

# Increasing prevalence of plant-fungal symbiosis over 195 years of climate change

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## Abstract

Many species' distributions and abundances have shifted 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. Alternatively, environmental change that causes declines in host or symbiont may disrupt the interaction. Microbes can be preserved within the dried plant tissue of herbarium specimens presenting an opportunity to quantify changes across broad temporal and spatial scales. We ask how the prevalence of a symbiont of grasses, *Epichloë* fungal endophytes, which can protect hosts from drought, has changed over time in response to climate change, and how these changes vary across the hosts' ranges. Specifically, we took seed samples from herbarium specimens of three grass host species collected over the last two centuries, quantified fungal hyphae presence within the seeds using microscopy, and evaluated spatial and temporal trends. Overall, endophytes have increased in prevalence over the last two centuries from 25% prevalence to 75% prevalence on average across the three hosts. We also found that the changes in prevalence were associated with observed changes in annual and seasonal climate drivers corresponding with each hosts' peak growing season. Thus we provide novel evidence for a cryptic biological response to climate change that may contribute to the resilience of the symbiosis.

## Introduction

Understanding how biotic interactions are altered by global climate change is a major goal of basic ecological research and conservation management [13, 33]. Documented responses to environmental change, such as shifts in species' distributions [4] and phenology [56], are typically blind to concurrent changes in associated biotic interactions. Empirically evaluating these biotic changes, whether interacting species shift in tandem with their partners or not [38], is crucial to predicting future climate change responses, but has been limited because, simply put, data on species interactions are limited, with few datasets extending over the long time scales of contemporary climate change [57].

Natural history collections, which were originally collected to study and preserve taxonomic diversity, present a unique opportunity to explore long-term changes in ecological interactions across broad spatial scales [51]. Natural history collections, built and maintained by the efforts of thousands of scientists, are invaluable time machines, primarily comprised of physical specimens of organisms along with information about the time and place of their collection. These specimens are samples preserving physical legacies of ecological processes and species' interactions from dynamically changing environments across time and space. Researchers have used plant collections to document climate change responses, including shifts in phenology [11, 53, 81], as well as in rates of pollination [29, 55], and herbivory [50]. However, focus has been lacking on long-term changes in a particularly common type of biotic interaction, microbial symbioses.

Microbial symbionts are common to all macroscopic organisms and can have important effects on their hosts' survival, growth and reproduction [49, 62]. Many microbial symbionts act as mutualists, engaging in reciprocally beneficial interactions with their hosts that ameliorate environmental stress. For example, bacterial symbionts of insects, such as *Wolbachia*, can improve their hosts' thermal tolerance [60, 74], and arbuscular mycorrhizal fungi, documented in 70-90% of families of land plants [54], allow their hosts to persist through drought conditions by improving water and nutrient uptake [17]. 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 [5]. Coral bleaching (the loss of symbiotic algae) due to temperature stress [71] 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 community composition of their algal symbionts [52]. How commonly and under what conditions microbial mutualisms deteriorate or strengthen under climate change remain unanswered questions, but 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 mutualists [58, 72, 77].

Understanding of how microbial symbioses are affected by climate change is additionally complicated by spatial heterogeneity in the direction and magnitude of environmental change [39]. 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 [78]. Additionally, symbionts are often unevenly distributed across their hosts' distribution. Facultative symbionts may be absent from portions of the host range [2], and hosts may engage with a diversity of partners (different species, or different locally-adapted strains of one species) across their environments [32, 63]. Identifying broader spatial trends in symbiont prevalence is therefore an important step in developing predictions of where to expect changes in the symbiosis in future climates.

*Epichloë* fungal endophytes are specialized symbionts of cool-season grasses, which have been documented in ~ 30% of cool-season grasses [44]. There are transmitted vertically from maternal plants to offspring through the grasses' seeds. Vertical transmission creates a feedback between the fitness of host and symbiont [28, 31, 64]. Over time, endophytes that act as mutualists for their hosts should rise in prevalence within a population. *Epichloë* are known to improve their hosts' drought tolerance [? ], and protect their hosts against herbivores [20] and pathogens [82] likely through the production of a diverse suite of alkaloids and other secondary metabo-

lites. The fitness feedback induced by vertical transmission leads to the prediction that endophyte prevalence should be high in populations where these fitness benefits are most important. Previous survey studies have documented large-scale spatial patterns in endophyte prevalence structured by environmental gradients [1, 9, 34, 69]. We predicted that prevalence should also track temporal changes in environmental drivers that elicit these fitness benefits. For example, endophyte-mediated drought tolerance should lead prevalence to increase in regions where precipitation declines over time. Early research on *Epichloë* used herbarium specimens to describe the broad taxonomic diversity of hosts [79]. Grasses are commonly identified and collected based on the presence of their reproductive structures meaning that collected specimens typically contain seeds, conveniently preserving the fungi along with their host plants on herbarium sheets. This creates the opportunity to leverage the unique spatio-temporal sampling of herbarium collections to examine the response of the symbiosis to historical climate change.

In this study, we assessed for the first time the long-term responses of endophyte symbiosis to climate change through the use of herbarium specimens of three *Epichloë* endophyte host species, *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*. We first address questions describing spatial and temporal trends in endophyte prevalence: (i) How does endophyte prevalence vary across space?, (ii) How has endophyte prevalence changed over time? and (iii) How have temporal changes in endophyte prevalence differed across space? We evaluated the predictive ability of this climate-implicit model using contemporary endophyte surveys as "out-of-sample test data, an important but rarely used strategy in ecological studies [73]. We then address how climate change may be driving these trends in endophyte prevalence by addressing the question: (iv) What is the relationship between variation in temporal trends in endophyte prevalence and changes in climate drivers? We examined 1,865 specimens collected across eastern North America between 1824 and 2019.

## Methods

### *Focal species*

Our surveys focused on three endophyte hosts, *Agrostis hyemalis*, *Agrostis perennans*, and *Elymus virginicus*. Both *Agrostis* species host *Epichloë amarillans* [19, 45], while *Elymus virginicus* typically hosts *Epichloë elymi* [18]. These C<sub>3</sub> grass species are commonly represented in natural history collections with broad distributions covering much the Eastern United States. *A. hyemalis* is a small short-lived perennial species that germinates in the spring and typically blooms between March and July (most common collection month: May). *A. perennans* is of similar stature but is longer lived than *Agrostis hyemalis* and blooms in late Summer and early Autumn (most common collection month: September). This species is relatively more sparsely distributed, tending to be found in shadier and more moist habitats while *A. hyemalis* is often found in open and recently disturbed ground. Both *Agrostis* species are recorded from throughout the Eastern US, but *A. perennans* has a slightly more northern distribution, whereas *A. hyemalis* is found rarely as far north as Canada and is listed as a rare plant in Minnesota. *E. virginicus* is a larger and relatively longer-lived species that is more broadly distributed than the *Agrostis* species. It begins flowering as early as March or April but continues throughout the Summer (most common collection month: July).

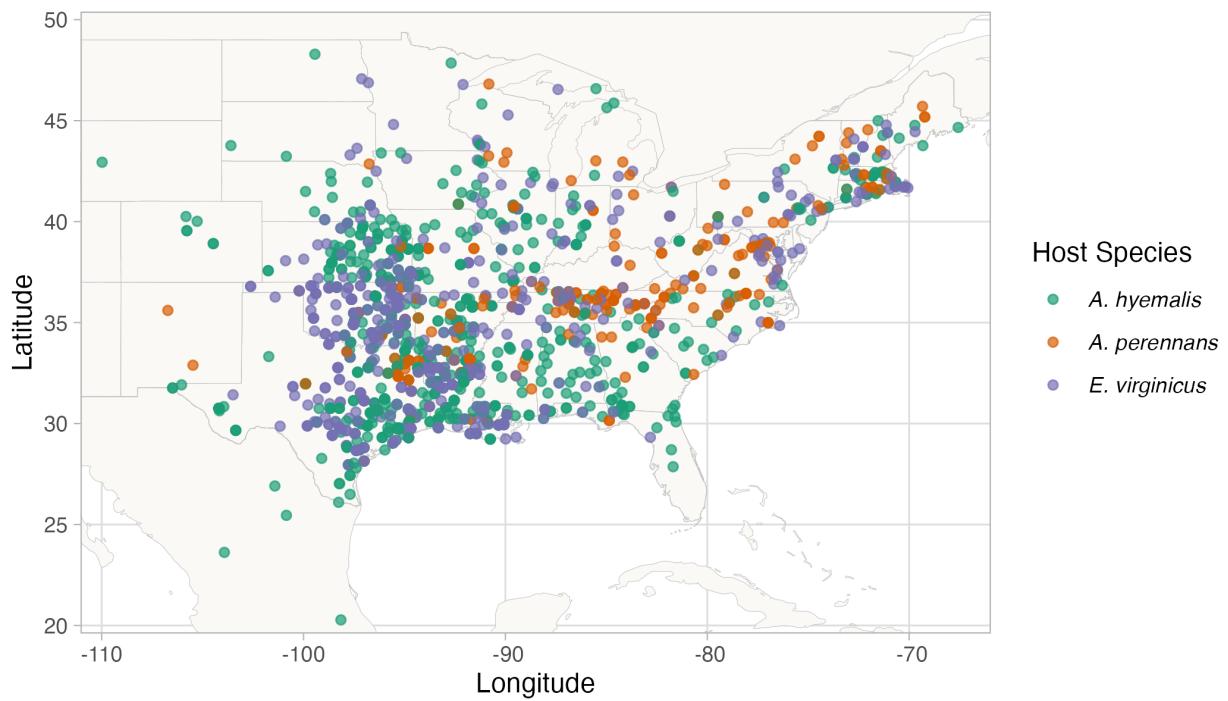
### *Herbarium surveys*

We visited herbaria between 2019 and 2022, including The Botanical Research Institute of Texas (which also houses the Vanderbilt University herbarium and the R. Dale Thomas Plant collection from the University of Louisiana at Monroe), the Louisiana State University Herbarium, the Mercer Botanic Garden Herbarium, Missouri Botanic Garden's Herbarium, the S.M. Tracy Herbarium at Texas A&M, the University of Kansas Herbarium, the Robert Bebb Herbarium at the University of Oklahoma, the Lundell Herbarium and the UT Herbarium at the University of Texas, and

Oklahoma State University Herbarium (See Table A1 for a summary of specimens includes from each collection). Our sampling plan was designed to minimize damage to these specimens. We chose our focal species in part because they are commonly represented in herbarium collections, and produce high numbers of 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 viable seeds fit for our purpose of identifying fungal endophytes.

After collecting seeds, we quantified the presence or absence of *Epichloë* fungal hyphae, which grow intercellularly, using microscopy. We first softened seeds with a 10% NaOH solution, then stained the seeds with aniline blue dye and squashed them under a microscope cover slip. We examined the squashed seeds for the presence of fungal hyphae at 100X magnification [6]. In some cases, the tissues examined during microscopy came from flowers or otherwise non-viable seeds, which were excluded from the seed counts for that specimen. On average we scored 4.7 seeds per specimen in *A. hyemalis*, 5 seeds per specimen in *A. perennans*, and 3.8 seeds per specimen in *E. virginicus*. Due to imperfect vertical transmission [3], it is possible that symbiotic host-plants produce a mixture of symbiotic and non-symbiotic seeds. We therefore designated each specimen as symbiotic if endophyte hyphae were observed in one or more seeds, or non-symbiotic if hyphae were observed in zero seeds. To capture uncertainty in the identification process, we recorded both a "liberal" and a "conservative" endophyte status for each plant. When we identified potential endophytes with unusual morphology, low uptake of stain, or a small amount of fungal hyphae across the scored seeds, we recorded a positive liberal status (more likely to be a true endophyte) and a negative conservative status (less likely to be a true endophyte). 89% of scored plants had matching liberal and conservative endophyte statuses, reflecting high confidence in endophyte identification. The following analyses presented in the main text used the liberal statuses, but we repeated all analyses with the conservative statuses which yielded qualitatively similar results (Figure A5). We excluded specimens for which information about the collection location and date were unavailable. In total, we quantified endophyte symbiosis for

964 *A. hyemalis* specimens collected between 1824 and 2019, 299 *A. perennans* specimens collected between 1863 and 2017, and 602 *E. virginicus* specimens collected between 1839 and 2017 (Fig. 1, Fig 2A). Each specimen was assigned geographic coordinates based on collection information recorded on the herbarium sheet using the geocoding functionality of the ggmap R package [40]. Many specimens had digitized collection information readily available, but for those that did not, we transcribed information from pictures of the specimens. Collections were geo-referenced to the nearest county centroid, or nearest municipality when that information was available. For a few of the oldest specimens, only information at the state level was available, and so we used the state centroid.



**Figure 1: Collection locations of herbarium specimens sampled for endophyte presence absence.** Color designates host species (*A. hyemalis* (green), *A. perennans* (orange), *E. virginicus* (purple))

## *Assessing spatial and temporal changes in endophyte prevalence*

To quantify spatial and temporal trends in endophyte prevalence, we used an approximate Bayesian method, Integrated Nested Laplace Approximation (INLA), to fit a spatio-temporal model of endophyte prevalence. Compared to MCMC Bayesian methods, INLA provides a computationally more efficient method of ascertaining model posteriors for certain models that can be formulated as latent Gaussian Models. [66]. Many common statistical models, including structured and unstructured mixed-effects models, can be represented as latent Gaussian Models. Fitting models with structured spatial effects is possible with MCMC sampling but can require long computation times, making INLA an effective alternative, which has been used to model spatial patterns in flowering phenology [80], habitat overlap between marine predators and prey [67], the distribution of temperate trees [30] as well as the population dynamics of endangered amphibians [42] and other ecological processes [10].

We modeled endophyte presence/absence ( $P$ ) as a Bernoulli response variable for specimen  $i$  of host species  $h$ .

$$P_{i,h} \sim \text{Bernoulli}(\hat{P}_{i,h}) \quad (1a)$$

$$\text{logit}(\hat{P}_h) = \beta_{0,h} + \beta_{1,h} * \text{year}_i + \quad (1b)$$

$$\beta_{2,h} * \text{year}_i * \text{lat}_i + \beta_{3,h} * \text{year}_i * \text{lon}_i + \beta_{4,h} * \text{year}_i * \text{lat}_i * \text{lon}_i + \quad (1c)$$

$$\chi + \omega + \phi \quad (1d)$$

The expected endophyte prevalence,  $\hat{P}$ , was modelled with intercepts specific to each host species ( $\beta_0$ ), slopes for changes over time ( $\beta_1$ ) as well as the interaction between time and the specimen's latitude and longitude ( $\beta_2$ ,  $\beta_3$ , and  $\beta_4$ ). To quantify spatial patterns in prevalence, we included a spatially-dependent random intercept,  $\phi$ . This random spatial effect was modeled as a stochastic partial differential equation (SPDE) that depended on a covariance matrix according to the proximity of each collection location [7, 48]. The covariance matrix is approximated using

a Matérn covariance function, with each data point assigned a location according to the nodes of a mesh of non-overlapping triangles across our study area (Fig A2). We accounted for potential biases introduced during the process of collecting specimens as well as in scoring ability by including random effects specific to each collector  $\chi$  and to each scorer  $\omega$ . We performed modeling fitting using the rINLA package, [47], with vague priors, and compared models with different sizes of mesh, which had little effect on the resulting model estimates. Posterior modes were stable indicating that numeric convergence was successful. We assessed model fit with graphical posterior predictive checks (Fig. A3). The model performed adequately at classifying the historical data (AUC = 0.77; Fig. A4).

We used data from contemporary surveys of on endophyte prevalence in *A. hyemalis* and *E. virginicus* as test data to evaluate predictions of the model. During these surveys, which took place between 2013 and 2020, we collected seeds from living plants from 63 populations(average number of plants sampled: 22.9) across a portion of each species' range (Fig A4). We quantified the endophyte status with staining microscopy as described for the herbarium surveys and calculated prevalence of endophytes within the population (proportion of symbiotic plants divided by the number of sampled plants). We compared the model's prediction for these locations to the observed population prevalence.

### *Assessing the role of climate drivers*

We assessed how the magnitude of climate change may have driven changes in endophyte prevalence by assessing correlations between changes in climate and changes in endophyte prevalence predicted from our spatial model at evenly spaced pixels across the study area. We first downloaded monthly temperature and precipitation rasters from the PRISM climate group [21] covering the time period between 1895 and 2020 using the 'prism' R package [36]. Prism provides reconstructions of historic climate variables across the United States by spatially-interpolating weather station data [26]. We calculated 30-year climate normals for annual and seasonal mean temperature and cumulative precipitation for the recent (1990 to 2020) and historic (1895 to 1925)

periods. We used three four-month seasons within the year (Spring: January, February, March, April; Summer: May, June, July, August; Autumn: September, October, November, December). This division of seasons allowed us to quantify differences in climate associated with the two "cool" seasons that shoulder summer when we expect our focal species to be most biologically active (*A. perennans*: Spring; *E. virginicus*: Spring and Summer; *A. perennans*: Fall). In addition to mean climate conditions, environmental variability in and of itself can influence population dynamics [75] and changes in variability are a key prediction of climate change models [39? ]. So we calculated the coefficient of variation during each period for each annual and seasonal climate driver as the interannual standard deviation divided by the mean across each 30-year period. We then took the difference between recent and historic periods for the mean and coefficient of variation for each climate driver (Fig. A5). Because initial analyses indicated a high degree of collinearity between seasonal and annual changes in temperature, we used annual temperature only, along with annual and seasonal precipitation, in the subsequent analysis. All together, this left us with measurements of change in 10 potential climate drivers: the mean and coefficient of variation of annual temperature, as well as the mean and coefficient of variation of cumulative annual precipitation, cumulative spring precipitation, cumulative summer precipitation, and cumulative autumn precipitation (Fig A8-A9).

We calculated the relative change in endophyte prevalence across the 1925 to 2020, i.e., the difference between predicted prevalence divided by the endophyte prevalence in 1925, because regions varied in their predicted starting prevalence. This time period connects the predicted change in prevalence with the endpoints of the available climate record. We then calculated the Pearson correlation coefficient between the relative change in endophyte prevalence and each climate driver.

## Results

### *Temporal and spatial trends*

We quantified temporal and spatial trends in endophyte prevalence while accounting for potential biases introduced by collectors and by individuals who quantified endophyte presence/absence with the use of random effects. We found no evidence that collector biases influenced our results (random effects values centered around 0; Fig 2A), while the identity of individual scorers did contribute to observed patterns in endophyte prevalence (3 of the 16 scorers were more likely than average to assign positive endophyte status, as indicated by 95% credible intervals that do not overlap 0) (Fig 2B). Interpretation of the data without accounting for this source of variation would lead to potentially biased interpretations of temporal and spatial trends.

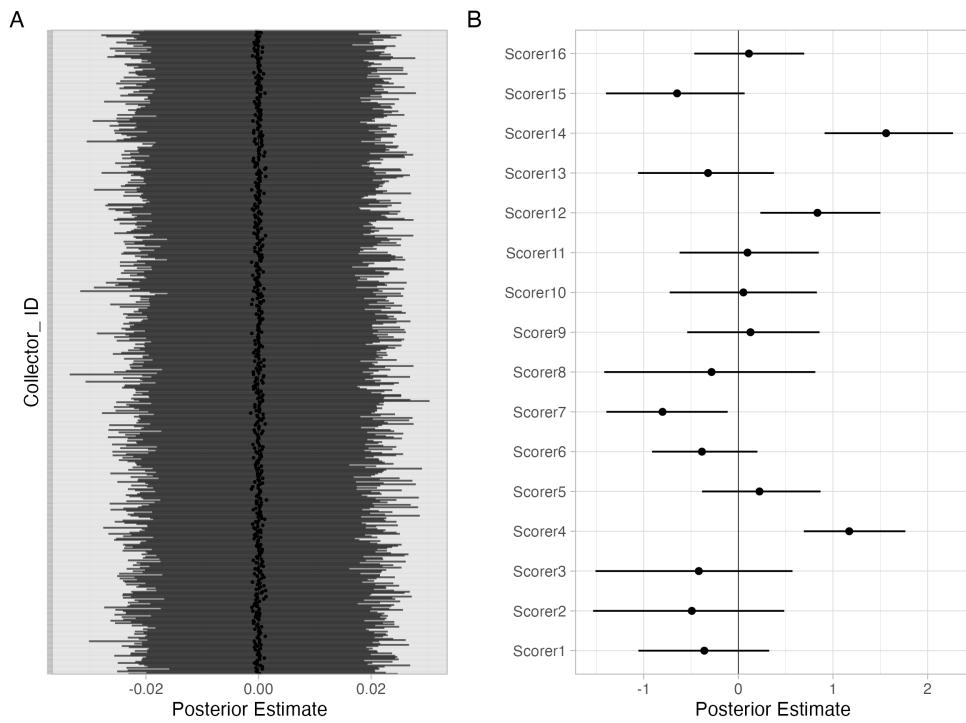
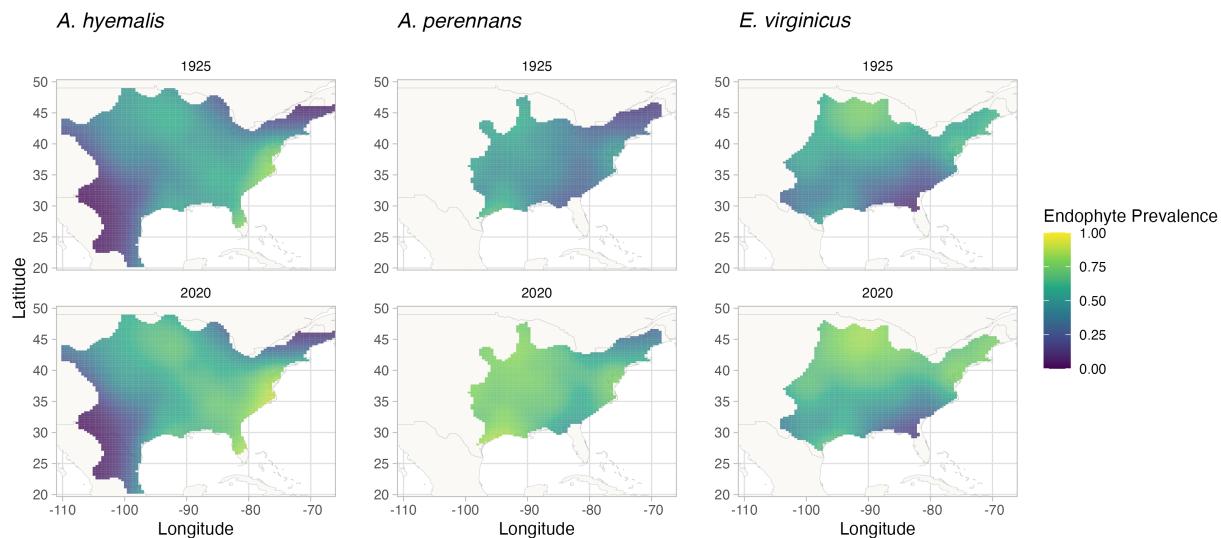


Figure 2: **Posterior estimates of (A) collector and (B) scorer random effects.** Points show the posterior mean along with 95% CI for random effects estimate from 532 collectors and 16 scorers.

## How does endophyte prevalence vary across space?

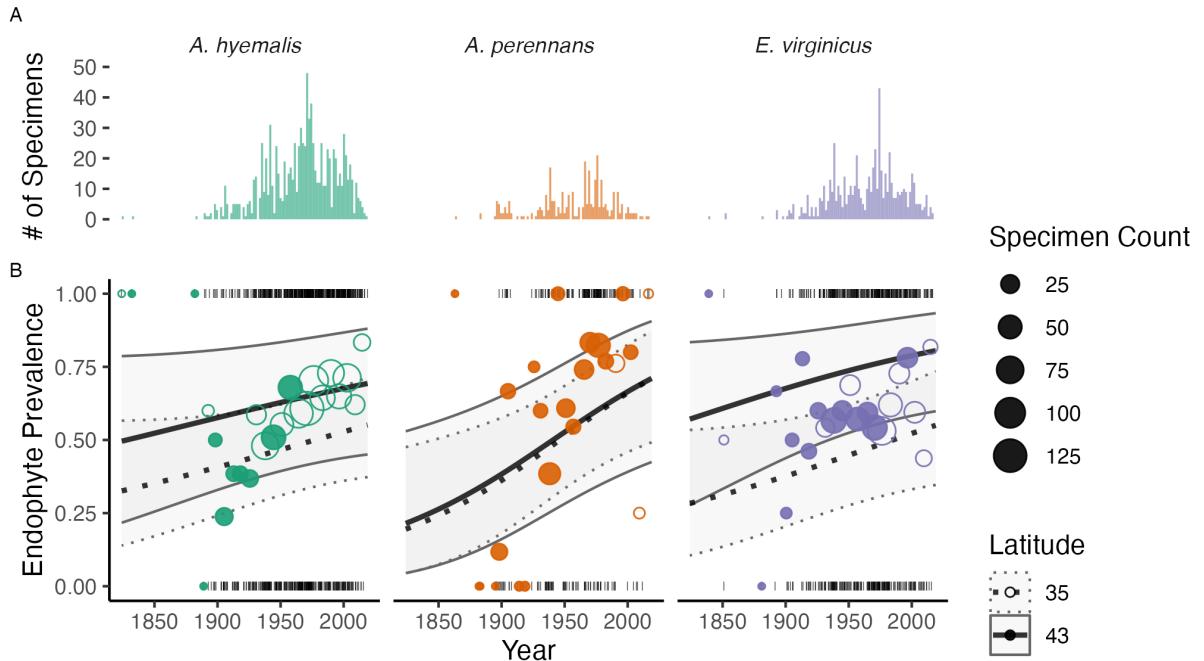
Across space, there were clear trends in endophyte prevalence which varied between species (Fig. 3). *Elymus virginicus* had higher prevalence towards the northern portions of its range. In contrast, *Agrostis hyemalis* had highest prevalence towards the center of its range with regions of low prevalence towards the northeast and towards its western range edge. *Agrostis perennans* had highest prevalence towards the western portion of its range edge. There is considerable uncertainty in the spatial pattern of endophyte prevalence (See Fig. A10 for projections of the 95% credible interval), however these broad spatial patterns are consistent across the low and high probability model predictions.



**Figure 3: Mean predicted endophyte prevalence for each host species (columns) in 1925 (top row) and 2020 (bottom row).** Color indicates mean predicted rate of endophyte prevalence across a convex boundary enscribing the collections locations of each species (excluding two disjoint collections of *A. perennans* to the west).

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

We found that endophyte prevalence increased within the examined specimens over the last two centuries for all three host species (Fig. 4). On average, *A. hyemalis* and *E. virginicus* both increased from 30 % to over 50% prevalence across the study region, and *A. perennans* increased from 15% to over 70% prevalence. Rerunning the analysis excluding specimens collected before 1900 when samples are much sparser (Fig. 2A), lead to qualitatively similar predictions, and so we continued analyses with the full dataset. Our model indicates a higher certainty that overall temporal trends are positive for *A. hyemalis* and *A. perennans* than for *E. virginicus* (99% probability of a positive overall year slope in *A. hyemalis*, 89% probability of a positive overall year slope in *A. perennans*, and 58% probability of a positive overall year slope in *E. virginicus*).

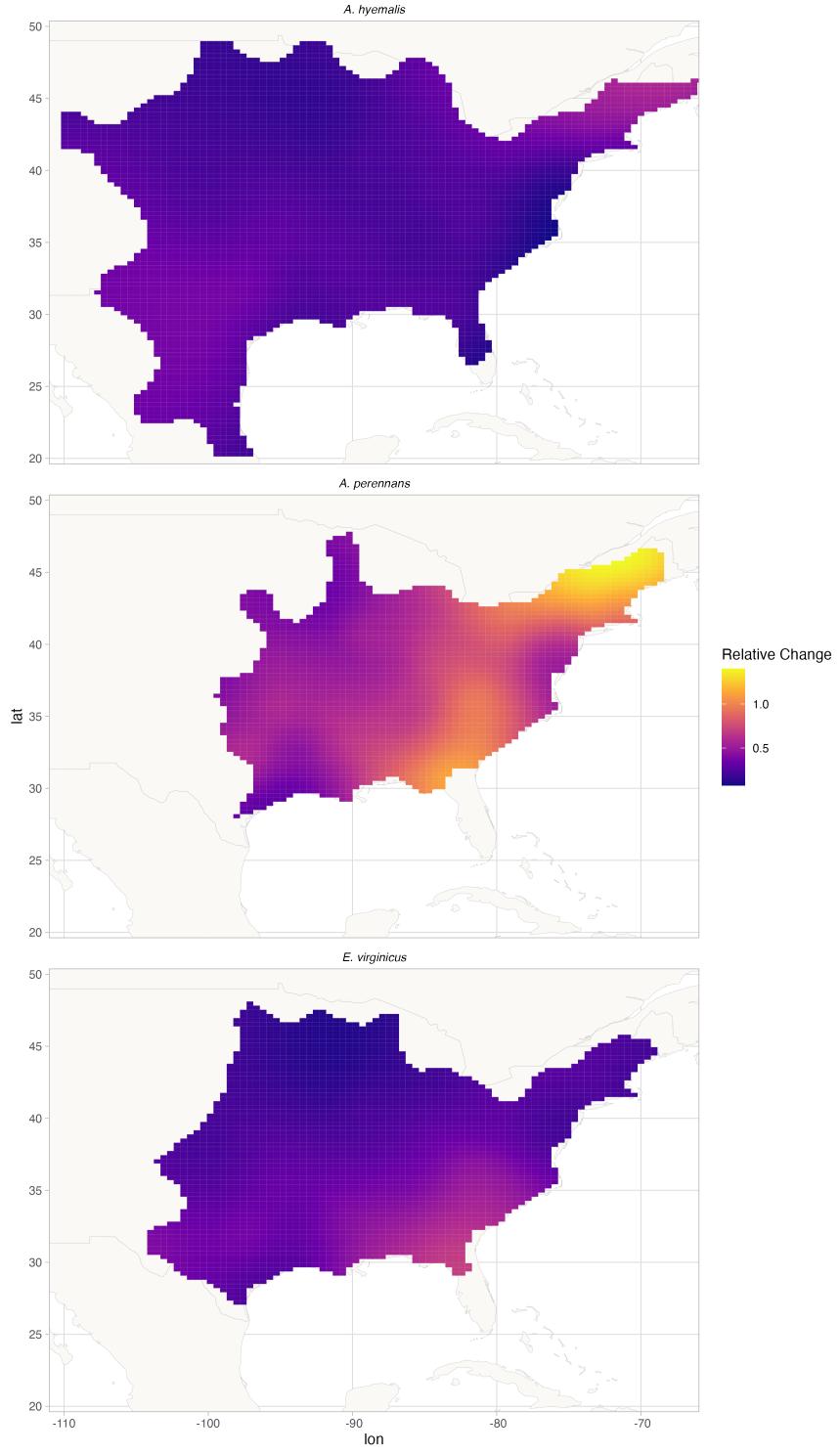


**Figure 4: Temporal trends in endophyte prevalence.** (A) Histograms show the frequency of collection through time for each host species. (B) Colored points are binned means of the observed endophyte presence/absence data (black dashes). Colors represent each host species and point size is determined by the number of specimens. Lines show predicted mean endophyte prevalence over the study period at low (dotted) high (solid) latitudes and along with 95% CI bands.

*How have temporal changes in endophyte prevalence differed across space?*

Our model revealed that while there was an overall increase in endophyte prevalence, these changes varied across the host species' ranges. Temporal trends vary with latitude and longitude, as evidenced by variation in the spatial pattern of endophyte prevalence through time in Fig. 3, and that temporal trends in Fig. 4 vary at different rates according to their latitude. In some regions, *A. perennans* experienced increases in percent prevalence by as much as 35 percentage points between 1925 and 2020, while *A. hyemalis* and *E. virginicus* experienced increases up to

around 15 percentage points. In other regions, there were smaller increases of only five percentage points for *E. virginicus* and fifteen percentage points for *A. perennans*. We calculated relative change in endophyte prevalence because regions differ in their initial endophyte prevalence.



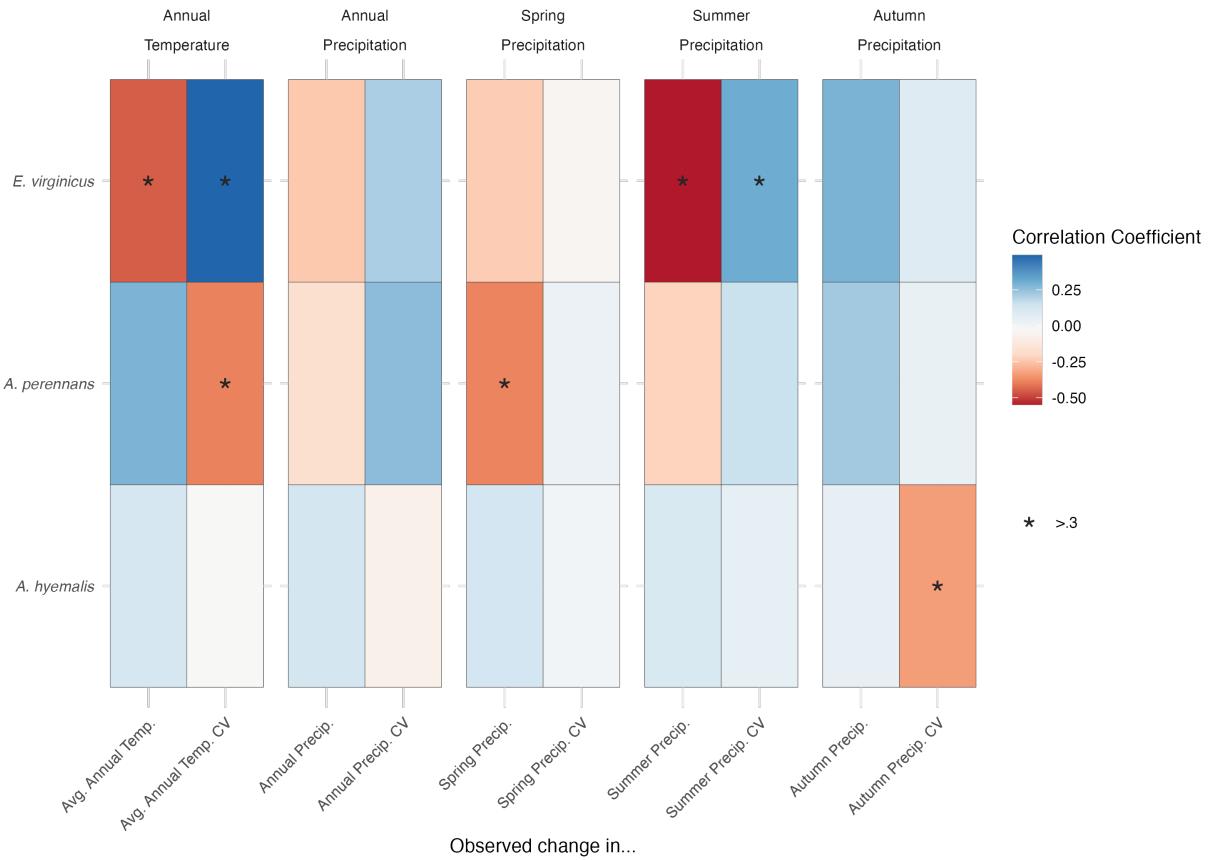
**Figure 5: Relative change in predicted prevalence for each host species.** Color indicates the relative change in predicted endophyte prevalence between 1925 and 2020 across the collection range.

This reveals locations that are hotspots of change in endophyte prevalence (Fig. 5). The overall magnitude of changes were greatest in *A. perennans*, particularly in the northeast and in the south, where endophytes were previously only found in low prevalence (Fig. 3). *A. hyemalis* and *E. virginicus* experience lower overall increases in prevalence, yet we can still identify hotspots of change in the northeast for *A. hyemalis* and in the southeast for *E. virginicus*.

### *The role of climate drivers*

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

We found that trends in endophyte prevalence were strongly associated with seasonal climate change drivers (Fig. 6). For the majority of the study region, the climate has become wetter and cooler over the last century (Fig. A5), a consequence of regional variation in global climate change [39]. Within the study region, spatially heterogeneous environmental changes were predictive of changes in endophyte prevalence. For example, strong increases in prevalence within *E. virginicus* were most associated (as indicated by correlation coefficients  $> .3$ ) with declines in Summer precipitation (a negative correlation in Fig. 4) as well as with increases in the year-to-year variability of Summer precipitation (a positive correlation in Fig. 4). Changes were also associated with reductions in average annual temperatures, and increases in year-to-year temperature variability. *A. perennans* endophyte prevalence increased most strongly in regions that experienced reduced spring precipitation and reduced variability in annual temperature. Although these correlations were weaker, changes in *A. perennans* endophyte prevalence were also associated with increased in increases in annual precipitation and increasing autumn precipitation. For *A. hyemalis*, endophyte prevalence increased most strongly in regions that experienced reductions in autumn precipitation variability.



**Figure 6: Correlations between changes in climate drivers and changes in endophyte prevalence.** Color denotes the Pearson correlation coefficient between the relative rate of change in endophyte prevalence and the change in annual mean temperature ( $^{\circ}\text{C}$ ) and total annual and seasonal precipitation (mm), as well as the change in the coefficient of variation of each climate driver. Positive correlation coefficients indicate that greater increases in a climate driver were associated with larger increases in endophyte prevalence, while negative values indicate that . Asteriks denote correlation coefficients  $.3$  or  $< -.3$ .

## Discussion

Our examination of historic plant specimens revealed a cryptic biotic reponse to climate change. For the three host species we examined, there have been clear increases in fungal endophyte

prevalence over the last two centuries. This increase suggests that *Epichloë* fungal endophytes are playing key adaptive<sup>1</sup> roles in response to climate change<sup>2</sup> and that the mutualism between grasses and their fungal endophytes has contributed to resilience for each partner species under the observed changes in climate. Spatial and temporal trends in endophyte prevalence varied between each species in ecologically meaningful ways<sup>3</sup>. Our spatially-explicit model predicted regions of both high and low endophyte prevalence, suggesting that symbiotic and non-symbiotic host plants have overlapping, but non-identical niche requirements.<sup>4</sup> Endophytes may expand the range of their hosts by allowing them to persist in environments where they otherwise could not [2, 41].<sup>5</sup> Understanding how microbial symbionts respond to climate change is a first step towards incorporating reciprocal host-symbiont interactions into predictions host range shifts.<sup>6</sup>

For *E. virginicus*, we found greater increases in prevalence in regions that have become drier during the Summer, the species' peak growing season.<sup>7</sup> Fitness benefits of the symbiosis under drought conditions could explain this increase and also explain high predicted prevalence towards the species' northern range edge coinciding with a strong latitudinal decline in precipitation<sup>8</sup>. Previous population surveys for endophytes in this species found similar latitudinal trends in prevalence [65, 69], but at smaller scales. Following previous work demonstrating drought benefits in a greenhouse manipulation with *A. hyemalis* [24], we had predicted that endophyte prevalence should similarly increase at a greater rate in regions that have seen reduced precipitation, yet we found the opposite trend in our herbarium surveys and that changes in prevalence for this species had the weakest correlations with climate drivers. Given that the

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<sup>1</sup>In what way are they adaptive?

<sup>2</sup>Climate change is not the only change over this time period, so be careful with attribution.

<sup>3</sup>vague

<sup>4</sup>This is a space-only result and interpretation, which should be better explained and motivated.

<sup>5</sup>Not sure this is right for the first paragraph.

<sup>6</sup>This paragraph should address the core question of whether climate change strengthens or disrupts mutualism.

<sup>7</sup>It is worth paying attention to both absolute and relative change. The AGPE results for example are notable because this is notable because this is the species with the greatest absolute change.

<sup>8</sup>Show this? There is also a longitudinal cline in precip - how do you interpret that difference?

study region has experienced relatively moderate<sup>9</sup> changes in precipitation and in temperature, potentially the magnitude of climate change has not been great enough to cause larger changes in endophyte prevalence for this species. Weak associations with drought could also be explained by climate-driven changes in the rate of imperfect transmission (the generation of non-symbiotic offspring from symbiotic hosts), which could counterbalance endophyte-mediated fitness benefits, and leading to stable intermediate prevalence rates [27]. To our knowledge, the response of the symbiosis in *A. perennans* to drought has not been examined experimentally, but in a separate greenhouse experiment, endophytes had a positive effect on reproduction under shaded, low-light conditions [25]. *Epichloë* endophytes have been connected to a suite of non-drought related fitness benefits including herbivore protection [15], salinity resistance [76], and mediation of the soil microbiome [61], potentially mediated by the diverse bioactive alkaloids and other signaling compounds they produce [68]. The strong increase in symbiotic *A. perennans* could be explained by these diverse benefits. Differences between the responses of each species underscore that while all of these C3 grasses share similar broad-scale distributions, each engages in unique biotic interactions and has unique niche requirements.<sup>10</sup>

Our analysis advances the use of herbarium specimens in global change biology in two ways. First and foremost, this is the first study to link long-term changes in microbial symbioses to changes in climate using specimens from natural history collections. The responses of microbial symbioses are a rich target for future studies within museum 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 [12, 14, 35, 37, 83]. Continued advances in capturing historic DNA and in filtering out potential contamination during specimen storage [8, 22, 59] will be imperative in the effort to scale up

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<sup>9</sup>Not sure what to make of “relatively moderate”.

<sup>10</sup>I think this is an interesting point worth elaborating on. On the one hand the three species are consistent in showing increases in prevalence, yet the mechanisms that explain those increases may be diverse and idiosyncratic.

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 microbial community. 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 [43]. **Second, we accounted 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.**<sup>11</sup> Spatial autocorrelation [80], potential biases introduced by the sampling habits of collectors [23], and variation between contemporary researchers during the collection of trait data, if not corrected for could lead to biased inference about the strength and direction of historic change. Fitting this model in a Bayesian framework allows for full propagation of uncertainty.<sup>12</sup>

Ultimately, a central goal of global change biology is to generate predictive insights into the future of natural systems. While this survey of historic endophyte prevalence is necessarily correlative, it serves as a foundation to develop better predictive models of the response of microbial symbioses to climate change. Combining the insights from this type of regional-scale survey with field experiments and physiological data could be invaluable. While we found that climate is strongly correlated with endophytes' temporal responses, we do not know why trends in prevalence were weak in some areas or how endophytes would respond to more extreme changes in climate. For example, transplanting symbiotic and non-symbiotic plants beyond the range edge of *A. hyemalis* could tell us whether persistent lack of endophytes in that area is a result of environmental conditions that lead the symbiosis to negative fitness consequences, or is a result of some historical contingency or dispersal limitation that has thus far limited the presence of symbiotic hosts from a region where they would otherwise flourish and provide resilience. While we observed evidence of mutualism resilience, more extreme environmental changes than those

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<sup>11</sup>I think you should say more about collector and observer effects in the results.

<sup>12</sup>Excellent paragraph.

observed in our study could potentially push one or both partners beyond their physiological limit, leading to the collapse of the mutualism. Our analysis thus far is agnostic to changes in the distributions of hosts. Mechanistic models could connect the responses of both host and symbionts to abiotic climate drivers integrating dispersal processes. Beyond host-microbe symbioses, building these types of models would work towards quantitatively attributing biotic responses to anthropogenically driven climate change, similar to methods in climate science and economics [16, 70].

## Acknowledgments

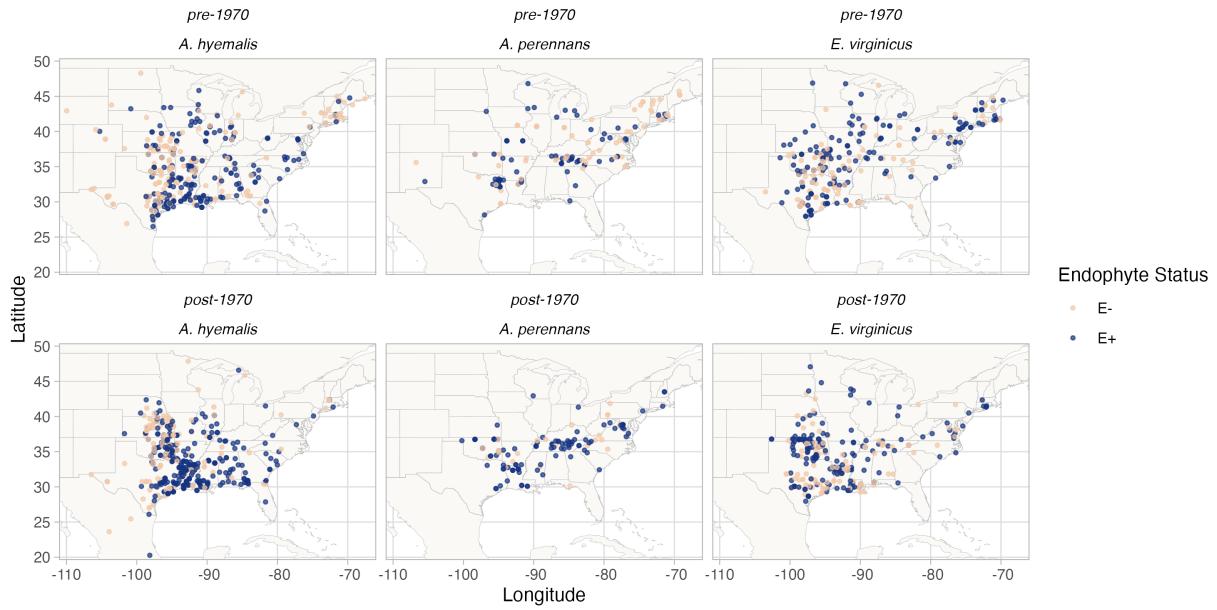
We thank Jessica Budke for help in drafting our initial destructive sampling plan, and to the many members of herbarium staff who facilitated our research visits, as well as to the hundreds of collectors who contributed to the natural history collections. This research was supported by funding from NSF grant () and from funding from the Texas Ecolab Program.

## Statement of Authorship

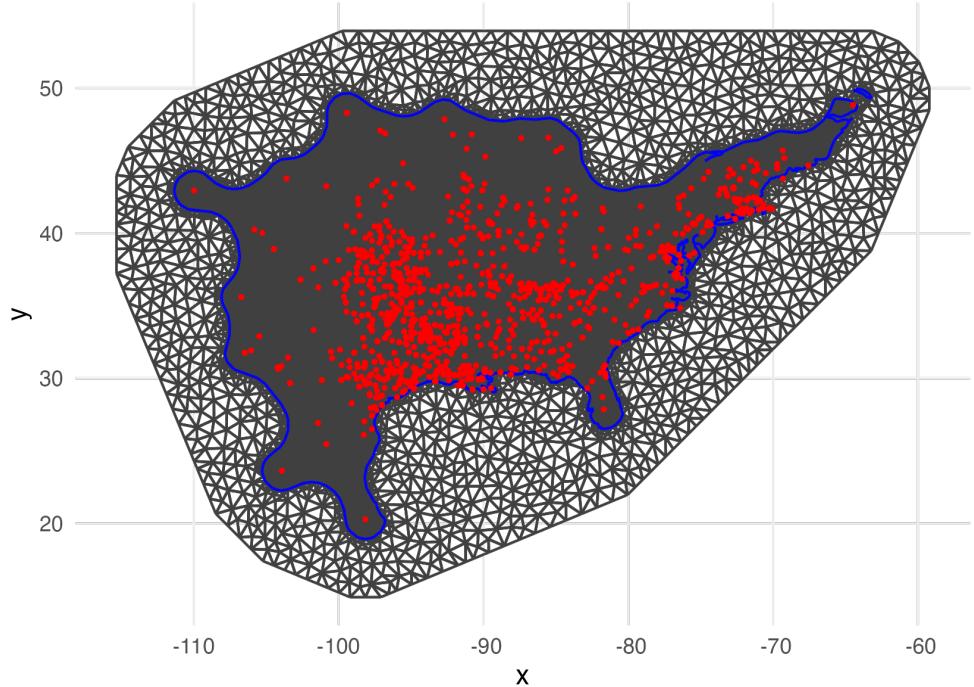
## Data and Code Availability

On initial submission, you may use this section to provide a URL for editors and reviewers that is 'private for peer review'. After acceptance, this section must be updated with correct, working DOIs for data deposits (typically on the Dryad Digital Repository, ? ) and code deposits (such as in Zenodo).

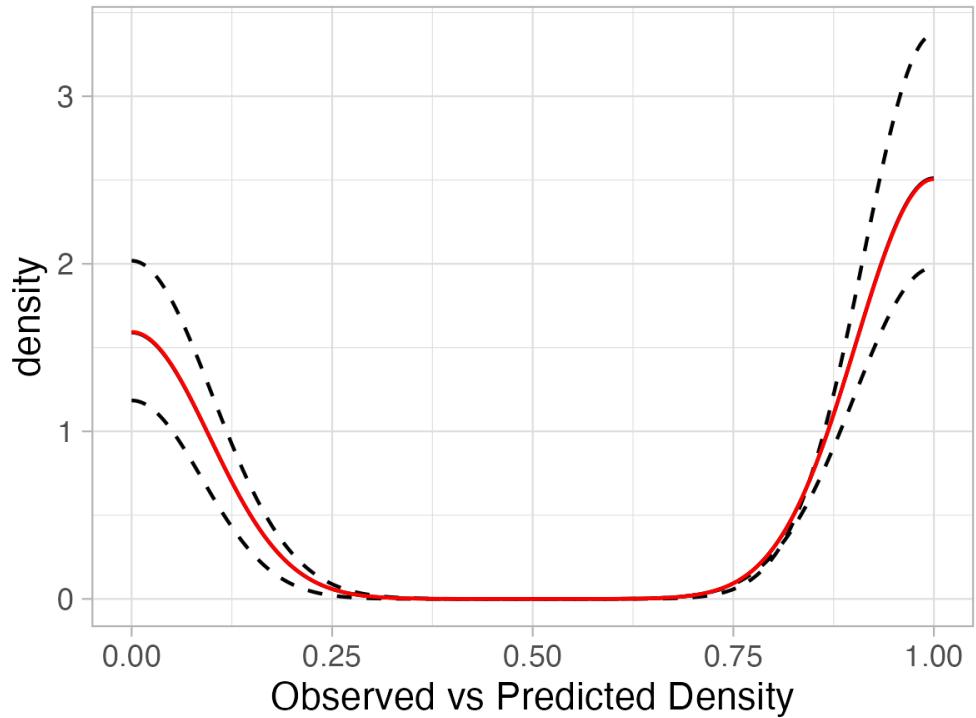
## Appendix A: Additional Methods and Parameters



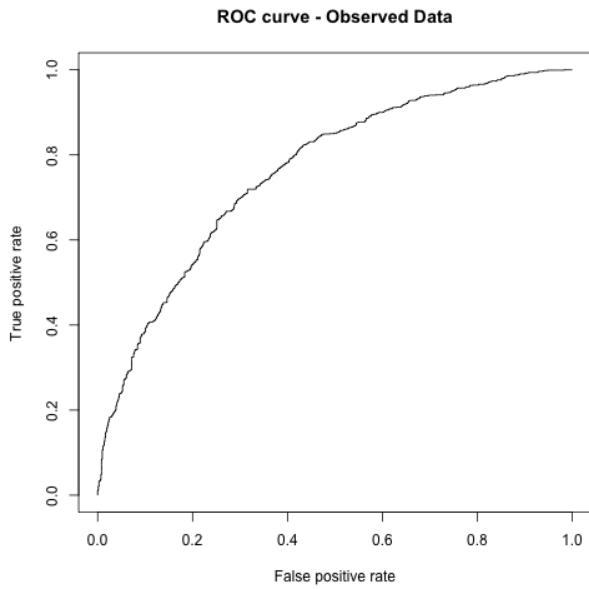
**Figure A1: Endophyte presence/absence in specimens of each host species.** Points show collection locations colored according to whether the specimen contained endophytes ( E+; blue points) or did not contain endophytes (E-, tan points) and are faceted based on collection period.



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



**Figure A3: Consistency between real data and simulated values indicate that the fitted model accurately describes the data.** Graph shows density curves for the observed data (red) along with the mean(solid) and 95% CI (dashed) of simulated values (black).



**Figure A4: ROC plot showing model performance classifying observations according to endophyte status.** The curves show adequate model performance for observed (top) and test (bottom) data. The AUC for each is 0.77.

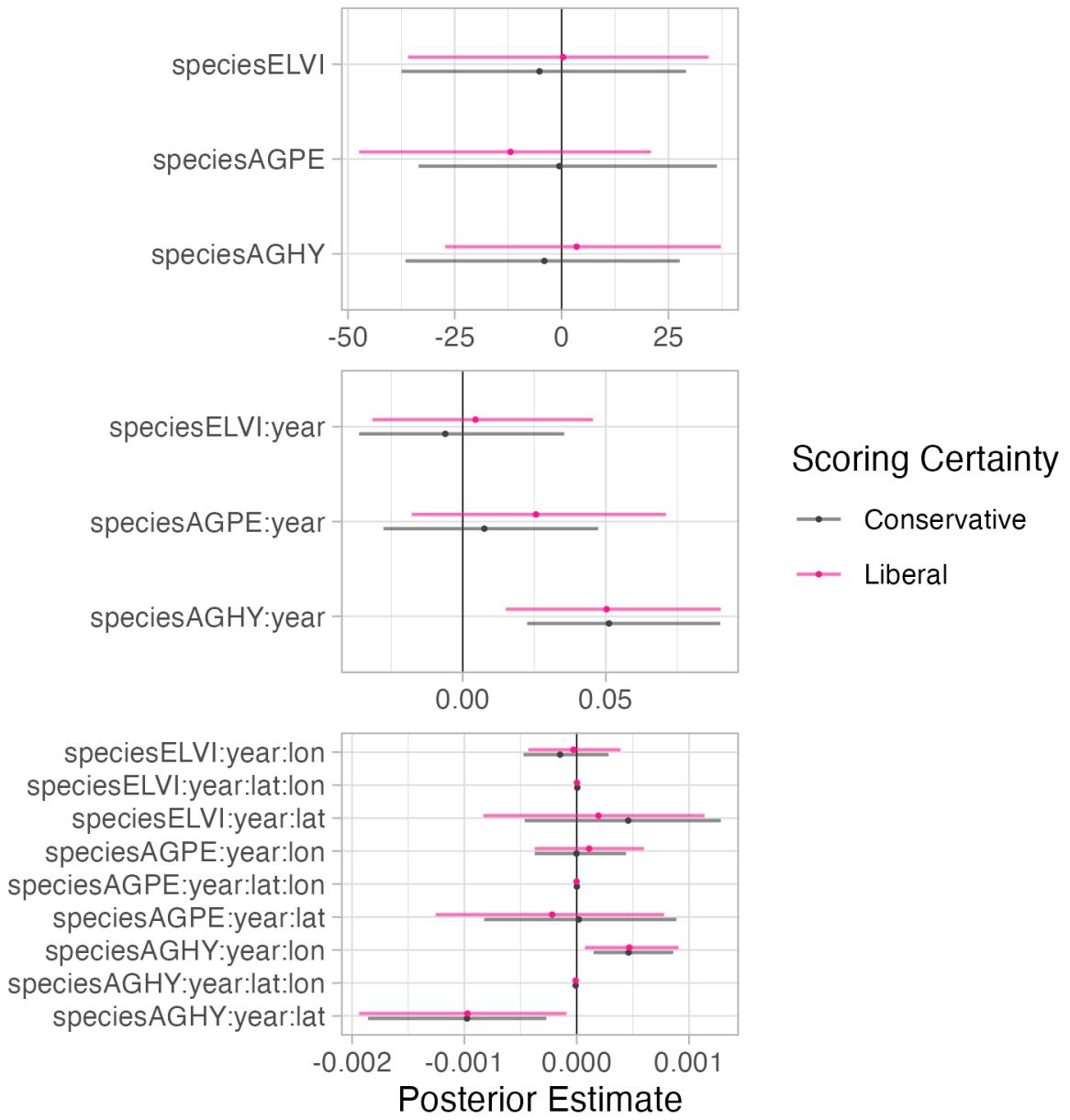


Figure A5: Comparison of posterior estimates of fixed effects when using Liberal or Conservative endophyte scores.

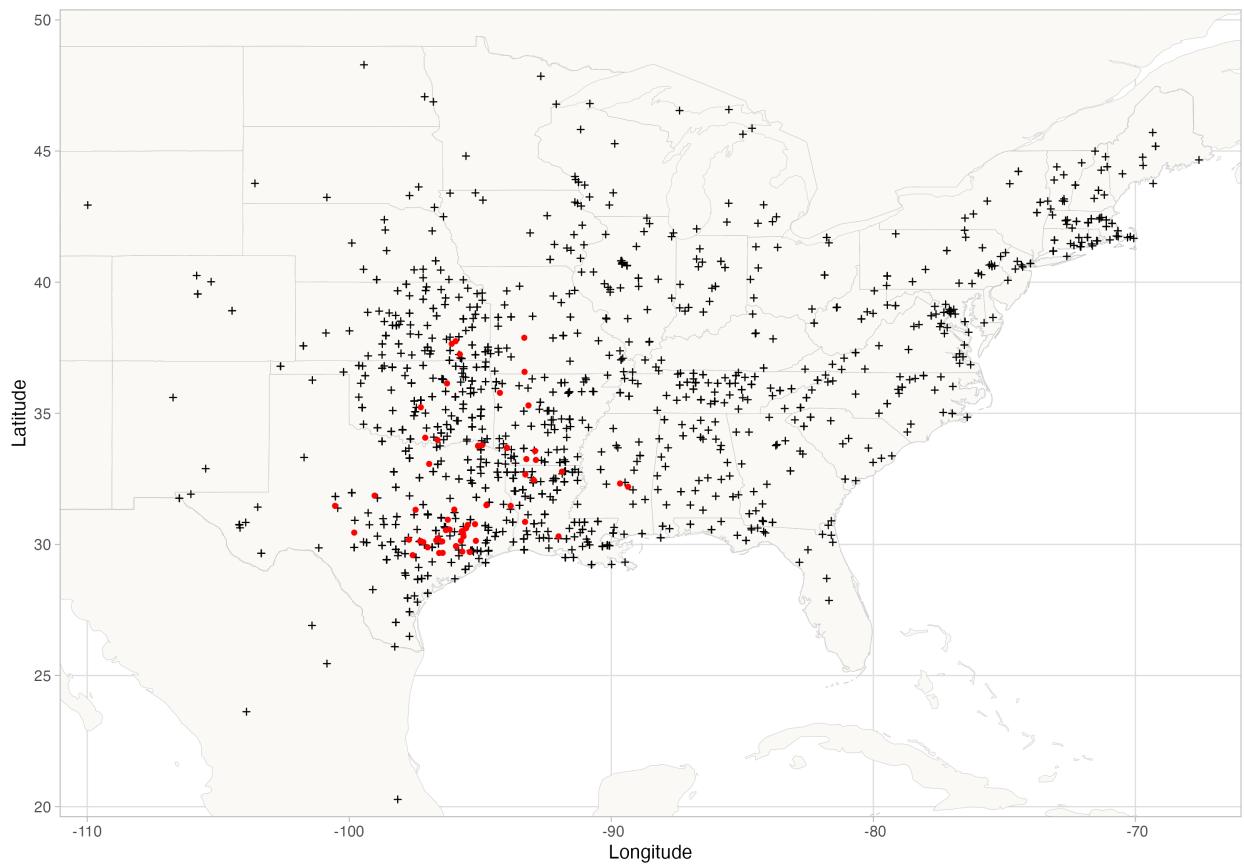
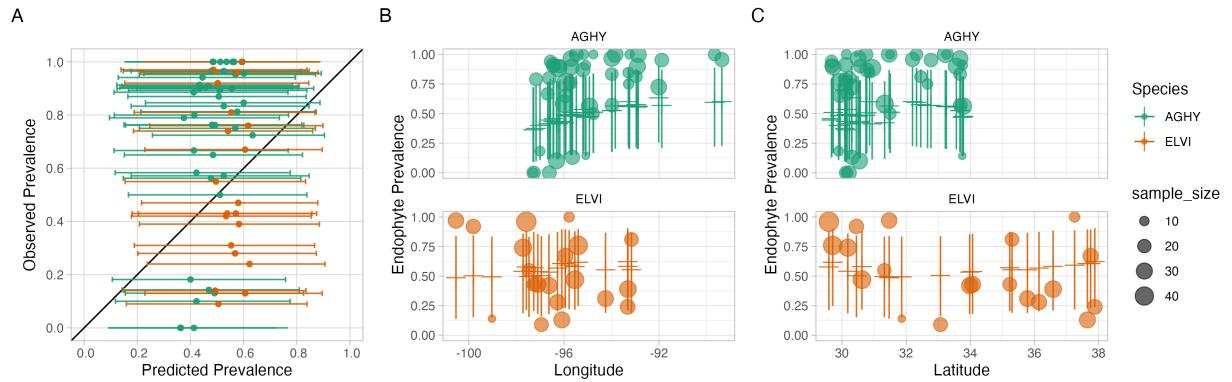
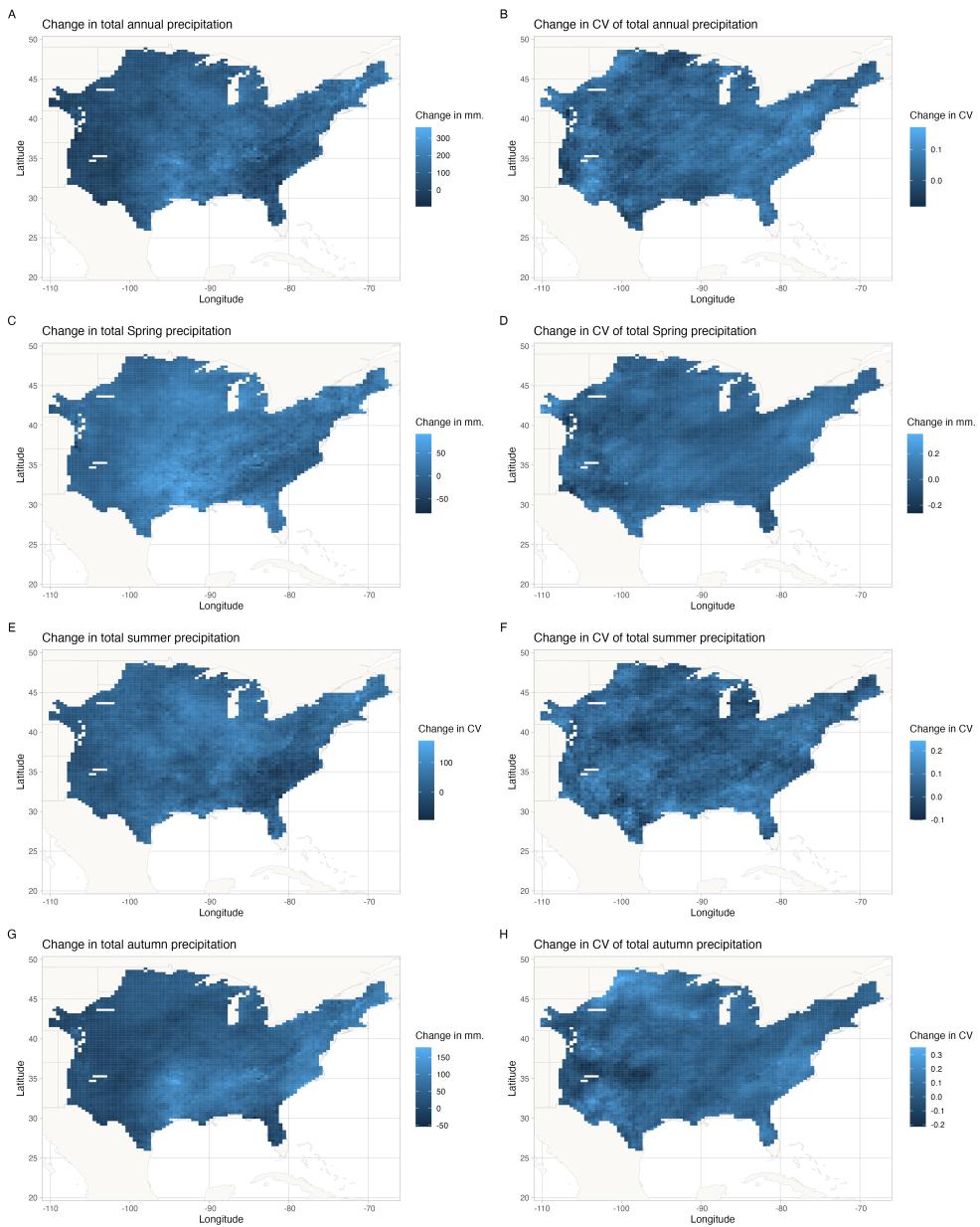


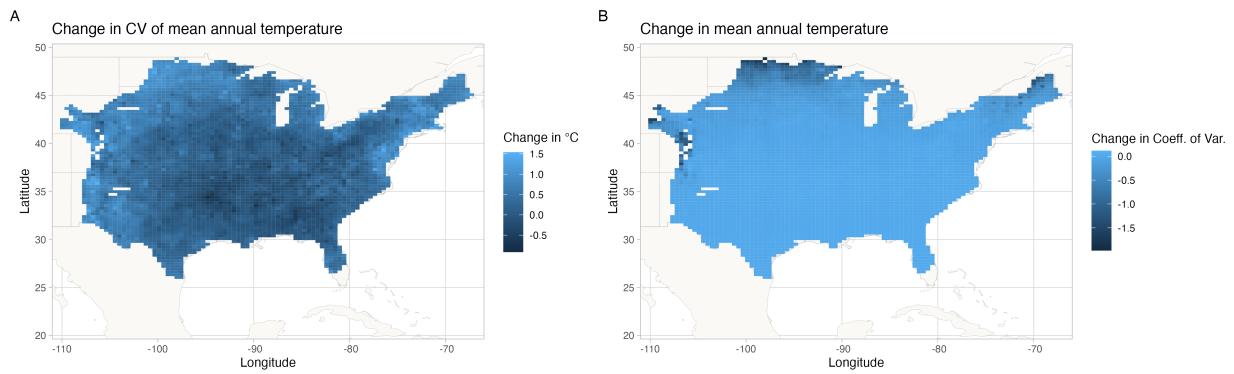
Figure A6: Locations of contemporary surveys of endophytes in *A. hyemalis* used as "test" data (red points), relative to the historical collection data (black crosses).



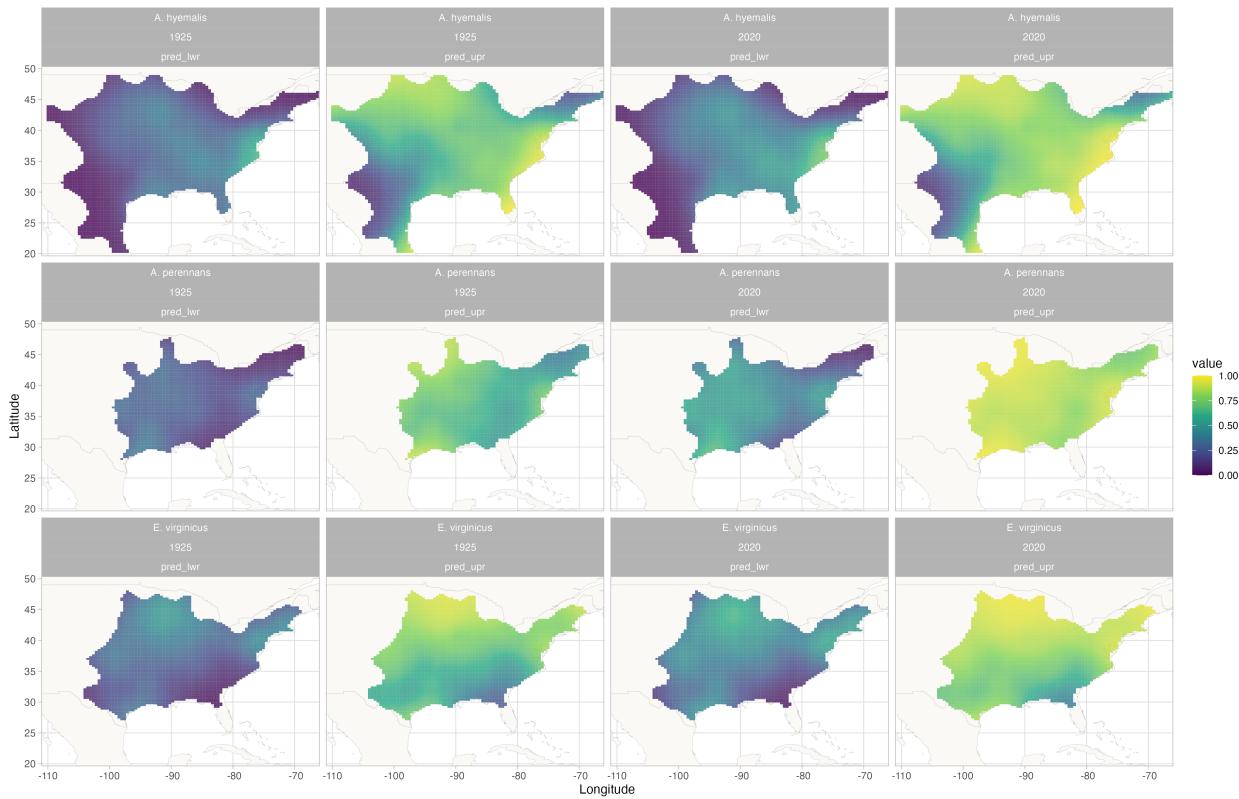
**Figure A7: Predicted vs observed endophyte prevalence for contemporary test data.** (A) The model, trained on historic herbarium collection data, performed modestly at predicting contemporary endophyte prevalence, as indicated by some overlap of predicted 95% CI with the 1:1 line, however contemporary test data generally had more variance between populations than model predictions. The model did recapitulate broader regional trends across (B) longitude and (C) latitude. Colors indicate host species, and point size in panels B and C reflect sample sizes of contemporary endophyte population surveys.



**Figure A8: Change in precipitation between the periods 1895-1925 and 1990-2020.** Color represents change in annual or seasonal total precipitation (A,C,E,G) and in the coefficient of variation of annual or seasonal total precipitation (B,D,F,H). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change were pulled from studies areas specific to each host species.



**Figure A9: Change in temperature between the periods 1895-1925 and 1990-2020.** Color represents change in annual mean temperature (A) and in the coefficient of variation of annual mean temperature (B). Maps show the study area of *A. hyemalis*. Map pixels used in correlation analysis with endophyte change were pulled from studies areas specific to each host species.



**Figure A10: Uncertainty associated with spatial trends in endophyte prevalence.** Color represents change in predicted endophyte prevalence. Panels show upper and lower 95% posterior probability for each host species between 1925 and 2020.

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Table A1: Summary of herbarium samples across collections

Herbarium Collection	AGHY	AGPE	ELVI
Botanical Research Institute of Texas	341	189	176
Louisiana State University	71	–	61
Mercer Botanic Garden	3	–	6
Missouri Botanic Garden	78	39	31
Texas A&M	73	–	49
University of Kansas	134	–	20
University of Oklahoma	65	30	91
University of Texas & Lundell	169	41	99
Oklahoma State University	30	–	69

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