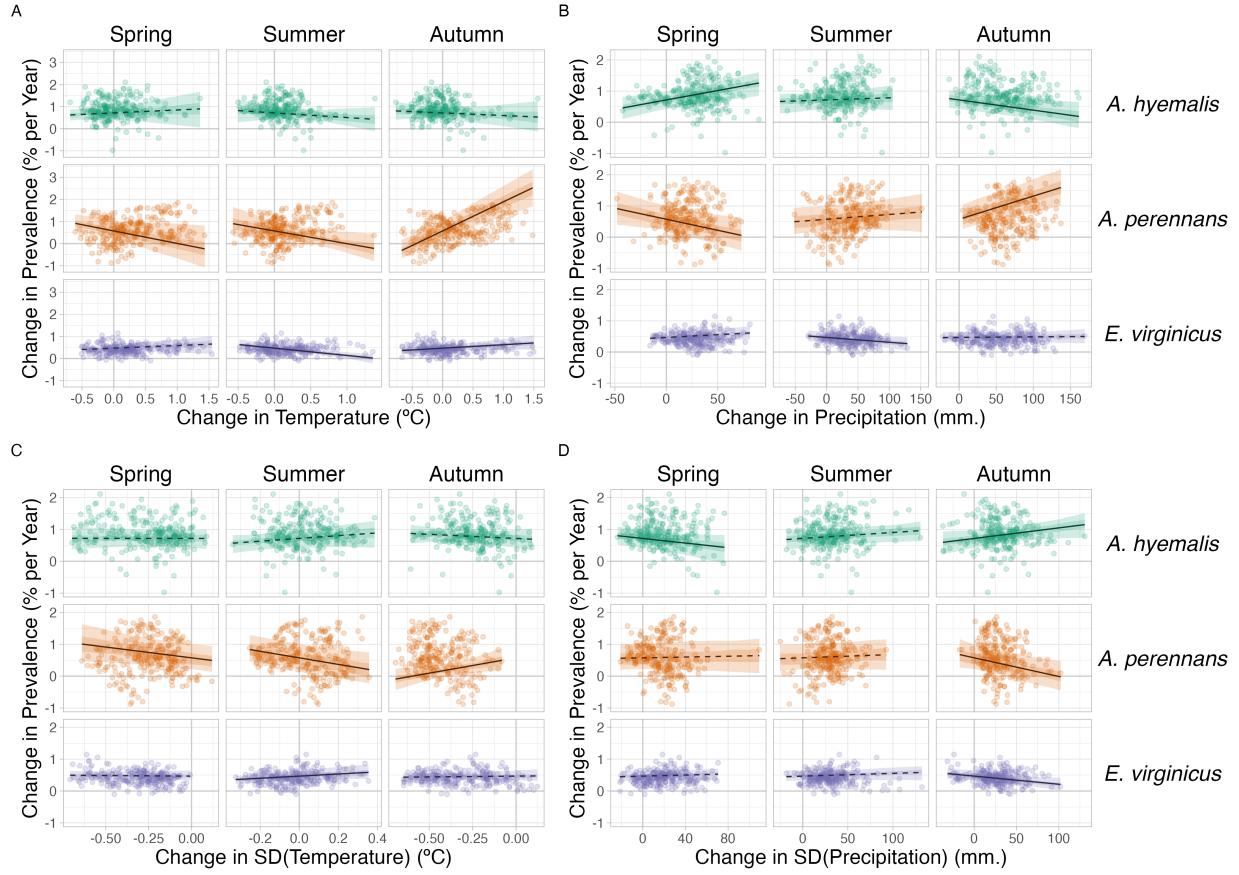
414     What is the relationship between variation in temporal trends in endophyte  
415     prevalence and changes in climate drivers?

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

421 region, climate changes were spatially variable; certain locations experienced increases in annual  
422 precipitation as large as 375 mm or decreases up to 54 mm across the last century, while annual  
423 temperature changes ranged from warming as great as 1.4 °C to cooling by 0.46 °C.

424 Spatially variable climate trends were predictive of trends in endophyte prevalence. For exam-  
425 ple, among the tested climate drivers, strong increases in endophyte prevalence for *A. perennans*  
426 were most strongly associated with increasing autumn precipitation and with increasing mean and  
427 variability in autumn temperature (greater than 97% posterior probabilities of positive slopes). For  
428 this species, each 1 °C increase in autumn temperature was associated with a 1.07 % greater increase  
429 per year in endophyte prevalence (Fig. 5A) and a 100 mm increase in precipitation was associated  
430 with a 0.8% greater increase per year in endophyte prevalence (Fig. 5B). This result aligns with  
431 the species' autumn active growing season, however other seasonal climate drivers were also posi-  
432 tively associated with increasing endophyte prevalence in this host species. In particular, we found  
433 cooler and drier springs and cooler summers to be associated with increasing endophyte prevalence  
434 (greater than 99% posterior probabilities of negative slopes), though these slopes were generally of  
435 smaller magnitude than those for autumn climate drivers. Changes in endophyte prevalence across  
436 the ranges of *A. hyemalis* and *E. virginicus* were less strongly driven by changes in climate. Like  
437 *A. perennans*, climate during peak growing season (spring for *A. perennans* and summer for *E. vir-*  
438 *ginicus*) emerged most commonly as drivers of changes in endophyte prevalence. Across the tested  
439 climate drivers, increases in mean spring precipitation were the strongest predictor of increasing  
440 trends in endophyte prevalence for *A. hyemalis* (Fig. 5B) (greater than 99% posterior probability  
441 of a positive slope). For this species, an increase of 100 mm in spring precipitation was associated  
442 with 0.6% per year stronger increases in endophyte prevalence relative to regions with no change in  
443 precipitation. The next greatest slopes were those associated with variability in spring precipitation  
444 (greater than 96% posterior probability of a negative slope), as well as in the mean and variabil-  
445 ity of autumn climate (greater than 98% probability of negative and positive slopes, respectively).  
446 Changes in endophyte prevalence in *E. virginicus* were not strongly associated with changes in most  
447 climate drivers, but regions with reduced variability in autumn precipitation (Fig. 5B) and with

448 cooler and more variable summer temperatures (Fig. 5A,C) experienced the largest increases in  
449 endophyte prevalence. Our analysis indicated relatively high confidence that these climate drivers  
450 influence endophyte prevalence shifts in *E. virginicus* (greater than 99% posterior probability of ei-  
451 ther negative or positive slopes respectively), however they translate, for example, to less than a  
452 0.4% decrease in endophyte prevalence per year for each  $1^{\circ}C$  of summer warming over the century.  
453 Repeating this analysis using all pixels across each species' distribution were qualitatively similar  
454 to these results.



**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% posterior 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.

455

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

456 Tests of the endophyte prevalence model's predictive performance, as quantified by AUC and by  
457 visual posterior predictive checks, indicated good predictive ability. Model performance was similar  
458 between historic herbarium specimens used as training data and the out-of-sample test data from  
459 contemporary surveys (AUC = 0.79 and 0.77 respectively; Fig. A5-A4). The model successfully  
460 captured broad regional trends in endophyte prevalence seen in the contemporary survey data,  
461 such as decline endophyte prevalence in *A. hyemalis* towards western longitudes (Fig. 6A) and an  
462 increase towards northern latitudes (Fig. 6B). It is noteable that model predictions for endophyte  
463 prevalence exhibited relatively little local geographic variation, whereas the out-of-sample survey  
464 data were highly variable with populations spanning 0% to 100% endophyte-symbiotic plants (Fig.  
465 6C), indicating population-to-population variation not captured in the endophyte prevalence model.

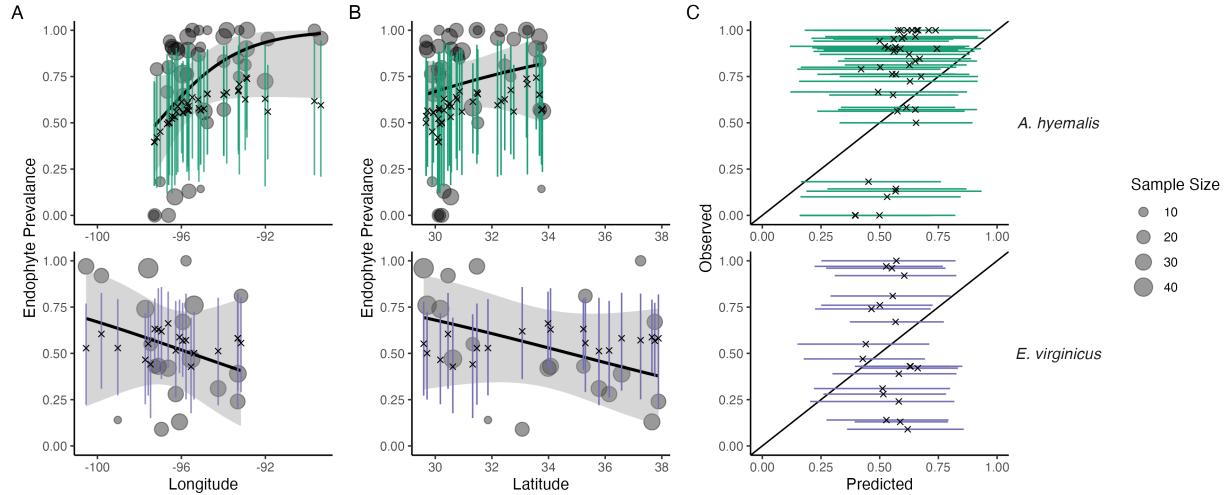


Figure 6: **Predictive performance for contemporary test data.** (A) The endophyte prevalence model, trained on historic herbarium collection data, performed modestly at predicting prevalence in contemporary population surveys. The model captured regional trends across (A) longitude and (B) latitude. 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 contemporary observed population prevalence vs. predicted endophyte prevalence shows that contemporary test data had more variance between populations than in model predictions.

## Discussion

466

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

473 prevalence within a population (Donald et al., 2021; Fine, 1975). We further found that trends  
474 in endophyte prevalence often varied across the host distribution in association with changes in  
475 climate drivers, consistent with the hypothesis that increases in endophyte prevalence are driven  
476 by context-dependent benefits to hosts that confer resilience under environmental change. Taken  
477 together, our results suggest an overall strengthening of host-symbiont mutualism over the last two  
478 centuries.

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

480 Differences across host species underscore that while all of these  $C_3$  grasses share similar broad-scale  
481 distributions, each engages in unique biotic interactions and has unique responses to environmental  
482 drivers. We identified hotspots of change for *A. perennans*, which was the species whose endophyte  
483 prevalence was most responsive to changes in climate drivers (Fig. 5). Predicted declines of 0.9%  
484 per year in the southern portion of its range and predicted increases of up to 2% per year in the  
485 north suggest a potential poleward range shift of endophyte-symbiotic plants (Fig. 3B); whether  
486 the overall host distribution is shifting in parallel is an exciting next question.

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

499 time for the species' germination. Previous work demonstrated drought benefits in a greenhouse  
500 manipulation with this host-symbiont pair (Davitt et al., 2011), and early life stages may be partic-  
501 ularly vulnerable to prolonged droughts. For *E. virginicus*, which experienced the weakest changes  
502 in endophyte prevalence overall (ranging between 1.1% increases and 0.2% decreases), we only found  
503 modest associations with changes in climate drivers. Surveys by Sneck et al. (2017), used as part  
504 of the test data in this study, identified a drought index (SPEI) that integrates precipitation with  
505 estimated evapotranspiration as an important predictor of contemporary endophyte prevalence in  
506 this species. The diverse relationships we detect between trends in endophyte prevalence and cli-  
507 mate drivers suggest a more complicated picture than the simple explanation that drought alone, as  
508 measured through changes in annual precipitation, causes increasing endophyte prevalence through  
509 context-dependent fitness benefits.

510 While we show consistent increasing trends in prevalence between the three species, the mecha-  
511 nisms that explain these changes may be diverse and idiosyncratic. First, climate change responses  
512 may depend on genotype-specific responses that are not considered in our current analysis. While  
513 *Epichloë* symbioses are highly specialized, surveys have demonstrated genotypic and chemotypic  
514 diversity of the symbionts among and within populations (Treindl et al., 2023; von Cräutlein et al.,  
515 2021). Genotypic variation in *Epichloë* endophytes, particularly in genes responsible for alkaloid  
516 production, produces "chemotypes" with differing benefits for hosts against insect or mammalian  
517 herbivores mediated by environmental conditions (Ambrose et al., 2014; Saikkonen et al., 2013;  
518 Schardl et al., 2012). Genotypic variation of the hosts themselves can also influence interaction out-  
519 comes (Gundel et al., 2011a; Parker et al., 2017). Whether hotspots of change in endophyte preva-  
520 lence reflect selection for genotype-pairings with particularly strong fitness benefits is an unanswered  
521 question. Additionally, *Epichloë* endophytes have been connected to a suite of non-drought related  
522 fitness benefits including herbivory defense (Brem and Leuchtmann, 2001), salinity resistance (Wang  
523 et al., 2020), and mediation of pathogens (Vikuk et al., 2019) and the soil microbiome (Roberts and  
524 Ferraro, 2015). Broad changes in the distribution and abundance of natural enemies (Côté et al.,  
525 2004), along with stresses from anthropogenic changes in land management and pollution (Sage,

526 2020) likely influence the benefits of symbiosis (Rudgers et al., 2020). Changing endophyte preva-  
527 lence results from the combination of net fitness benefits playing out across the heterogeneous map  
528 of a changing climate and and its interactive effects on other anthropogenic drivers. Host species  
529 experience a world that is made increasingly stressful by a combination of global change drivers,  
530 and while historic trends that we observed suggest that symbiotic fitness benefits have helped mit-  
531 iate this stress, it is possible that at yet higher levels of stress, increasing costs of the mutualism  
532 could lead to declines in endophyte prevalence. It is also know that stressful conditions can reduce  
533 the rate of successful transmission of *Epichloë*<sup>2</sup> endophytes from mother plant to offspring (Gundel  
534 et al., 2011b), a likely consequences of host sanctions on costly symbionts or symbiont failure to  
535 successfully colonize seeds (Afkhami and Rudgers, 2008; Gundel et al., 2011c). While we did not  
536 investigate differences in the rate of transmission across these historic specimens due to low numbers  
537 of sampled seeds per individual, it would be valuable to conduct deeper sampling for a subset of  
538 specimens with known endophyte status and investigate how transmission itself may respond to  
539 environmental change. More extreme climate stresses, which are expected more frequently in the  
540 future (Seneviratne et al., 2021) could shift the balance of interactions costs and benefits. Identify-  
541 ing ‘tipping points’ of mutualism breakdown under increasing environmental stress is an important  
542 area of future inquiry.

543 Our results indicate that *Epichloë* symbiosis has likely improved host fitness in stressful en-  
544 vironments leading to increasing prevalence. What is less clear is how this will influence future  
545 range shifts. Based on our analysis, it is likely that the symbiosis will facilitate range shifts for  
546 hosts by improving population growth at range edges. Previous population surveys (Rudgers and  
547 Swafford, 2009; Semmartin et al., 2015; Sneck et al., 2017) attributed environment-dependent gra-  
548 dients in endophyte prevalence to symbiont-derived fitness benefit’s allowing hosts to persist in  
549 environments where they otherwise could not (Afkhami et al., 2014; Kazenel et al., 2015). How-  
550 ever, symbiont-facilitated range shifts require that endophytes be present in the populations to be

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<sup>2</sup> I think this statement is too strong given that there is also evidence that stress promotes transmission – see Pedro Gundel’s paper on *Poa autumnalis*.

able to contribute to population growth. For example, the arid western range edge of *A. hyemalis* has had historically low endophyte prevalence (Fig. 4), and dispersal of symbionts may limit the capacity for range shifts. A range edge population with no endophytes would require dispersal of symbionts to occur, either via symbiotic seeds or horizontally-transmitted fungal spores, before the symbiosis could result in an expanding distribution (Fowler et al., 2023) At the same time, we found that endophyte prevalence has increased most quickly in regions with historically low endophyte prevalence (Fig. A11), suggesting strong selection for symbiotic hosts when they are present. These factors potentially contribute to the ability of the host species to track its environmental niche. Another interesting question is the degree to which symbiotic and non-symbiotic hosts, which occupy overlapping but distinct niches, are likely to experience distribution shifts in tandem or at different rates in the future.

## 562 *Steps towards forecasts of host-microbe symbioses*

The combination of a spatially-explicit model and historic herbarium specimens allowed us to identify regions of both increasing and decreasing endophyte prevalence. We see several next steps toward the goal of predicting host and symbiont niche-shifts in response to future climate change. While the model successfully predicted large-scale spatial trends observed in the out-of-sample contemporary population surveys, these data contained more population-to-population variability in prevalence than could be explained by the model. We interpret this to mean that the model captures coarse-scale spatial and temporal trends reasonably well, but is not equipped to capture local-scale nuances that generate population-to-population differences. Validating our model predictions with this test, a rare extra step in collections-based studies, allows us to identify ways in which the model's out-of-sample predictive ability could be improved. Lack of information on local variability in symbiont prevalence may simply be a feature of data derived from herbarium specimens. Natural history collectors sample one or a few specimens from local populations, and these observations are aggregated by the model to derive broad-scale estimates. This suggests that increasing local replication should be a factor considered in future collection efforts of natural history

577 specimens, balancing the required time and effort along with limitations on storage space within  
578 collections. Herbarium collections were predominately used for taxonomic research in the past,  
579 but use of specimens to understand ongoing global change would benefit from increased collection  
580 efforts and expansion of herbarium collections. An alternative validation test would be to hold-out  
581 samples from the historic data set. Such a test would more clearly match the conditions of the  
582 training data (i.e., in spatial scale and climate conditions), however the trade-off between training  
583 and testing the model with a limited number of sampled specimens held us back from exploring this  
584 option. Splitting datasets can negatively impact model estimates, and the choice of how to split  
585 the data for model validation is not trivial (Bergmeir and Benítez, 2012; James et al., 2013).

586 Another key consideration in forecasting the dynamics of host-microbe symbioses is the spatial  
587 scale of both specimen georeferencing and available climate data. For this analysis, most specimen  
588 localities were assigned coordinates at county or city centroids, and the climate data examined  
589 was on 4 km grid cells. Georeferencing of specimens as accurately as possible is a key priority  
590 of herbarium specimen digitization efforts (Davis, 2023; Soltis, 2017). While the INLA modeling  
591 approach that we used allows for predictions at arbitrarily small spatial scales, and would simplify  
592 connecting model predictions to the scale of a given climate driver, the coarse scale inherent to our  
593 analysis may obscure some local-scale ecological processes. Poor predictive ability at local scales in  
594 this grass-endophyte system is not surprising, as previous studies have found that local variation  
595 (e.g., in soil conditions, in microclimate), even to the scale of hundreds of meters can structure  
596 endophyte-host niches (Gundel et al., 2024; Kazenel et al., 2015). Local adaptation in either the  
597 host or symbiont to microclimate or soil conditions could cause populations to differ from broad  
598 regional trends. The choice of prior distributions for spatially-varying random effects also impacts  
599 the model's flexibility to capture spatial trends. Our exploration of model sensitivity to prior choice  
600 (presented in the *Supplemental Methods*) reveals qualitatively similar results across a broad range  
601 of priors. An important next step would be integrating data from local and regional scales through  
602 modeling to constrain estimates of local and regional variation.

603 Predicting future niche-shifts of hosts and symbionts will require considering the coupled dynam-

604 ics of host-symbiont dispersal in addition to fitness benefits. For example, transplanting symbiotic  
605 and non-symbiotic plants beyond the range edge of *A. hyemalis* could tell us whether low endophyte  
606 prevalence in that area (Fig. 4A) is a result of environmental conditions that lead the symbiosis  
607 to have negative fitness consequences, or is a result of some historical contingency or dispersal lim-  
608 itation that has thus far limited the presence of symbiotic hosts from a region where they would  
609 otherwise flourish and provide resilience. Incorporating available climatic and soil layers as covari-  
610 ates is another obvious step that could improve predictions. These steps will bridge gaps that often  
611 exist between large but broad bioclimatic and biodiversity data and small but high-resolution data  
612 on biotic interactions, and move towards the goal of predicting the dynamics of microbial symbioses  
613 under climate change (Isaac et al., 2020; Miller et al., 2019).

614 *Herbaria for global change research*

615 Our analysis advances the use of herbarium specimens in global change biology in two ways.  
616 First and foremost, this is one of a growing number of studies to examine microbial symbiosis  
617 using specimens from natural history collections, and the first, to our knowledge, to link long-term  
618 changes in symbioses to changes in climate. The responses of microbial symbioses are a rich target  
619 for future studies within historic specimens, particularly those that take advantage of advances  
620 in sequencing technology. While we used relatively coarse presence/absence data based on fungal  
621 morphology, other studies have examined historic plant microbiomes using molecular sequencing  
622 and sophisticated bioinformatics techniques, but these studies have so far been limited to relatively  
623 few specimens at limited spatial extents (Bearchell et al., 2005; Bieker et al., 2020; Bradshaw et al.,  
624 2021, 2023; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al., 2015). Much of this work  
625 highlights the important role that historic specimens can play in tracking pathogens, a particularly  
626 important area as climate change facilitates the spread of new diseases (Ristaino, 2020; Singh et al.,  
627 2023) Continued advances in capturing historic DNA and in filtering out potential contamination  
628 during specimen storage (Bakker et al., 2020; Daru et al., 2019; Raxworthy and Smith, 2021) will  
629 be imperative in the effort to scale up these efforts. This scaling up will be essential to be able  
630 to quantify changes not just in the prevalence of symbionts, but also in symbionts' intraspecific  
631 variation and evolutionary responses to climate change, as well as in changes in the wider host  
632 microbiome. With improved molecular insights from historic specimens, we could ask whether  
633 the broad increases in endophytes that we have identified reflect selection for particular genetic  
634 strains or chemotypes and how this selection varies across space. Answering these questions as well  
635 as the unknown questions that future researchers may ask also reiterates the value in capturing  
636 meta-information during ongoing digitization efforts at herbaria around the world and during the  
637 accession of newly collected specimens (Edwards et al.; Lendemer et al., 2020).

638 The second major advance in this analysis is in accounting for several potential biases in the data  
639 observation process that may be common to many collections-based research questions by using a

640 spatially-explicit random effects model. Potential biases introduced by the sampling habits of col-  
641 lectors (Daru et al., 2018), and variation between contemporary researchers during the collection of  
642 trait data, if not corrected for could lead to over-confident inference about the strength and direction  
643 of historic change (Fig. 2). Previous studies that have quantified the effects of collector biases typ-  
644 ically find them to be small (Davis et al., 2015; Meineke et al., 2019), and we similarly did not find  
645 that collector has a strong effect on the results of our analysis, but that scorer identity did impact  
646 results. It is difficult to distinguish whether the impact of scorers was driven by true differences  
647 in scorers' biases or by unintended spatial or temporal clustering of the specimens examined by  
648 each scorer (Clayton et al., 1993; Urdangarin et al., 2023). By under-weighting endophyte-positive  
649 samples that are clustered spatially or by collector or observer, the endophyte prevalence model is  
650 appropriately accounting for nuisance variables and providing a conservative inference of endophyte  
651 change relative to the raw data. Spatial autocorrelation is another phenomenon likely common  
652 in data derived from herbarium specimens (Willems et al., 2022), which our spatially-explicit anal-  
653 ysis models among samples. Beyond spatial autocorrelation of outcomes, systematic differences in  
654 sampling across space can result in spatial bias.

655 One strength of herbaria as vehicles for global change research is the relative ease with which  
656 specimens from many distinct geographic locations can be examined. We visited just nine institu-  
657 tions in the central southern United States, and we were able to sample seeds from specimens across  
658 an area spanning over 300,000 sq. km, including specimens from Mexico and Canada. Despite this  
659 advantage, the specimens we examined are concentrated in the south-central United States, with  
660 fewer specimens in the rapidly warming northeastern United States reflecting the regional focus of  
661 herbaria. We provide a simulation analysis exploring the potential impact of spatially and tempo-  
662 rally biased sampling (Appendix A - Supporting Methods). We found that the spatially-varying  
663 coefficient model had a strong ability to re-capitulate temporal trends across space in simulated  
664 data, and that this result was robust to relatively high levels of spatial bias (80% of data missing  
665 from one spatial region). Simulation analyses that extend this work to consider the myriad ways  
666 herbarium data may be biased (i.e. testing different spatial arrangements and scales of spatial

667 bias, or testing different sample sizes) would be extremely valuable (Daru et al., 2018; Erickson and  
668 Smith, 2021; Gaul et al., 2020; Meineke and Daru, 2021; Schmidt et al.).

669 *Conclusion*

670 Ultimately, a central goal of global change biology is to generate predictive insights into the future of  
671 natural systems on a rapidly changing planet. Beyond host-microbe symbioses, detecting ecological  
672 responses to anthropogenic global change and attributing their causes would inform public policy  
673 decision-makers and adaptive management strategies. Natural history specimens, such as the plant  
674 hosts examined in this study, have a clear role to play in informing global change biodiversity  
675 science, including building understanding of the dynamics of host-symbiont interactions (Davis,  
676 2023). This survey of historic endophyte prevalence is necessarily correlative, yet it serves as a  
677 foundation to develop better predictive models of the response of microbial symbioses to climate  
678 change. Combining the insights from this type of regional-scale survey with field experiments and  
679 physiological performance data could be invaluable to identify mechanisms driving shifts in host-  
680 symbiont dynamics. Evidence is strong that certain dimensions of climate change correlated with  
681 endophytes' temporal responses, however we do not know why trends in prevalence were weak  
682 in some areas or how endophytes would respond to more extreme changes in climate. The "time  
683 machine" of natural history collections revealed evidence of mutualism resilience for grass-endophyte  
684 symbioses in the face of environmental change, but more extreme changes could potentially push  
685 one or both partners beyond their physiological limits, leading to the collapse of the mutualism;  
686 more research is needed to understand what those limits might be.

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697 **Statement of Authorship**

698 J.C.F. contributed to research conception, data collection, data analysis, and led manuscript draft-  
699 ing. J.M. contributed to data analysis and manuscript revisions. T.E.X.M. contributed to research  
700 conception, data collection, data analysis, and manuscript revisions.

701 **Data and Code Availability**

702 Data from this publication can be found through a publicly available repository  
703 (<https://doi.org/10.5061/dryad.rn8pk0pn0>). Code for analyses can be found through a publicly  
704 available repository (<https://github.com/joshuacfowler/EndoHerbarium>) that will be permanently  
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## Appendix A

1068

1069     *Appendix to "Increasing Prevalence of plant-fungal symbiosis across two*  
1070                 *centuries of environmental change"*

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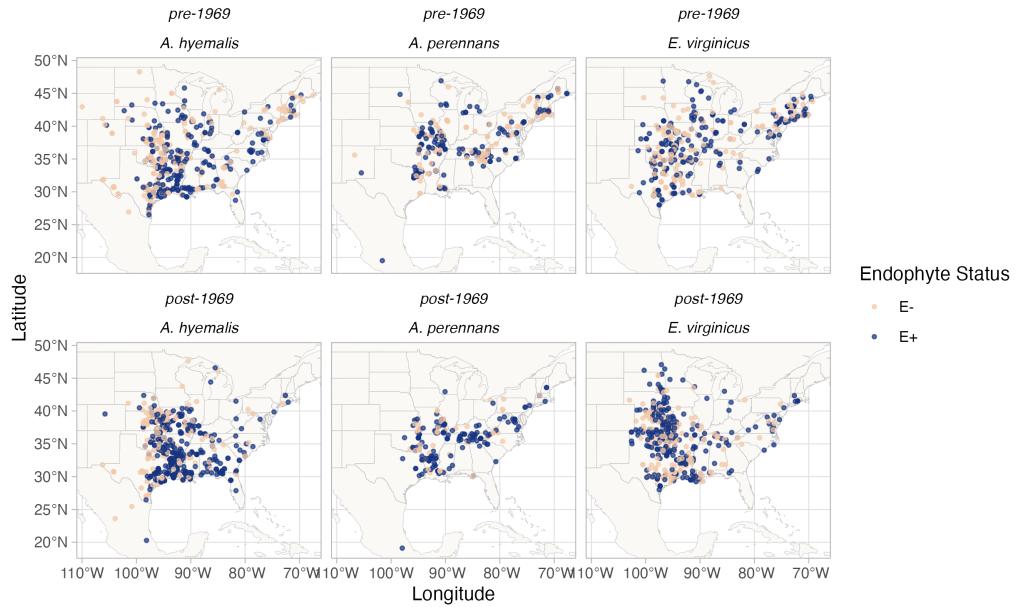
1078     \* Corresponding author; e-mail: jcf221@miami.edu.

1079     **Contents:**

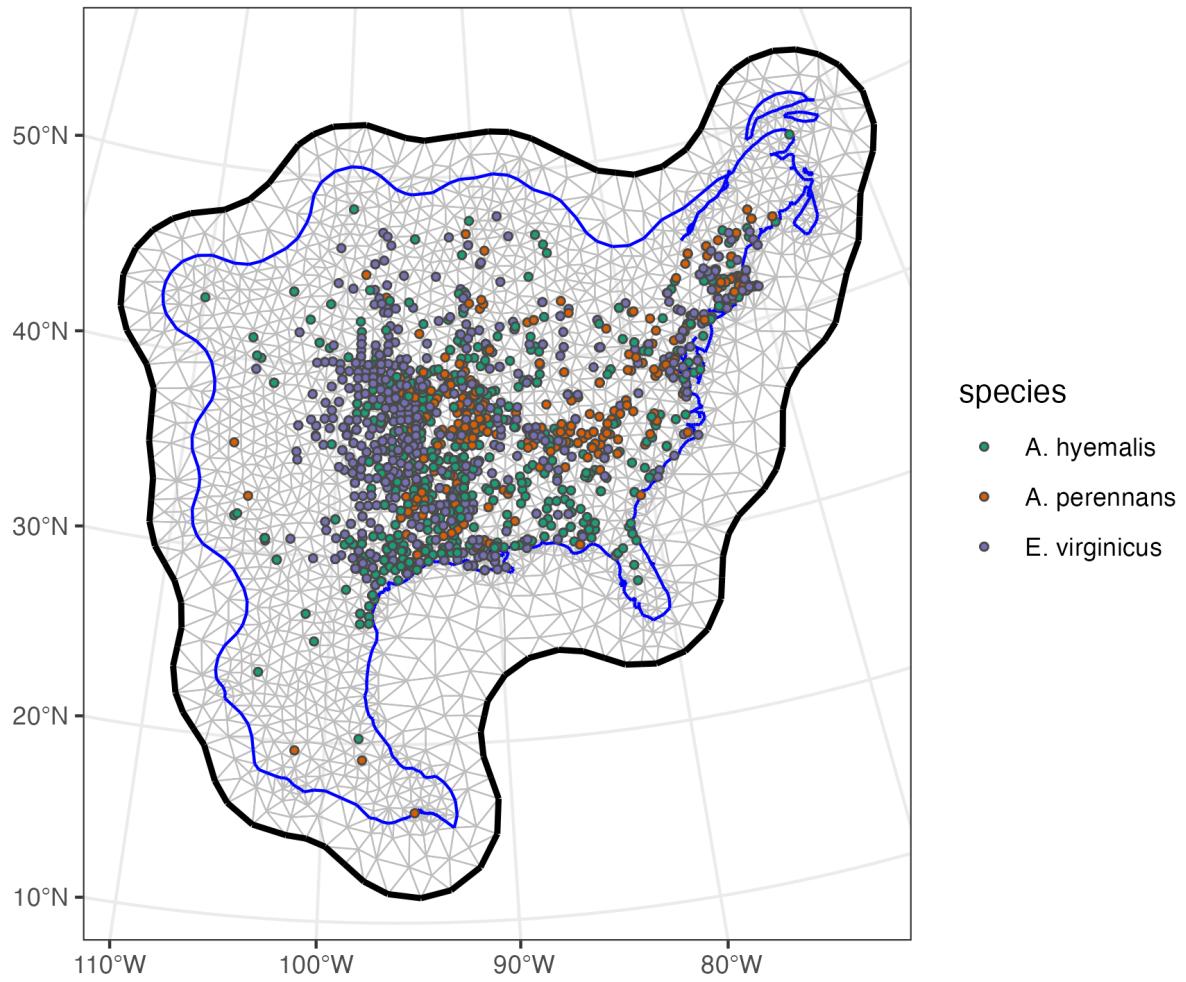
1080     Appendix A includes: Figure A1 - Figure A15, Table A1, and Supporting Methods).

1081

## 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.

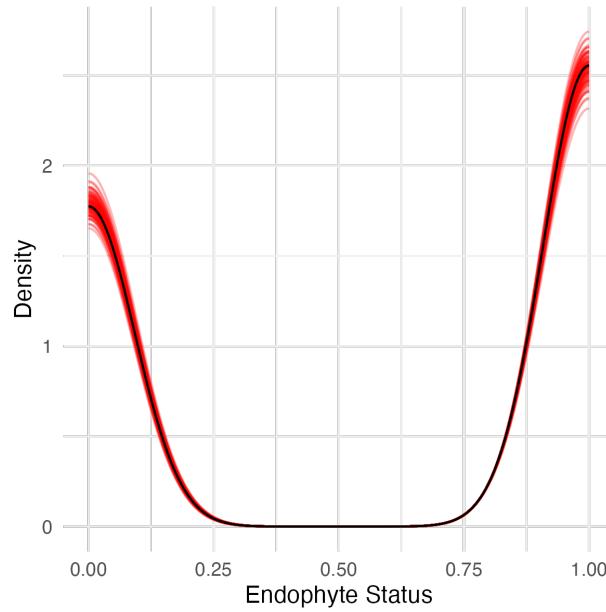
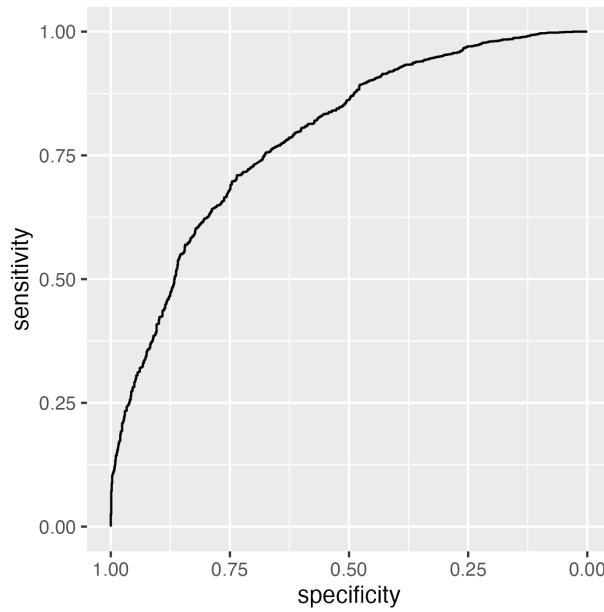
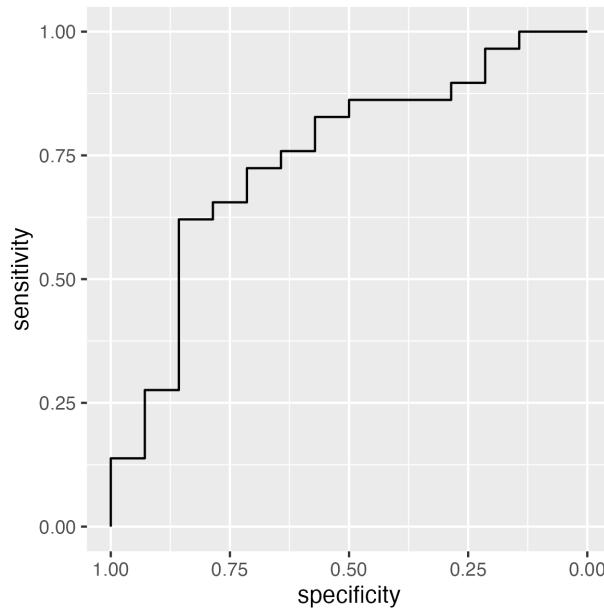


Figure A3: **Graphical posterior predictive check of the endophyte prevalence model fit.**

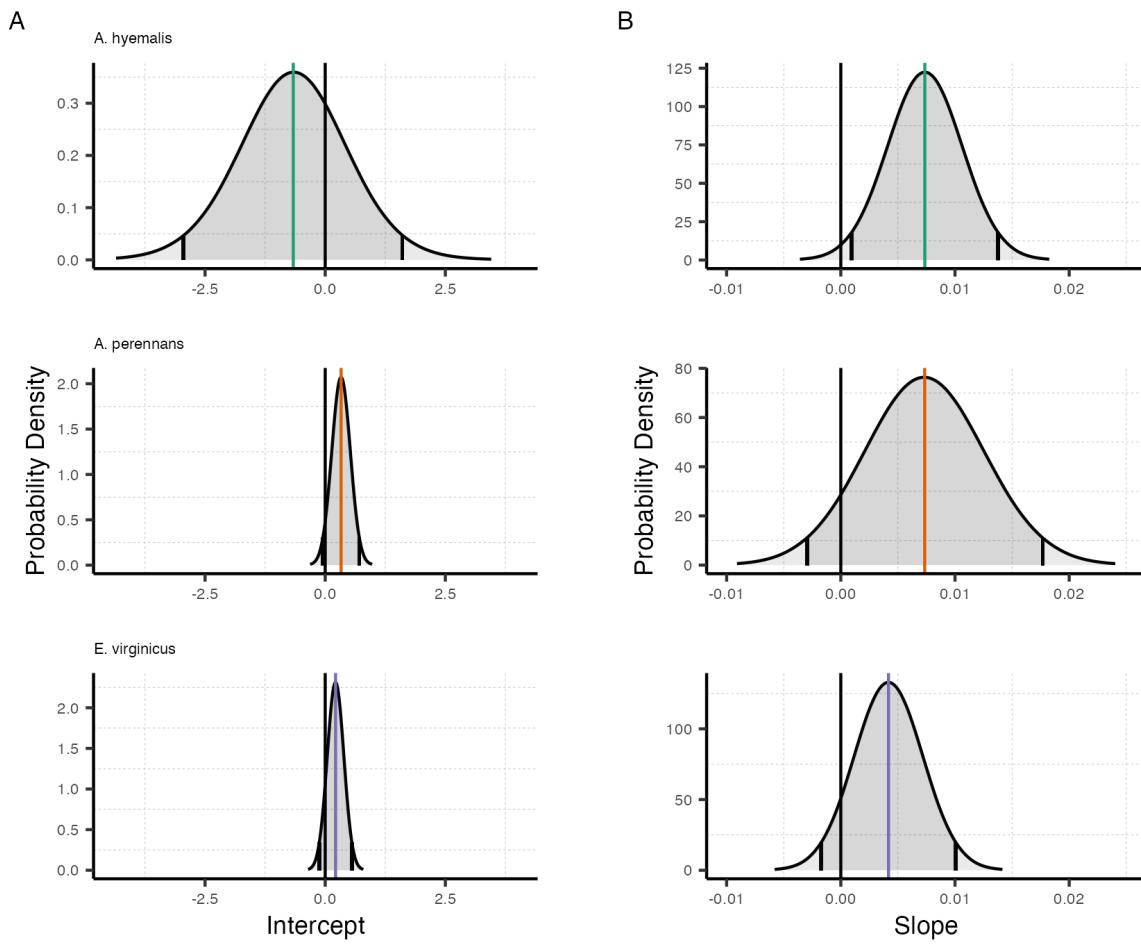
Consistency between observed data and predicted values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (black) along with 100 predicted datasets (red).



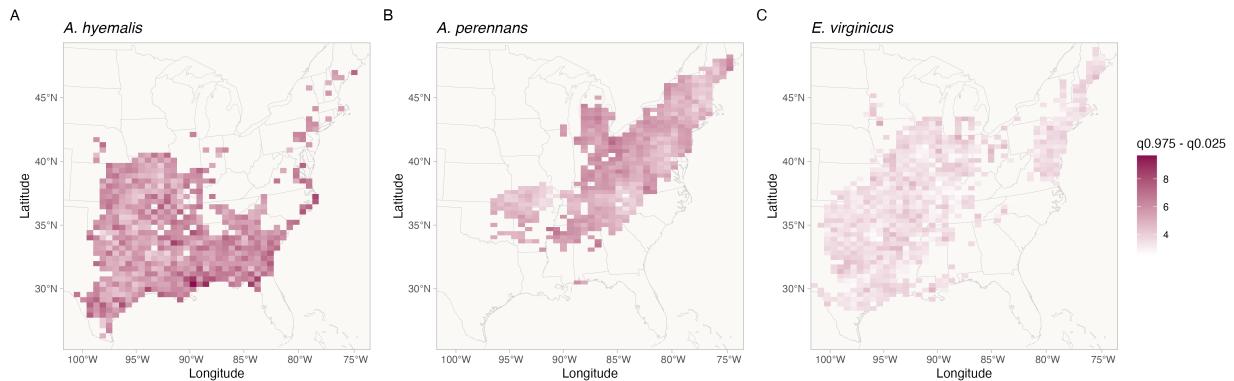
**Figure A4: ROC plot showing performance of the endophyte prevalence model in classifying observations according to endophyte status within the in-sample training data from herbarium collections.** The curves show adequate model performance for observed data. The AUC value is 0.79.



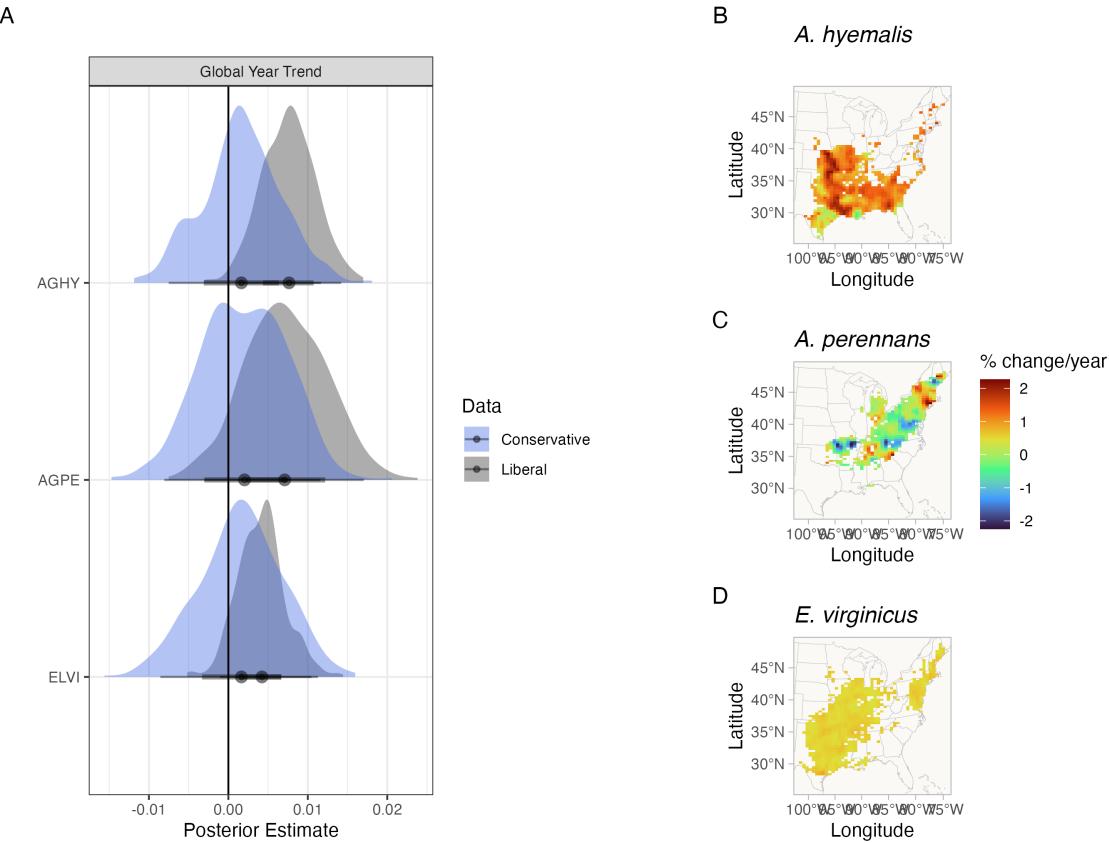
**Figure A5: ROC plot showing performance of the endophyte prevalence model in classifying observations according to endophyte status within the out-of-sample test data from contemporary surveys.** The curves show adequate model performance for test data. The AUC value is 0.77.



**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 ( $T$  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.

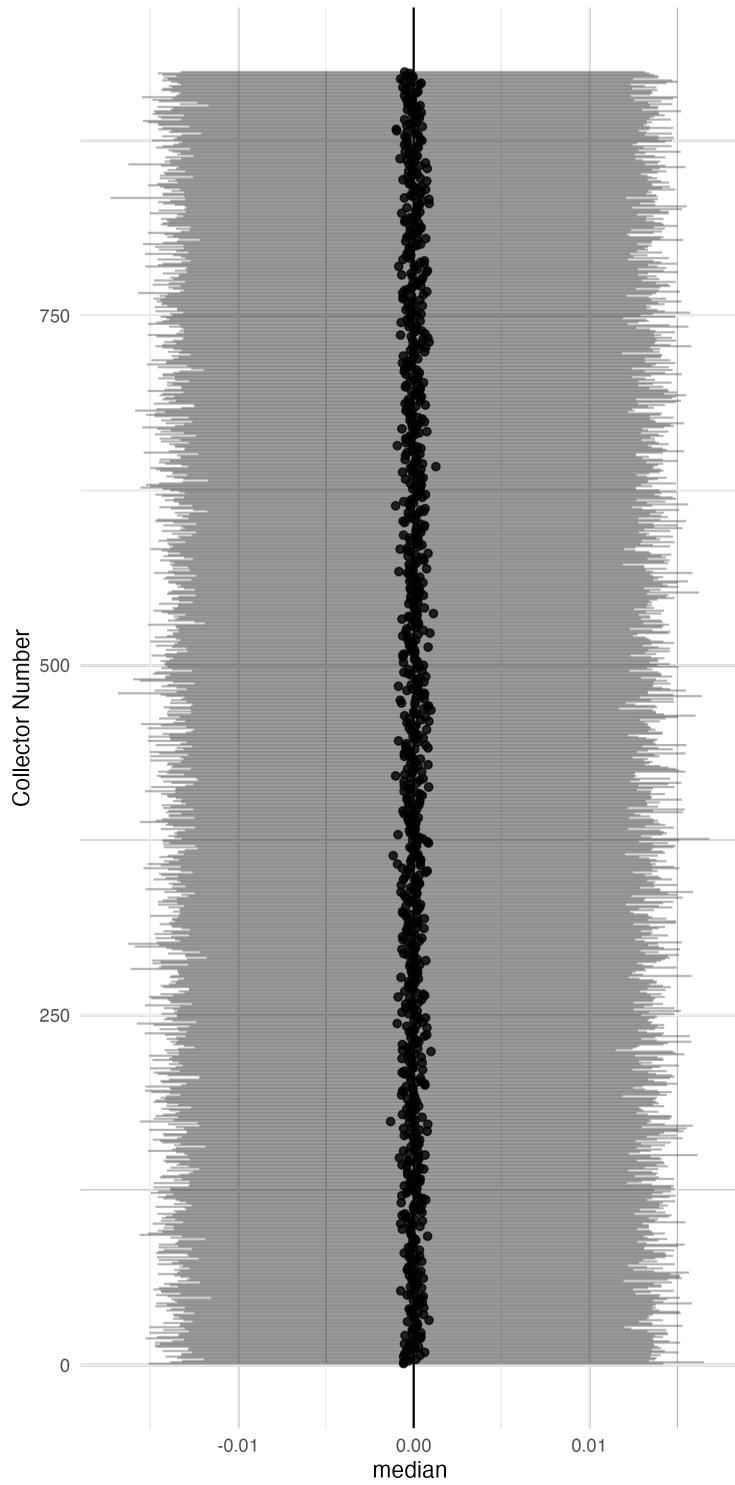


Figure A9: **Posterior estimates of collector random effects from endophyte prevalence model.** Collector random effects are denoted  $\chi$  in Eqn. 1 and represent variance associated with researchers who collected historic herbarium specimens. Points show posterior median along with 95% CI for each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

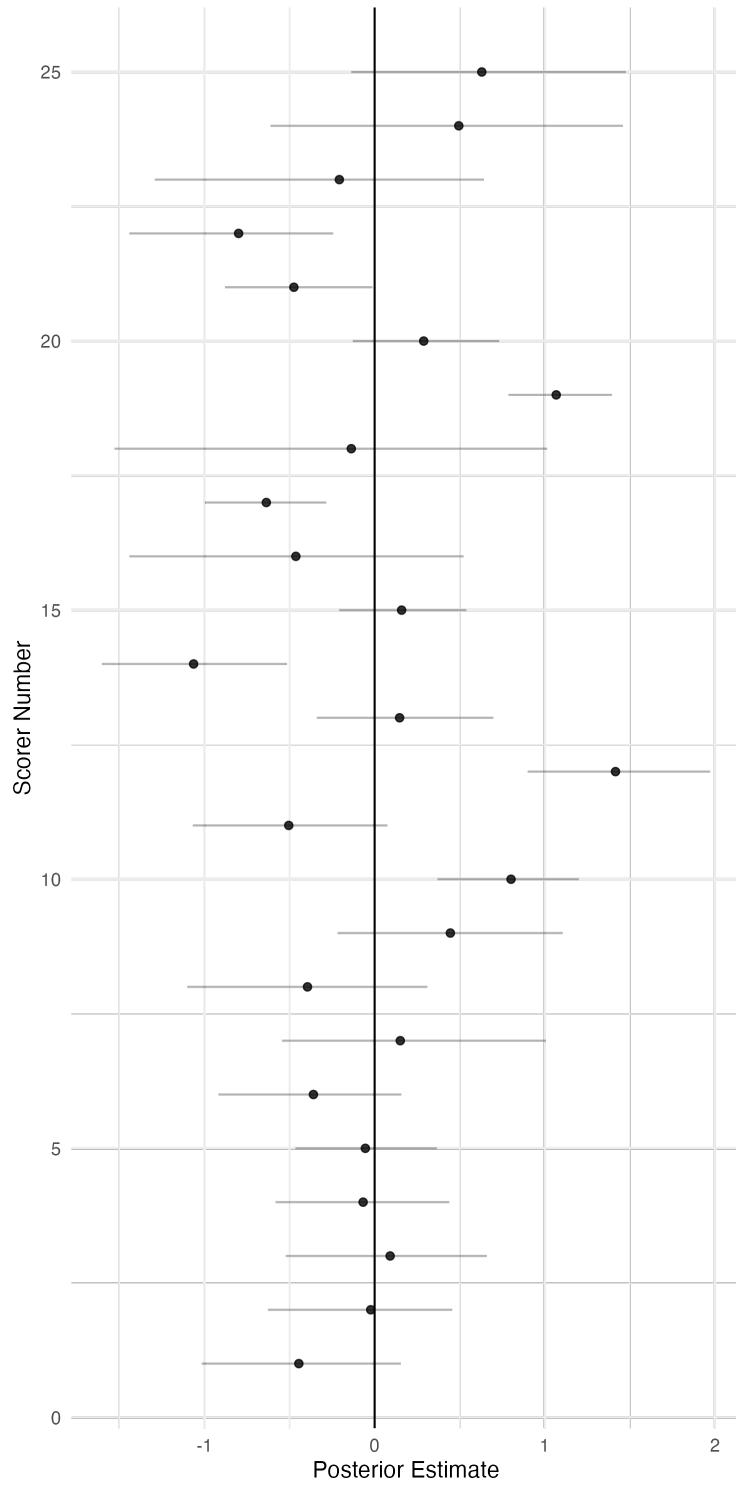
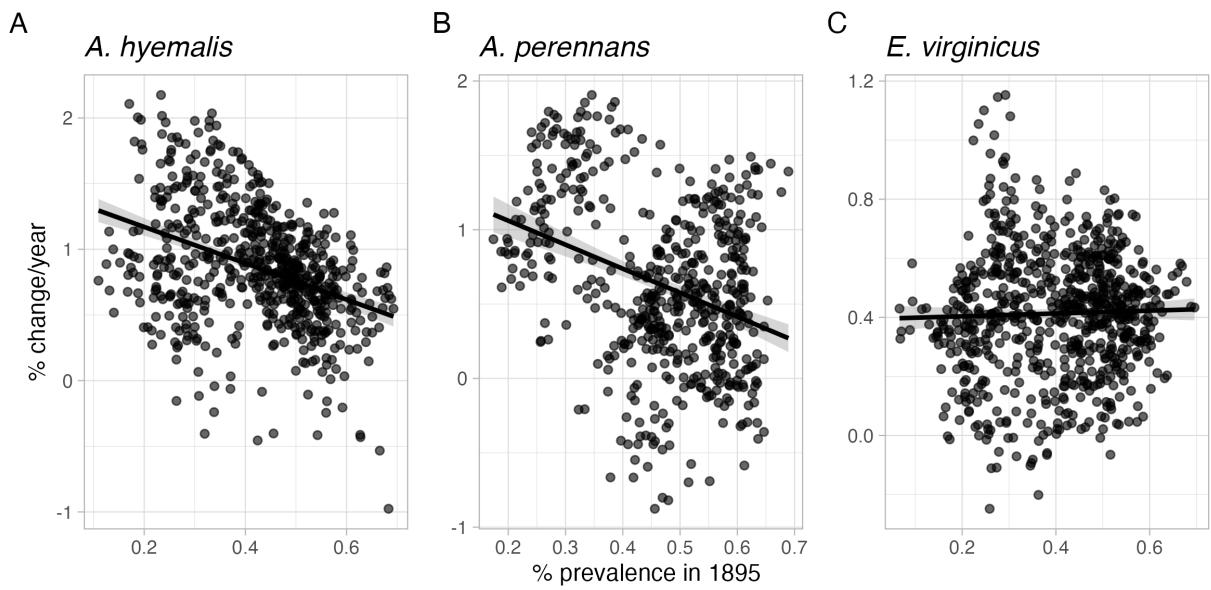
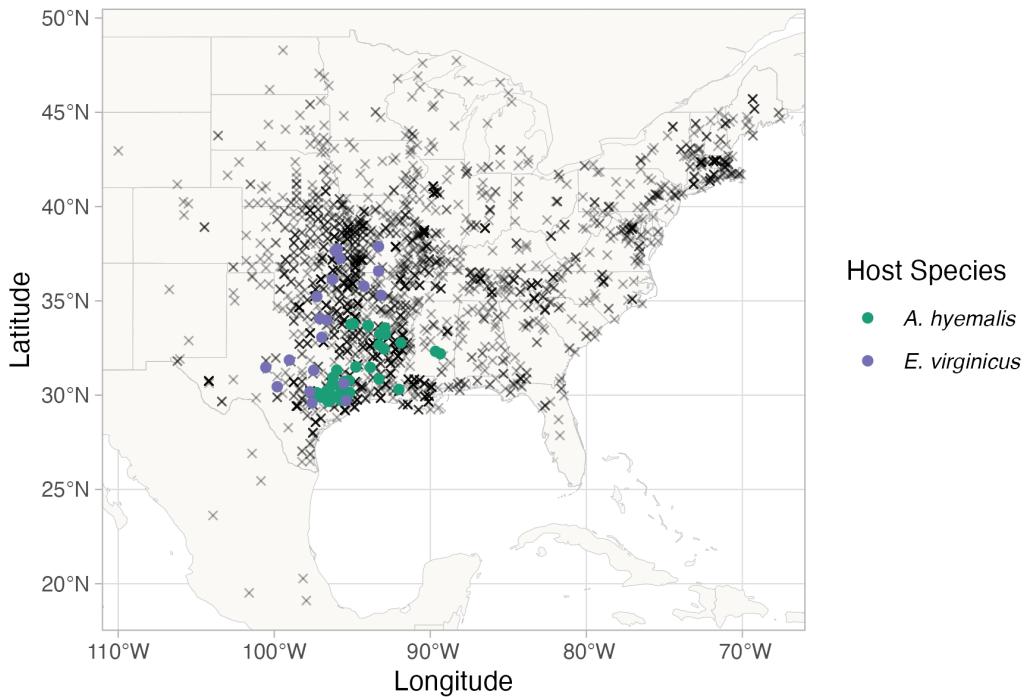


Figure A10: **Posterior estimates of scorer random effects from endophyte prevalence model.** Scorer random effects are denoted  $\omega$  in Eqn. 1 and represent variance associated with researchers who identified *Epichloë* endophytes within herbarium specimen tissue samples. Points show posterior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

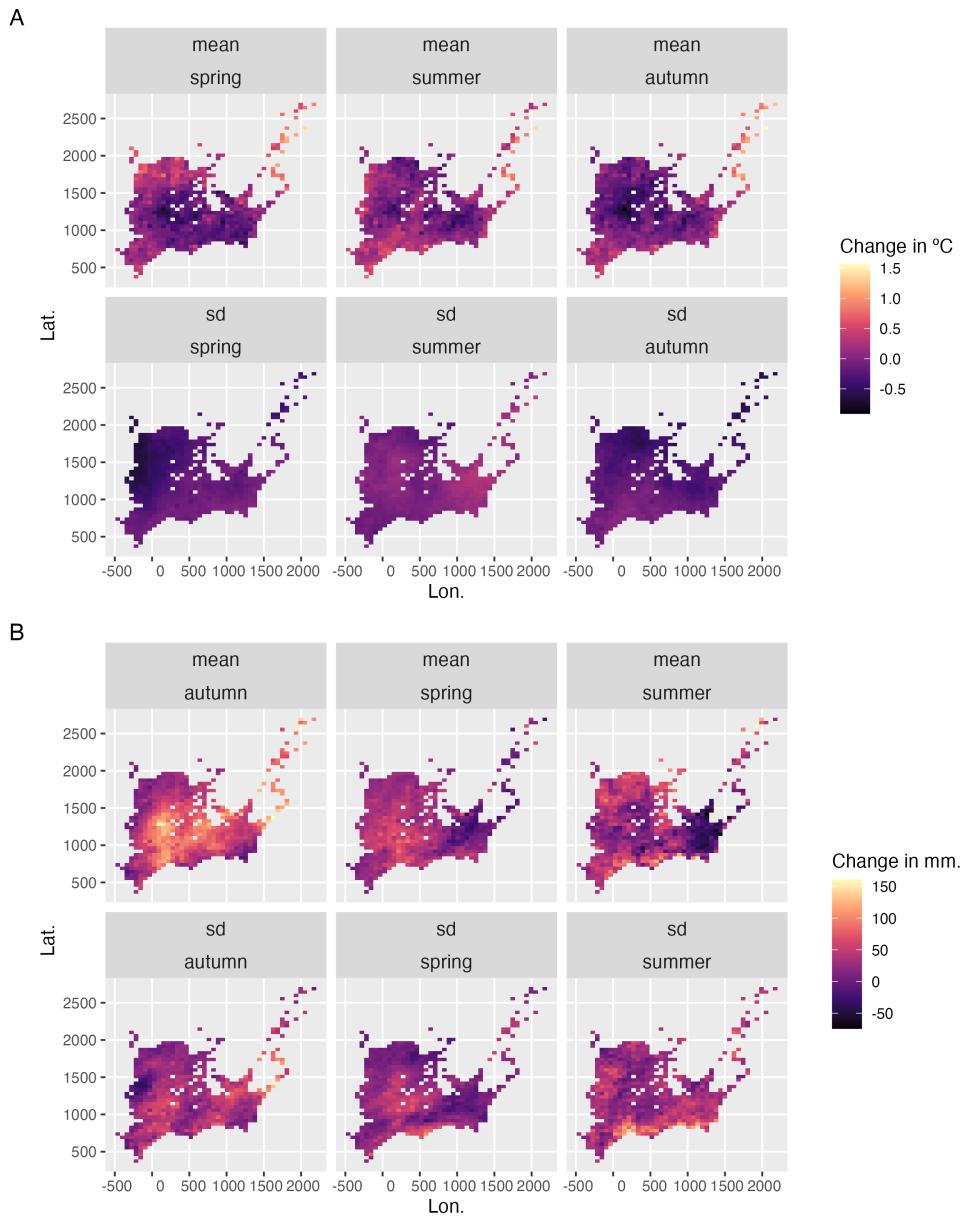


Figure A13: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *A. hyemalis*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution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

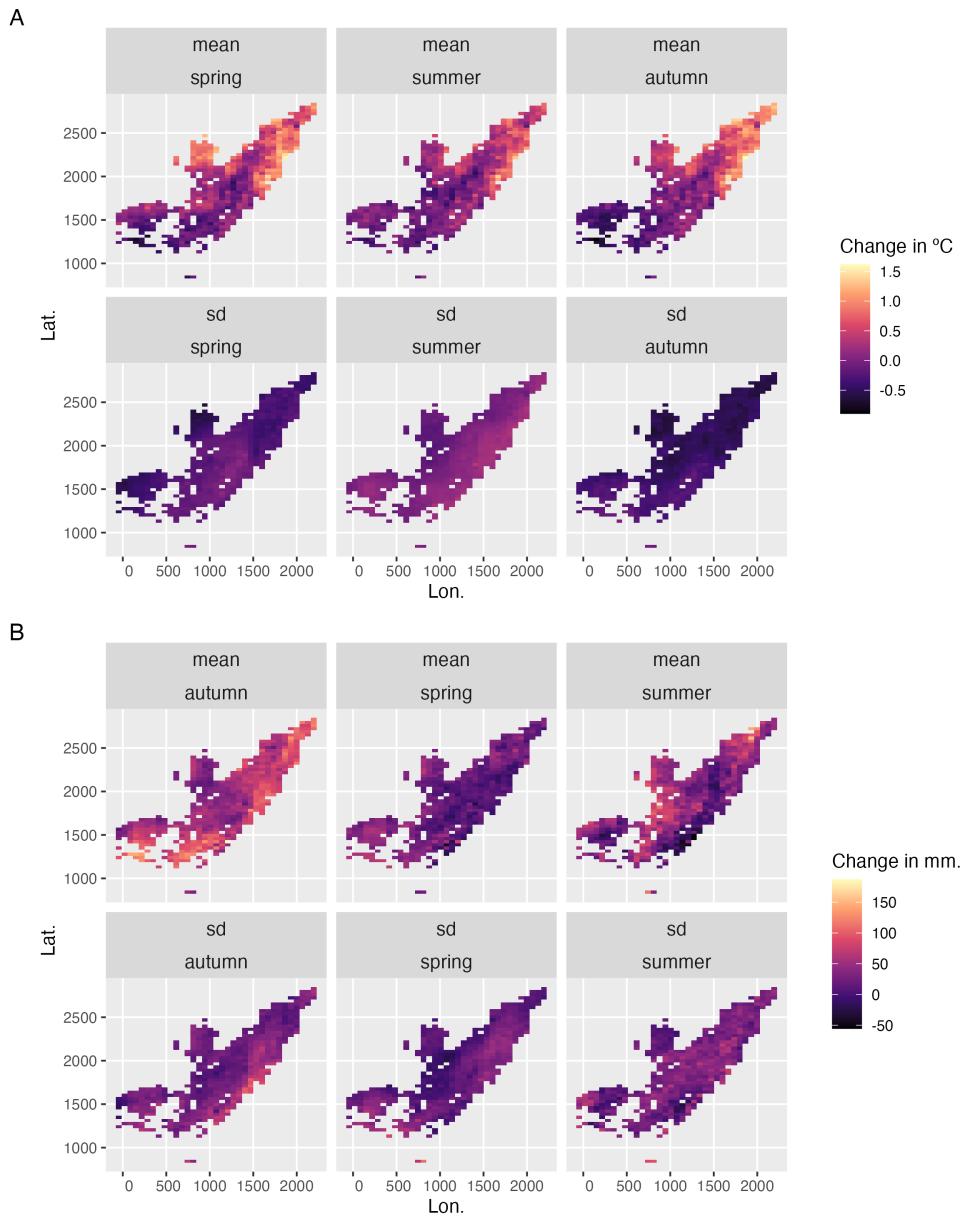


Figure A14: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *A. perennans*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution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

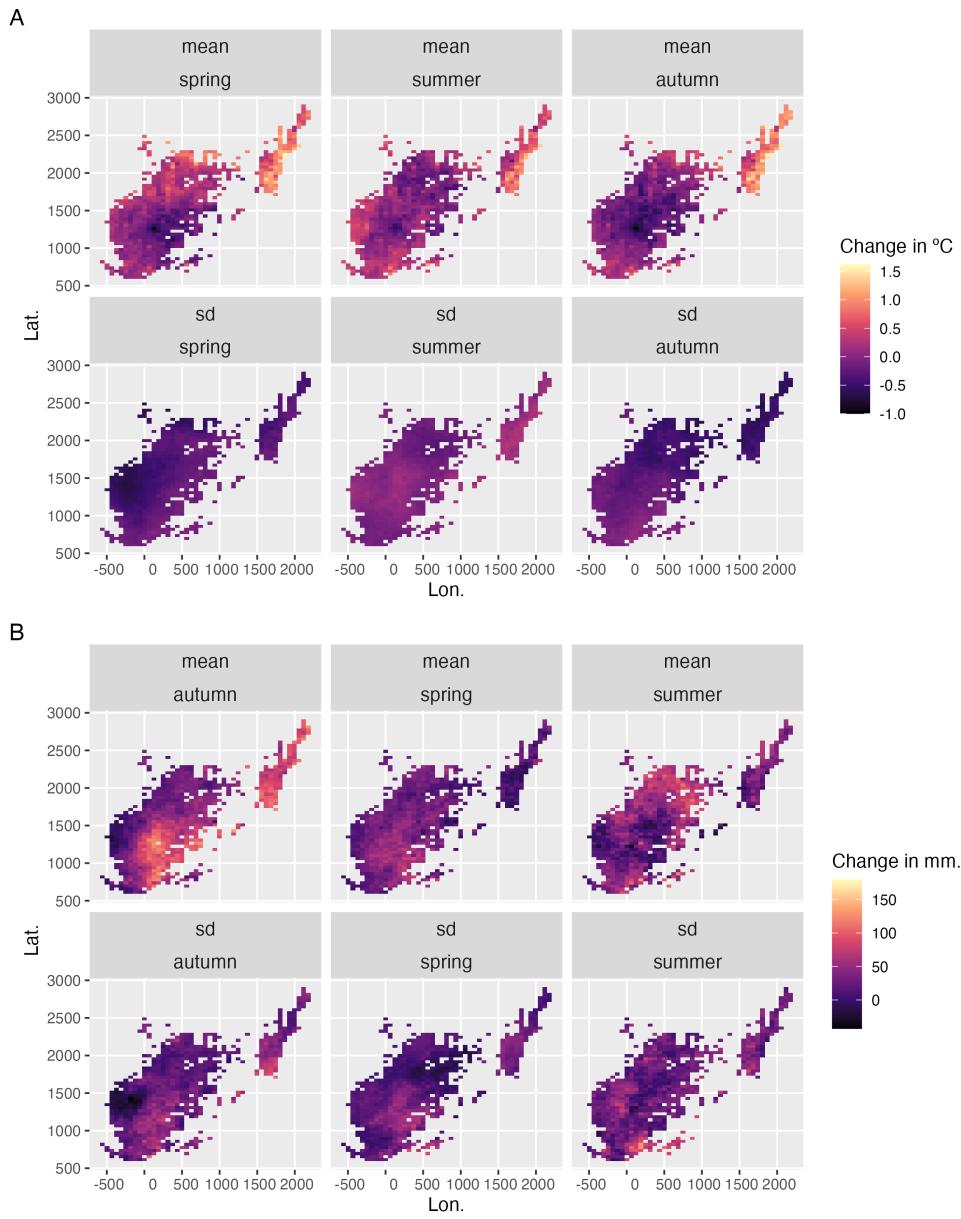


Figure A15: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *E. virginicus*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution 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	0	6
Missouri Botanic Garden	210	205	122
Texas A&M	100	0	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

1083

## Supporting Methods

1084

### *ODMAP Protocol*

1085 [Overview](#)

1086 **Model purpose:** Mapping current distribution of *Epichloë* host species.

1087 **Target species:** *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*.

1088 **Study area:** Eastern North America

1089 **Spatial extent:** -125.0208, -66.47917, 24.0625, 49.9375 (xmin, xmax, ymin, ymax).

1090 **Spatial resolution:** 0.04166667, 0.04166667 (x, y).

1091 **Temporal extent:** 1990 to 2020.

1092 **Boundary:** Natural.

1093 [Data](#)

1094 **Observation type:** Occurrence records from Global Biodiversity Information Facility and

1095 herbarium collection across eastern North America. We used 713 occurrences records for *Agrostis*  
1096 *hyemalis*, 656 occurrence records for *Agrostis perennans* and 2338 for *Elymus virginicus*.

1097 **Response data type:** occurrence record, presence-only.

1098 **Coordinate reference system:** WGS84 coordinate reference system (EPSG:4326 code)

1099 **Climatic data:** raster data extracted from PRISM; 30-year normal mean and standard deviation  
1100 of temperature and of precipitation for three four-month seasons within the year (Spring: January,  
1101 February, March, April; Summer: May, June, July, August; Autumn: September, October,  
1102 November, December).

1103 **Model**

1104 **Model assumption:** We assumed that the target species are at equilibrium with their environ-  
1105 ment.

1106 **Algorithms:** Maximum entropy (maxent)

1107 **Workflow:** We described the workflow in the methods section of the manuscript.

1108 **Software:** All statistics were performed using Maxent 3.3.4 and R4.3.1 with packages terra, usdm,  
1109 spThin and dismo.

1110 **Code availability:** Available through this link: <https://github.com/joshuacfowler/EndoHerbarium>

1111 **Data availability:** Data was accessed through open-source R packages *rgbif*. *A. hyemalis*  
1112 (GBIF.Org, 2025a), *A. perennans* (GBIF.Org, 2025b), *E. virginicus* (GBIF.Org, 2025c)

1113 **Assessment**

1114 We used AUC to test model performance.

1115 **Prediction**

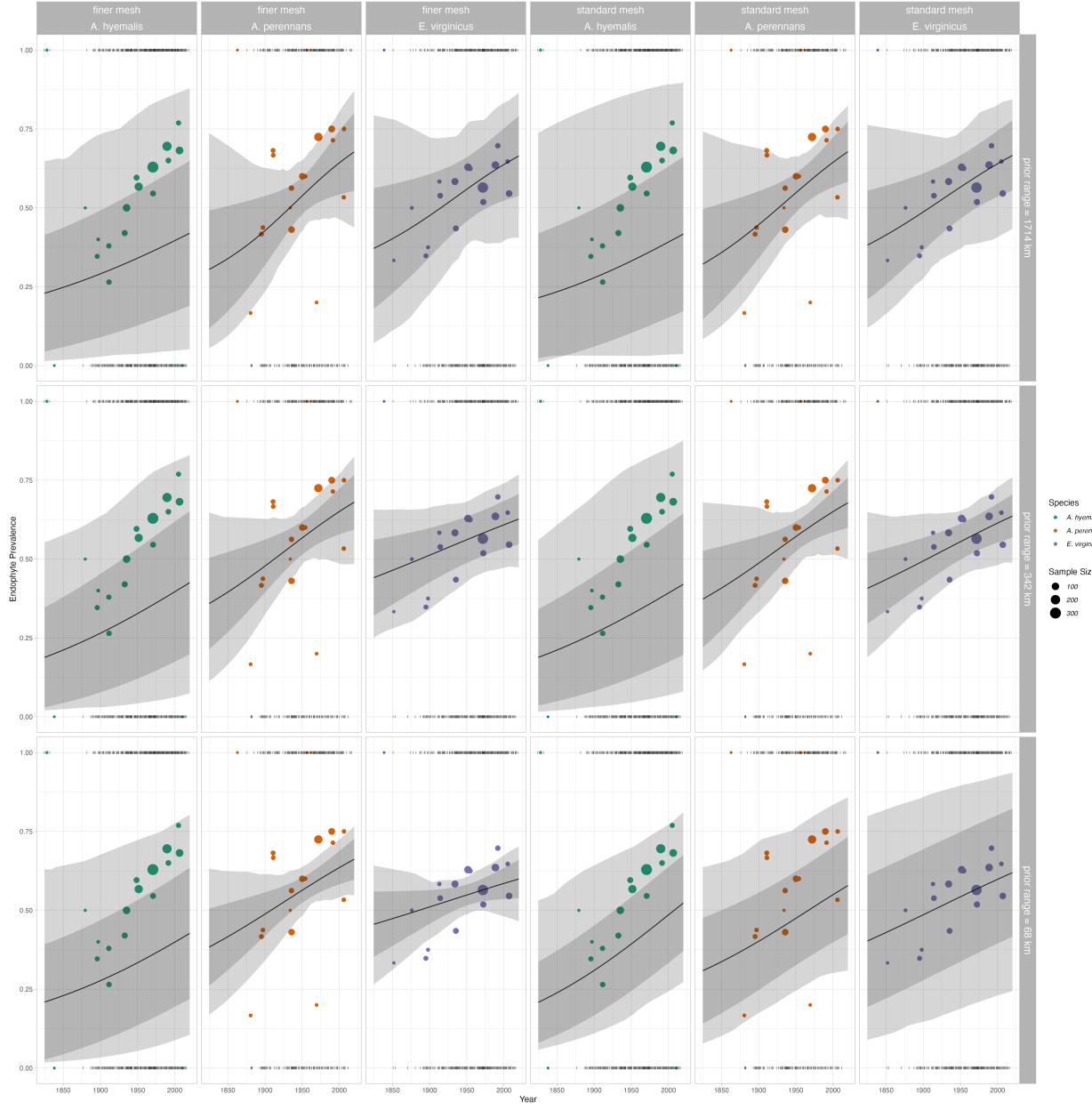
1116 We predicted the probability of presence of the host species as a binary maps (presence or absence)

1117 *Mesh and Prior Sensitivity Analysis*

1118 To test the influence that the triangulation mesh and choice of priors has on results, we compared  
1119 model results across a range of meshes and priors. We re-ran our model for the mesh used in main  
1120 body of the text (Fig. A2), which we refer to as the "standard mesh", and with a mesh with smaller

minimum vertices (finer mesh). Finer scale meshes increase computation time. For each of these meshes, we ran the model with a range of priors defining the spatial range of our spatial random effects: 342km (the prior used for presented results), as well as ranges five times smaller (68 km) and five times larger (1714 km). We found generally that these choices did not alter the direction of model predictions, but did influence the associated uncertainty and magnitude of some effects.

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

1128 For spatially-varying temporal trends, we found that models with different priors predicted  
1129 consistent spatial patterns in temporal trends, although the range of this prediction varied depending  
1130 on the prior and mesh (Fig. A17 - A18). One noteworthy result of this analysis is that combinations  
1131 of prior choice and mesh can introduce instability in model fitting. This is evident in A17 panel B  
1132 and A18 panel B, where the prior range is smaller than the minimum vertex length of the mesh.  
1133 Model fitting takes an extended time period and the model struggles to identify variation across  
1134 space. Results with a set of prior ranges (Fig. A17 - A and C; Fig. A18 - A and C) result in  
1135 models that estimate trends across space of the same direction and order of magnitude, although  
1136 the "smoothness" of these predictions vary.

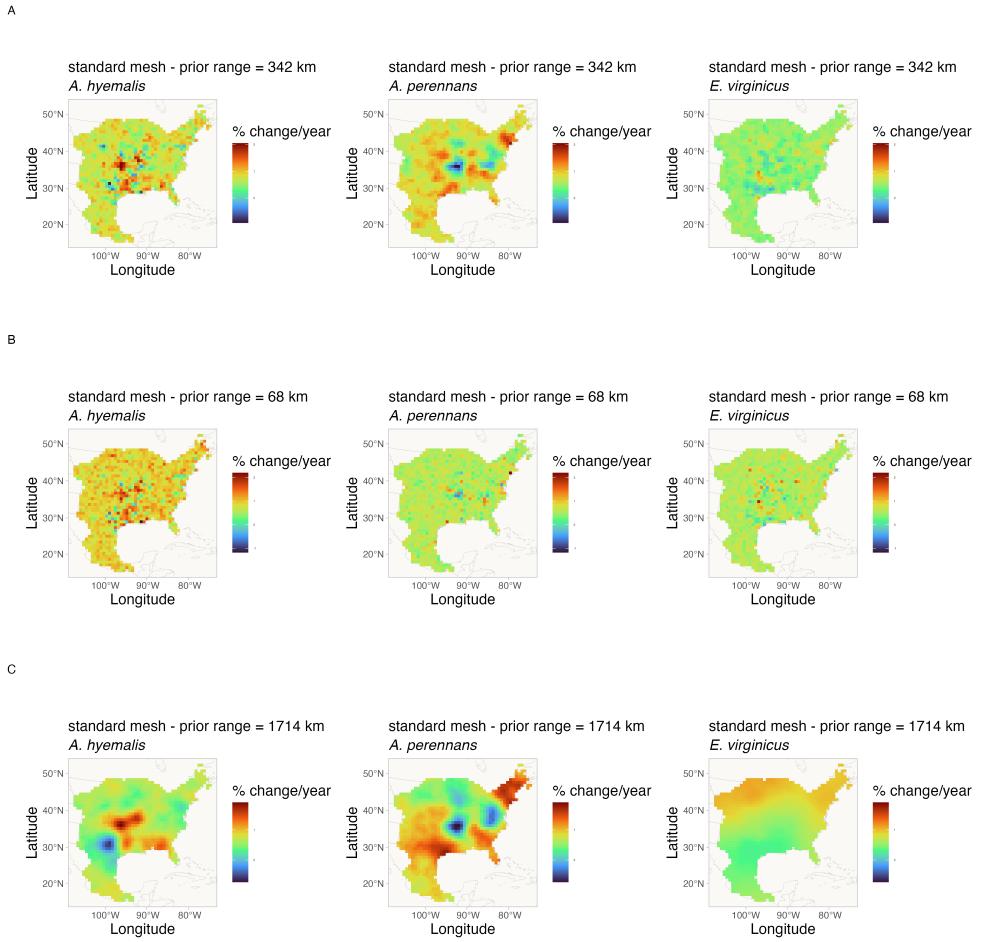
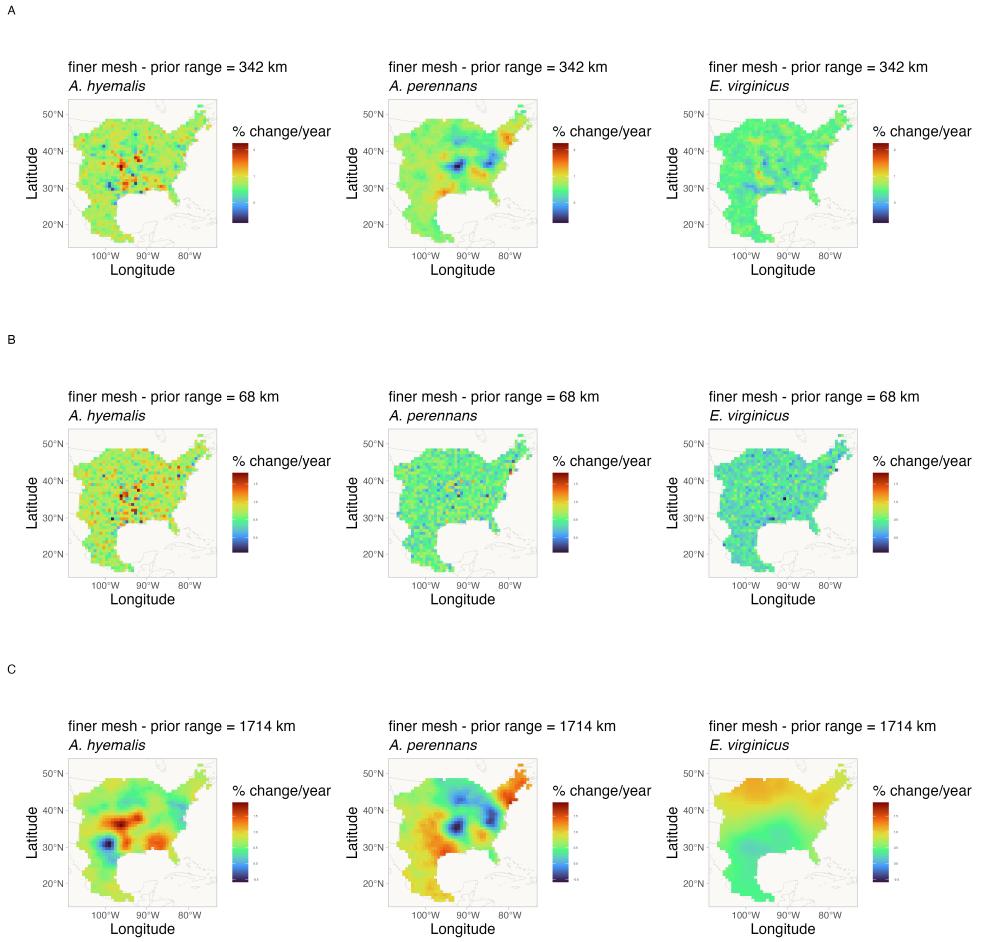


Figure A17: **Spatially-varying trends in endophyte prevalence evaluated for the endophyte prevalence model with different range priors on spatially structured random effects, and for the "standard" mesh.** Data used in model fitting is the same across all panels and as in the main text. Shading indicates 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.

1137

## *Spatially-biased Sample Size Simulation Analysis*

1138 To examine how data that is unevenly distributed across host distributions may influence interpreta-  
1139 tion of spatially-varying coefficients, we performed a simulation analysis. Our focal species, *Agrostis*  
1140 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*, are widely distributed grasses across the east-  
1141 ern United States that host *Epichloë* fungal endophytes. For logistical reasons, our sampling visits  
1142 to herbaria focused on herbaria in the central southern U.S., which resulted in unevenly distributed  
1143 data across each host species' range. This is particularly noteable for *Agrostis perennans* which has  
1144 the most northern distribution and relatively fewer total collected specimens compared to the other  
1145 focal species. Thus, a significant portion in the northeast of this species' range is relatively sparsely  
1146 sampled. Our analysis presented in the main text identified this region as having strong increase in  
1147 endophyte prevalence. Future visits to herbaria with regional focuses in the Northeastern US would  
1148 certainly garner new specimens that could provide valuable insights into shifting host and symbiont  
1149 distributions.

1150 *Simulation of spatially-biased symbiont occurrence data*

1151 We simulated datasets with varying levels of missing-ness to examine how this missing-ness influ-  
1152 enced the estimation of spatially-varying trend estimates. We first generated 300 data points for  
1153 each of three hypothetical species at random positions across an area approximating the scale of  
1154 our focal data. Each data point was randomly assigned a year of collection across 200 years. We  
1155 then simulated data from a Bernoulli process with trends alternating across nine regions (Fig. A19)  
1156 in a 3X3 grid pattern. This grid pattern was intended to create a complex spatial layout of trends,  
1157 where trends were either an increase of 1% per year or a decrease of 1% per year.

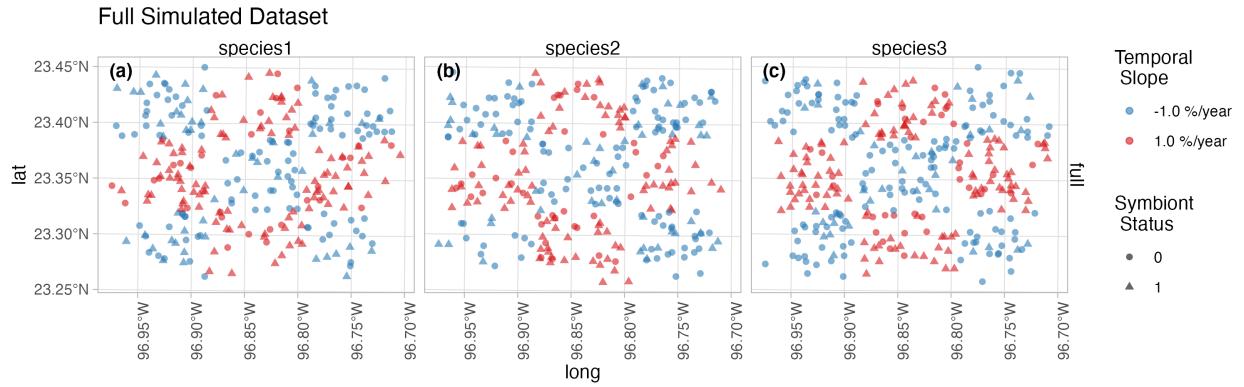
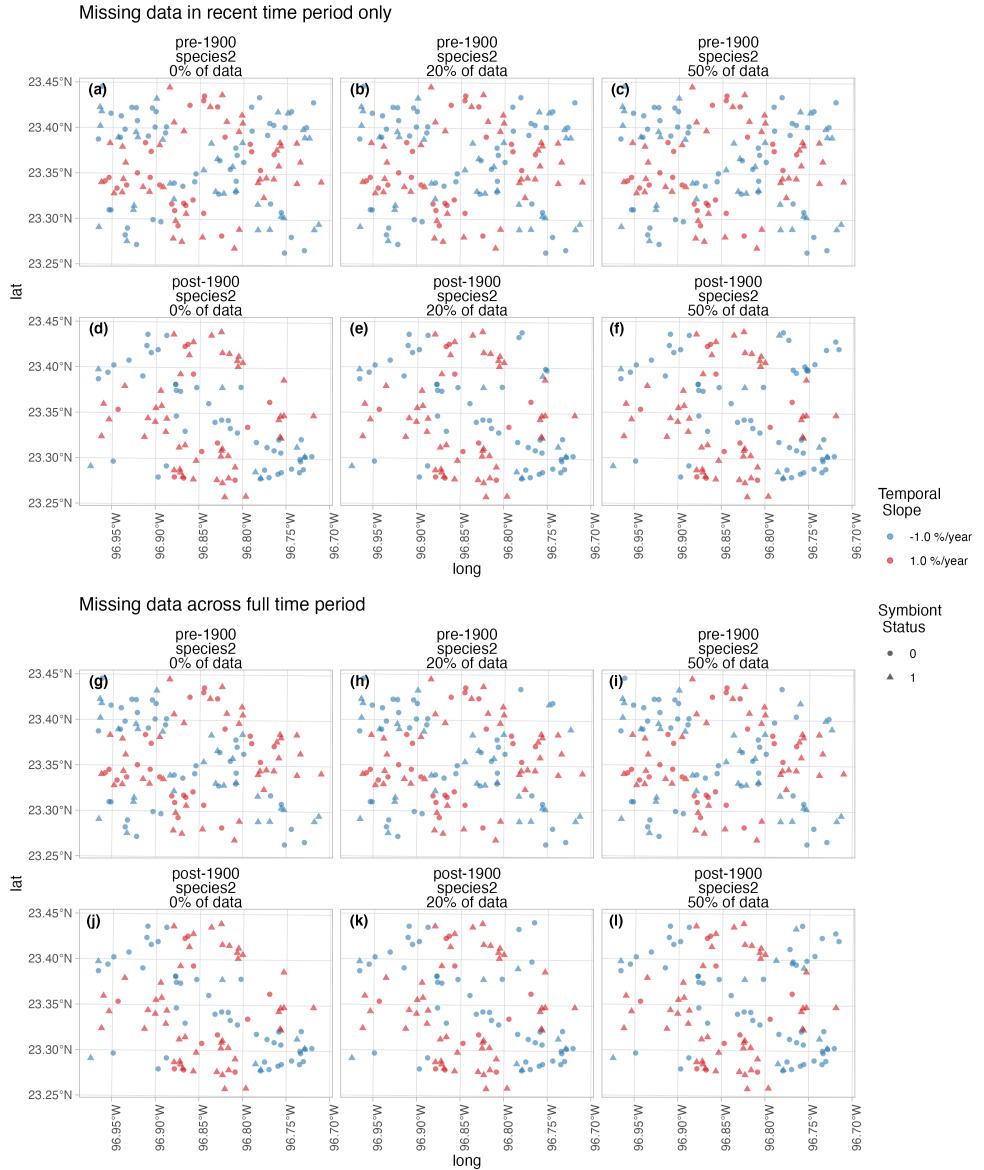


Figure A19: **Full simulated dataset of symbiotic association with spatially-varying temporal trends.** Color indicates the slope parameter used to simulate trends in endophyte status across nine "regions" for three species. Data are assigned collection years across a period of 200 years. Shape indicates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

From this full data, we generated six additional datasets with missing-ness in the northeast region of the simulated data for hypothetical species 2. The data remained the same for Species 1 and for species 3 across all datasets. For these six datasets, we removed data points at random in six ways: 0% of datapoints in northeast region, 0% of recent datapoints, only 20% of datapoints, only 20% of recent datapoints, only 50% of datapoints, and only 50% of recent datapoints (Fig. A20). We define the datapoints as part of the recent time period if they occur later than the median 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.

1165 *Statistical analysis*

1166 We analyzed each dataset with a model given by Eqn. A1 similar in construction to that used in  
1167 our central analysis.

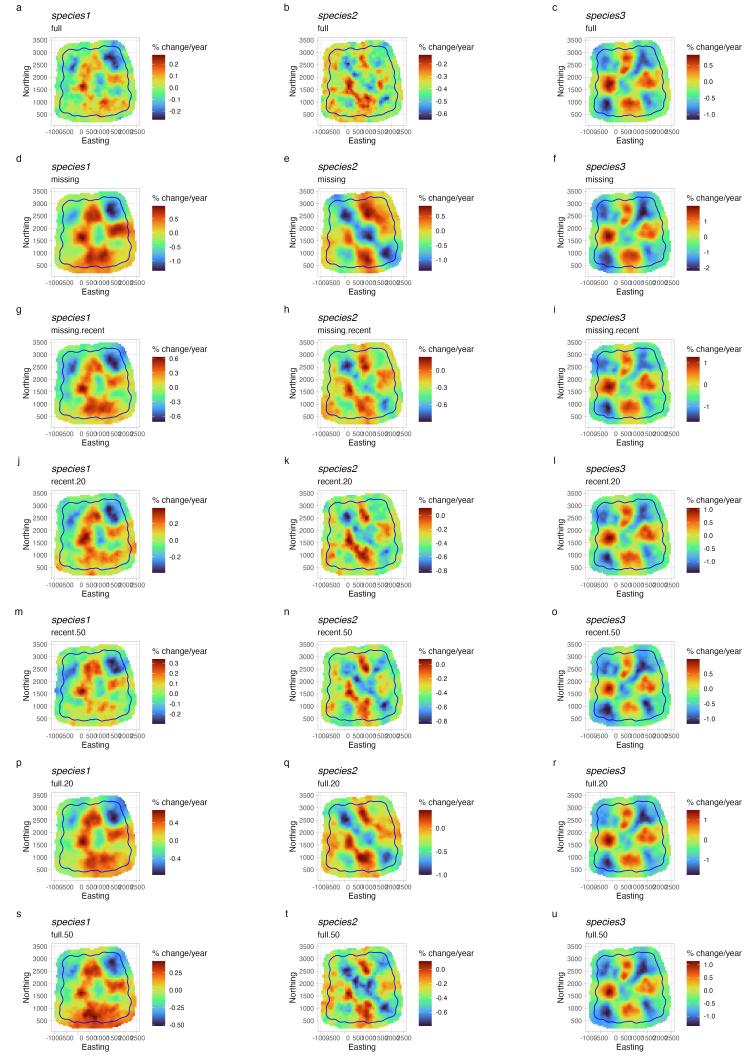
$$\text{logit}(\hat{P}_{h,i}) = A_h + T_h * \text{year}_i + \alpha_{h,l_i} + \tau_{h,l_i} * \text{year}_i + \delta_{l_i} \quad (\text{A1})$$

1168 Where symbiont presence/absence of the  $i^{th}$  specimen ( $P_{h,i}$ ) was modeled as a Bernoulli re-  
1169 sponse variable with expected probability of symbiont occurrence  $\hat{P}_{h,i}$  for each host species  $h$ . We  
1170 modeled  $\hat{P}_{h,i}$  as a linear function of intercept  $A_h$  and slope  $T_h$  defining the global trend in endophyte  
1171 prevalence specific to each host species as well as with spatially-varying intercepts  $\alpha_{h,l_i}$  and slopes  
1172  $\tau_{h,l_i}$  associated with location ( $l_i$ , the unique latitude-longitude combination of the  $i$ th observation).  
1173 Similar to the SVC model of our central analysis (Eqn. 1), we estimated a shared variance term  
1174 with the spatially-dependent random effect  $\delta_{l_i}$ , intended to account for residual spatial variation.  
1175 However in this analysis we omit i.i.d.-random effects terms associated with collector and scorer  
1176 identity ( $\chi_{c_i}$  and  $\omega_{s_i}$  in Eqn. 1) for the sake of simplicity.

1177 *Influence of spatially-biased sampling on model interpretation*

1178 Our analysis of the full simulated data shows that our model is suitably flexible to capture complex  
1179 spatial patterns in temporal trends (Fig. A21 a-c). Beyond this, the model also qualitatively  
1180 captures the spatial patterns in temporal trends even with large amounts of data missingness (i.e.  
1181 missing up to 80% of the datapoints (Fig. A21 p-r)).

1182 While this analysis is not an exhaustive examination of the influence of sampling bias on our  
1183 results for several reasons (including not examining how different strengths in temporal trends,  
1184 different spatial arrangements of missing-ness influence model estimates, or different sample sizes  
1185 influence results), it demonstrates that the spatially-varying modelling framework implemented in  
1186 INLA we employ can suitably recover regional trends even with significant spatially-bias within  
1187 data collection, and further the analysis is likely robust to temporally-structured bias (missing data



**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.

1188 within recent collection period). Future work could more fully explore the scenarios that cause  
1189 this ability to break down. We expect this simulation reflects what may be a common scenario for  
1190 research investigating global change using natural history specimens. Collection effort by trained  
1191 taxonomists and professional collectors peaked in the past, and collections contain relatively fewer  
1192 modern specimens in many regions. Additionally, most global change research necessarily involves  
1193 accessing many specimens across collections. Research efforts such as ours will be unable to access  
1194 every specimen from all possible collections. Ongoing digitization efforts will make it possible to  
1195 more clearly assess how much data is missing from a particular study compared to the actual  
1196 holdings of natural history collections, but ultimately, the decision of what data and collections to  
1197 include is a question of sample size and study design.