

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

<sup>3</sup> Joshua C. Fowler<sup>1,2\*</sup>

Jacob Moutouama<sup>1</sup>

Tom E. X. Miller<sup>1</sup>

<sup>4</sup> 1. Rice University, Department of BioSciences, Houston, Texas 77006; 2. University of Miami,  
<sup>5</sup> Department of Biology, Miami, Florida;

<sup>6</sup> \* Corresponding author; e-mail: jcf221@miami.edu.

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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 analysis 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. We identified greater local-scale variability in endophyte prevalence in contemporary data compared to model predictions based on historic data, 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

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

46 Natural history specimens, which were originally collected to study and preserve taxonomic  
47 diversity, present a unique opportunity to explore long-term changes in **biodiversity** and ecological  
48 interactions across broad spatial and temporal scales (Davis, 2023; Meineke et al., 2018). Natural  
49 history collections, built and maintained by the efforts of thousands of scientists, are invaluable  
50 time machines, primarily comprised of physical specimens of organisms along with information  
51 about the time and place of their collection. These specimens often preserve physical legacies of  
52 ecological processes and species' interactions from dynamically changing environments across time  
53 and space (Lendemer et al., 2020). For example, previous researchers have examined the flowers,  
54 pollen grains, and leaves of specimens within plant collections (herbaria) collected across time to  
55 document shifts in reproductive phenology (Berg et al., 2019; Park et al., 2019; Willis et al., 2017),  
56 pollination (Duan et al., 2019; Pauw and Hawkins, 2011), and herbivory (Meineke et al., 2019)  
57 related to anthropogenic climate change. However, few previous studies have leveraged biological  
58 collections to examine climate change-related shifts in a particularly common type of interaction:  
59 microbial symbiosis.

60 Microbial symbionts are common to all macroscopic organisms and can have important effects  
61 on their hosts' survival, growth and reproduction (McFall-Ngai et al., 2013; Rodriguez et al., 2009).

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

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

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

104       Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic diversity  
105 of host species that harbor these symbionts (White and Cole, 1985), establishing that endophyte  
106 symbiosis could be identified in plant tissue from as early as 1851. However, no subsequent studies,  
107 to our knowledge, have used the vast resources of biological collections to quantitatively assess  
108 spatio-temporal trends in endophyte prevalence and their environmental correlates. Previous work  
109 has used herbarium specimens to identify the origins and population genomics of plant diseases such  
110 as *Phytophthora*, the Irish potato famine pathogen (Ristaino et al., 2001; Ristaino, 2002; Yoshida et al.,  
111 2013), and have been proposed as vehicles to track other emerging plant pathogens (Bradshaw et al.,  
112 2021; Ristaino, 2020). Grasses are commonly collected and identified based on the presence of their  
113 reproductive structures, meaning that preserved specimens typically contain seeds, conveniently  
114 preserving the fungi along with their host plants on herbarium sheets. This creates the opportunity  
115 to leverage the unique spatio-temporal sampling of herbarium collections to examine the response

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

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

136

## Methods

137

### *Focal species*

138 Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis perennans*,  
139 and *Elymus virginicus* that host *Epichloë* symbionts. These cool-season grass species are commonly  
140 represented in natural history collections with broad distributions covering much the eastern United

<sup>141</sup> States (Fig. 1). Cool-season grasses grow during the cooler temperatures of spring and autumn  
<sup>142</sup> due to their reliance on  $C_3$  photosynthesis. *A. hyemalis* is a small short-lived perennial species  
<sup>143</sup> that germinates in late winter/early spring and typically flowers between March and July (most common  
<sup>144</sup> collection month: May). *A. perennans* is of similar stature but is longer lived than *Agrostis hyemalis*  
<sup>145</sup> and flowers in late summer and early autumn (most common collection month: September). *A.*  
<sup>146</sup> *perennans* is more sparsely distributed, tending to be found in shadier and moister habitats, while  
<sup>147</sup> *A. hyemalis* is commonly found in open and recently disturbed habitats. Both *Agrostis* species are  
<sup>148</sup> recorded from throughout the Eastern US, but *A. perennans* has a slightly more northern distri-  
<sup>149</sup> bution, whereas *A. hyemalis* is found rarely as far north as Canada and is listed as a rare plant  
<sup>150</sup> in Minnesota. *E. virginicus* is a larger and relatively longer-lived species that is more broadly dis-  
<sup>151</sup> tributed than the *Agrostis* species. It begins flowering as early as March or April but continues  
<sup>152</sup> throughout the summer (most common collection month: July).

<sup>153</sup> Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001; Leuchtmann et al., 2014),  
<sup>154</sup> while *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl, 2002). The fungal sym-  
<sup>155</sup> bionts primarily reproduce asexually and are passed from mother to offspring by vertical transmis-  
<sup>156</sup> sion through seeds. These traits contribute to highly specialized interactions between symbiont and  
<sup>157</sup> host. Some host species have been shown to partner with multiple symbiont species or strains, and in  
<sup>158</sup> some cases multiple symbiont lineages can co-exist within a host population (Mc Cargo et al., 2014).  
<sup>159</sup> However, surveys have typically found limited *Epichloë* genotypic diversity within host populations  
<sup>160</sup> (Treindl et al., 2023). Across host populations, concentrations of biologically-active alkaloids and  
<sup>161</sup> the genes associated with their production vary substantially (Schardl et al., 2012). In this analysis,  
<sup>162</sup> we focus on the presence/absence of *Epichloë* symbionts, and we discuss potential implications of  
<sup>163</sup> symbiont genotypic diversity in the Discussion.

<sup>164</sup> *Herbarium surveys*

<sup>165</sup> We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens included  
<sup>166</sup> from each collection). With permission from herbarium staff, we acquired seed samples from 1135

*A. hyemalis* specimens collected between 1824 and 2019, 357 *A. perennans* specimens collected between 1863 and 2017, and 854 *E. virginicus* specimens collected between 1839 and 2019 (Fig. 1, Fig. 2A, Fig. A1). We chose our focal species in part because they are commonly represented in herbarium collections and produce many seeds, meaning that small samples would not diminish the value of the specimens for future studies. We collected 5-10 seeds per specimen after examining the herbarium sheet under a dissecting microscope to ensure that we collected mature seeds, not florets or unfilled seeds, fit for our purpose of identifying fungal endophytes with microscopy. We excluded specimens for which information about the collection location and date were unavailable.

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

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

194 sampling on our results through a simulation analysis (Appendix A - Supporting Methods).

195 After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hyphae  
196 in each specimen using microscopy. We first softened seeds with a 10% NaOH solution, then stained  
197 the seeds with aniline blue-lactic acid stain and squashed them under a microscope cover slip. We  
198 examined the squashed seeds for the presence of fungal hyphae at 200-400X magnification (Bacon  
199 and White, 2018). On average we scored 4.7 intact seeds per specimen of *A. hyemalis*, 4.2 seeds  
200 per specimen of *A. perennans*, and 3.8 seeds per specimen of *E. virginicus*; we scored 10,342 seeds  
201 in total. Due to imperfect vertical transmission, the production of symbiont-free offspring from  
202 symbiotic hosts (Afkhami and Rudgers, 2008), it is possible that symbiotic host-plants produce a  
203 mixture of symbiotic and non-symbiotic seeds. We therefore designated a specimen as endophyte-  
204 symbiotic if *Epichloë* hyphae were observed in one or more of its seeds, or non-symbiotic if *Epichloë*  
205 hyphae were observed in none of its seeds. To capture uncertainty in the endophyte identification  
206 process, we recorded both a "liberal" and a "conservative" endophyte score for each plant specimen.  
207 When we confidently identified endophytes within a specimen's seeds, we assigned matching liberal  
208 and conservative scores. When we identified potential endophytes with unusual morphology, low  
209 uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a positive  
210 identification for the liberal score and a negative identification for the conservative score. 89% of  
211 scored plants had matching liberal and conservative scores, reflecting high confidence in endophyte  
212 status. The following analyses used the liberal status, however repeating all analyses with the  
213 conservative status yielded qualitatively similar results (Fig. A8).

214 *Modeling spatial and temporal changes in endophyte prevalence*

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

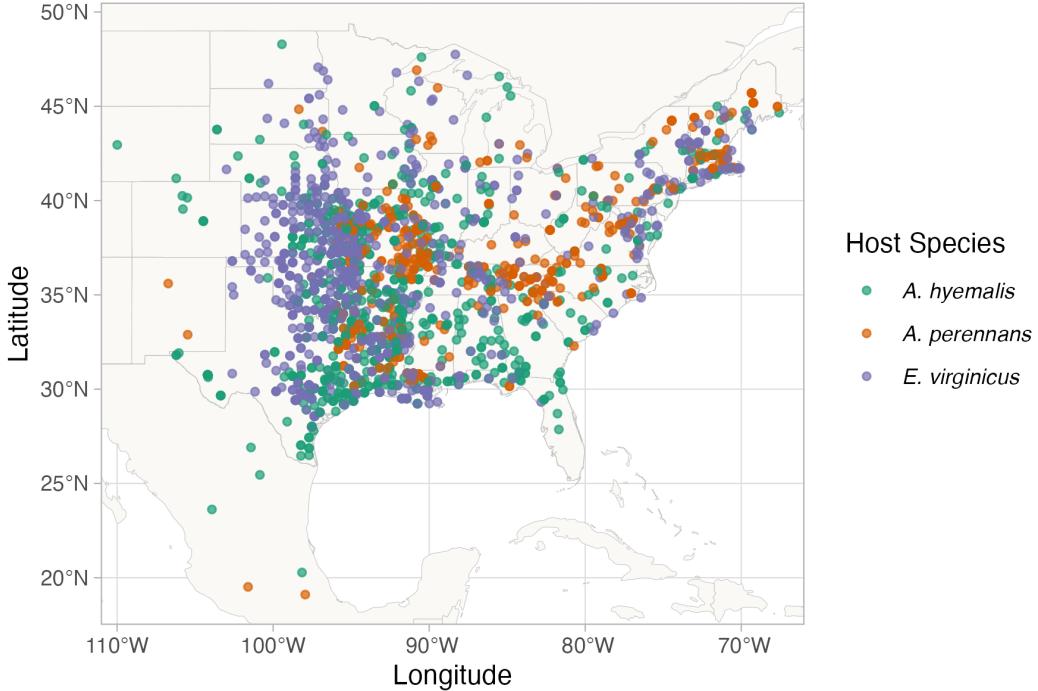


Figure 1: Collection locations of herbarium specimens sampled for *Epichloë* endophytes.

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.

(INLA), to construct spatio-temporal models of endophyte prevalence. INLA provides a computationally efficient method of ascertaining parameter posterior distributions for certain models that can be formulated as latent Gaussian Models (Rue et al., 2009). Many common statistical models, including structured and unstructured mixed-effects models, can be represented as latent Gaussian Models. We incorporated spatial heterogeneity into this analysis using spatially-structured intercept and slope parameters implemented as stochastic partial differential equations (SPDE) to approximate a continuous spatial Gaussian process. This SPDE approach is a flexible method of smoothing across space while explicitly accounting for spatial dependence between data-points (Bakka et al., 2018; Lindgren et al., 2011). Fitting models with structured spatial effects is possible with MCMC sampling but can require long computation times, making INLA an effective alternative. This ap-

230 proach has been used to model spatial patterns in flowering phenology (Willems et al., 2022), the  
231 abundance of birds (Meehan et al., 2019) and butterflies (Crossley et al., 2022), the distribution of  
232 temperate trees (Engel et al., 2022) as well as the population dynamics of endangered amphibians  
233 (Knapp et al., 2016) and other ecological processes (Beguin et al., 2012).

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

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

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

251 We performed model fitting using the inlabru R package (Bachl et al., 2019). Global intercept  
252 and slope parameters,  $A$  and  $T$ , were given vague priors. Collector and scorer random effects,  $\chi$  and  
253  $\omega$  respectively, were centered at 0 with precision parameters assigned penalized complexity (PC)  
254 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 spatial-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 of the data and generate predictions across the entire study region. The SPDE approach is flexible enough that it can capture smooth trends across space that are informed by the data rather than by spatial regions chosen *a priori* by researchers. However this flexibility also invites the risk of overfitting, as with other non-linear modeling approaches (Lapeyrolerie and Boettiger, 2023; Ramampiandra et al., 2023; Ward et al., 2014). Priors for the Matérn covariance function, termed "range" and "variance", define how proximity effects decay with distance. The choice of priors for these types of spatial models is an area of active research (Bakka et al., 2018; Simpson et al., 2017), but another advantage of the INLA approach is that its computational efficiency allows for sensitivity analyses. Results presented in the main text reflect a prior range of 342 kilometers (i.e. a 50% probability of estimating a range less than 342 kilometers). We tested a range of values (from 68 kilometers to 1714 kilometers) and meshes (presented in the Supporting Methods), finding that while the magnitude and uncertainty of effects varied, model results were qualitatively similar, i.e. the same direction of effects across space. We assessed model fit with visual posterior predictive checks (A3) and measurements of AUC (Figs. A4-A5) (Gelman and Hill, 2006). Through results and discussion that follow, we refer to the model described in this section as the “endophyte prevalence model”.

281                   *Modeling distributions of host species*

282   The herbarium records did not encompass the entirety of each host species' range. Therefore, we  
283   used additional data sources to model the geographic distribution of each host species, with two  
284   goals: (1) generate realistic maps on which we could project the predictions of the INLA model,  
285   and (2) use the geographic distributions to test for relationships between climate change drivers and  
286   trends in endophyte prevalence. We followed the ODMAP (overview, data, model, assessment,  
287   prediction) protocol (Crossley et al., 2022) (see Supporting Methods). In short, we used presence-  
288   only observations of each host species from Global Biodiversity Information Facility (GBIF) between  
289   1990 to 2020 (713 occurrence records for *A. hyemalis* (GBIF.Org, 2025a), 656 occurrence records  
290   for *A. perennans* (GBIF.Org, 2025b), and 2338 occurrence records for *A. virginicus* (GBIF.Org,  
291   2025c)). We fit maximum entropy (MaxEnt) models using the maxent function in the R package  
292   dismo (Hijmans et al., 2017) using following seasonal climate predictors (1990-2020 climate normals):  
293   mean and standard deviation of spring, summer, and autumn temperature, and mean and standard  
294   deviation of spring, summer, and autumn cumulative precipitation. We generated 10,000 pseudo-  
295   absences as background points, and split the occurrence data into 75% for model training and 25%  
296   for model testing. The performance of models was evaluated with AUC (Jiménez-Valverde, 2012).  
297   We found AUC values of 0.862, 0.838, 0.821 respectively for *Agrostis hyemalis*, *Agrostis perennans*,  
298   and *Elymus virginicus* indicating good model fit to data. We used the training sensitivity (true  
299   positive rate) and specificity (true negative rate) to set a threshold for transforming the continuous  
300   predicted probabilities into binary presence - absence host distribution maps on which we projected  
301   INLA predictions of endophyte prevalence (Liu et al., 2005).

302                   *Assessing the role of climate drivers*

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

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

327 We then evaluated whether areas that have experienced the greatest changes in endophyte preva-  
328 lence (hotspots of endophyte change) are associated with high degrees of change in climate (hotspots  
329 of climate change) To do so, we modeled the fitted, spatially-varying slopes of endophyte change  
330 through time ( $\tau_{[h]l}$ ) as a linear function of environmental covariates, with a Gaussian error distri-  
331 bution for a set of pixels across each host distribution. The continuous SPDE approach taken from  
332 our endophyte prevalence model allows us to generate predictions of temporal trends in prevalence

333 at arbitrarily many pixels across each host distribution. Balancing computation time with resolu-  
334 tion, we generated predicted trends for 546, 645, and 753 pixels across each host distribution for *A.*  
335 *perennans*, *A. hyemalis*, and *E. virginicus* respectively ( pixel dimensions: *A. perennans* = 65 km  
336 x 36 km; *A. hyemalis* = 61km x 45 km; *E. virginicus* = 62 km x 40 km ). Fitting regressions to  
337 many pixels across the study region risks artificially inflating confidence in our results due to large  
338 sample sizes, and so we performed this analysis using only a random subsample of 250 pixels across  
339 the study region; other sizes of subsample yielded similar results. Data from each host species were  
340 analyzed separately. Throughout the results and discussion that follow, we refer to this analysis as  
341 the “*post hoc* climate regression analysis”.

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

343 We evaluated the predictive ability of the endophyte prevalence model using both in-sample train-  
344 ing data from the herbarium surveys, and with out-of-sample test data, an important but rarely  
345 used strategy in ecological studies (Lee et al., 2024; Tredennick et al., 2021). We generated out-of-  
346 sample test data from contemporary surveys of endophyte prevalence in natural populations of *A.*  
347 *hyemalis* and *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted  
348 in 2013 as described in Sneck et al. (2017), and surveys of *A. hyemalis* took place between 2015 and  
349 2020. Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation in  
350 endophyte prevalence towards its range edge, while surveys of *E. virginicus* were designed to cover  
351 latitudinal variation. In total, we visited 43 populations of *A. hyemalis* and 20 populations of *E.*  
352 *virginicus* across the south-central US, with emphasis on Texas and neighboring states (Fig A12).  
353 During surveys, we collected seeds from up to 30 individuals per population (average number of  
354 plants sampled per population: 22.9); note that this sampling design provided greater local depth  
355 of information than the herbarium records, where only one plant was sampled at each locality. We  
356 quantified the endophyte status of each individual with microscopy as described for the herbarium  
357 surveys (with 5-10 seeds scored per individual), and calculated the prevalence of endophytes within  
358 the population (proportion of plants that were endophyte-symbiotic). For each population, we com-

359 pared the observed fraction of endophyte-symbiotic hosts to the predicted probability of endophyte  
360 occurrence  $\hat{P}$  derived from the model for that location and year. The contemporary survey period  
361 (2013–2020) is at the most recent edge of the time period encompassed by the historical specimens  
362 used for model fitting.

363 **Results**

364 *How has endophyte prevalence changed over time?*

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

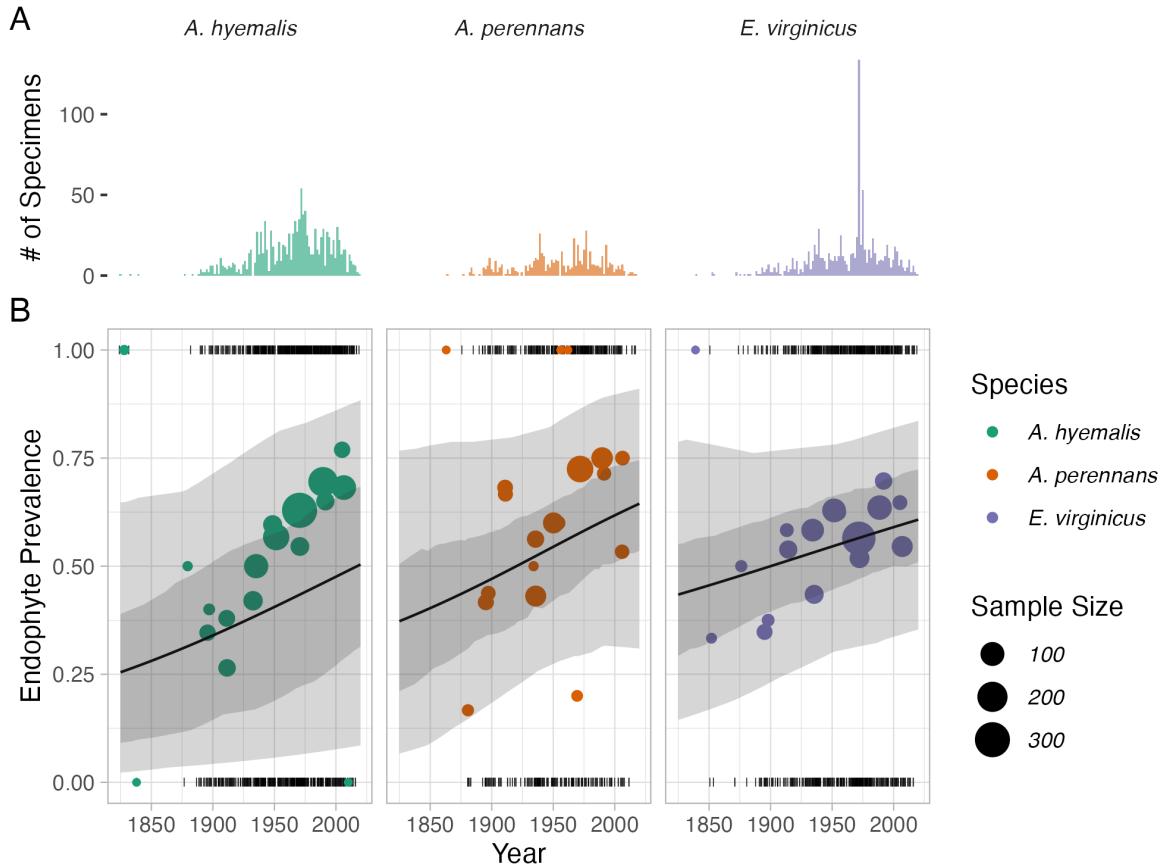


Figure 2: **Temporal trends in endophyte prevalence.** (A) Histograms show the frequency of scored specimens through time for each host species. (B) Lines show mean endophyte prevalence predicted by the endophyte prevalence model over the study period along with the 50% and 95% CI bands incorporating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

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

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

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

382 While there was an overall increase in endophyte prevalence, our model revealed hotspots and  
383 coldspots of change across the host species' ranges, which are mapped in Fig. 3 across geographic  
384 ranges predicted by MaxEnt species distribution models. In some regions, posterior mean esti-  
385 mates of spatially varying temporal trends indicate that *A. hyemalis* and *A. perennans* experienced  
386 increases in prevalence by as much as 2% per year over the study period, while *E. virginicus* ex-  
387 perienced increases up to around 1% per year. Both *Agrostis* species show areas of strong increase  
388 and areas of declining prevalence, while *E. virginicus* had an overall weaker and geographically  
389 more homogeneous increase in endophyte prevalence. Notably, endophytes are predicted to have  
390 increased most strongly towards the western range edge of *A. hyemalis* (Fig. 3A) and across the  
391 northeastern US for *A. perennans* (Fig. 3B). Broad increases in prevalence on average, along with  
392 increases towards range edges that had low historic prevalence result in range expansions of the  
393 symbiosis for both *Agrostis* species (Fig. 4). Increases in prevalence were strongest in regions with  
394 low historic prevalence for the *Agrostis* species (Fig. A11 A-B), but for *E. virginicus* trends did  
395 not differ according to historic prevalence (A11 C). Posterior estimates of uncertainty in spatially  
396 varying slopes indicate that these hotspots of change may have experienced increases of up to 5%  
397 per year while declines in prevalence may be as great as -4% per year for *A. hyemalis* and *A.*  
398 *perennans*. For *E. virginicus*, uncertainty ranges between 3.5% increases and 2.5% decreases (Fig.  
399 A7).

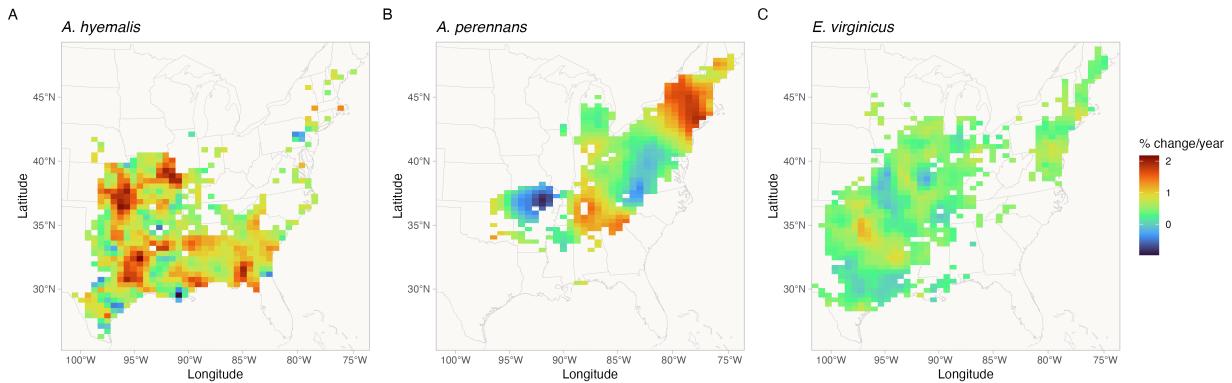


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species (A, *A. hyemalis*; B, *A. perennans*; C, *E. virginicus*). Spatially-varying trends are estimated from the endophyte prevalence model. Color indicates the relative change in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

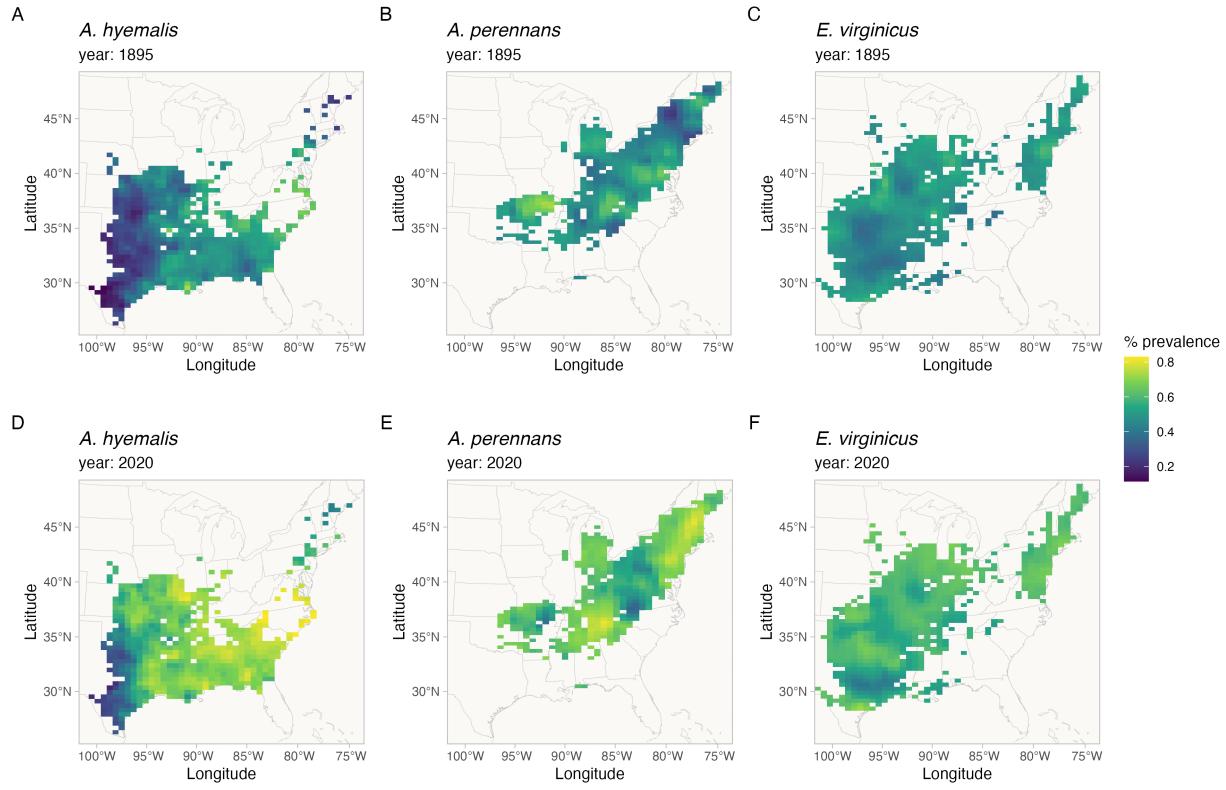


Figure 4: Predicted endophyte prevalence for each host species in 1895 and 2020. Predictions of prevalence come from the endophyte prevalence model. Color indicates the posterior mean endophyte prevalence for *A. hyemalis* (A,D), *A. perennans* (B,E), and *E. virginicus* (C,F). Map lines delineate study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

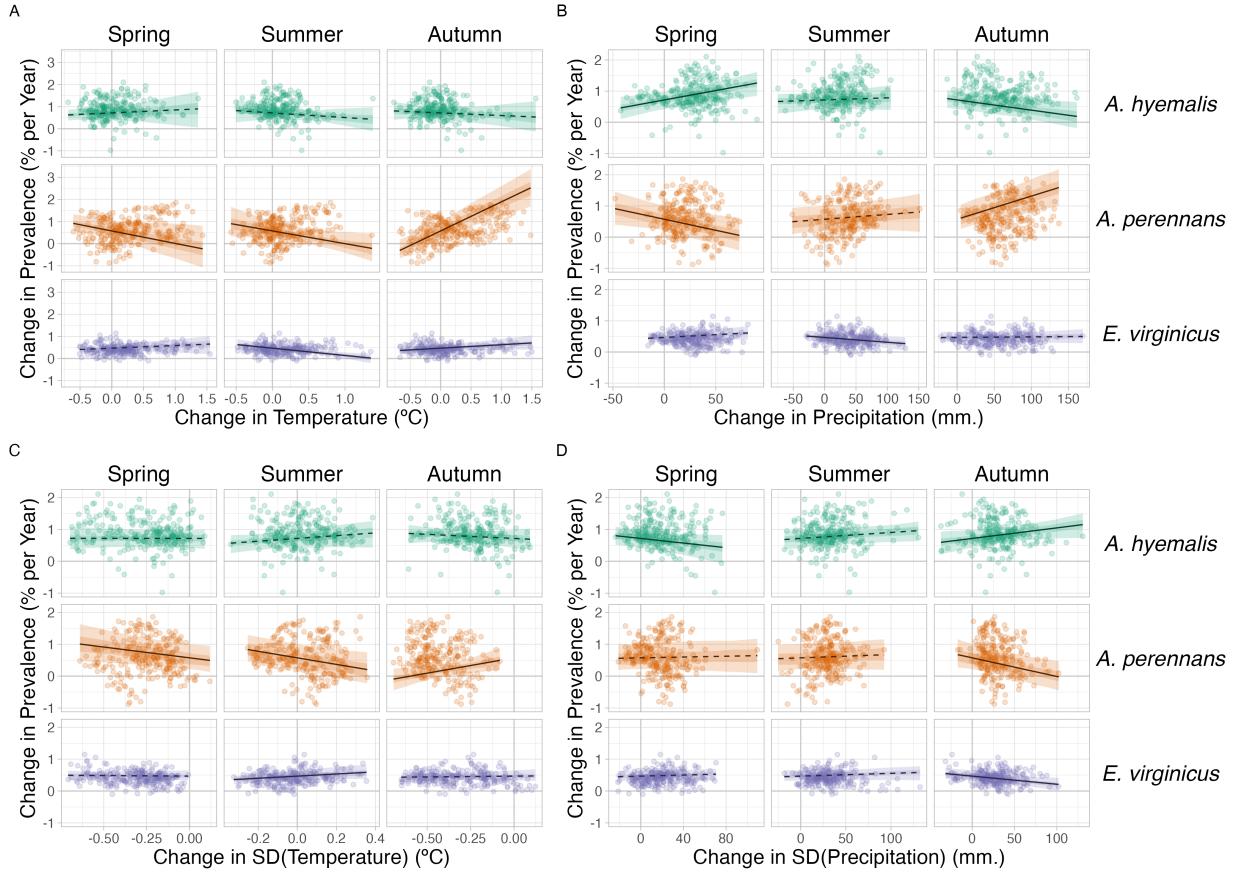
400     What is the relationship between variation in temporal trends in endophyte  
401     prevalence and changes in climate drivers?

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

407 changes were spatially variable; certain locations experienced increases in annual precipitation as  
408 large as 375 mm or decreases up to 54 mm across the last century, while annual temperature changes  
409 ranged from warming as great as 1.4 °C to cooling by 0.46 °C. Spatially variable climate trends were  
410 predictive of trends in endophyte prevalence. For example, strong increases in endophyte prevalence  
411 for *A. perennans* were most strongly associated with increasing autumn precipitation and with  
412 increasing mean and variability in autumn temperature (greater than 97% posterior probabilities  
413 of positive slopes). For this species, each 1 °C increase in autumn temperature was associated with  
414 a 1.07 % greater increase per year in endophyte prevalence (Fig. 5A) and a 100 mm increase in  
415 precipitation was associated with a 0.8% greater increase per year in endophyte prevalence (Fig.  
416 5B). This result aligns with the species' autumn active growing season, however other seasonal  
417 climate drivers were also associated with increasing endophyte prevalence in this host species. In  
418 particular, we found cooler and drier springs and cooler summers to be associated with increasing  
419 endophyte prevalence (greater than 99% posterior probabilities of negative slopes), though these  
420 slopes were generally of smaller magnitude than those for autumn climate drivers.

421 Changes in endophyte prevalence across the ranges of *A. hyemalis* and *E. virginicus* were less  
422 strongly driven by changes in climate. Like *A. perennans*, climate during peak growing season  
423 (spring for *A. perennans* and summer for *E. virginicus*) emerged most commonly as drivers of  
424 changes in endophyte prevalence. Increases in mean spring precipitation were the strongest predictor  
425 of increasing trends in endophyte prevalence for *A. hyemalis* (Fig. 5B) (greater than 99% posterior  
426 probability of a positive slope). For this species, an increase of 100 mm in spring precipitation was  
427 associated with 0.6% per year stronger increases in endophyte prevalence relative to regions with  
428 no change in precipitation. The next greatest slopes were those associated with variability in spring  
429 precipitation (greater than 96% posterior probability of a negative slope), as well as in the mean  
430 and variability of autumn climate (greater than 98% probability of negative and positive slopes,  
431 respectively). Changes in endophyte prevalence in *E. virginicus* were not strongly associated with  
432 changes in most climate drivers, but regions with reduced variability in autumn precipitation (Fig.  
433 5B) and with cooler and more variable summer temperatures (Fig. 5A,C) experienced the largest

434 increases in endophyte prevalence. Our analysis indicated relatively high confidence that these  
435 climate drivers influence endophyte prevalence shifts in *E. virginicus*(greater than 99% posterior  
436 probability of either negative or positive slopes respectively), however they translate, for example,  
437 to less than a 0.4% decrease in endophyte prevalence per year for each 1°C of summer warming  
438 over the century. Repeating this analysis using all pixels across each species' distribution were  
439 qualitatively similar 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.

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

441    Tests of the endophyte prevalence model's predictive performance as quantified by AUC and by  
442    visual posterior predictive checks, indicated good predictive ability. Model performance was similar  
443    between historic herbarium specimens used as training data and the out-of-sample test data from  
444    contemporary surveys (AUC = 0.79 and 0.77 respectively; Fig. A5-A4). The model successfully  
445    captured broad regional trends in endophyte prevalence seen in the contemporary survey data,  
446    such as decline endophyte prevalence in *A. hyemalis* towards western longitudes (Fig. 6A) and an  
447    increase towards northern latitudes (Fig. 6B). It is noteable that model predictions for endophyte  
448    prevalence exhibited relatively little local geographic variation, whereas the out-of-sample survey  
449    data were highly variable with populations spanning 0% to 100% endophyte-symbiotic plants (Fig.  
450    6C), indicating population-to-population variation not captured in the endophyte prevalence model.

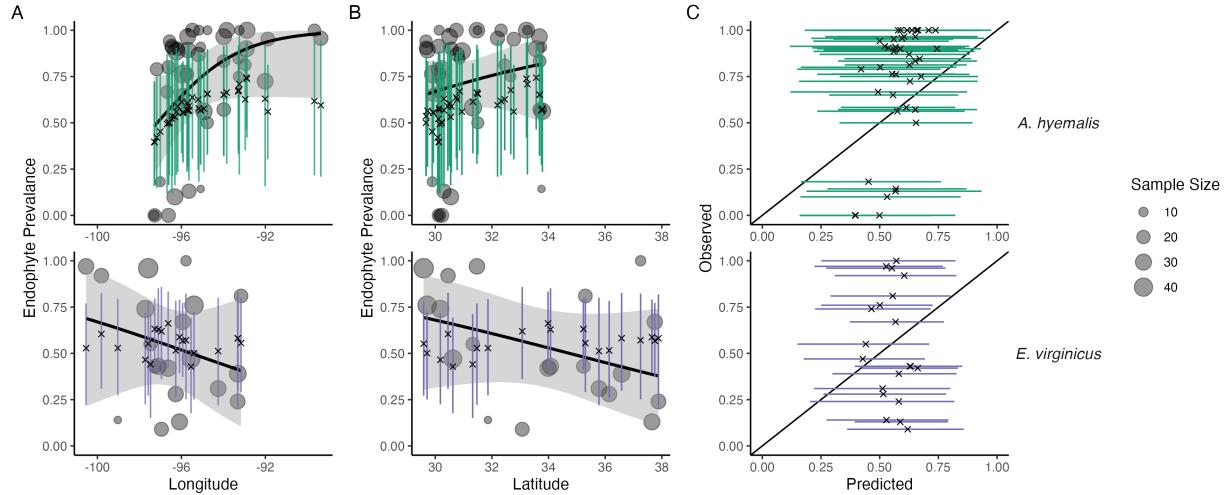


Figure 6: **Predictive performance for contemporary test data.** (A) The endophyte prevalence model, trained on historic herbarium collection data, performed modestly at predicting prevalence in contemporary population surveys. The model captured regional trends across (A) longitude and (B) latitude. 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

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

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

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

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

471 Based on previous work demonstrating that endophytes can shield their hosts from drought  
472 stress (reviewed in Decunta et al. (2021)), we generally predicted that drought conditions would be  
473 a driver of increasing endophyte prevalence. In contrast to this expectation, increasing prevalence  
474 for *A. perennans* was associated with both increasing autumn temperature and precipitation (Fig.  
475 5). To our knowledge, the response of the symbiosis in *A. perennans* to drought has not been  
476 examined experimentally, but in a greenhouse experiment, endophytes had a positive effect on host  
477 reproduction under shaded, low-light conditions (Davitt et al., 2010). Our results also hint that it  
478 may be useful to investigate whether lagged climate effects are important predictors of host fitness  
479 in this system (Evers et al., 2021). Endophyte prevalence of the autumn-flowering *A. perennans* was  
480 strongly linked with decreasing spring precipitation, and that of the spring-flowering *A. hyemalis*  
481 was associated with decreasing autumn precipitation (Fig. 5B). For *A. hyemalis*, endophytes could  
482 be playing a role helping hosts weather autumn-season droughts, which is likely also an important  
483 time for the species' germination. Previous work demonstrated drought benefits in a greenhouse

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

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

511 increasingly stressful by the combination of these global change drivers, and while historic trends  
512 that we observed suggest that symbiotic fitness benefits have helped mitigate this stress, it is likely  
513 that at some higher leveler of stress, increasing costs of maintaining the mutualism could lead to  
514 declines in endophyte prevalence. Changing endophyte prevalence results from the combination of  
515 net fitness benefits playing out across the heterogeneous map of a changing climate and and its  
516 interactive effects on other anthropogenic drivers.

517 Our results indicate that *Epichloë* symbiosis has likely improved host fitness in stressful en-  
518 vironments leading to increasing prevalence. What is less clear is how this will influence future  
519 range shifts. Based on our analysis, it is likely that the symbiosis will facilitate range shifts for  
520 hosts by improving population growth at range edges. Previous population surveys (Rudgers and  
521 Swafford, 2009; Semmarin et al., 2015; Sneck et al., 2017) attributed environment-dependent gra-  
522 dients in endophyte prevalence to symbiont-derived fitness benefit's allowing hosts to persist in  
523 environments where they otherwise could not (Afkhami et al., 2014; Kazenel et al., 2015). However,  
524 symbiont-facilitated range shifts require that endophytes be present in the populations to be able  
525 to contribute to population growth. The arid western range edge of *A. hyemalis* has had histor-  
526 ically low endophyte prevalence (Fig. 4), and while prevalence has increased most quickly in the  
527 regions with historically low endophyte prevalence (Fig. A11), the complete absence of endophytes  
528 at range edges in this species would make it impossible for prevalence to increase without dispersal  
529 of symbiotic seeds (Fowler et al., 2023). These factors potentially contribute to the ability of the  
530 host species to track its environmental niche. Another interesting question is the degree to which  
531 symbiotic and non-symbiotic hosts, which occupy overlapping but distinct niches, are likely to ex-  
532 perience distribution shifts in tandem or at different rates in future. More extreme climate stresses,  
533 which are expected more frequently in the future (Seneviratne et al., 2021), have the potential to  
534 alter the costs and benefits of the interaction. The past indicates a resilient symbiosis, but it will  
535 be crucial to understand whether more extreme future climate conditions could tip this interaction  
536 to deteriorate.

537 *Steps towards forecasts of host-microbe symbioses*

538 The combination of a spatially-explicit model and historic herbarium specimens allowed us to  
539 identify regions of both increasing and decreasing endophyte prevalence, however we see several  
540 next steps toward the goal of predicting host and symbiont niche-shifts in response to future cli-  
541 mate change. While the model recreated the large-scale spatial trends observed in contemporary  
542 population surveys, test data contained more population-to-population variability in prevalence than  
543 could be explained by the model. We interpret this to mean that the model captures coarse-scale  
544 spatial and temporal trends reasonably well, but is not equipped to capture local-scale nuances that  
545 generate population-to-population differences. Validating our model predictions with this test, a  
546 rare extra step in collections-based studies, allows us to identify ways in which the model's out-of-  
547 sample predictive ability could be improved. Lack of information on local variability in symbiont  
548 prevalence may simply be a feature of data derived from herbarium specimens. Natural history  
549 collectors sample one or a few specimens from local populations, and these observations are aggre-  
550 gated by the model to derive broad-scale estimates. This suggests that increasing local replication  
551 should be a factor considered in future collection efforts of natural history specimens, balancing the  
552 required time and effort along with limitations on storage space within collections. An alternative  
553 validation test would be to hold-out samples from within the historic data set. Such a test would  
554 more clearly provide a set of data that matched the conditions of the training data (i.e in spatial  
555 scale and climate conditions) missing within the contemporary test data, however a relatively lim-  
556 ited number of sampled specimens held us back from exploring this option. Splitting datasets can  
557 negatively impact model estimates, and the choice of how to split the data for model validation is  
558 not trivial (Bergmeir and Benítez, 2012; James et al., 2013). From a modeler's perspective, this  
559 also urges advocacy for increased collection efforts and expansion of herbarium collections.

560 Another key consideration in forecasting the dynamics host-microbe symbioses is the spatial  
561 scale of both specimen georeferencing and available climate data. For this analysis, most specimen  
562 localities were assigned coordinates at county or city centroids, and the climate data examined

563 was on 4 km grid cells. Georeferencing of specimens as accurately as possible is a key priority  
564 of herbarium specimen digitization efforts (Davis, 2023; Soltis, 2017). While the INLA modeling  
565 approach that we used allows for predictions at arbitrarily small spatial scales, and would simplify  
566 connecting model predictions to the scale of a given climate driver, the coarse scale inherent to our  
567 analysis may obscure some local-scale ecological processes. Poor predictive ability at local scales in  
568 this grass-endophyte system is not surprising, as previous studies have found that local variation,  
569 even to the scale of hundreds of meters can structure endophyte-host niches (Gundel et al., 2024;  
570 Kazenel et al., 2015). An important step would be integrating data from local and regional scales  
571 through modeling to constrain estimates of local and regional variation.

572 Predicting future niche-shifts of hosts and symbionts will require considering the coupled dynam-  
573 ics of host-symbiont dispersal in addition to fitness benefits. For example, transplanting symbiotic  
574 and non-symbiotic plants beyond the range edge of *A. hyemalis* could tell us whether low endophyte  
575 prevalence in that area (Fig. 4A) is a result of environmental conditions that lead the symbiosis  
576 to have negative fitness consequences, or is a result of some historical contingency or dispersal lim-  
577 itation that has thus far limited the presence of symbiotic hosts from a region where they would  
578 otherwise flourish and provide resilience. Another interesting question is whether local adaption,  
579 in either host or symbiont may influence future range shifts. Incorporating available climatic and  
580 soil layers as covariates is another obvious step that could improve predictions. These steps will  
581 bridge gaps that often exist between large but broad bioclimatic and biodiversity data and small but  
582 high-resolution data on biotic interactions, and move towards the goal of predicting the dynamics  
583 of microbial symbioses under climate change (Isaac et al., 2020; Miller et al., 2019).

584 *Herbaria for global change research*

585 Our analysis advances the use of herbarium specimens in global change biology in two ways.  
586 First and foremost, this is one of a growing number of studies to examine microbial symbiosis using  
587 specimens from natural history collections, and the first, to our knowledge, to link long-term changes  
588 in the symbioses to changes in climate. The responses of microbial symbioses are a rich target for  
589 future studies within historic specimens, particularly those that take advantage of advances in

sequencing technology. While we used relatively coarse presence/absence data based on fungal morphology, other studies have examined historic plant microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these studies have so far been limited to relatively few specimens at limited spatial extents (Bearchell et al., 2005; Bieker et al., 2020; Bradshaw et al., 2021, 2023; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al., 2015). Much of this work highlights the important role that historic specimens can play in tracking pathogens, a particularly important area as climate change facilitates the spread of new diseases (Ristaino, 2020; Singh et al., 2023) Continued advances in capturing historic DNA and in filtering out potential contamination during specimen storage (Bakker et al., 2020; Daru et al., 2019; Raxworthy and Smith, 2021) will be imperative in the effort to scale up these efforts. This scaling up will be essential to be able to quantify changes not just in the prevalence of symbionts, but also in symbionts' intraspecific variation and evolutionary responses to climate change, as well as in changes in the wider host microbiome. With improved molecular insights from historic specimens, we could ask whether the broad increases in endophytes that we have identified reflect selection for particular genetic strains or chemotypes and how this selection varies across space. Answering these questions as well as the unknown questions that future researchers may ask also reiterates the value in capturing meta-information during ongoing digitization efforts at herbaria around the world and during the accession of newly collected specimens (Edwards et al.; Lendemer et al., 2020).

The second major advance in this analysis is in accounting for several potential biases in the data observation process that may be common to many collections-based research questions by using a spatially-explicit random effects model. Potential biases introduced by the sampling habits of collectors (Daru et al., 2018), and variation between contemporary researchers during the collection of trait data, if not corrected for could lead to over-confident inference about the strength and direction of historic change (Fig. 2). Previous studies that have quantified the effects of collector biases typically find them to be small (Davis et al., 2015; Meineke et al., 2019), and we similarly did not find that collector has a strong effect on the results of our analysis, but that scorer identity did impact results. It is difficult to distinguish whether the impact of scorers was driven by true differences

617 in scorers' biases or by unintended spatial or temporal clustering of the specimens examined by  
618 each scorer (Clayton et al., 1993; Urdangarin et al., 2023). By under-weighting endophyte-positive  
619 samples that are clustered spatially or by collector or observer, the endophyte prevalence model is  
620 appropriately accounting for nuisance variables and providing a conservative inference of endophyte  
621 change relative to the raw data. Spatial autocorrelation is another phenomenon likely common in  
622 data derived from herbarium specimens (Willems et al., 2022), which our spatially-explicit analysis  
623 models among samples. Beyond spatial autocorrelation of outcomes, systematic differences in sam-  
624 pling across space can result in spatial bias. One strength of herbaria as vehicles for global change  
625 research is the relative ease with which specimens from many distinct geographic locations can be  
626 examined. We visited just nine institutions in the central southern United States, and we were able  
627 to sample seeds from specimens across an area spanning over 300,000 sq. km, including specimens  
628 from Mexico and Canada. Despite this advantage, the specimens we examined are concentrated in  
629 the south-central United States, with fewer specimens in the rapidly warming northeastern United  
630 States reflecting the regional focus of herbaria. We provide a simulation analysis exploring the  
631 potential impact of spatially and temporally biased sampling (Appendix A - Supporting Methods).  
632 We found that the spatially-varying coefficient model had a strong ability to re-capitulate temporal  
633 trends across space in simulated data, and that this result was robust to relatively high levels of  
634 spatial bias (80% of data missing from one spatial region). Simulation analyses that extend this  
635 work to consider the myriad ways herbarium data may be biased (i.e. testing different spatial ar-  
636 rangements and scales of spatial bias, or testing different sample sizes) would be extremely valuable  
637 (Daru et al., 2018; Erickson and Smith, 2021; Gaul et al., 2020; Meineke and Daru, 2021; Schmidt  
638 et al.).

### 639 Conclusion

640 Ultimately, a central goal of global change biology is to generate predictive insights into the future of  
641 natural systems on a rapidly changing planet. Beyond host-microbe symbioses, detecting ecological  
642 responses to anthropogenic global change and attributing their causes would inform public policy  
643 decision-makers and adaptive management strategies. Natural history specimens, such as the plant

644 hosts examined in this study have a clear role to play in informing global change biodiversity science,  
645 including building understanding of the dynamics of host-symbiont interactions (Davis, 2023). This  
646 survey of historic endophyte prevalence is necessarily correlative, yet it serves as a foundation to  
647 develop better predictive models of the response of microbial symbioses to climate change. By  
648 comparing detected ecological responses with alternative mechanistic simulations of the past, we  
649 could attribute their cause, in a manner similar to methods from climate science and economics  
650 (Carleton and Hsiang, 2016; Stott et al., 2010; Trenberth et al., 2015). Combining the insights from  
651 this type of regional-scale survey with field experiments and physiological performance data could be  
652 invaluable to identify mechanisms driving shifts in host-symbiont dynamics. Evidence is strong that  
653 certain dimensions of climate change correlated with endophytes' temporal responses, however we  
654 do not know why trends in prevalence were weak in some areas or how endophytes would respond  
655 to more extreme changes in climate. The "time machine" of natural history collections revealed  
656 evidence of mutualism resilience for grass-endophyte symbioses in the face of environmental change,  
657 but more extreme changes could potentially push one or both partners beyond their physiological  
658 limits, leading to the collapse of the mutualism; more research is needed to understand what those  
659 limits might be.

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669

## Statement of Authorship

670 J.C.F. contributed to research conception, data collection, data analysis, and led manuscript draft-  
671 ing. J.M. contributed to data analysis and manuscript revisions. T.E.X.M. contributed to research  
672 conception, data collection, data analysis, and manuscript revisions.

673

## Data and Code Availability

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

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1018

## Appendix A

1019

1020     *Appendix to "Increasing Prevalence of plant-fungal symbiosis across two*  
1021                 *centuries of environmental change"*

1022     **Authors:**

1023     Joshua C. Fowler<sup>1,2\*</sup>

1024     Jacob Moutouama<sup>1</sup>

1025     Tom E. X. Miller<sup>1</sup>

1026

1027     1. Rice University, Department of BioSciences, Houston, Texas 77006

1028     2. University of Miami, Department of Biology, Miami, Florida

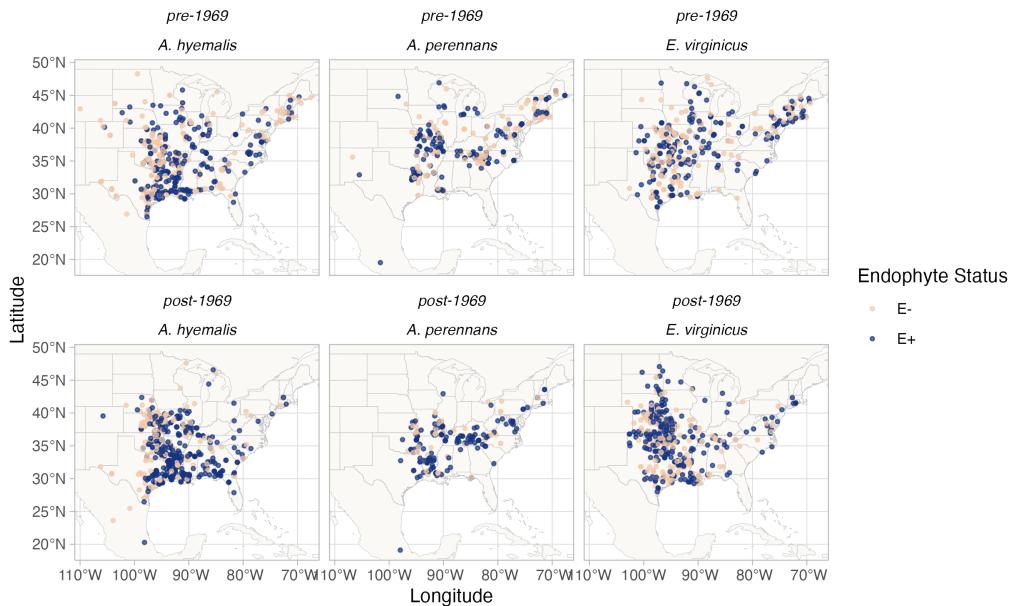
1029     \* Corresponding author; e-mail: jcf221@miami.edu.

1030     **Contents:**

1031     Appendix A includes: Figure A1 - Figure A15, Table A1, and Supporting Methods).

1032

## 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.

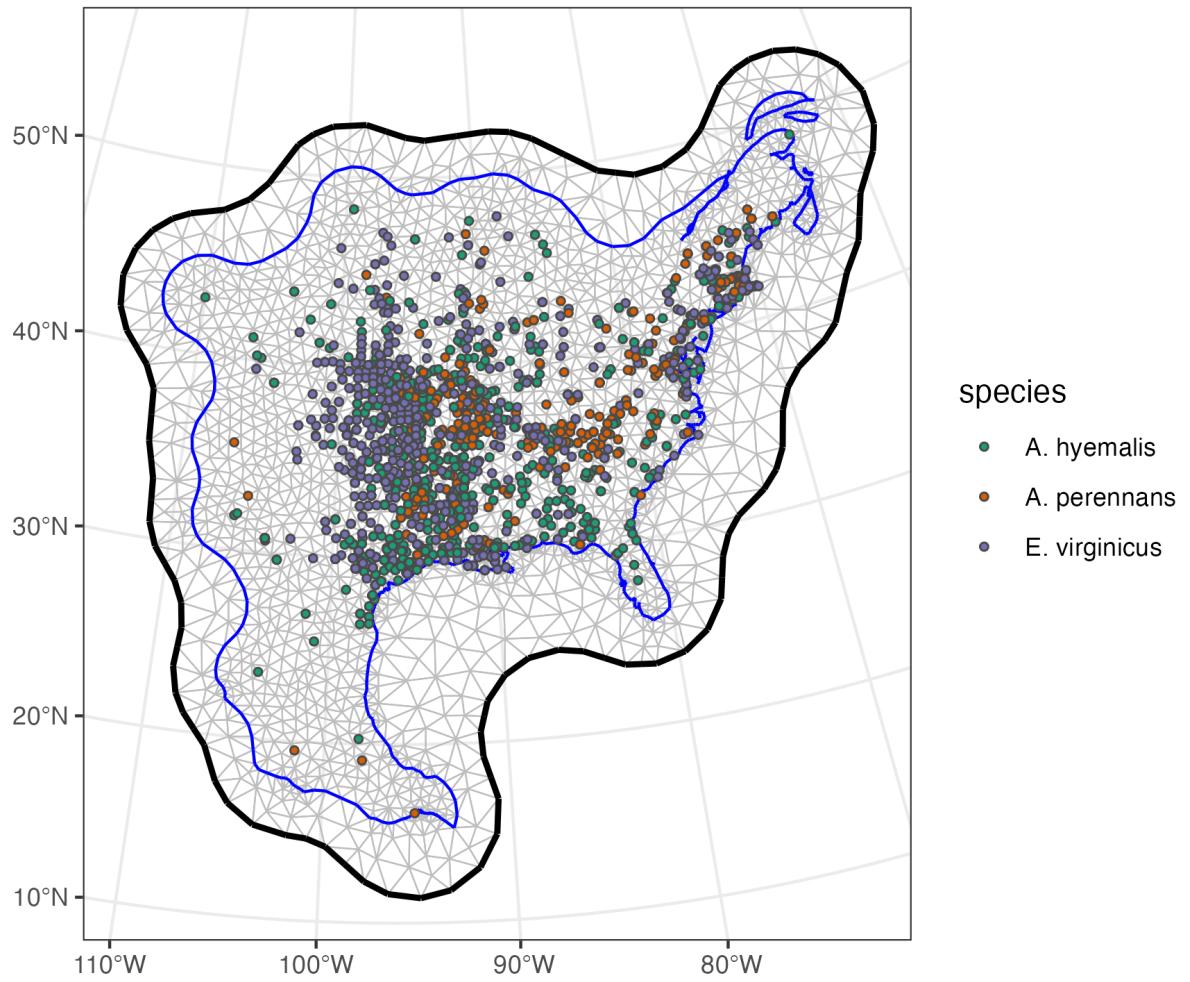


Figure A2: **Triangulation mesh used to estimate spatial dependence between data points.**

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.

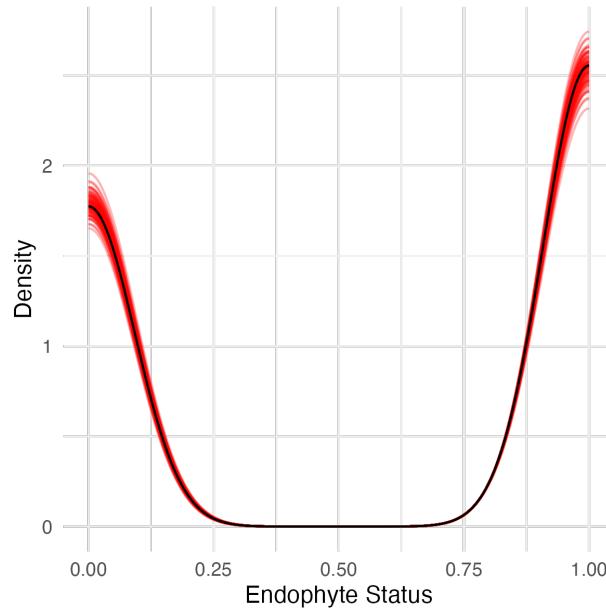
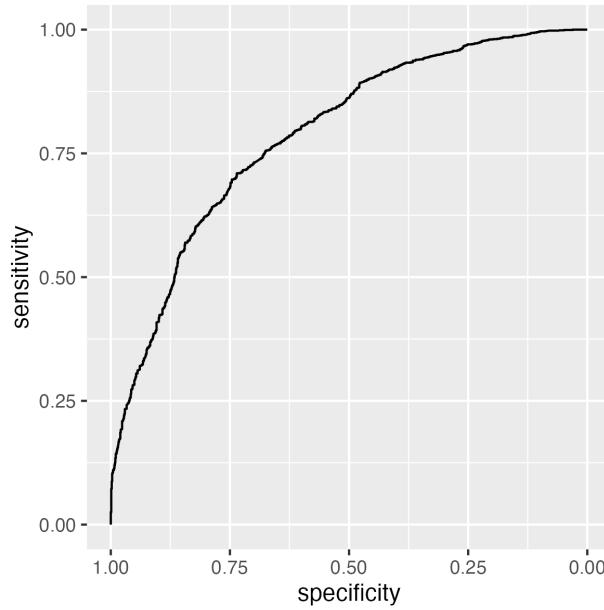
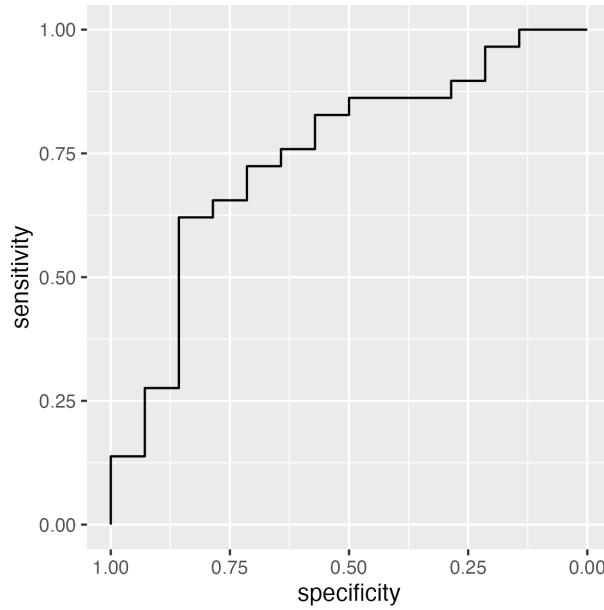


Figure A3: **Graphical posterior predictive check of the endophyte prevalence model fit.**

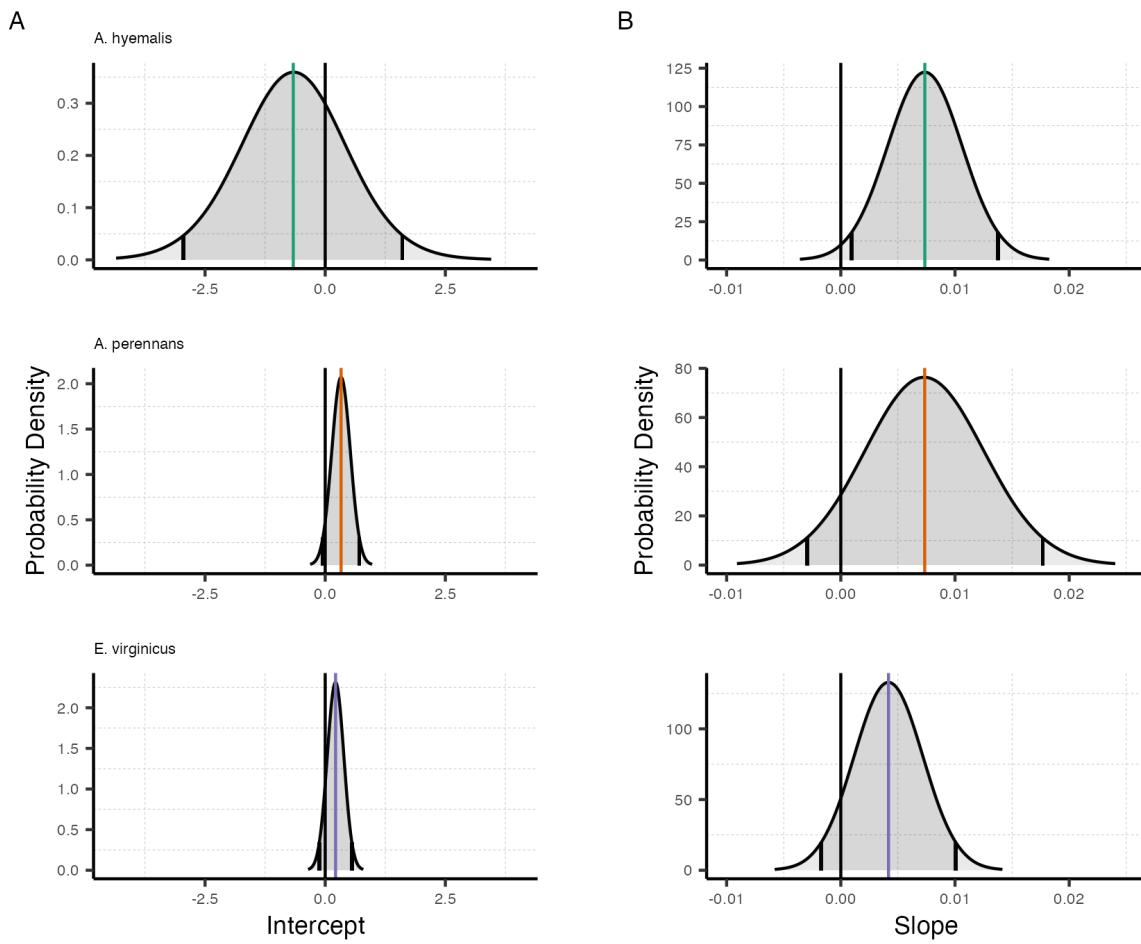
Consistency between observed data and predicted values indicate that the fitted model accurately describes the data. Graph shows density curves for the observed data (black) along with 100 predicted datasets (red).



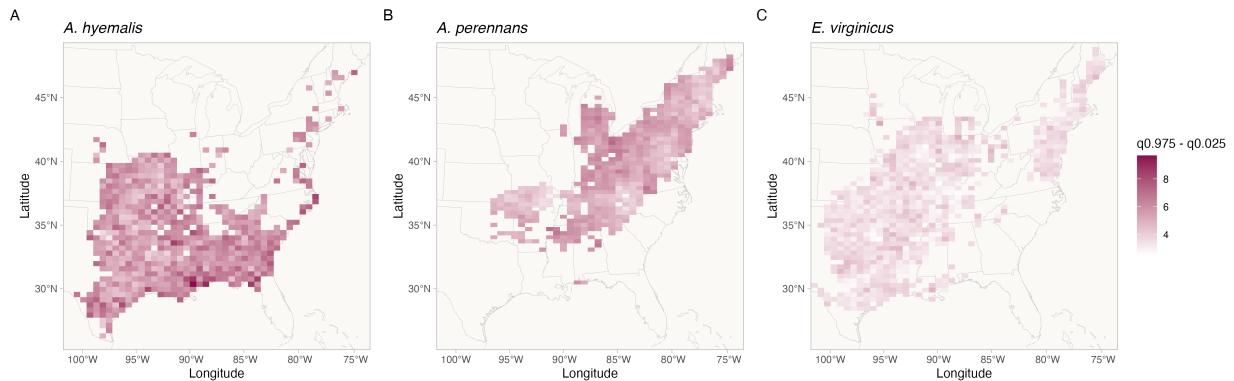
**Figure A4: ROC plot showing performance of the endophyte prevalence model in classifying observations according to endophyte status within the in-sample training data from herbarium collections.** The curves show adequate model performance for observed data. The AUC value is 0.79.



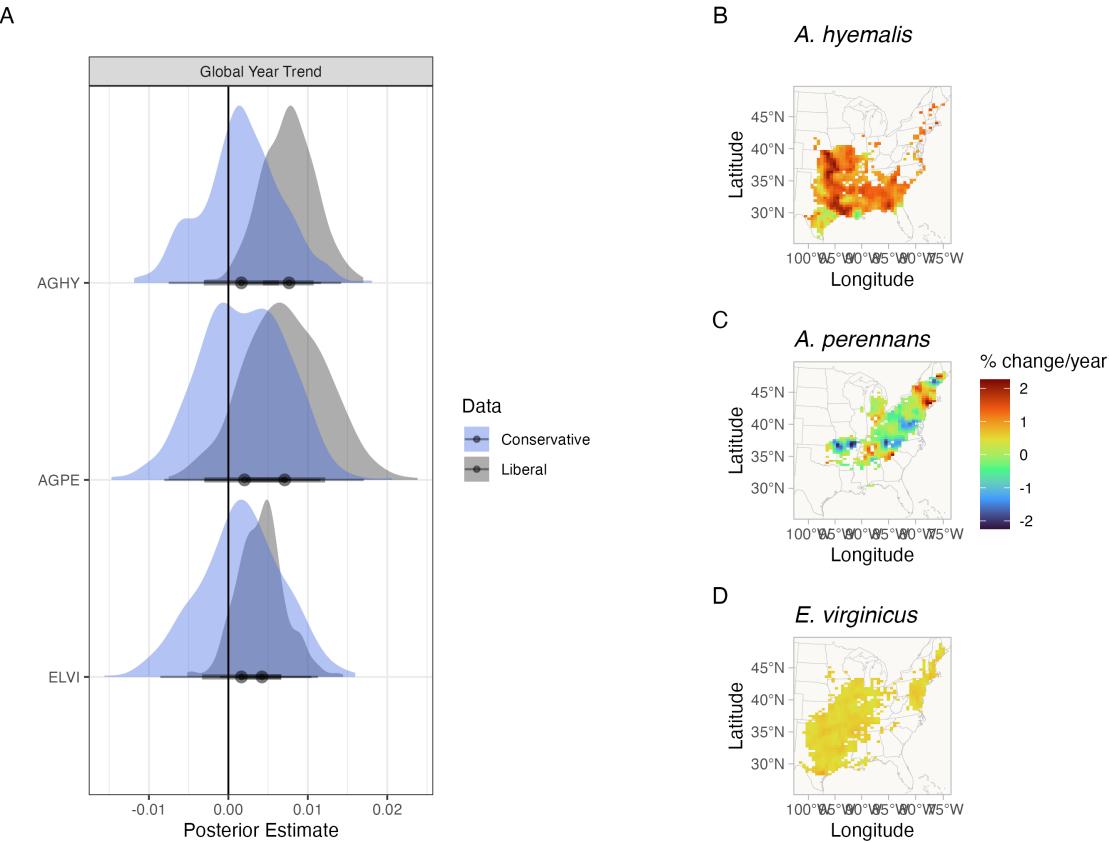
**Figure A5: ROC plot showing performance of the endophyte prevalence model in classifying observations according to endophyte status within the out-of-sample test data from contemporary surveys.** The curves show adequate model performance for test data. The AUC value is 0.77.



**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.

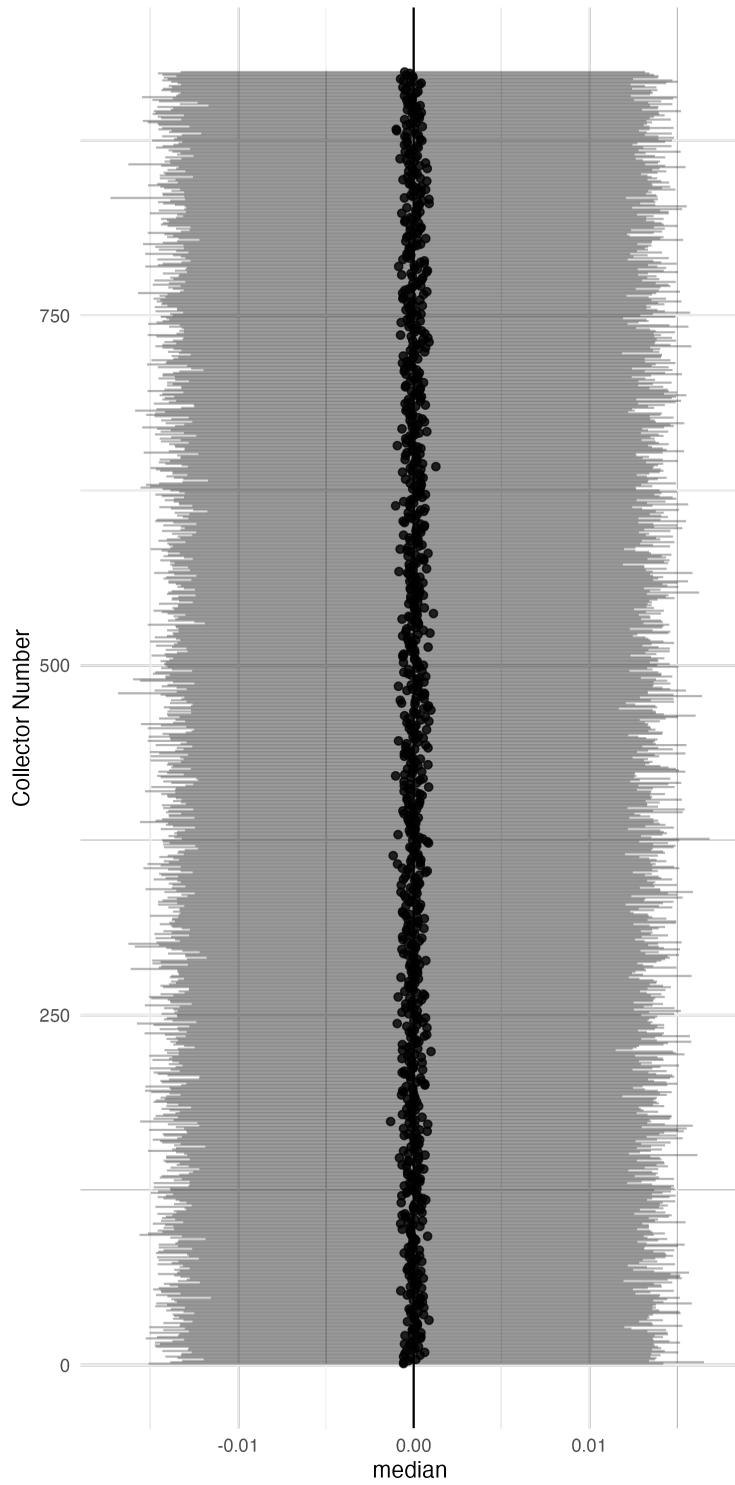
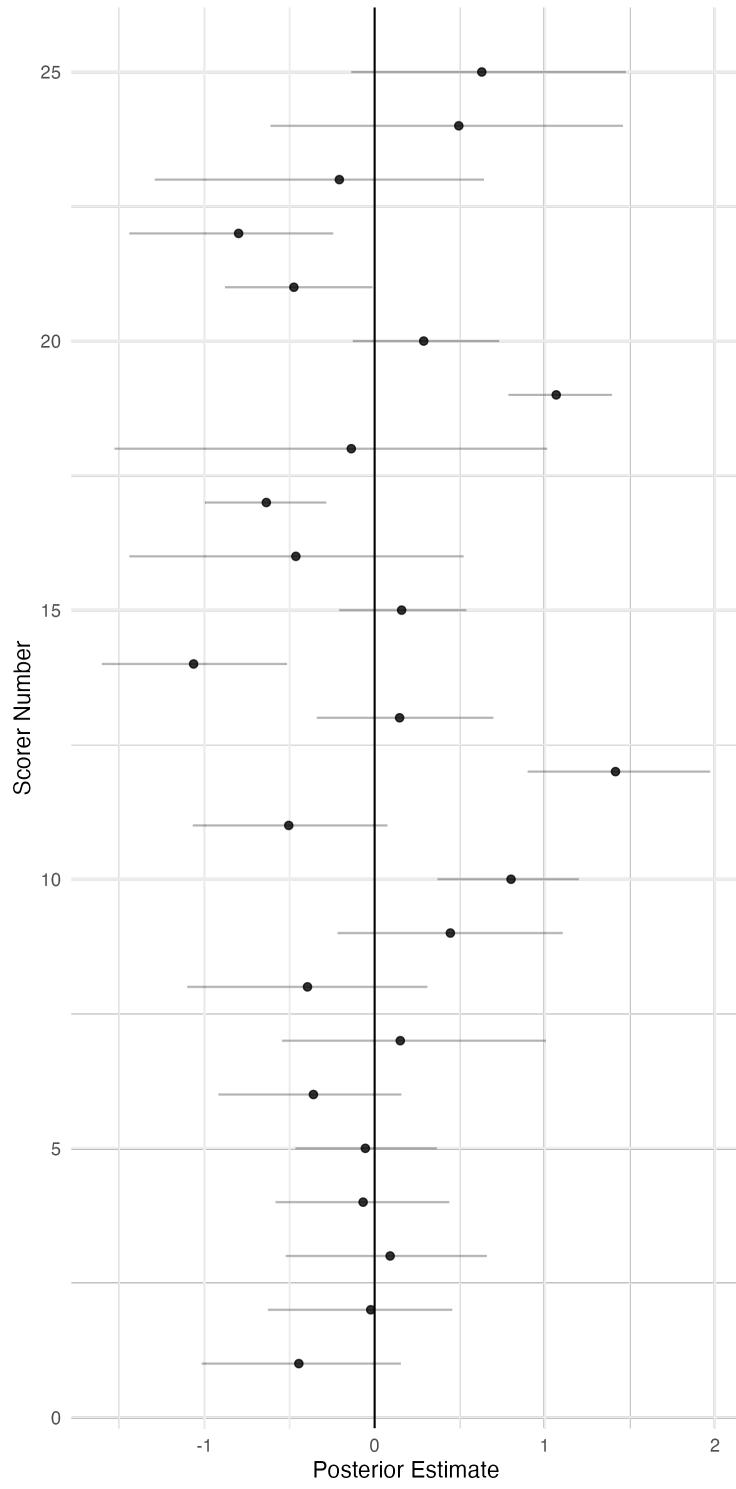
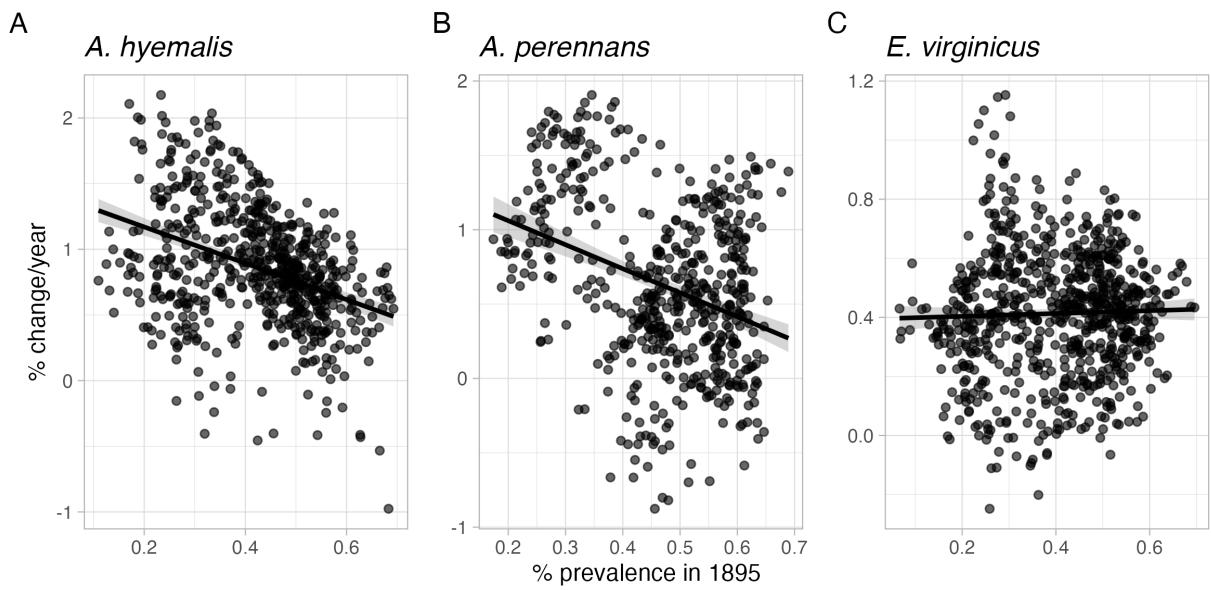


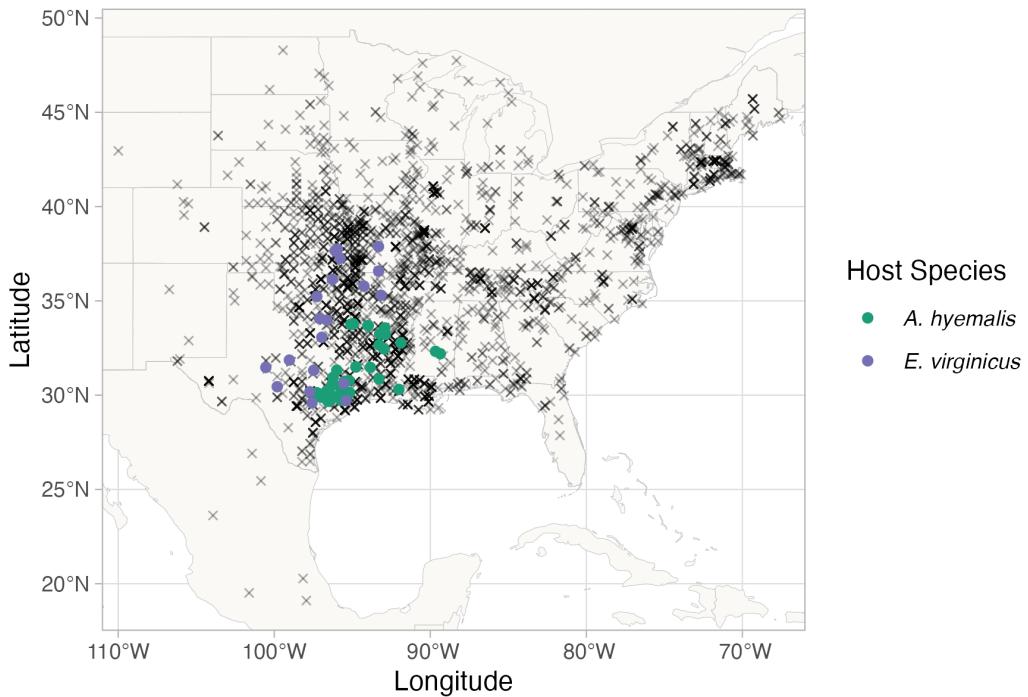
Figure A9: **Posterior estimates of collector random effects from endophyte prevalence model.** Collector random effects are denoted  $\chi$  in Eqn. 1 and represent variance associated with researchers who collected historic herbarium specimens. Points show posterior median along with 95% CI for each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

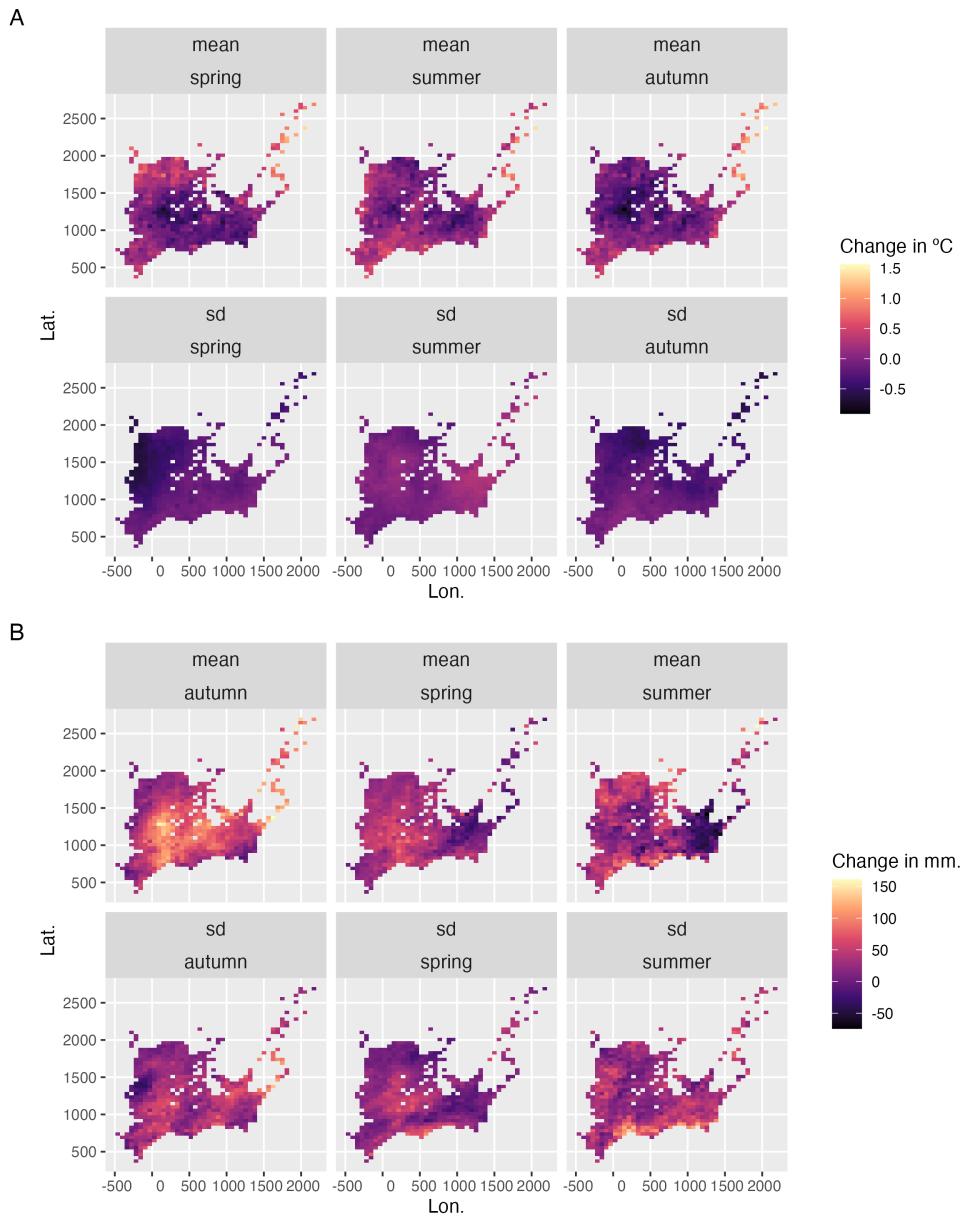
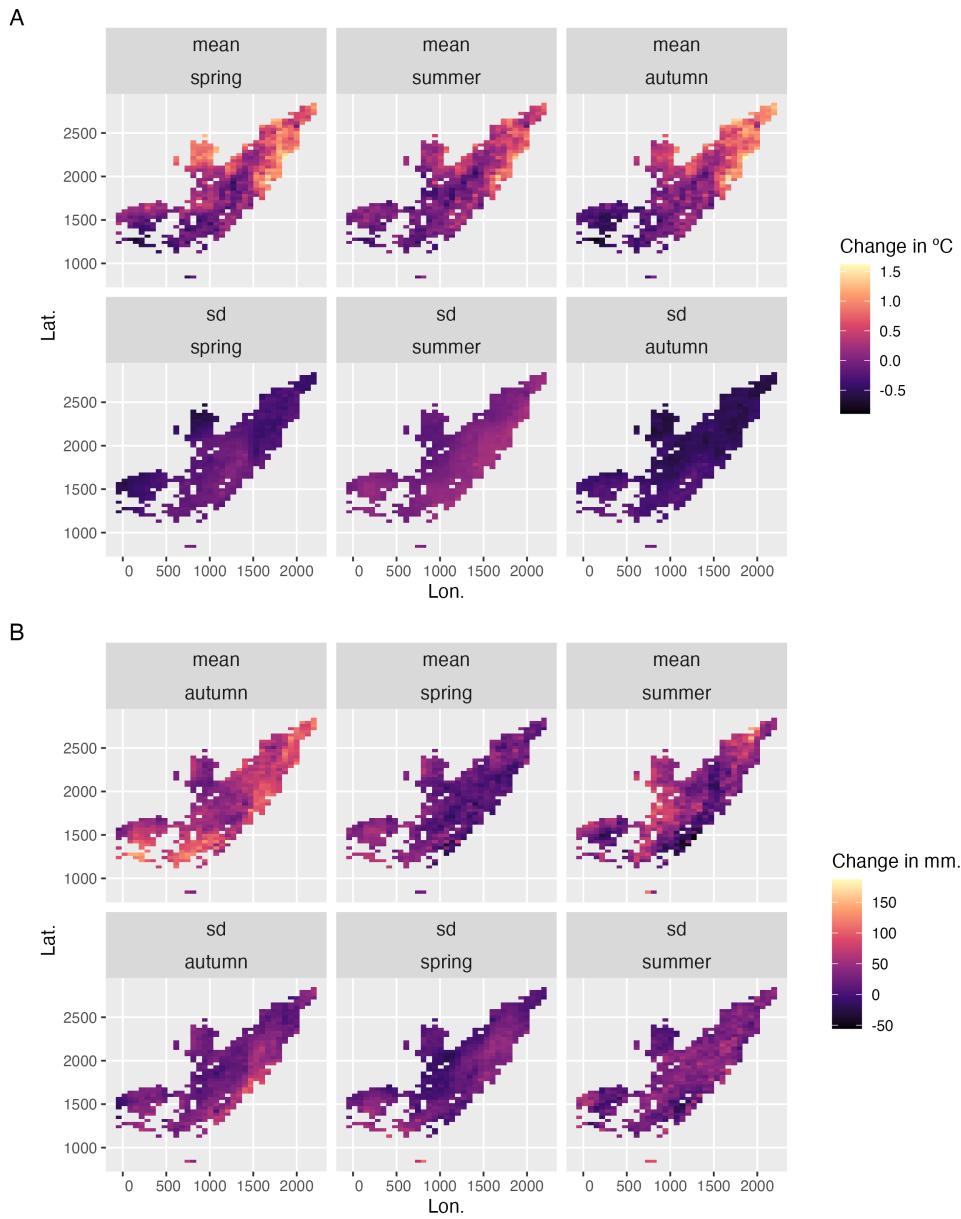


Figure A13: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *A. hyemalis*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution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

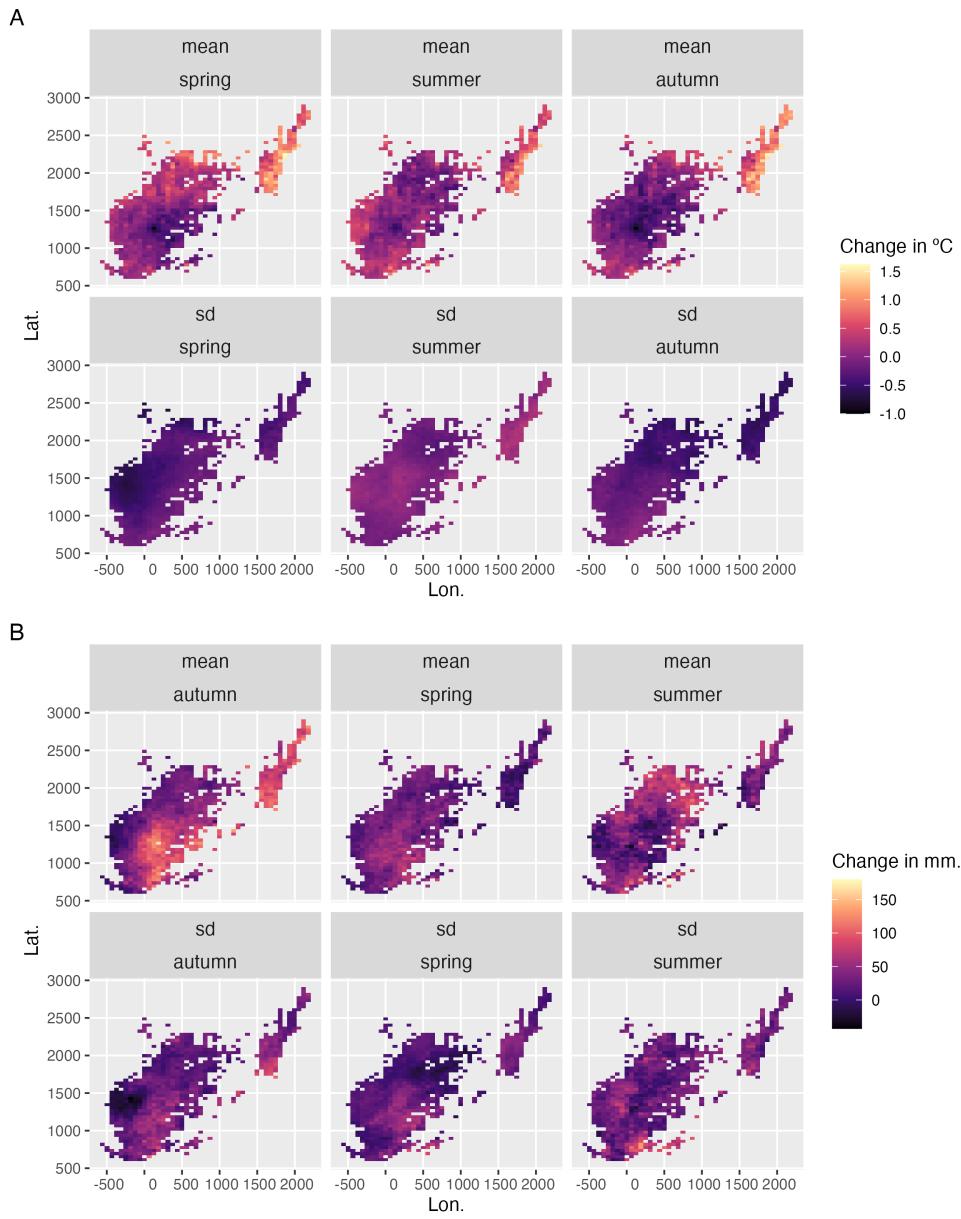


Figure A15: **Change in seasonal climate variables between the periods 1895-1925 and 1990-2020 across the distribution of *E. virginicus*.** Color represents change in (A) seasonal temperature ( $^{\circ}\text{C}$ ) and (B) seasonal precipitation (mm.). Maps show pixels covering the modeled distribution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

1034

## Supporting Methods

1035

### *ODMAP Protocol*

1036 [Overview](#)

1037 **Model purpose:** Mapping current distribution of *Epichloë* host species.

1038 **Target species:** *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*.

1039 **Study area:** Eastern North America

1040 **Spatial extent:** -125.0208, -66.47917, 24.0625, 49.9375 (xmin, xmax, ymin, ymax).

1041 **Spatial resolution:** 0.04166667, 0.04166667 (x, y).

1042 **Temporal extent:** 1990 to 2020.

1043 **Boundary:** Natural.

1044 [Data](#)

1045 **Observation type:** Occurrence records from Global Biodiversity Information Facility and

1046 herbarium collection across eastern North America. We used 713 occurrences records for *Agrostis*  
1047 *hyemalis*, 656 occurrence records for *Agrostis perennans* and 2338 for *Elymus virginicus*.

1048 **Response data type:** occurrence record, presence-only.

1049 **Coordinate reference system:** WGS84 coordinate reference system (EPSG:4326 code)

1050 **Climatic data:** raster data extracted from PRISM

1051 **Model**

1052 **Model assumption:** We assumed that the target species are at equilibrium with their environment.

1053

1054 **Algorithms:** Maximum entropy (maxent)

1055 **Workflow:** We described the workflow in the method section of the manuscript.

1056 **Software:** All statistics were performed using Maxent 3.3.4 and R4.3.1 with packages terra, usdm,  
1057 spThin and dismo.

1058 **Code availability:** Available through this link: <https://github.com/joshuacfowler/EndoHerbarium>

1059 **Data availability:** Data was accessed through open-source R packages *rgbif*. *A. hyemalis*  
1060 (GBIF.Org, 2025a), *A. perennans* (GBIF.Org, 2025b), *E. virginicus* (GBIF.Org, 2025c)

1061 **Assessment**

1062 We used AUC to test model performance.

1063 **Prediction**

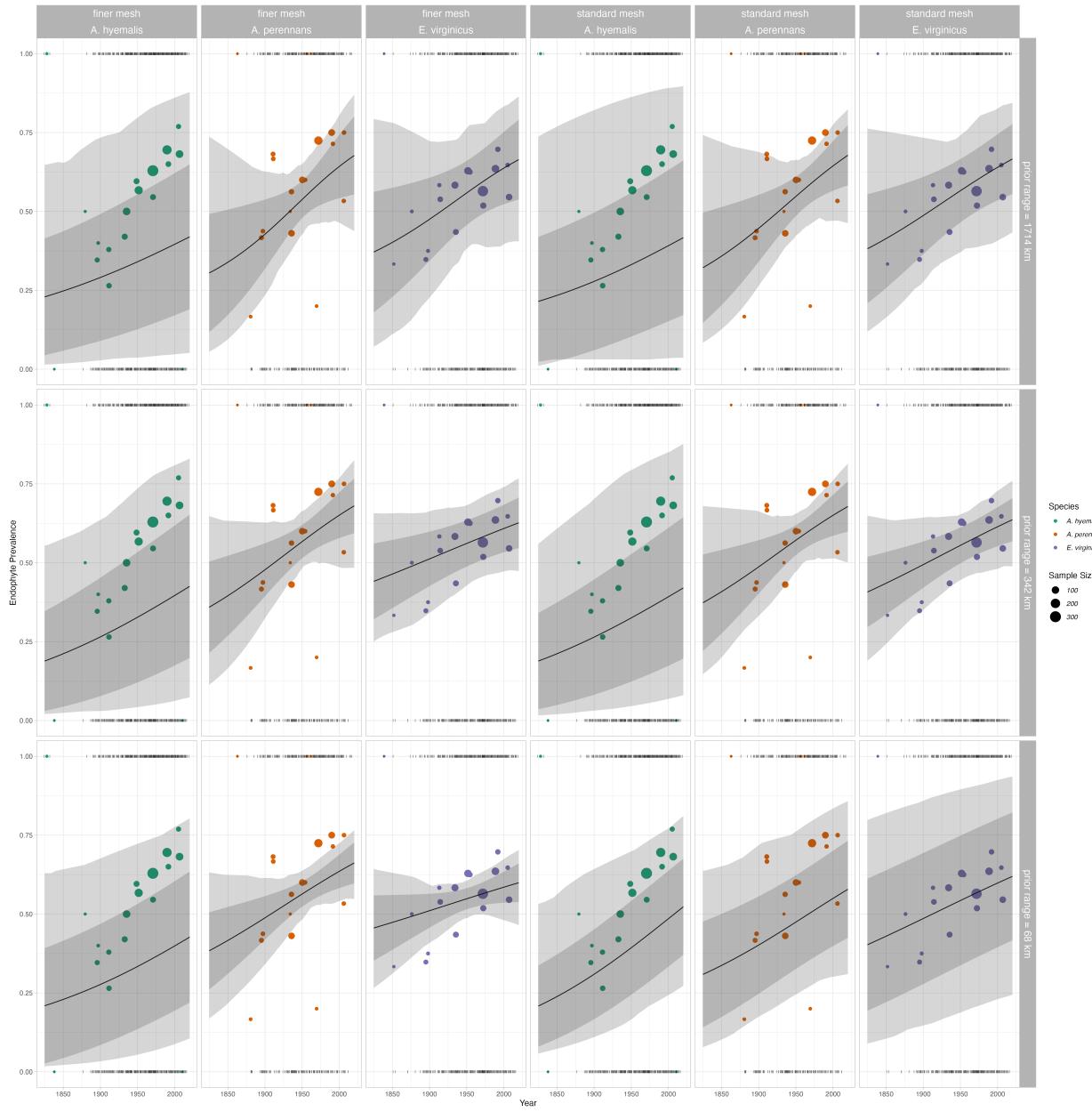
1064 We predicted the probability of presence of the host species as a binary maps (presence or absence)

### 1065 *Mesh and Prior Sensitivity Analysis*

1066 To test the influence that the triangulation mesh and choice of priors has on results, we compared  
1067 model results across a range of meshes and priors. We re-ran our model for the mesh used in main  
1068 body of the text (Fig. A2), which we refer to as the "standard mesh", and with a mesh with smaller  
1069 minimum vertices (finer mesh). Finer scale meshes increase computation time. For each of these  
1070 meshes, we ran the model with a range of priors defining the spatial range of our spatial random  
1071 effects: 342km (the prior used for presented results), as well as ranges five times smaller (68 km)

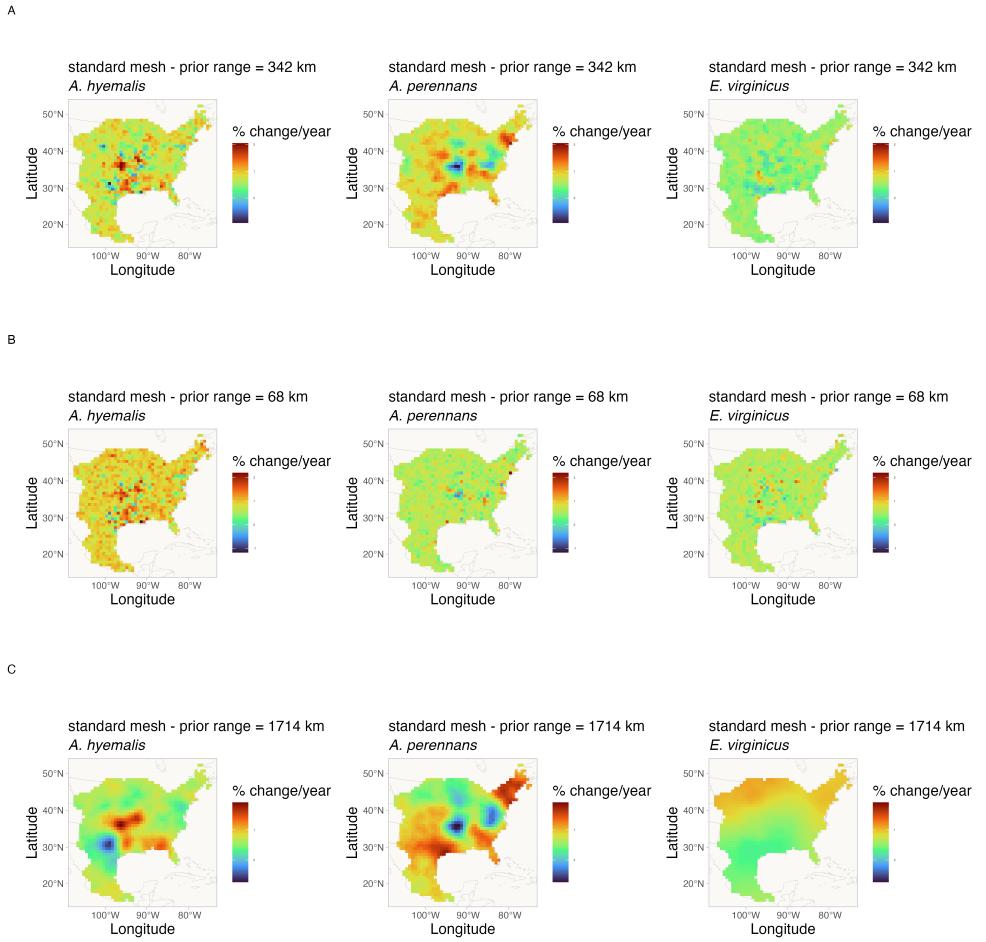
1072 and five times larger (1714 km). We found generally that these choices did not alter the direction  
1073 of model predictions, but did influence the associated uncertainty and magnitude of some effects.

1074 For overall temporal trends, we found that models with differing priors predicted consistently  
1075 positive relationships over time (Fig. A16).



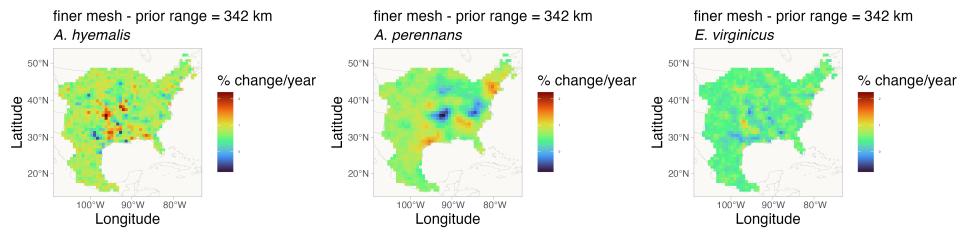
**Figure A16: Overall trend in endophyte prevalence evaluated for endophyte prevalence models with different range priors on spatially structured random effects, and for two different triangulation meshes.** Data used in model fitting is the same across all panels and as in the main text. Note that these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

1076 For spatially-varying temporal trends, we found that models with different priors predicted  
1077 consistent spatial patterns in temporal trends, although the range of this prediction varied depending  
1078 on the prior and mesh (Fig. A17 - A18). One noteworthy result of this analysis is that combinations  
1079 of prior choice and mesh can introduce instability in model fitting. This is evident in A17 panel B  
1080 and A18 panel B, where the prior range is smaller than the minimum vertex length of the mesh.  
1081 Model fitting takes an extended time period and the model struggles to identify variation across  
1082 space. Results with a set of prior ranges (Fig. A17 - A and C; Fig. A18 - A and C) result in  
1083 models that estimate trends across space of the same direction and order of magnitude, although  
1084 the "smoothness" of these predictions vary.

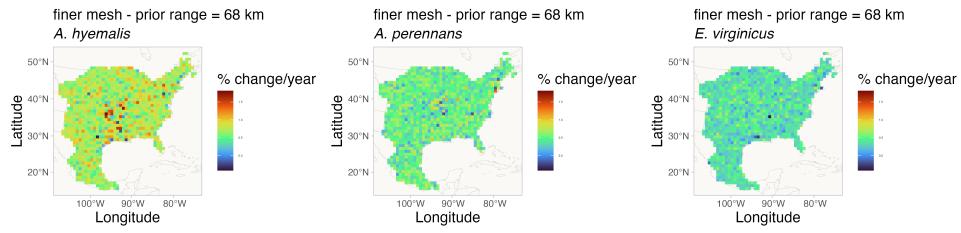


**Figure A17: Spatially-varying trends in endophyte prevalence evaluated for the endophyte prevalence model with different range priors on spatially structured random effects, and for the "standard" mesh.** Data used in model fitting is the same across all panels and as in the main text. Shading indicates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

A



B



C

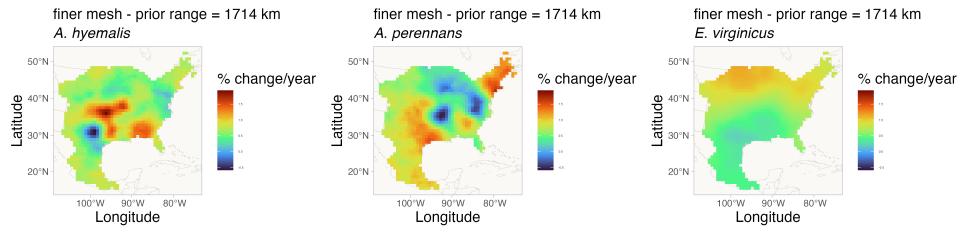


Figure A18: **Spatially-varying trends in endophyte prevalence evaluated for the endophyte prevalence model with different range priors on spatially structured random effects, and for the "finer" mesh.** Data used in model fitting is the same across all panels and as in the main text. Shading indicates 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.

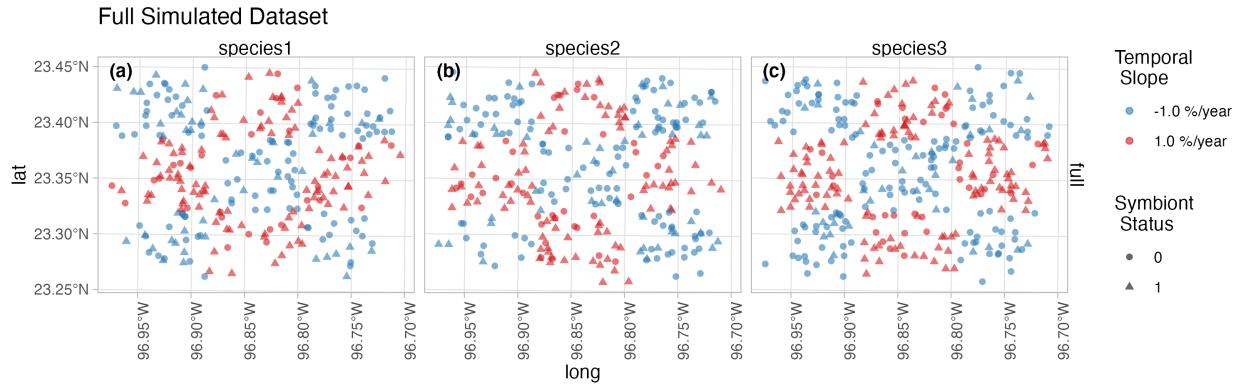
1085

## *Spatially-biased Sample Size Simulation Analysis*

1086 To examine how data that is unevenly distributed across host distributions may influence interpreta-  
1087 tion of spatially-varying coefficients, we performed a simulation analysis. Our focal species, *Agrostis*  
1088 *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*, are widely distributed grasses across the east-  
1089 ern United States that host *Epichloë* fungal endophytes. For logistical reasons, our sampling visits  
1090 to herbaria focused on herbaria in the central southern U.S., which resulted in unevenly distributed  
1091 data across each host species' range. This is particularly noteable for *Agrostis perennans* which has  
1092 the most northern distribution and relatively fewer total collected specimens compared to the other  
1093 focal species. Thus, a significant portion in the northeast of this species' range is relatively sparsely  
1094 sampled. Our analysis presented in the main text identified this region as having strong increase in  
1095 endophyte prevalence. Future visits to herbaria with regional focuses in the Northeastern US would  
1096 certainly garner new specimens that could provide valuable insights into shifting host and symbiont  
1097 distributions.

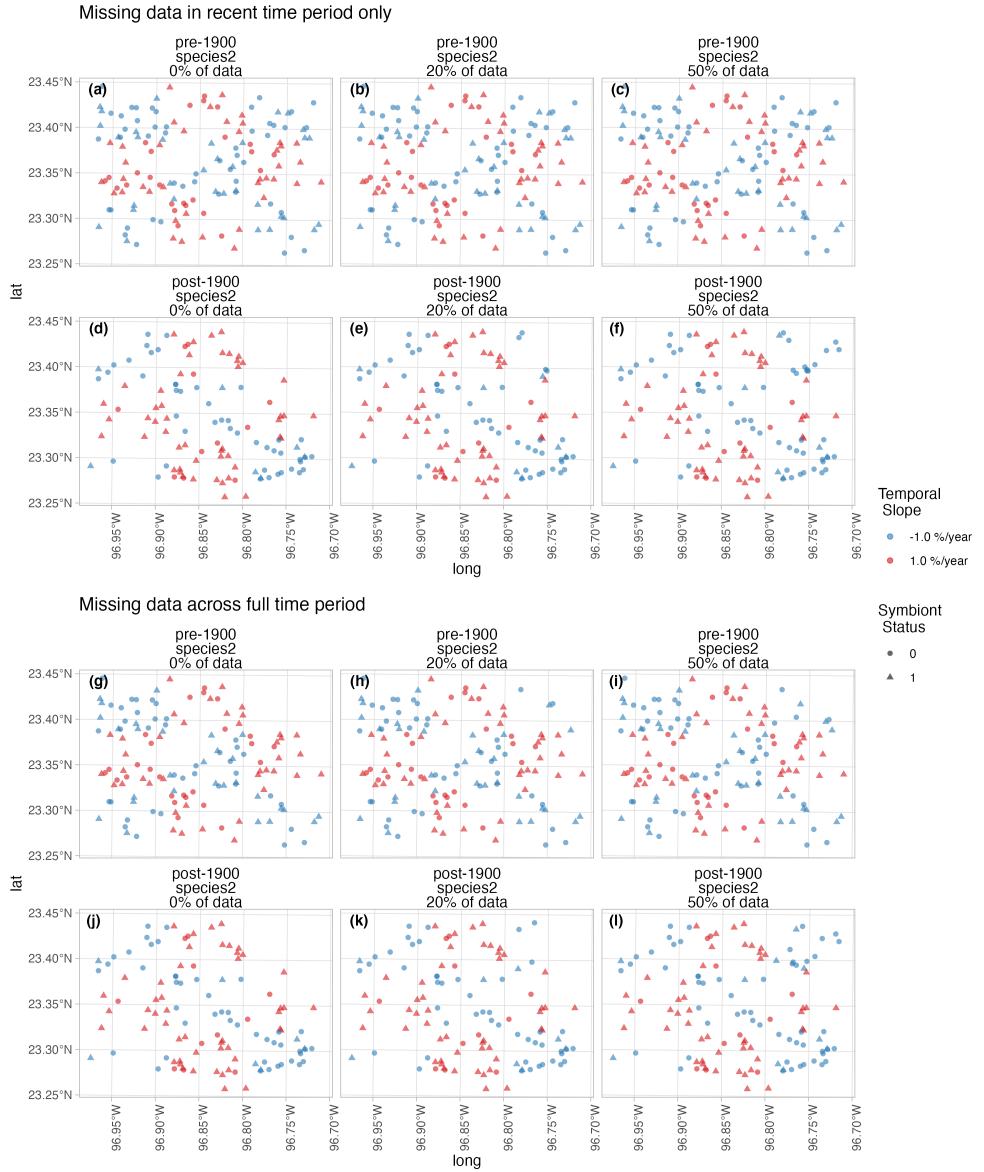
1098 *Simulation of spatially-biased symbiont occurrence data*

1099 We simulated datasets with varying levels of missing-ness to examine how this missing-ness influ-  
1100 enced the estimation of spatially-varying trend estimates. We first generated 300 data points for  
1101 each of three hypothetical species at random positions across an area approximating the scale of  
1102 our focal data. Each data point was randomly assigned a year of collection across 200 years. We  
1103 then simulated data from a Bernoulli process with trends alternating across nine regions (Fig. A19)  
1104 in a 3X3 grid pattern. This grid pattern was intended to create a complex spatial layout of trends,  
1105 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.

1113 *Statistical analysis*

1114 We analyzed each dataset with a model given by Eqn. A1 similar in construction to that used in  
1115 our central analysis.

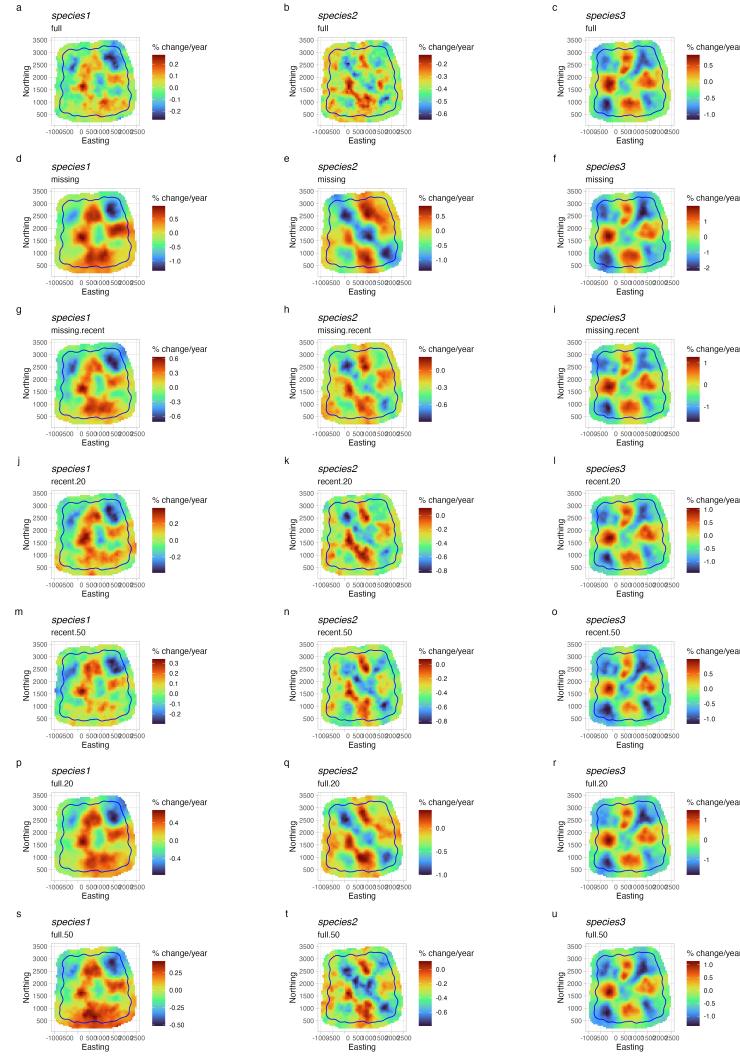
$$\text{logit}(\hat{P}_{h,i}) = A_h + T_h * \text{year}_i + \alpha_{h,l_i} + \tau_{h,l_i} * \text{year}_i + \delta_{l_i} \quad (\text{A1})$$

1116 Where symbiont presence/absence of the  $i^{th}$  specimen ( $P_{h,i}$ ) was modeled as a Bernoulli re-  
1117 sponse variable with expected probability of symbiont occurrence  $\hat{P}_{h,i}$  for each host species  $h$ . We  
1118 modeled  $\hat{P}_{h,i}$  as a linear function of intercept  $A_h$  and slope  $T_h$  defining the global trend in endophyte  
1119 prevalence specific to each host species as well as with spatially-varying intercepts  $\alpha_{h,l_i}$  and slopes  
1120  $\tau_{h,l_i}$  associated with location ( $l_i$ , the unique latitude-longitude combination of the  $i$ th observation).  
1121 Similar to the SVC model of our central analysis (Eqn. 1), we estimated a shared variance term  
1122 with the spatially-dependent random effect  $\delta_{l_i}$ , intended to account for residual spatial variation.  
1123 However in this analysis we omit i.i.d.-random effects terms associated with collector and scorer  
1124 identity ( $\chi_{c_i}$  and  $\omega_{s_i}$  in Eqn. 1) for the sake of simplicity.

1125 *Influence of spatially-biased sampling on model interpretation*

1126 Our analysis of the full simulated data shows that our model is suitably flexible to capture complex  
1127 spatial patterns in temporal trends (Fig. A21 a-c). Beyond this, the model also qualitatively  
1128 captures the spatial patterns in temporal trends even with large amounts of data missingness (i.e  
1129 missing up to 80% of the datapoints (Fig. A21 p-r)).

1130 While this analysis is not an exhaustive examination of the influence of sampling bias on our  
1131 results for several reasons (including not examining how different strengths in temporal trends,  
1132 different spatial arrangements of missing-ness influence model estimates, or different sample sizes  
1133 influence results), it demonstrates that the spatially-varying modelling framework implemented in  
1134 INLA we employ can suitably recover regional trends even with significant spatially-bias within  
1135 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.

1136 within recent collection period). Future work could more fully explore the scenarios that cause  
1137 this ability to break down. We expect this simulation reflects what may be a common scenario for  
1138 research investigating global change using natural history specimens. Collection effort by trained  
1139 taxonomists and professional collectors peaked in the past, and collections contain relatively fewer  
1140 modern specimens in many regions. Additionally, most global change research necessarily involves  
1141 accessing many specimens across collections. Research efforts such as ours will be unable to access  
1142 every specimen from all possible collections. Ongoing digitization efforts will make it possible to  
1143 more clearly assess how much data is missing from a particular study compared to the actual  
1144 holdings of natural history collections, but ultimately, the decision of what data and collections to  
1145 include is a question of sample size and study design.