

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

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## Abstract

Species' distributions and abundances are shifting in response to climate change. Most species harbor microbial symbionts that have the potential to influence these responses. Mutualistic microbial symbionts may provide resilience to environmental change by protecting their hosts from increasing stress. However, environmental change that disrupts these interactions may lead to declines in hosts and/or symbionts. Symbionts preserved within herbarium specimens offer a unique opportunity to quantify changes in microbial symbiosis across broad temporal and spatial scales.<sup>1</sup> We asked how the prevalence of seed-transmitted fungal symbionts of grasses (*Epichloë* endophytes), which can protect hosts from abiotic stress, have changed over time in response to climate change, and how these changes vary across host species' ranges. Specifically, we analyzed 2,346 herbarium specimens of three grass host species (*Agrostis hyemalis*, *Agrostis perennans*, *Elymus virginicus*) collected over the past two centuries (1824 – 2019) for the presence or absence of *Epichloë* symbiosis, and evaluated spatial and temporal trends in endophyte prevalence<sup>2</sup>. We found that endophytes increased in prevalence over the last two centuries from ca. 25% prevalence to ca. 75% prevalence, on average, across three host species. We also found that changes in prevalence were associated with observed changes in seasonal climate drivers; notably, increasing precipitation corresponding to each host species' peak growing season and changes in off-peak season variability in precipitation<sup>3</sup>. Our analysis performed favorably in an out-of-sample predictive test with contemporary survey data, a rare extra step in collections-based research. However, we identified greater local-scale variability in endophyte prevalence in contemporary data compared to what our model could predict 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 context-dependent benefits that confer a fitness advantage to symbiotic hosts

<sup>1</sup>If you need to cut words, I think some of this could be condensed.

<sup>2</sup>Could cut, overlaps with previous sentence.

<sup>3</sup>I would rephrase this to better communicate the sign of the association, and thus the inferred causality.

<sup>36</sup> under environmental change.

<sup>37</sup> Abstract : 287 words

## Introduction

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

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

60 Microbial symbionts are common to all macroscopic organisms and can have important ef-  
61 fects on their hosts' survival, growth and reproduction (McFall-Ngai et al., 2013; Rodriguez et al.,  
62 2009). Many microbial symbionts act as mutualists, engaging in reciprocally beneficial interac-  
63 tions with their hosts that can ameliorate environmental stress. For example, bacterial symbionts

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

Understanding of how microbial symbioses are affected by climate change is additionally complicated by spatial heterogeneity in the direction and magnitude of environmental change (IPCC, 2021). Beneficial symbionts are likely able to shield their hosts from environmental stress in locations that experience a small degree of change, but symbionts in locations that experience changes of large magnitude may be pushed beyond their physiological limits (Webster et al., 2008). Additionally, symbionts are often unevenly distributed across their hosts' distribution. Facultative symbionts may be absent from portions of the host range (Afkhami et al., 2014), and hosts may engage with a diversity of partners (different symbiont species or locally-adapted strains) across their environments (Frade et al., 2008; Rolshausen et al., 2018).<sup>4</sup> Identifying broader spatial trends in symbiont prevalence is therefore an important step in developing predictions for where to expect changes in the symbiosis in future climates.

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<sup>4</sup>I would encourage self-citation here!

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

104       Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic di-  
105      versity of host species that harbor these symbionts (White and Cole, 1985), establishing that  
106      endophyte symbiosis could be identified in plant tissue from as early as 1851. However, no  
107      subsequent studies, to our knowledge, have used the vast resources of biological collections to  
108      quantitatively assess spatio-temporal trends in endophyte prevalence and their environmental  
109      correlates. Grasses are commonly collected and identified based on the presence of their re-  
110      productive structures, meaning that preserved specimens typically contain seeds, conveniently  
111      preserving the fungi along with their host plants on herbarium sheets. This creates the oppor-  
112      tunity to leverage the unique spatio-temporal sampling of herbarium collections to examine the  
113      response of the symbiosis to historical climate change. However, the predictive ability derived  
114      from historical analyses is rarely tested against contemporary data (Lee et al., 2024). Critically  
115      evaluating whether insights from historical reconstruction are predictive of variation across con-  
116      temporary populations is a crucial step for the field to move from reading signatures of the past

<sup>117</sup> to forecasting ecological dynamics into the future.

<sup>118</sup> In this study, we assessed the long-term responses of endophyte symbiosis to climate change  
<sup>119</sup> through the use of herbarium specimens of three North American host grass species (*Agrostis*  
<sup>120</sup> *hyemalis*, *Agrostis perennans*, and *Elymus virginicus*). We first address questions describing spa-  
<sup>121</sup> tial and temporal trends in endophyte prevalence: (i) How has endophyte prevalence changed  
<sup>122</sup> over the past two centuries? and (ii) How spatially variable are temporal trends in endophyte  
<sup>123</sup> prevalence across eastern North America? We then address how climate change may be driv-  
<sup>124</sup> ing trends in endophyte prevalence by asking: (iii) What is the relationship between temporal  
<sup>125</sup> trends in endophyte prevalence and associated changes in climate drivers? We predicted that  
<sup>126</sup> aggregate endophyte prevalence would increase over time in tandem with climate warming, and  
<sup>127</sup> that hotspots of endophyte change would correspond spatially to hotspots of climate change.  
<sup>128</sup> Finally, we evaluated the performance of models built on data from historic specimens with  
<sup>129</sup> an out-of-sample test, using data on endophyte prevalence from contemporary surveys of host  
<sup>130</sup> populations. To answer these questions we examined a total of 2,346 specimens collected across  
<sup>131</sup> eastern North America between 1824 and 2019.<sup>5</sup>

## <sup>132</sup> Methods

### <sup>133</sup> Focal species

<sup>134</sup> Our surveys focused on three native North American grasses: *Agrostis hyemalis*, *Agrostis peren-*  
<sup>135</sup> *nans*, and *Elymus virginicus*. Both *Agrostis* species host *Epichloë amarillans* (Craven et al., 2001;  
<sup>136</sup> Leuchtmann et al., 2014), while *Elymus virginicus* typically hosts *Epichloë elymi* (Clay and Schardl,  
<sup>137</sup> 2002). These C<sub>3</sub> grass species are commonly represented in natural history collections with broad  
<sup>138</sup> distributions covering much the eastern United States (Fig. ??). *A. hyemalis* is a small short-lived  
<sup>139</sup> perennial species that germinates in spring and typically flowers between March and July (most

<sup>5</sup>Since the preceding sentence is about the out of sample test, maybe include here the numbers of individuals and populations that the historical predictions were tested against?

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

<sup>149</sup> *Herbarium surveys*

<sup>150</sup> We visited nine herbaria between 2019 and 2022 (see Table A1 for a summary of specimens in-  
<sup>151</sup> cluded from each collection). With permission from herbarium staff, we acquired seed samples  
<sup>152</sup> from 1135 *A. hyemalis* specimens collected between 1824 and 2019, 357 *A. perennans* specimens  
<sup>153</sup> collected between 1863 and 2017, and 854 *E. virginicus* specimens collected between 1839 and  
<sup>154</sup> 2019 (Fig. 1, Fig. 2A, Fig. A1). We chose our focal species in part because they are commonly  
<sup>155</sup> represented in herbarium collections, and produce high numbers of seeds, meaning that small  
<sup>156</sup> samples would not diminish the value of the specimens for future studies. We collected 5-10  
<sup>157</sup> seeds per specimen after examining the herbarium sheet under a dissecting microscope to en-  
<sup>158</sup> sure that we collected mature seeds, not florets or unfilled seeds, fit for our purpose of identifying  
<sup>159</sup> fungal endophytes with microscopy. We excluded specimens for which information about the  
<sup>160</sup> collection location and date were unavailable. Each specimen was assigned geographic coor-  
<sup>161</sup> dinates based on collection information recorded on the herbarium sheet using the geocoding  
<sup>162</sup> functionality of the ggmap R package (Kahle et al., 2019). Many specimens had digitized collec-  
<sup>163</sup> tion information readily available, but for those that did not, we transcribed information printed  
<sup>164</sup> on the herbarium sheet. Collections were geo-referenced to the nearest county centroid, or near-  
<sup>165</sup> est municipality when that information was available. **For a few of the oldest specimens, only**

<sup>166</sup> information at the state level was available, and so we used the state centroid.<sup>6</sup>

<sup>167</sup> After collecting seed samples, we quantified the presence or absence of *Epichloë* fungal hy-  
<sup>168</sup> phae in each specimen using microscopy. We first softened seeds with a 10% NaOH solution,  
<sup>169</sup> then stained the seeds with aniline blue-lactic acid stain and squashed them under a microscope  
<sup>170</sup> cover slip. We examined the squashed seeds for the presence of fungal hyphae at 200-400X  
<sup>171</sup> magnification (Bacon and White, 2018). In some cases, the tissues examined during microscopy  
<sup>172</sup> came from flowers or otherwise non-viable seeds, which were excluded for that specimen.<sup>7</sup> On  
<sup>173</sup> average we scored 4.7 seeds per specimen of *A. hyemalis*, 4.2 seeds per specimen of *A. perennans*,  
<sup>174</sup> and 3.8 seeds per specimen of *E. virginicus*; we scored 10,342 seeds in total. . Due to imper-  
<sup>175</sup> fect vertical transmission (Afkhami and Rudgers, 2008), it is possible that symbiotic host-plants  
<sup>176</sup> produce a mixture of symbiotic and non-symbiotic seeds. We therefore designated a specimen  
<sup>177</sup> as endophyte-symbiotic if *Epichloë* hyphae were observed in one or more of its seeds, or non-  
<sup>178</sup> symbiotic if *Epichloë* hyphae were observed in none of its seeds. To capture uncertainty in the  
<sup>179</sup> endophyte scoring process, we recorded both a "liberal" and a "conservative" endophyte status  
<sup>180</sup> for each plant specimen. When we identified potential endophytes with unusual morphology,  
<sup>181</sup> low uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a  
<sup>182</sup> positive liberal status (more likely to be endophyte-positive) and a negative conservative status  
<sup>183</sup> (less likely to be endophyte-positive). 89% of scored plants had matching liberal and conservative  
<sup>184</sup> scores, reflecting high confidence in endophyte status. The following analyses used the liberal  
<sup>185</sup> status, but we repeated all analyses with the conservative status which yielded qualitatively  
<sup>186</sup> similar results (Fig. A8).

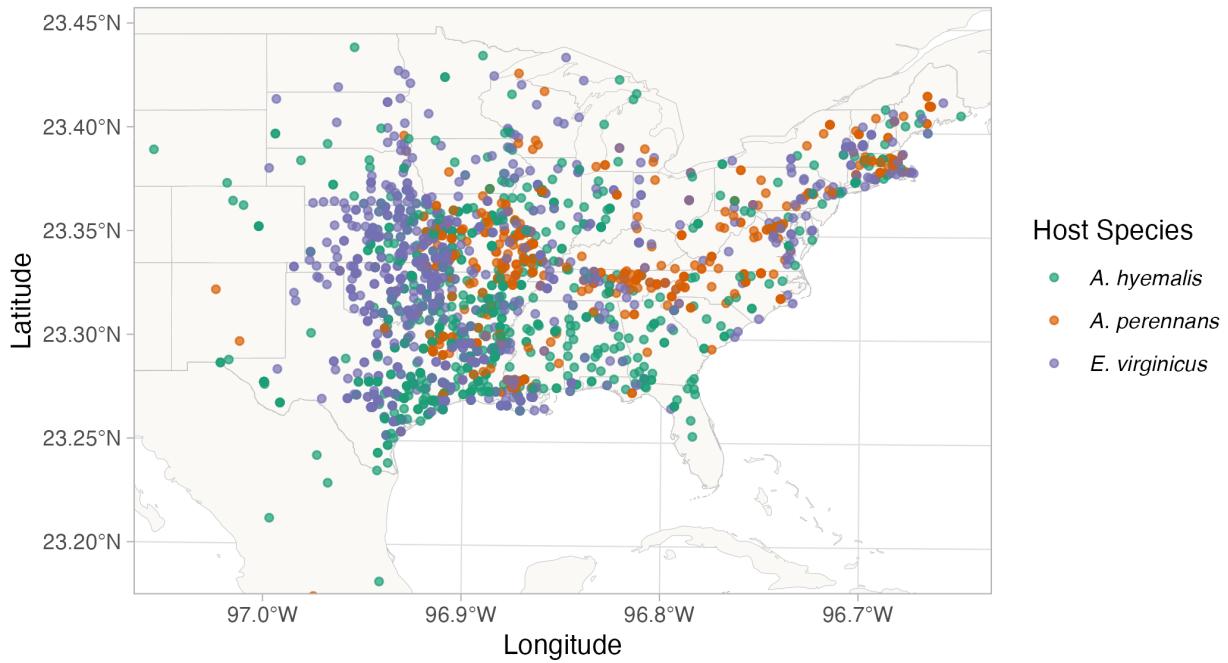
### <sup>187</sup> Modeling spatial and temporal changes in endophyte prevalence

<sup>188</sup> We assessed spatial and temporal changes in endophyte prevalence across each host distribution,  
<sup>189</sup> quantifying the "global" temporal trends aggregated across space, and then examining spatial

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<sup>6</sup> Say how many?

<sup>7</sup> Could cut, since you already say you only took good seeds.



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

190 heterogeneity in the direction and magnitude of endophyte change (hotspots and coldspots)  
 191 across the spatial extent of each host's distribution. To account for the spatial non-independence  
 192 of geo-referenced occurrences, we used an approximate Bayesian method, Integrated Nested  
 193 Laplace Approximation (INLA), to construct spatio-temporal models of endophyte prevalence.  
 194 INLA provides a computationally efficient method of ascertaining parameter posterior distribu-  
 195 tions for certain models that can be formulated as latent Gaussian Models (Rue et al., 2009).  
 196 Many common statistical models, including structured and unstructured mixed-effects models,  
 197 can be represented as latent Gaussian Models. We incorporated spatial heterogeneity into this  
 198 analysis using spatially-structured intercept and slope parameters implemented as stochastic  
 199 partial differential equations (SPDE) to approximate a continuous spatial Gaussian process. This  
 200 SPDE approach is a flexible method of smoothing across space while explicitly accounting for  
 201 spatial dependence between data-points (Bakka et al., 2018; Lindgren et al., 2011). Fitting models

202 with structured spatial effects is possible with MCMC sampling but can require long computa-  
 203 tion times, making INLA an effective alternative. This approach has been used to model spatial  
 204 patterns in flowering phenology (Willems et al., 2022), the abundance of birds (Meehan et al.,  
 205 2019) and butterflies (Crossley et al., 2022), the distribution of temperate trees (Engel et al., 2022)  
 206 as well as the population dynamics of endangered amphibians (Knapp et al., 2016) and other  
 207 ecological processes (Beguin et al., 2012).<sup>8</sup>

208 We estimated global and spatially-varying trends in endophyte prevalence using a joint-  
 209 likelihood model. For each host species  $h$ , endophyte presence/absence of the  $i^{th}$  specimen ( $P_{[h]i}$ )<sup>9</sup>  
 210 was modeled as a Bernoulli response variable with expected probability of endophyte occurrence  
 211  $\hat{P}_{[h]i}$ . We modeled  $\hat{P}_{[h]i}$  as a linear function of intercept  $A_{[h]i}$ <sup>10</sup> and slope  $T_{[h]}$  defining the global  
 212 trend in endophyte prevalence specific to each host species as well as with spatially-varying  
 213 intercepts  $\alpha_{[h_1]l[i]}$  and slopes  $\tau_{[h_1]l[i]}$  associated with location ( $l[i]$ , the unique latitude-longitude  
 214 combination of the  $i^{th}$  observation). The joint-model structure allowed us to “borrow strength”  
 215 across species in the estimation of shared variance terms for the spatially-dependent random ef-  
 216 fect  $\delta_{l[i]}$ , intended to account for residual spatial variation, and  $\chi_{c[i]}$  and  $\omega_{s[i]}$  i.i.d.-random effects  
 217 indexed for each collector identity ( $c[i]$ ), and scorer identity ( $s[i]$ ) of the  $i^{th}$  specimen.<sup>11</sup>

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

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

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

218 By including random effects for collectors and scorers, we accounted for “nuisance” variance  
 219 that may bias predictions for changes in endophyte prevalence. Previous work suggests that  
 220 behavior of historical botanists may introduce biases into ecological inferences made from historic

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<sup>8</sup>Probably not necessary if you need space.

<sup>9</sup>Not obvious to me why you use square brackets here.

<sup>10</sup>I don't think there should be an  $i$  subscript here. You need  $i$  only where there is a covariate unique to the  $i^{th}$  observation.

<sup>11</sup>I don't think you need three expressions here. If you just index host as  $h$  it is clear that some pieces are unique to host  $h$  and others are shared across hosts.

<sup>221</sup> collections (Kozlov et al., 2020). Prolific collectors who contribute thousands of specimens may  
<sup>222</sup> be more or less likely to collect certain species, or specimens with certain traits (Daru et al., 2018).  
<sup>223</sup> Similarly, the process of scoring seeds for hyphae involved several student researchers who, even  
<sup>224</sup> with standardized training, may vary in their likelihood of positively identifying *Epichloë*.

<sup>225</sup> We performed model fitting using the inlabru R package (Bachl et al., 2019). Global intercept  
<sup>226</sup> and slope parameters A, and T, were given vague priors. Scorer and collector random effects,  
<sup>227</sup>  $\chi$  and  $\omega$ , were given penalized complexity priors, with distributions approximating a Normal  
<sup>228</sup> distribution with standard deviation of 5. Each spatially-structured parameter depended on a  
<sup>229</sup> covariance matrix according to the proximity of each pair of collection locations (Bakka et al.,  
<sup>230</sup> 2018; Lindgren et al., 2011). The covariance matrix was approximated using a Matérn covariance  
<sup>231</sup> function, with each data point assigned a location according to the nodes of a mesh of non-  
<sup>232</sup> overlapping triangles encompassing the study area (Fig. A2). We assessed model fit with visual  
<sup>233</sup> posterior predictive checks (A3) and measurements of AUC (Figs. A4-A5). Priors for the Matérn  
<sup>234</sup> covariance function, termed "range" and "variance", define how proximity effects decay with  
<sup>235</sup> distance. **Priors for results presented in the main text reflect a range of 342 kilometers.** We  
<sup>236</sup> found that model results were somewhat sensitive to the choices of priors for these parameters,  
<sup>237</sup> so we tested a range of values (from 68 kilometers to 2160 kilometers) and meshes (Supplemental  
<sup>238</sup> Material), finding that model results were qualitatively similar, i.e. the same direction of effects  
<sup>239</sup> across space, but that the magnitude and uncertainty varied.<sup>12</sup>

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<sup>12</sup>I struggled here. I tried to make some edits but I don't think I understand the methods well enough to edit correctly. It is not clear how the distances you quote should be interpreted (locations within 68 or 2160 km are more likely to have the same endo status just due to proximity?), or whether they should be interpreted at all. The text seems to say both the priors do not matter, but also that they do. I have not looked at what is in the supplement yet, but if you present results there based on different priors it should be easy to write this in a way that succinctly says "priors mattered a little bit, and if you care you can look at the supplement for a prior sensitivity analysis."

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

241    We evaluated the predictive ability of the model using both in-sample training data from the  
242    herbarium surveys, and with out-of-sample test data, an important but rarely used strategy in  
243    ecological studies (Lee et al., 2024; Tredennick et al., 2021). We generated out-of-sample test  
244    data from contemporary surveys of endophyte prevalence in natural populations of *A. hyemalis*  
245    and *E. virginicus* in Texas and the southern US. Surveys of *E. virginicus* were conducted in 2013  
246    as described in Sneck et al. (2017), and surveys of *A. hyemalis* took place between 2015 and  
247    2020. Population surveys of *A. hyemalis* were initially designed to cover longitudinal variation  
248    in endophyte prevalence towards its range edge, while surveys of *E. virginicus* were designed to  
249    cover latitudinal variation. In total, we visited 43 populations of *A. hyemalis* and 20 populations  
250    of *E. virginicus* across the south-central US, with emphasis on Texas and neighboring states (Fig  
251    A11). During surveys, we collected seeds from up to 30 individuals per population (average  
252    number of plants sampled per population: 22.9); note that this sampling design provided greater  
253    local depth of information than the herbarium records, where only one plant was sampled at  
254    each locality. We quantified the endophyte status of each individual with staining microscopy  
255    as described for the herbarium surveys (with 5-10 seeds scored per individual), and calculated  
256    the prevalence of endophytes within the population (proportion of plants that were endophyte-  
257    symbiotic). For each population, we compared the observed fraction of endophyte-symbiotic  
258    hosts to the predicted probability of endophyte occurrence  $\hat{P}$  derived from the model for that  
259    location and year. The contemporary survey period (2013-2020) is at the most recent edge of the  
260    time period encompassed by the historical observations used for model fitting.

261                    *Assessing the role of climate drivers*

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

265 loaded monthly temperature and precipitation rasters from the PRISM climate group (Daly and  
266 Bryant, 2013) covering the time period between 1895 and 2020 using the 'prism' R package (Hart  
267 and Bell, 2015). Prism provides reconstructions of historic climate variables across the United  
268 States by spatially-interpolating weather station data (Di Luzio et al., 2008). We calculated 30-year  
269 climate normals for seasonal mean temperature and cumulative precipitation for the recent (1990  
270 to 2020) and historic (1895 to 1925) periods. We used three four-month seasons within the year  
271 (Spring: January, February, March, April; Summer: May, June, July, August; Autumn: September,  
272 October, November, December). This division of seasons allowed us to quantify differences in  
273 climate associated with the two "cool" seasons, when we expected our focal species to be most  
274 biologically active (*A. hyemalis* flowering phenology: spring; *E. virginicus*: spring and summer; *A.  
275 perennans*: autumn). In addition to mean climate conditions, environmental variability itself can  
276 influence population dynamics (Tuljapurkar, 1982) and changes in variability are a key prediction  
277 of climate change models (IPCC, 2021; Stocker et al., 2013). Therefore, we calculated the standard  
278 deviation for each annual and seasonal climate driver across each 30-year period. We then took  
279 the difference between recent and historic periods for the mean and standard deviation for each  
280 climate driver (Figs. A12-A14). All together, we assessed twelve potential climate drivers: the  
281 mean and standard deviation of spring, summer, and autumn temperature, as well as the mean  
282 and standard deviation of spring, summer, and autumn cumulative precipitation.

283 To evaluate whether areas that have experienced the greatest changes in endophyte preva-  
284 lence (hotspots of endophyte change) are associated with high degrees of change in climate  
285 (hotspots of climate change), we modeled the fitted, spatially-varying slopes of endophyte change  
286 through time ( $\tau_{[h]l}$ ) as a linear function of environmental covariates, with a Gaussian error dis-  
287 tribution.<sup>13</sup> Data from each host species was analyzed separately. Fitting regressions to many  
288 pixels across the study region risks artificially inflating confidence in our results due to large sam-  
289 ple sizes, and so we performed this analysis using only a random subsample of 250 pixels across

<sup>13</sup>If I am reading the results figure correctly it looks like you force an intercept of zero, and I am not sure why you would want to do that.

290 the study region; other sizes of subsample yielded similar results.

291 *Modeling distributions of host species*

292 Because the herbarium records did not encompass the entirety of these host species' ranges,  
293 we additionally modeled the geographic distribution of each host species to generate realistic  
294 maps on which we could project the predictions of the INLA model. We followed the ODMAP  
295 (overview, data, model, assessment, prediction) protocol (Crossley et al., 2022) (see Supporting  
296 Methods). In short, we used presence-only observations of each host species from Global Biodi-  
297 versity Information Facility (GBIF) **between 1990 to 2020**<sup>14</sup>. We fit maximum entropy (MaxEnt)  
298 models using the maxent function in the R package dismo (Hijmans et al., 2017) using the same  
299 set of seasonal climate predictors considered above: **mean and standard deviation of spring,**  
300 **summer, and autumn temperature, and mean and standard deviation of spring, summer, and**  
301 **autumn cumulative precipitation.**<sup>15</sup> We generated 10,000 pseudo-absences as background points,  
302 and split the occurrence data into 75% for model training and 25% for model testing. The perfor-  
303 mance of models was evaluated with AUC (Jiménez-Valverde, 2012). **We found AUC values of**  
304 **0.862, 0.838, 0.821 respectively for *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*.**<sup>16</sup> To  
305 convert the continuous predicted probabilities into binary presence - absence maps on which we  
306 projected INLA predictions, we used the training sensitivity (true positive rate) and specificity  
307 threshold (true negative rate) (Liu et al., 2005).

---

<sup>14</sup>I am not sure why 1990-2020. From our conversations, I thought the SDM was based on all records. At this point I would not change anything.

<sup>15</sup>Were these 1990-2020 normals?

<sup>16</sup>Can we make this sentence more evaluative? I think AUC > 0.8 is very good, yes?

## Results

### 309 *How has endophyte prevalence changed over time?*

310 Across >2300 herbarium specimens dating back to 1824, we found that prevalence of *Epichloë*  
311 endophytes increased over the last two centuries for all three grass host species (Fig. 2). On  
312 average, endophytes of *A. perennans* and *E. virginicus* increased from ~ 40 % to 70% prevalence  
313 across the study region, and *A. hyemalis* increased from ~ 25% to over 50% prevalence. Our  
314 model indicates a high certainty that overall temporal trends are positive across species (99%  
315 probability of a positive overall year slope in *A. hyemalis*, 92% probability of a positive overall  
316 year slope in *A. perennans*, and 91% probability of a positive overall year slope in *E. virginicus*)  
317 (Fig. A6). The model appears to under-predict the observed increase in endophyte prevalence  
318 relative to the data, particularly for *A. hyemalis* (Fig. 2A), but the model is accounting for random  
319 effects and spatial non-independence that are not readily seen in the figure. By under-weighting  
320 endophyte-positive samples that are clustered spatially or by collector or observer, the INLA  
321 model is appropriately accounting for nuisance variables and providing a conservative inference  
322 of endophyte change relative to the raw data.

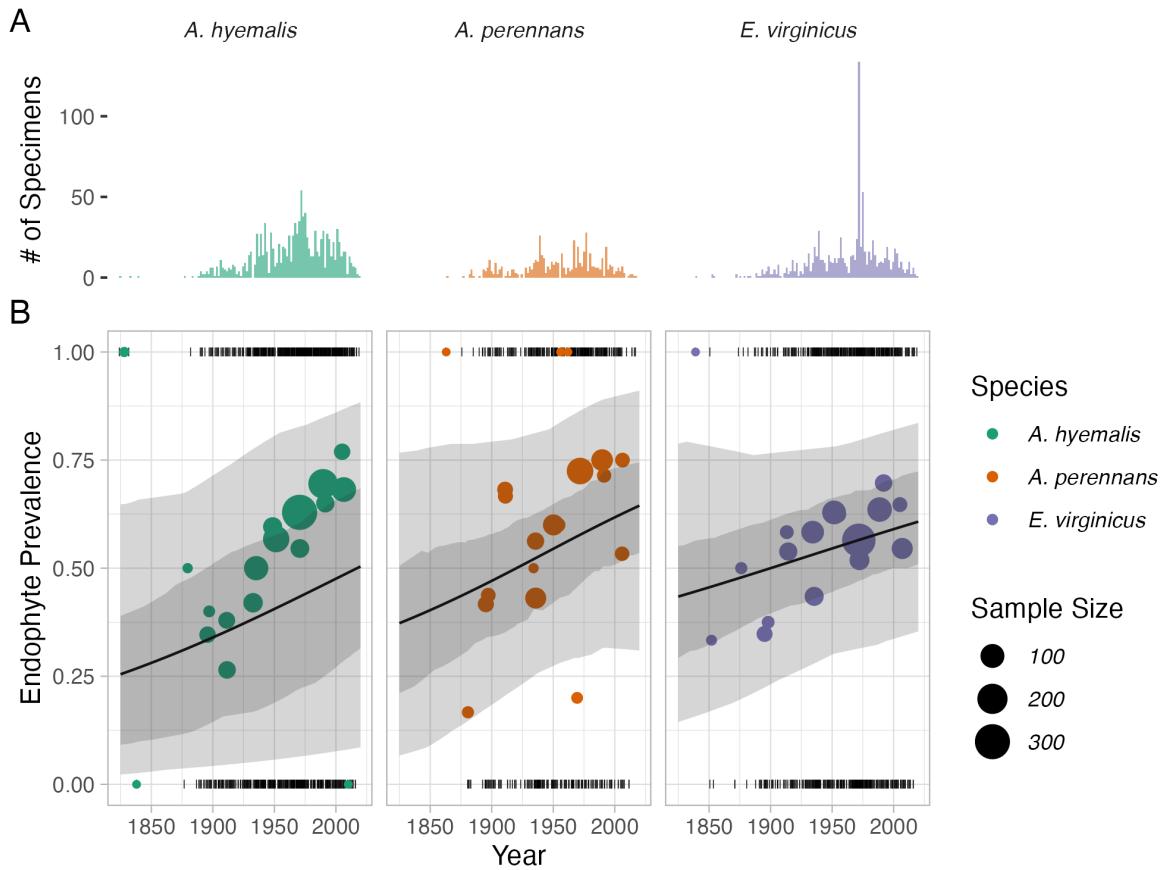


Figure 2: Temporal trends in endophyte prevalence. (A) Histograms show the frequency of scored specimens through time for each host species. (B) Lines show predicted mean endophyte prevalence over the study period along with the 50% and 95% CI bands incorporating uncertainty associated with collector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

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

While there was an overall increase in endophyte prevalence, our model revealed hotspots and coldspots of change across the host species' ranges, which are mapped in Fig. 3 across geographic ranges predicted by MaxEnt species distribution models. In some regions, posterior mean esti-

327 mates of spatially varying temporal trends indicate that *A. hyemalis* and *A. perennans* experienced  
 328 increases in prevalence by as much as 2% per year over the study period, while *E. virginicus* expe-  
 329 rienced increases up to around 1% per year. Both *Agrostis* species show areas of strong increase  
 330 and areas of declining prevalence, while *E. virginicus* had an overall weaker and geographically  
 331 more consistent increase in endophyte prevalence. Notably, endophytes increased most strongly  
 332 towards the western range edge of *A. hyemalis* (Fig. 3A) and across the northeastern US for *A.*  
 333 *perennans* (Fig. 3B). Posterior estimates of uncertainty in spatially varying slopes indicate that  
 334 these hotspots of change may have experienced increases of up to 5% per year while declines  
 335 in prevalence may be as great as 4% per year for *A. hyemalis* and *A. perennans*. For *E. virginicus*,  
 336 uncertainty ranges between 3.5% increases and 2.5% decreases (Fig. A7).

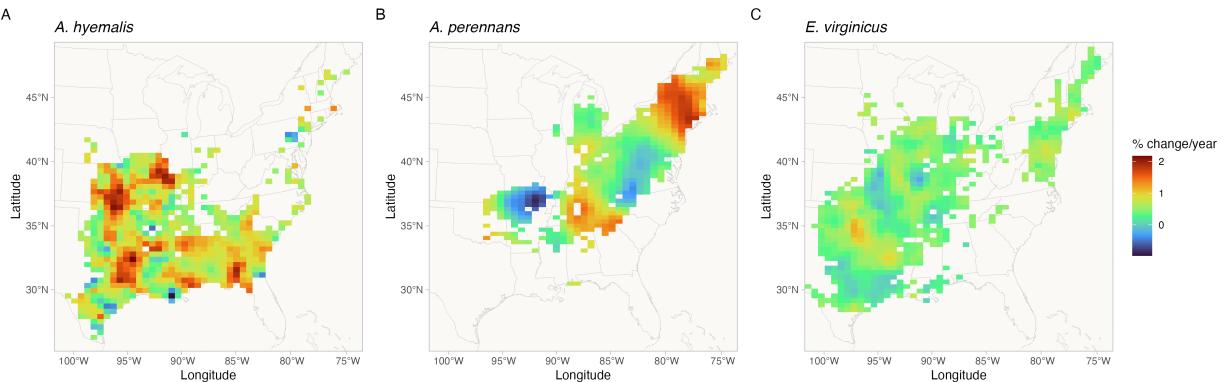


Figure 3: Predicted posterior mean of spatially-varying slopes representing change in endophyte prevalence for each host species. Color indicates the relative change in predicted endophyte prevalence.

337 *What is the relationship between variation in temporal trends in endophyte*  
 338 *prevalence and changes in climate drivers?*

339 <sup>17</sup> We found that trends in endophyte prevalence were strongly associated with seasonal climate  
340 change drivers (Fig. 4). For the majority of the study region, **the climate has become wetter and**  
341 **cooler over the last century**<sup>18</sup> (Fig. A12-A14), a consequence of regional variation in global climate  
342 change (IPCC, 2021).<sup>19</sup> Spatial variation in climate trends were predictive of trends in endophyte  
343 prevalence. For example, strong increases in endophyte prevalence for *A. perennans* were most  
344 strongly associated with change in autumn climate drivers that coincide with its active growing  
345 season.<sup>20</sup> For this species, regions experiencing warmer and wetter autumn climates showed  
346 particularly strong relationships with increasing endophyte prevalence, however other seasonal  
347 drivers may also contribute (drier springs and cooler summers). Trends in endophyte prevalence  
348 for *A. hyemalis* were most strongly associated with changes in precipitation and precipitation  
349 variability. Endophyte prevalence in *A. hyemalis* increased the most in regions that experienced  
350 greater increases in spring precipitation, as well as regions with changing variability in spring  
351 and autumn precipitation. **Changes in endophyte prevalence in *E. virginicus* were not strongly**  
352 **associated with changes in most climate drivers**<sup>21</sup>, but regions of reduced variability in autumn  
353 precipitation were associated with weak reductions in prevalence. **Correlations**<sup>22</sup> assessed using  
354 all pixels across each species' distribution were qualitatively similar to these results (Fig. A11).

---

<sup>17</sup>I think this section would be strengthened by greater statistical support. It is really just eye-balling lines. Can we say something more quantitative? See my comment about posteriors of slopes – that is the type of information I have in mind, and maybe we could embed some of this in the text.

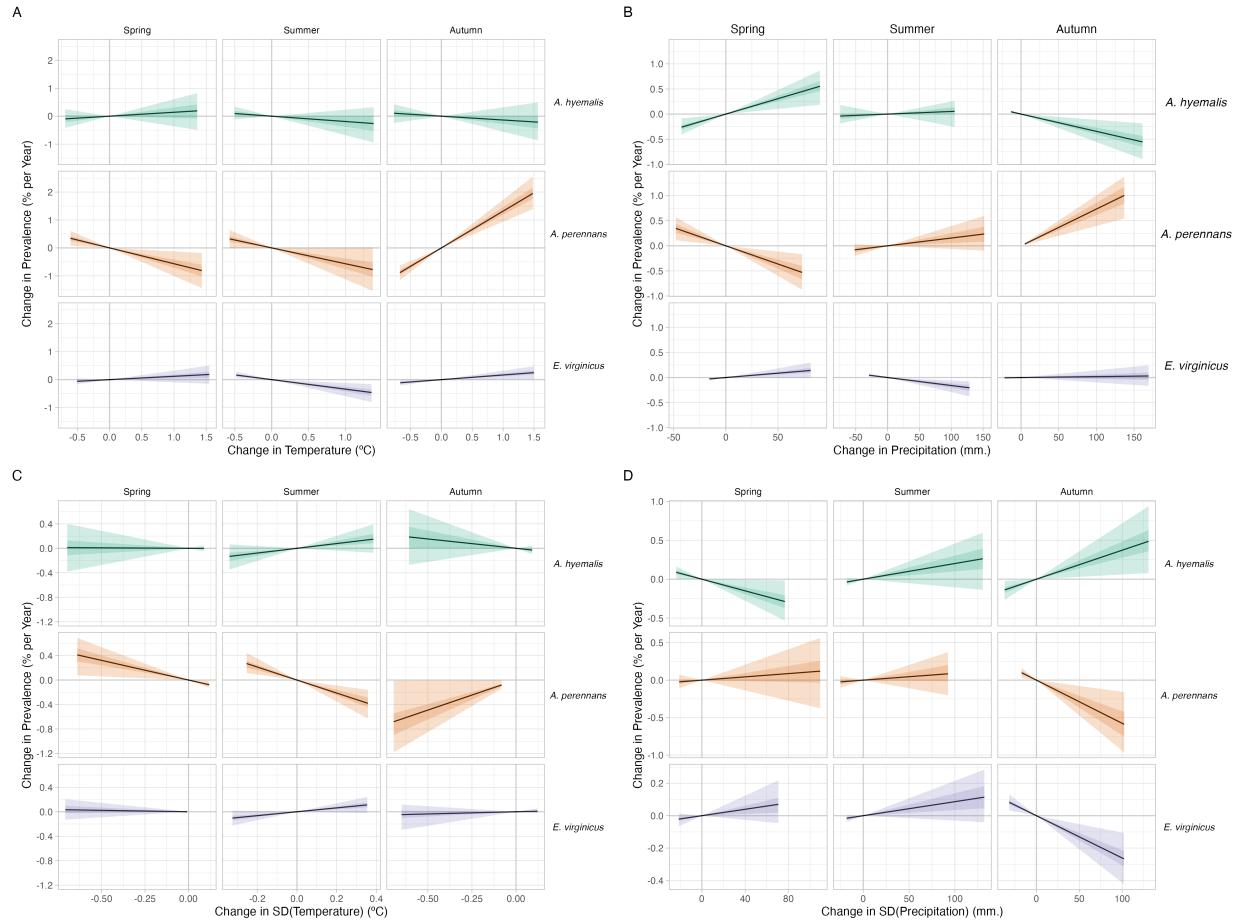
<sup>18</sup>Can you provide the average change in degrees celsius and mm precip across the range?

<sup>19</sup>Comment on these supplement figures: perhaps for the revision, I think a color scale that diverges at zero would be helpful here.

<sup>20</sup>Here and throughout the results, please reference specific figures and panels for statements like this so readers can see what you see. Regarding the figure, since it is just a bunch of regression lines and we only care about the slopes, it might be more effective to just show posteriors of the slopes - probably not worth making that change at this point, and maybe it's ugly.

<sup>21</sup>Notice that my edits here importantly change the meaning of the sentence and, as far as I understand, make it more correct.

<sup>22</sup>I am not sure why you describe these as "correlations". The methods makes these sound like linear regressions.



**Figure 4: Relationships between changes in seasonal climate drivers and predicted marginal trends in endophyte prevalence.** Lines show relationships between seasonal climate (A: mean temperature, B: cumulative precipitation, C: standard deviation in temperature, D: standard deviation in precipitation) and spatially-varying trends in endophyte prevalence for each host species, along with 50 and 95% CI.

### 355 Performance on test data

356 <sup>23</sup> We found that model performance, as quantified by AUC, was similar between historic herbarium specimens used as training data and the out-of-sample test data from contemporary surveys  
 357 (0.79 and 0.77 respectively; Fig. A5-A4). The model successfully captured broad regional trends

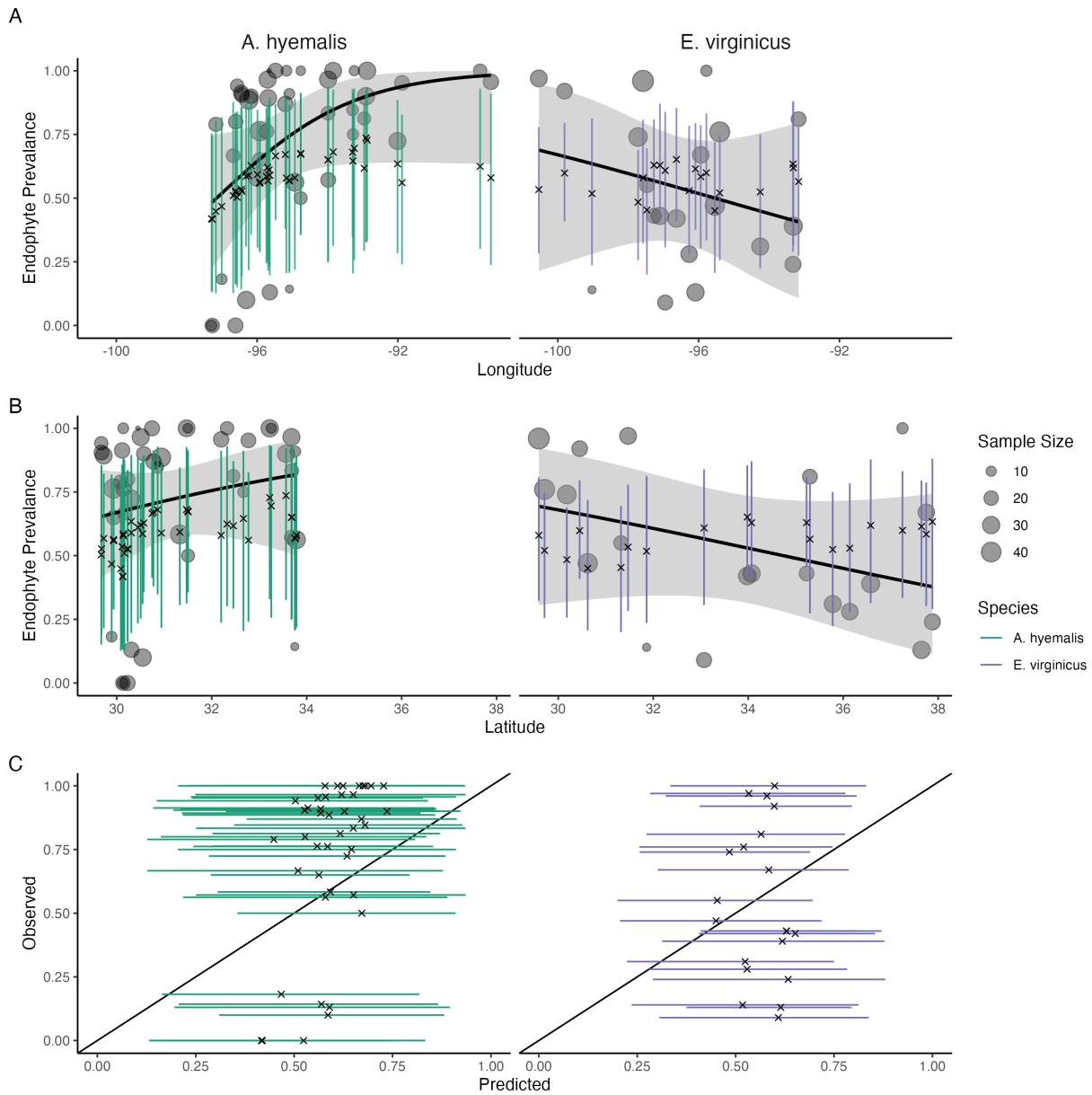
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<sup>23</sup>The order of the results does not match the order of the methods.

359 in endophyte prevalence seen in the contemporary survey data, such as decline endophyte preva-  
360 lence in *A. hyemalis* towards western longitudes (Fig. 5A) and northern latitudes (Fig. 5B). How-  
361 ever, model predictions for endophyte prevalence exhibited relatively little geographic variation,  
362 whereas the out-of-sample survey data were maximally variable with populations spanning 0%  
363 to 100% endophyte-symbiotic plants (Fig. 5C)<sup>24</sup>. We interpret this to mean that the model cap-  
364 tures coarse-scale spatial and temporal trends reasonably well, but is not equipped to capture  
365 local-scale nuances that generate population-to-population differences.

---

<sup>24</sup>*Love the figure but I think the legend can be improved. It does not describe the content very well.*



**Figure 5: Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporary endophyte prevalence in *A. hyemalis*, as indicated by some overlap of predicted 95% CI with the 1:1 line, however contemporary test data generally had more variance between populations than model predictions. The model did recapitulate broader regional trends across (B) longitude and (C) latitude. Point size in panels B and C reflect sample sizes of contemporary endophyte population surveys.

366 Assessing collector and scorer influences on predicted endophyte prevalence

367 Our modeling effort quantified temporal and spatial trends in endophyte prevalence while ac-  
368 counting for potential biases introduced by collectors and by individual scorers who quantified  
369 endophyte presence/absence within specimens. We found no evidence that collector biases in-  
370 fluenced our results. Collector random effects were consistently small (Fig. A9), and models fit  
371 with and without this random effect provide qualitatively similar results. The identity of indi-  
372 vidual scorers did contribute to observed patterns in endophyte prevalence. For example, 3 of  
373 the 25 scorers were more consistently likely than average to assign positive endophyte status, as  
374 indicated by 95% credible intervals greater than zero) (Fig. A10). It is difficult to distinguish  
375 whether this was driven by true differences in scorers biases during the seed scoring process or  
376 by unintended spatial or temporal clustering of the specimens scored by each scorer (Clayton  
377 et al., 1993; Urdangarin et al., 2023). Interpreting our models with the inclusion of variance as-  
378 sociated with the scorer effect thus provides conservative estimates of the absolute magnitude of  
379 changes in endophyte prevalence.

## Discussion

381 Our examination of historic plant specimens revealed cryptic shifts in microbial symbiosis over  
382 the last two centuries. For the three host species we examined, there have been strong increases  
383 in prevalence of fungal endophytes. We interpret increases in prevalence of *Epichloë*, which are  
384 vertically transmitted, as adaptive changes that improve the fitness of their hosts under increas-  
385 ing environmental stress. This interpretation is in line with theory predicting that the positive  
386 fitness feedback caused by vertical transmission leads beneficial symbionts to rise in prevalence  
387 within a population (Donald et al., 2021; Fine, 1975). We further found that trends in endophyte  
388 prevalence varied across the distribution of each species in association with changes in climate  
389 drivers, suggesting that the increases in endophyte prevalence are driven by context-dependent  
390 benefits to hosts that confer resilience under environmental change. Taken together, this suggests

<sup>391</sup> an overall strengthening of host-symbiont mutualism over the last two centuries.

<sup>392</sup> Differences across host species underscore that while all of these  $C_3$  grasses share similar  
<sup>393</sup> broad-scale distributions, each engages in unique biotic interactions and has unique responses to  
<sup>394</sup> environmental drivers. We identified hotspots of change for *A. perennans*, which was the species  
<sup>395</sup> that experienced the strongest absolute changes<sup>25</sup> in endophyte prevalence (Fig. 3). Declines in  
<sup>396</sup> the southern portion of its range and increases in the north suggest a potential poleward range  
<sup>397</sup> shift of endophyte-symbiotic plants (whether the overall host distribution is shifting in parallel  
<sup>398</sup> is an exciting next question). Based on previous work demonstrating that endophytes can shield  
<sup>399</sup> their hosts from drought stress (Decunta et al., 2021)<sup>26</sup>, we generally predicted that drought con-  
<sup>400</sup> ditions would be a driver of increasing endophyte prevalence. In contrast to this expectation,  
<sup>401</sup> increasing prevalence for this species<sup>27</sup> was associated with increasing autumn temperature and  
<sup>402</sup> precipitation (Fig. 4). To our knowledge, the response of the symbiosis in *A. perennans* to drought  
<sup>403</sup> has not been examined experimentally, but in a greenhouse experiment, endophytes had a pos-  
<sup>404</sup> tive effect on host reproduction under shaded, low-light conditions (Davitt et al., 2010). Our  
<sup>405</sup> results also hint that it may be useful to investigate whether lagged climate effects are important  
<sup>406</sup> predictors of host fitness in this system (Evers et al., 2021). Endophyte prevalence of the spring-  
<sup>407</sup> flowering *A. hyemalis* was most strongly linked to increasing variability in precipitation across  
<sup>408</sup> summer and autumn.<sup>28</sup> Endophytes could be playing a role helping hosts weather autumn-  
<sup>409</sup> season droughts while the species is dormant.<sup>29</sup> Previous work has demonstrated drought  
<sup>410</sup> benefits in a greenhouse manipulation with this species (Davitt et al., 2011), and early life stages

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<sup>25</sup>remind readers what the absolute change was

<sup>26</sup>I would cite this as “reviewed in” or something like that, so this is not read as a single case study

<sup>27</sup>I have now forgotten which species you are talk about, since the previous sentence is about grass-endophyte interactions more generally. I think this paragraph can be edited to make it clearer which are general statements and which refer to specific species (and which ones).

<sup>28</sup>I do not see this in the figure. I would argue you are over-interpreting the SD effects. The ppt amount effects look stronger, and these are in autumn and spring which lines up with this species’ growing season. Marion’s paper showed that wet autumn conditions can neutralize the benefits of the symbiosis, which I think is consistent with your result.

<sup>29</sup>Not dormant in autumn. They probably start germinating in October.

411 may be particularly vulnerable to prolonged droughts. For *E. virginicus*, which experienced the  
412 most modest changes in endophyte prevalence overall, we only modest associations with changes  
413 in climate drivers. Surveys by Sneck et al. (2017), used as part of the test data in this study, identi-  
414 fied a drought index (SPEI) that integrates precipitation with estimated evapotranspiration as an  
415 important predictor of endophyte prevalence. *Epichloë* endophytes have also been connected to  
416 a suite of non-drought related fitness benefits including herbivore protection (Brem and Leucht-  
417 mann, 2001), salinity resistance (Wang et al., 2020), and mediation of the soil microbiome (Roberts  
418 and Ferraro, 2015). These effects are potentially mediated by the diverse bioactive alkaloids and  
419 other signaling compounds they produce (Saikkonen et al., 2013). Increases in symbionts could  
420 be explained, at least in part, by these diverse benefits that may help hosts weather a world  
421 made increasingly stressful by changes in climate and other anthropogenically introduced stres-  
422 sors. While we show consistent increasing trends in prevalence between the three species, the  
423 mechanisms that explain these changes may be diverse and idiosyncratic.<sup>30</sup>

424 Our spatially-explicit model predicted regions of both high and low endophyte prevalence,  
425 suggesting that symbiotic and non-symbiotic host plants have overlapping, but non-identical  
426 niche requirements.<sup>31</sup> Endophytes fitness benefits potentially explain the spatial distribution of  
427 prevalence by allowing their hosts to persist in environments where they otherwise could not  
428 (Afkhami et al., 2014; Kazenel et al., 2015)<sup>32</sup>. For example, fitness benefits of the symbiosis could

---

<sup>30</sup>This paragraph was a little hard to read and I think does not really nail the points that I think we should make here, IMO. I would open to paragraph setting up the idea that the correlations you detected have some support in previous experimental studies, then elaborate. Then I would acknowledge that other factors can elicit benefits of symbiosis, and these factors may ALSO be changing. The takeaway, for me and perhaps for readers more generally, is that we are very confident endophytes are increasing, we have some confidence that is associated with (and perhaps due to) climate change, but a lot has changed over the past 200 years (climate, urbanization, nitrogen deposition, invasive species, deer!) and we need follow-up studies and experiments to gain a better mechanistic understanding of what has driven these changes, which will also allow us to build better forecasts about the future.

<sup>31</sup>I think this paragraph tries to do too much relative to this topic sentence. In general I think you could use stronger topics sentences to give these Discussion paragraphs greater focus.

<sup>32</sup>cite Eco Monograph

429 explain historically low prevalence in *A. hyemalis* towards its western range edge coinciding  
430 with a strong aridity gradient.<sup>33</sup> Previous population surveys for endophytes found similar  
431 regional trends in prevalence for endophyte host species (Rudgers and Swafford, 2009; Sneck  
432 et al., 2017)<sup>34</sup>. While the model recreated the large-scale spatial trends observed in contemporary  
433 population surveys, test data contained more population-to-population variability in prevalence.  
434 Validating our model predictions in this way, a rare extra step in collections-based studies, allows  
435 us to evaluate places to improve the model's out-of-sample predictive ability, which will be  
436 particularly important for predicting host and symbiont niche-shifts under future climate change.  
437 Lack of information on local variability may simply be a feature of data derived from herbarium  
438 specimens. They are samples from local populations, but they are single specimens that are  
439 aggregated to derive broad-scale model estimates. Poor predictive ability at local scales in this  
440 grass-endophyte system is not surprising, as previous studies have found that local variation,  
441 even to the scale of hundreds of meters can structure endophyte-host niches (Kazenel et al.,  
442 2015). Other studies have found factors including land-use history (Vikuk et al., 2019) and the  
443 biotic environment, including herbivory (Rudgers et al., 2016), and host genotype Sneck et al.  
444 (2017), to be important predictors of endophyte ecology. Incorporating available climatic and soil  
445 layers as covariates is an obvious first step that could improve predictions.<sup>35</sup> Another important  
446 step would be integrating data from local and regional scales through modeling to constrain  
447 estimates of local and regional variation. These steps will bridge gaps that often exist between  
448 large but broad bioclimatic and biodiversity data and small but local data on biotic interactions,  
449 and move towards the goal of predicting the dynamics of microbial symbioses under climate  
450 change (Isaac et al., 2020; Miller et al., 2019).

451 Our analysis advances the use of herbarium specimens in global change biology in two ways.

---

<sup>33</sup>But above you say that AGHY gets benefits under drought.

<sup>34</sup>I would add the semimartin study.

<sup>35</sup>This is not where my mind went. I was thinking more local replication/redundancy, e.g. multiple specimens from the same population. Your idea is getting at explaining the local variation, but right now the model is not even seeing the local variation because we (rightly I think) prioritized larger-scale geographic coverage.

452 First and foremost, this is the first study to link long-term changes in microbial symbioses to  
453 changes in climate using specimens from natural history collections. The responses of micro-  
454 bial symbioses are a rich target for future studies within museum specimens, particularly those  
455 that take advantage of advances in sequencing technology.<sup>36</sup> While we used relatively coarse  
456 presence/absence data based on fungal morphology, other studies have examined historic plant  
457 microbiomes using molecular sequencing and sophisticated bioinformatics techniques, but these  
458 studies have so far been limited to relatively few specimens at limited spatial extents (Bieker  
459 et al., 2020; Bradshaw et al., 2021; Gross et al., 2021; Heberling and Burke, 2019; Yoshida et al.,  
460 2015). Continued advances in capturing historic DNA and in filtering out potential contamina-  
461 tion during specimen storage (Bakker et al., 2020; Daru et al., 2019; Raxworthy and Smith, 2021)  
462 will be imperative in the effort to scale up these efforts. This scaling up will be essential to  
463 be able to quantify changes not just in the prevalence of symbionts, **but also in symbionts' in-**  
464 **traspecific variation and evolutionary responses to climate change**<sup>37</sup>, as well as in changes in the  
465 wider microbial community. Answering these questions as well as the unknown questions that  
466 future researchers may ask also reiterates the value in capturing meta-information during ongo-  
467 ing digitization efforts at herbaria around the world and during the accession of newly collected  
468 specimens (Edwards et al.; Lendemer et al., 2020). Second, we accounted for several potential  
469 biases in the data observation process that may be common to many collections-based research  
470 questions by using a spatially-explicit random effects model. Spatial autocorrelation (Willem's  
471 et al., 2022), potential biases introduced by the sampling habits of collectors (Daru et al., 2018),  
472 and variation between contemporary researchers during the collection of trait data, if not cor-  
473 rected for could lead to over-confident inference about the strength and direction of historic  
474 change (I would cite the AGHY figure as an example). **Previous studies that have quantified the**

---

<sup>36</sup>*This is not the place for it, but this sentence made me think about genetic variation in the endophytes. We see presence-absence changing but the endophytes themselves could also be changing ("chemotypes") which is something we could possibly detect if we could get fungal DNA out of the samples. Maybe worth mentioning this somewhere.*

<sup>37</sup>*OK maybe this is the place for it. I would add a sentence about how endophytes can vary by chemotype and different chemotypes can confer different benefits.*

475 effects of collector biases typically find them to be small (Davis et al., 2015; Meineke et al., 2019),  
476 and we similarly did not find that collector has a strong effect on the results of our analysis.<sup>38</sup>

477 Ultimately, a central goal of global change biology is to generate predictive insights into the  
478 future of natural systems on a rapidly changing planet. While this survey of historic endophyte  
479 prevalence is necessarily correlative, it serves as a foundation to develop better predictive mod-  
480 els of the response of microbial symbioses to climate change. Combining the insights from this  
481 type of regional-scale survey with field experiments and physiological data **could be invalu-**  
482 **able.**<sup>39</sup> While we found that certain dimensions of climate change were strongly correlated  
483 with endophytes' temporal responses, we do not know why trends in prevalence were weak  
484 in some areas or how endophytes would respond to more extreme changes in climate. **For ex-**  
485 **ample, transplanting symbiotic and non-symbiotic plants beyond the range edge of *A. hyemalis***  
486 **could tell us whether persistent low endophyte prevalence in that area is a result of environ-**  
487 **mental conditions that lead the symbiosis to negative fitness consequences, or is a result of some**  
488 **historical contingency or dispersal limitation that has thus far limited the presence of symbi-**  
489 **otic hosts from a region where they would otherwise flourish and provide resilience.**<sup>40</sup> While  
490 the "time machine" of natural history collections revealed evidence of mutualism resilience for  
491 grass-endophyte symbioses in the face of environmental change, more extreme changes could  
492 potentially push one or both partners beyond their physiological limits, leading to the collapse of  
493 the mutualism; more research is needed to understand what those limits might be. **Our analysis**  
494 **thus far is agnostic to changes in the distributions of hosts. Mechanistic models could connect**  
495 **the responses of both host and symbionts to abiotic climate drivers, integrating dispersal pro-**

---

<sup>38</sup> This seems to undercut the previous sentence, but its also a place where you could mention that scorer effects DID matter, so this is worth incorporating when possible.

<sup>39</sup> Vague.

<sup>40</sup> This does not fit well here. I would move earlier where you talk about geographic trends. I also want to highlight that there is an important difference between associations of endophyte prevalence and geographic variation in climate (as in this AGHY example), and associations between CHANGES in endophyte prevalence and CHANGES in climate (the lines figure). Too often the manuscript blurs this distinction, and it happens here.

496 cesses.<sup>41</sup> Beyond host-microbe symbioses, building these types of models would work towards  
497 quantitatively attributing biotic responses to anthropogenically driven climate change, similar to  
498 methods in climate science and economics (Carleton and Hsiang, 2016; Stott et al., 2010).<sup>42</sup>

499 **Acknowledgments**

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

508 **Statement of Authorship**

509 J.C.F. contributed to research conception, data collection, data analysis, and led manuscript draft-  
510 ing. J.M. contributed to data analysis and manuscript revisions. T.E.X.M. contributed to research  
511 conception, data collection, data analysis, and manuscript revisions.

512 **Data and Code Availability**

513 Data from this publication will be made publically available upon acceptance and before that  
514 upon request.<sup>43</sup> Code for analyses can be found through a publicly available repository (<https://github.com/josh>)  
515 that will be permanently archived upon publication.

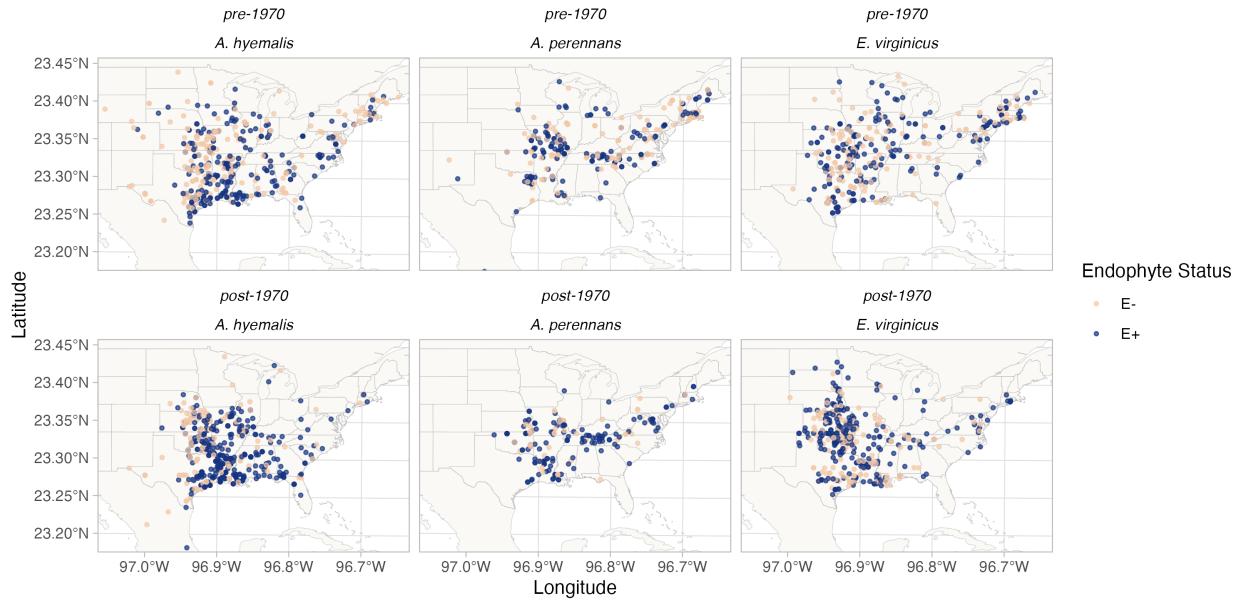
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<sup>41</sup>Does not feel like it fits well as a concluding point.

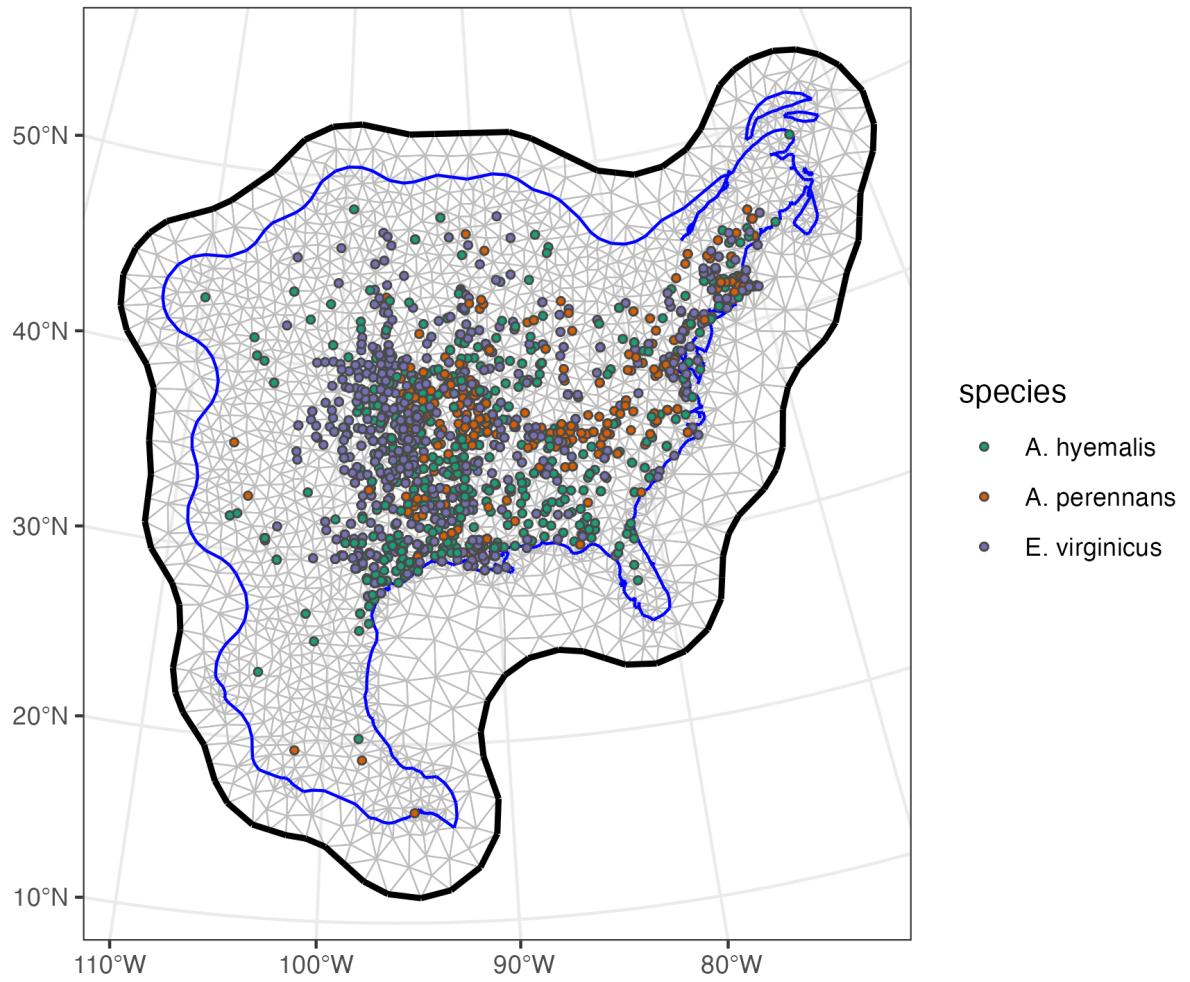
<sup>42</sup>Not knowing these citations, I am not really sure what you are referring to. This does not feel like a natural point to end on.

<sup>43</sup>CHeck journal requirements. It is generally better to make data and code available during peer review.

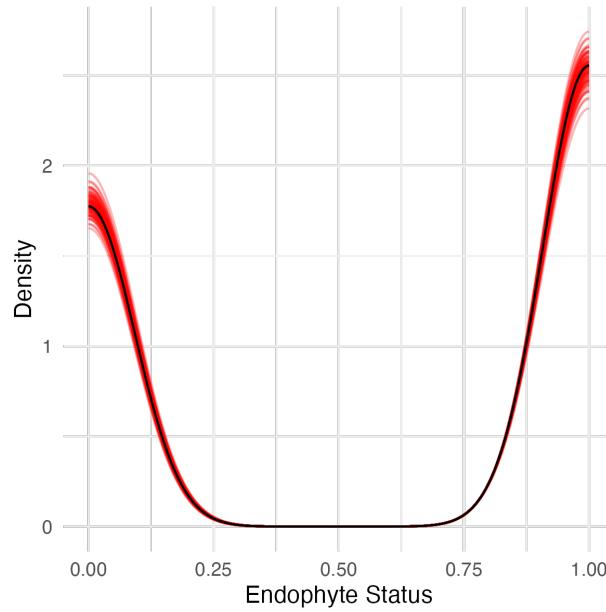
## Appendix A



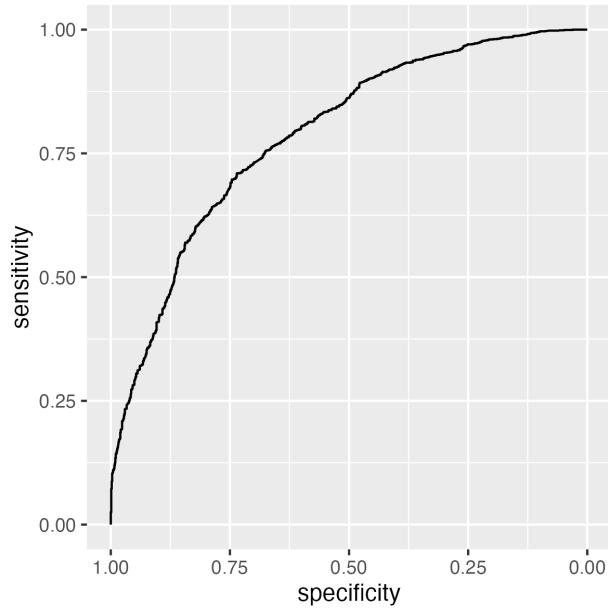
**Figure A1: Endophyte presence/absence in specimens of each host species.** Points show collection locations colored according to whether the specimen contained endophytes ( E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.



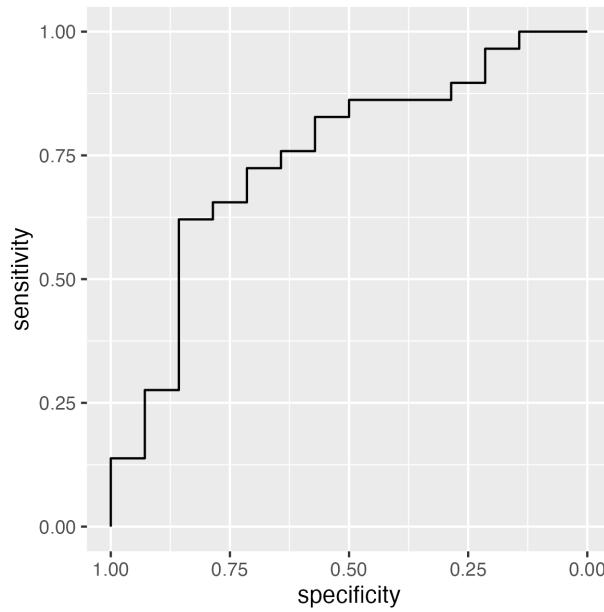
**Figure A2: Triangulation mesh used to estimate spatial dependence between data points.** Grey lines indicate edges of triangles used to define distances between observations. Colored points indicate locations of sampled herbarium specimens for each host species, and the blue line shows the convex hull 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: Consistency between real data and simulated values indicate that the fitted model accurately describes the data.** Graph shows density curves for the observed data (black) along with 100 simulated datasets (red).



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



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

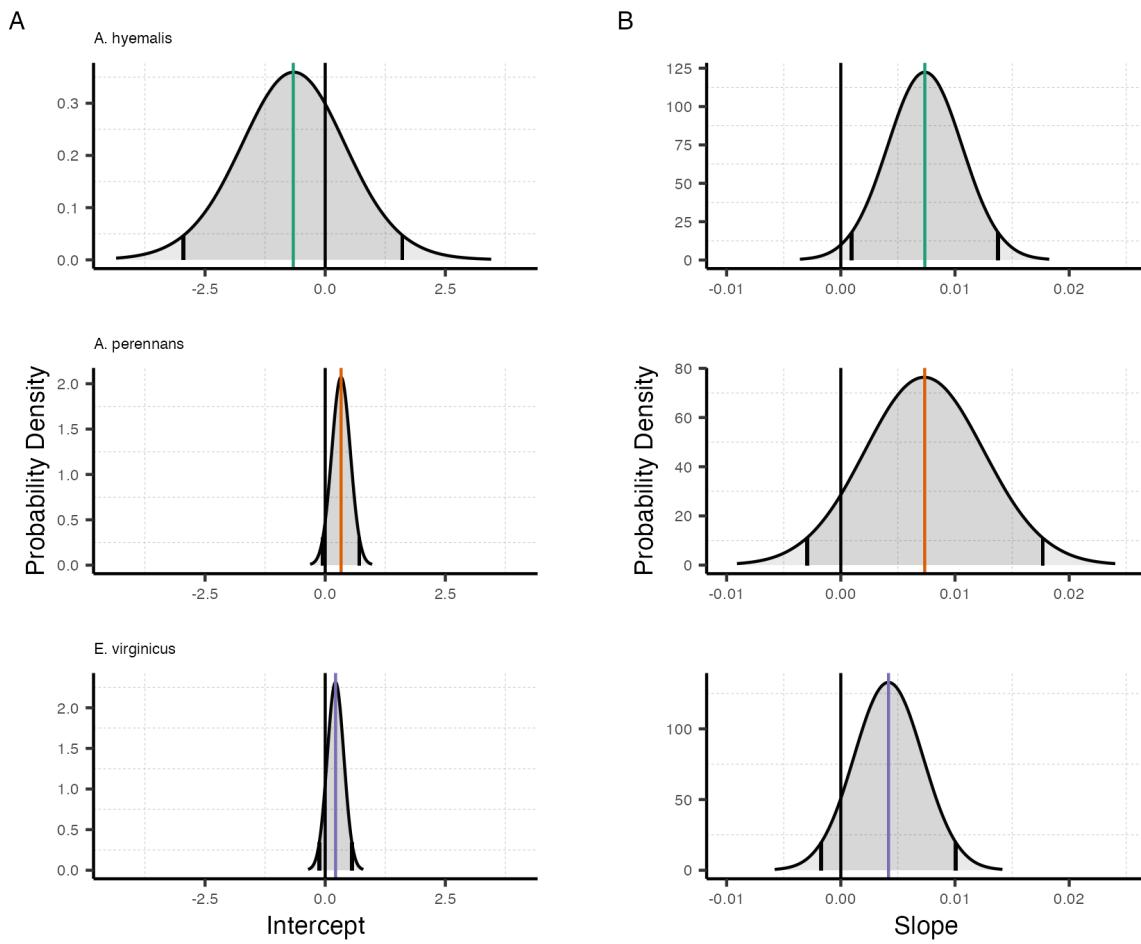


Figure A6: Density curves show the probability density along with mean (colored line) and 95% CI (black lines) for the (A) intercept and (B) slope terms, **A** and **T** respectively. Colors represent each host species

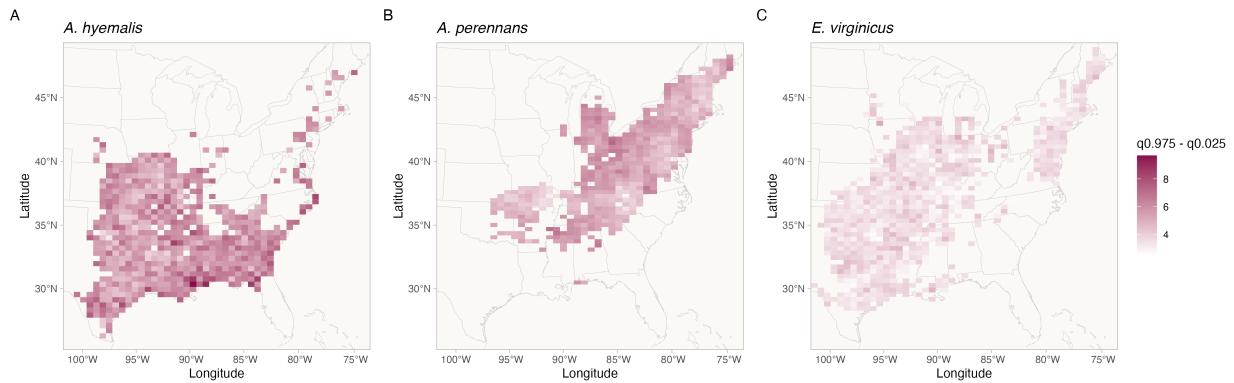


Figure A7: Shading represents the range of the 95% posterior credible interval for spatially varying slopes,  $\tau$ .

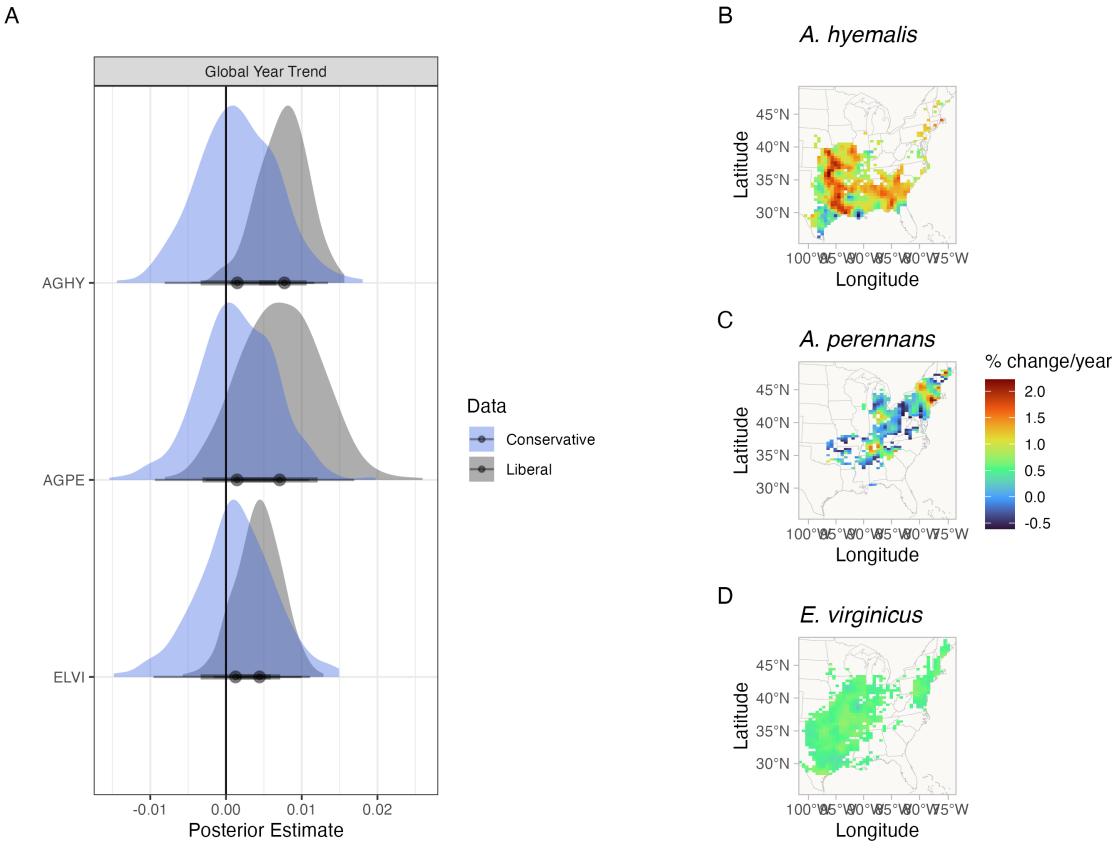


Figure A8: Comparison of liberal versus conservative endophyte scores on modeled outcomes. (A) Posterior estimates of global temporal trend for models fit to liberal scores (grey) and to conservative scores (blue). Maps show the spatially varying temporal trend estimates from model fit to conservative scores for (B) *A. hyemalis*, (C) *A. perennans*, and (D) *E. virginicus*. Note that the color scale differs between this visualization and Fig. 3.

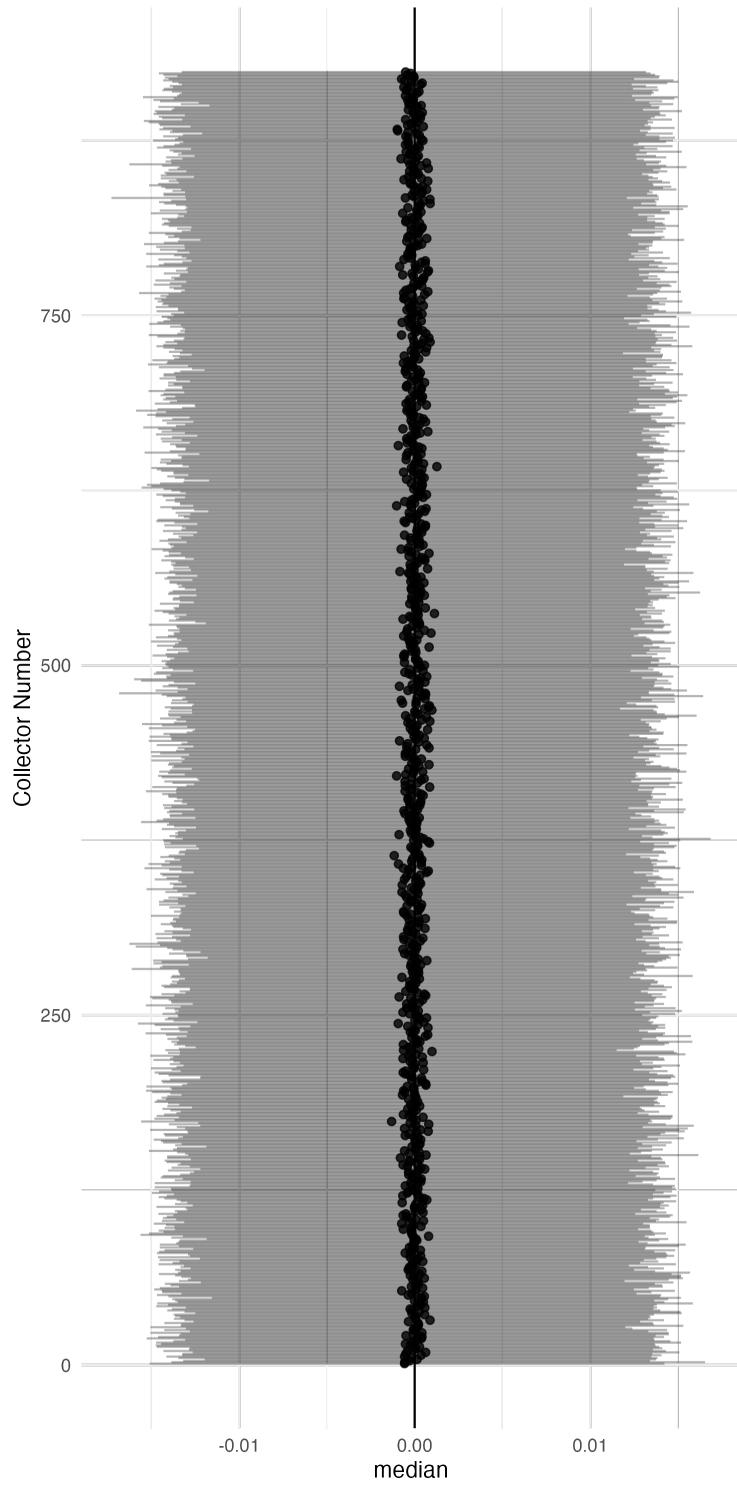


Figure A9: **Posterior estimates of collector random effects.** Points show posterior median along with 95% CI for each of 924 individual collectors.

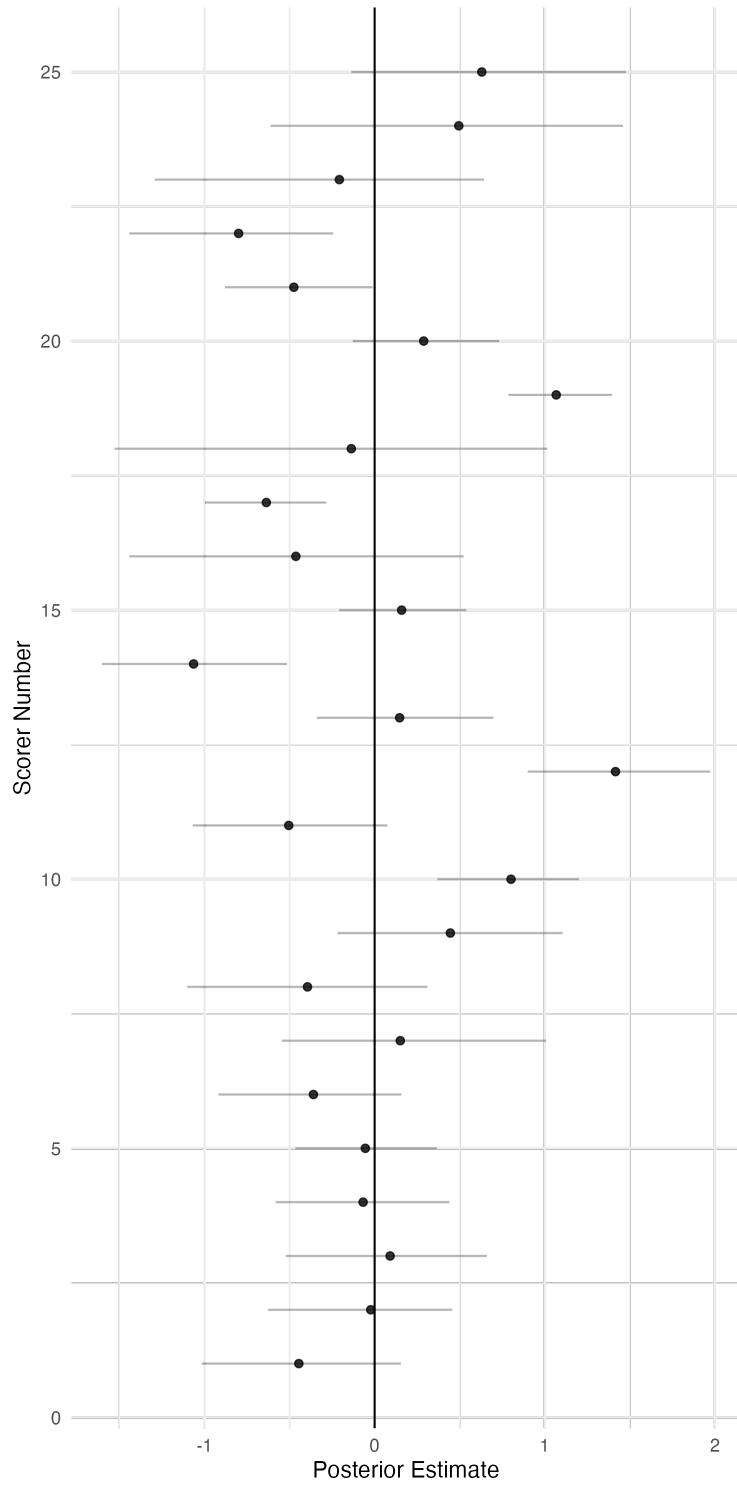


Figure A10: **Posterior estimates of scorer random effects.** Points show posterior median along with 95% CI for each of 25 individual collectors.

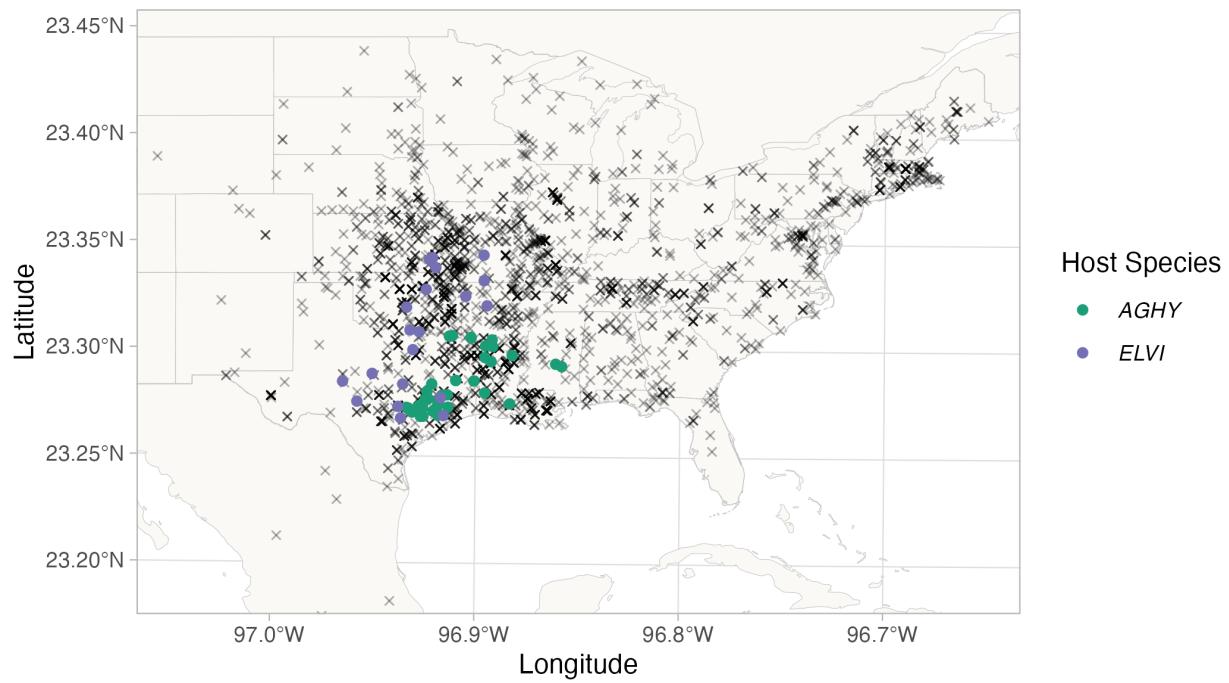
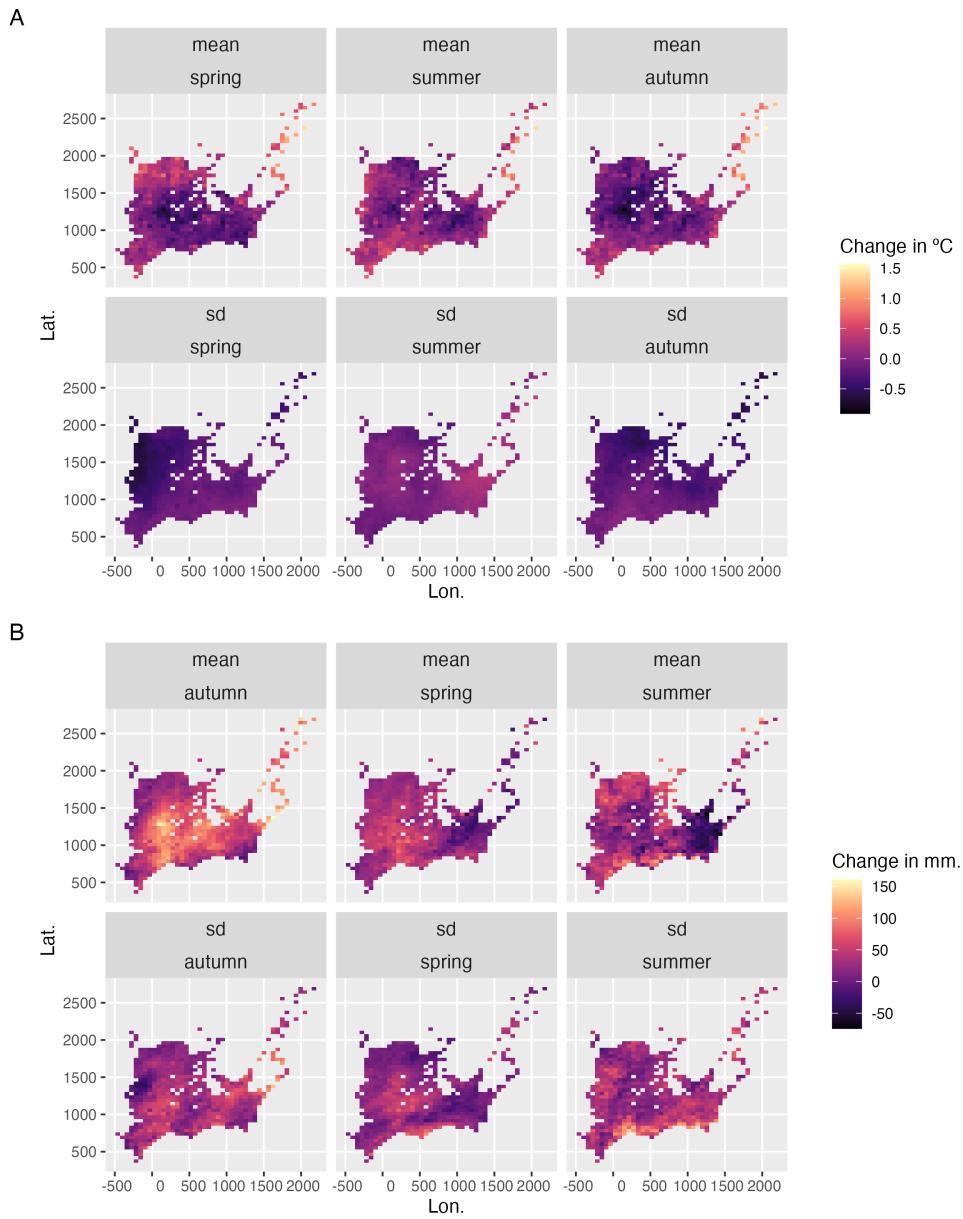
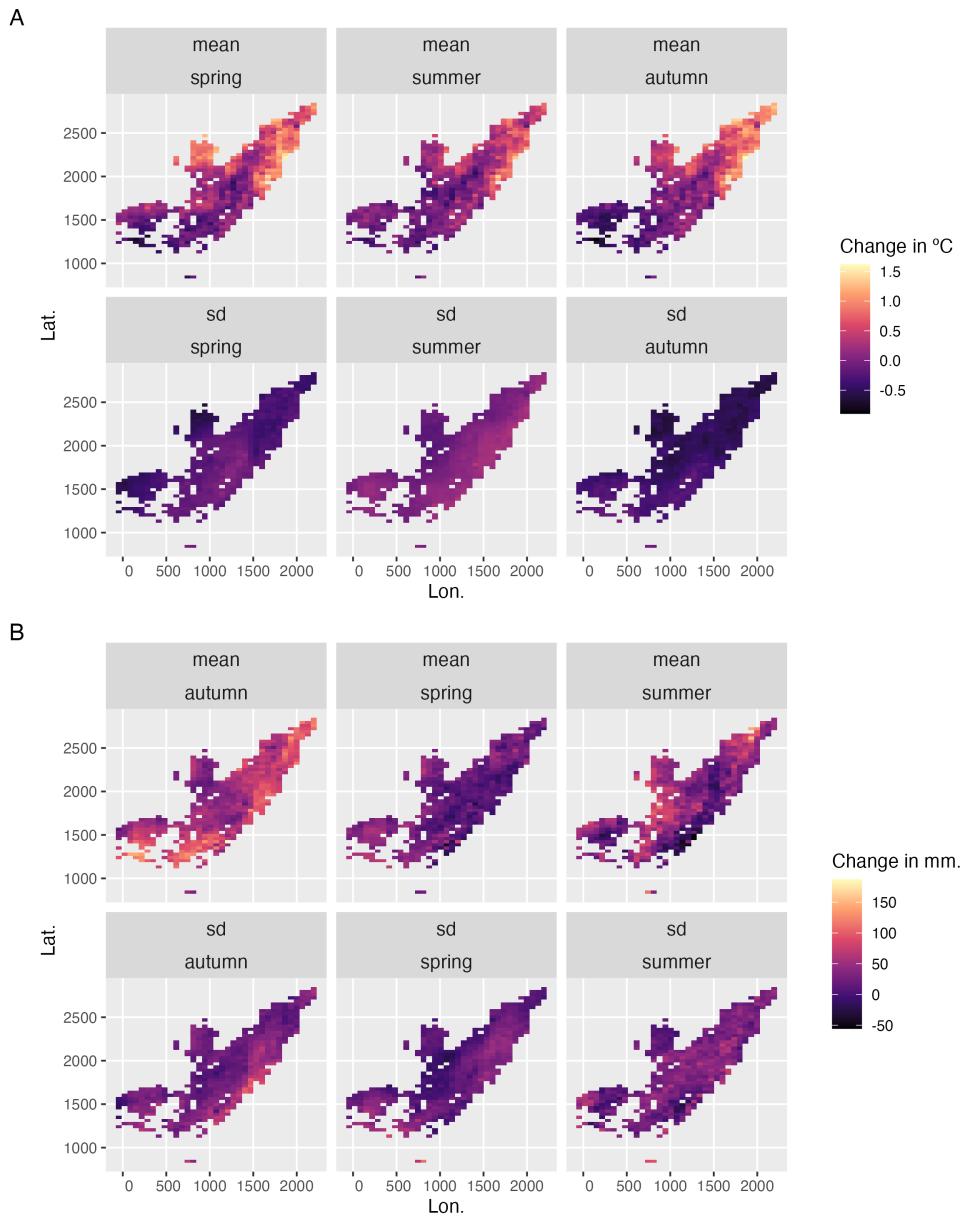


Figure A11: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).

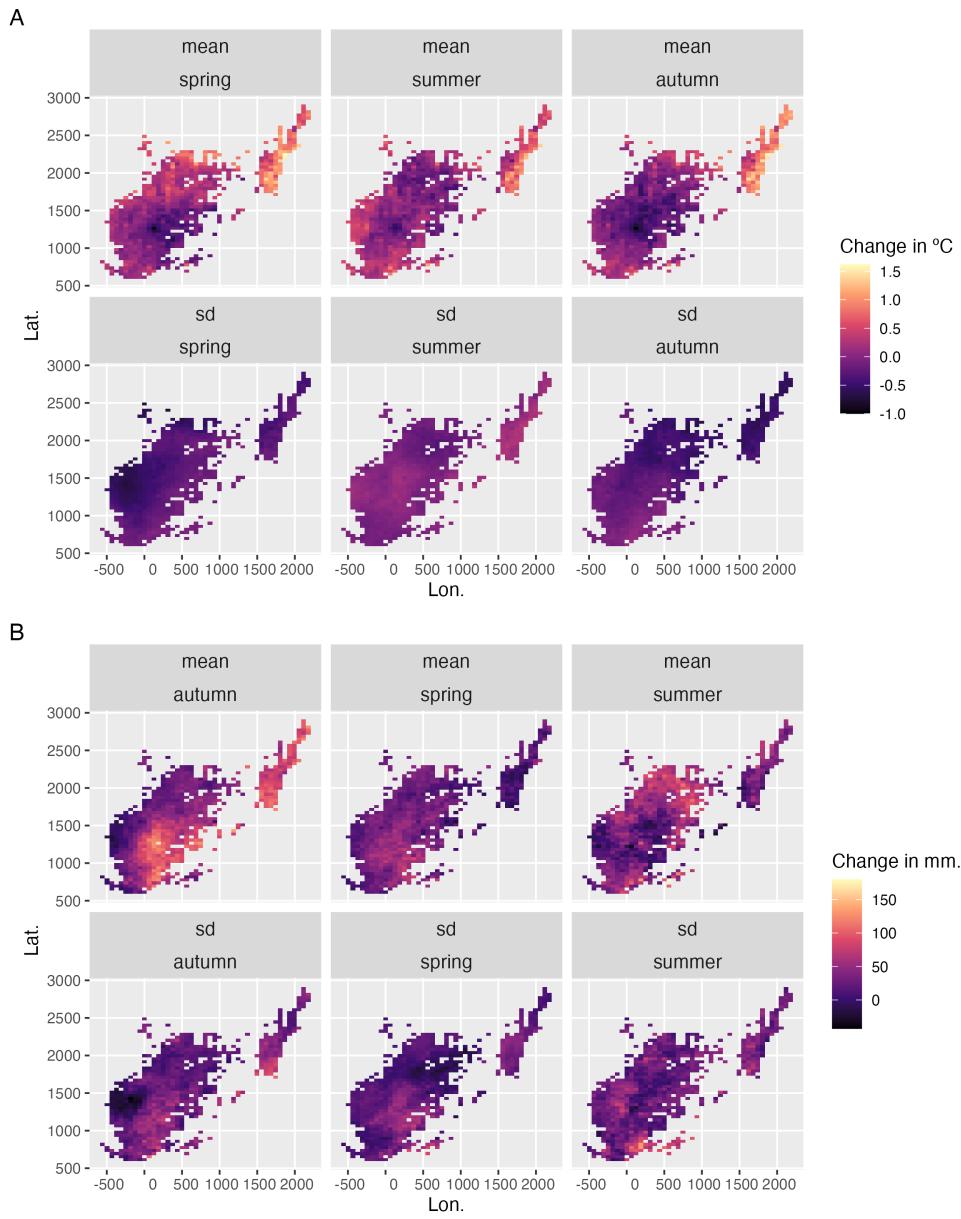


**Figure A12: Change in seasonal climate variables between the periods 1895-1925 and 1990-2020.** Color represents change in (A) seasonal temperature and (B) seasonal precipitation. Maps show pixels covering the modeled distribution of *A. hyemalis* used in post-hoc climate correlation analysis.



**Figure A13: Change in seasonal climate variables between the periods 1895-1925 and 1990-2020.**

Color represents change in (A) seasonal temperature and (B) seasonal precipitation. Maps show pixels covering the modeled distribution of *A. perennans* used in post-hoc climate correlation analysis.



**Figure A14: Change in seasonal climate variables between the periods 1895-1925 and 1990-2020.** Color represents change in (A) seasonal temperature and (B) seasonal precipitation. Maps show pixels covering the modeled distribution of *E. virginicus* used in post-hoc climate correlation analysis.

Table A1: Summary of herbarium samples across collections

Herbarium Collection	AGHY	AGPE	ELVI
Botanical Research Institute of Texas	350	190	198
Louisiana State University	72	38	62
Mercer Botanic Garden	3	–	6
Missouri Botanic Garden	210	205	122
Texas A&M	100	–	72
University of Kansas	134	34	197
University of Oklahoma	85	34	95
University of Texas & Lundell	183	91	102
Oklahoma State University	51	10	74

517

## Supporting Methods

518

### ODMAP Protocol

519 **Overview**

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

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

522 **Study area:** Eastern North America

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

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

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

526 **Boundary:** Natural.

527 **Data**

528 **Observation type:** Occurrence records from Global Biodiversity Information Facility and herbar-

529 ium collection across eastern North America. We used 713 occurrences records for *Agrostis hye-*

530 *malis*, 656 occurrence records for *Agrostis perennans* and 2338 for *Elymus virginicus*.

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

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

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

534 **Model**

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

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

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

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

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

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

543 **Assessment**

544 We used AUC to test model performance.

545 **Prediction**

546 We predicted the probability of presence of the host species as a binary maps (presence or ab-

547 sence)

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