

Manuscript GCB-25-0529 — Response to reviewers

August 3, 2025

To the editorial board,

Thank you for the opportunity to submit a second revision of our manuscript to *Global Change Biology*. The reviewers provided positive feedback on our previous submission while offering some further guidance to clarify our methods and improve our work's connections to the literature. In response to their comments, we have revised our manuscript to address key modeling assumptions and choices, and to highlight connections to the quickly growing field of research that uses herbarium specimens to study biological responses to global change. We detail these changes in the point-by-point responses below. All of our changes are denoted in the revised manuscript with **Mahogany font** and referenced below with line numbers.

With this revision, we hope you agree that our work is fit for publication in *Global Change Biology*.

Sincerely,

Joshua Fowler

Jacob Moutouama

Tom Miller

Response to Reviewer 3

Comment 1: *“The manuscript presents an intriguing analysis of long-term changes in *Epichloë* endophyte prevalence in grass species using herbarium specimens. The study’s findings, which indicate an increase in endophyte prevalence correlated with climate change, are potentially significant for understanding plant-microbe interactions in the context of environmental change. The use of herbarium specimens to assess these changes over an extended timescale is a novel and valuable approach. However, I have some concerns that require clarification.”*

Response: We thank Reviewer 3 for their constructive criticisms. In our point-by-point responses, we detail how we have incorporated them into our paper.

Comment 2: *“Statistics: (1) The approximate Bayesian spatially-varying coefficients model is a sophisticated approach. However, the manuscript would benefit from a more detailed explanation of the model’s assumptions, limitations, and sensitivity to prior specifications. ”*

Response: We thank Reviewer 3 for acknowledging the strength of our statistical approach. In response to this comment, we have edited our methods section to provide context on the Integrated Nested Laplace Approximation (INLA) approach and its assumptions, which have been enumerated by the teams who developed this method. In addition, we point out key areas where we expect this may influence our results.

Line(s): 266 - 272

Comment 3: *“(2) The justification for selecting specific prior distributions for the Matérn covariance function should be clarified. A sensitivity analysis exploring the impact of different prior choices on the results is presented in the Appendix, but a more thorough discussion of these results should be included in the main text. ”*

Response: We have added clarification about the assumptions of the Matérn covariance function, and about our choice of priors within the Methods section. In addition, we have added material to our Discussion that highlights the important inferences provided by the existing sensitivity analysis we present in our supplemental materials.

Line(s): 261 - 265

Line(s): 273 - 276

Line(s): 576 - 579

Comment 4: “ *Climate Data: (1) The analysis correlates changes in endophyte prevalence with changes in climate drivers. However, the choice of climate variables (mean and standard deviation of seasonal temperature and precipitation) needs to be better justified. For example, are these variables the most ecologically relevant for the host species and endophytes? (2) The spatial resolution of climate data may not align with the scale of ecological processes influencing endophyte prevalence. Consider discussing the potential implications of this mismatch.*”

Response: We appreciate these suggestions. We have edited our methods section to more clearly explain the rationale behind our analysis of climate drivers. Specifically, we expected there to be differences across seasons in the magnitude of climate change in temperature and precipitation because these seasons connect to the biology and natural history of our focal species. In addition, we have added to our discussion to acknowledge the potential importance of local scale ecological processes that may influence our analysis. We agree that the potential mismatch in scales is a limitation to herbarium studies in general, compounded not just by the scale of the climate data, but also by the spatial scale of georeferencing used to connect herbarium specimens to localities. We have added discussion material that acknowledges the implications of this scale mismatch.

Line(s): 313 - 321

Line(s): 564 - 571

Comment 5: “ *Out-of-Sample Validation: The out-of-sample predictive test with contemporary survey data is a strength of the study. However, the manuscript notes that the model exhibited relatively little local geographic variation, whereas the survey data were highly variable. This discrepancy suggests that local-scale factors not captured by the model may be important drivers of endophyte prevalence. A discussion of these potential factors (e.g., soil conditions, microclimate, local adaptation) and how they could be incorporated into future models would be valuable.*”

Response: We agree with Reviewer 3 that there is a wide array of other local-scale factors that would make valuable future directions, and the Discussion section of our previous submission touched on this already. We have expanded the existing material on this subject.

Line(s): 574 - 576

Comment 6: *“The contemporary survey period (2013-2020) is at the most recent edge of the time period encompassed by the historical specimens used for model fitting. It would be beneficial to acknowledge and discuss any potential limitations associated with using contemporary data for out-of-sample validation, particularly if climate conditions or other environmental factors have changed significantly during this period.”*

Response: This is a great point. In response to this comment, we have revised our Discussion section to include further discussion of the potential implications of the out-of-sample validation test, particularly highlighting that the data do differ in scale and potentially climate conditions, and how this might inform future modeling efforts. We discuss the main potential alternative validation test – leaving out test data from within the historic dataset. In addition, we now cite research that evaluates the importance of alternative choices of test-data time-period across datasets.

Line(s): 558 - 563

Comment 7: *“The manuscript interprets increases in *Epichloë* prevalence as adaptive changes that improve host fitness under increasing environmental stress. While this interpretation is reasonable, it is important to acknowledge that other factors (e.g., changes in land management practices, dispersal limitations) could also contribute to the observed trends.”*

Response: Thank you for urging us to further explore our interpretation of changes in endophyte prevalence. We agree with the reviewer that causal mechanisms underlying these changes in prevalence should be interpreted with caution. In fact, we have recently submitted an additional manuscript that explores the potential role of other anthropogenic global change drivers, including land use and nitrogen deposition. While our previous submission already touched on these ideas in the Discussion, in response to this comment, we have revised our Discussion to more clearly communicate the potential for other factors to contribute to these trends.

Line(s): 513 - 523

Comment 8: *“The manuscript mentions that *Epichloë* endophytes have been connected to a suite of non-drought related fitness benefits, including herbivory defense, salinity resistance, and mediation of pathogens and the soil microbiome. Consider discussing how these factors might interact with climate change to influence endophyte preva-*

lence. ”

Response: This is a great point that non-climatic factors may interact with changing climate, and is also connected to Reviewer 3’s Comment 7. And so we have edited our discussion material to more clearly communicate the potential for interactions between climate drivers and other factors that elicit beneficial effects of endophyte symbiosis.

Line(s): 516 - 516

Response to Reviewer 4

Comment 9: *“Fowler et al. examined herbarium specimens of three grass species spanning 200 years to investigate the persistence of Epichloë symbioses. This innovative and well-executed study highlights the underutilized value of herbarium collections and is well-suited for publication in Global Change Biology.”*

Response: We thank Reviewer 4 for their positive evaluation of our study, and we agree that herbarium collections are uniquely valuable yet underused in studies of biological responses to global change.

Comment 10: *“The authors have responded thoroughly to previous reviewer comments. My only additional suggestion is to expand the discussion of how herbaria are increasingly being used in contemporary research. While the manuscript includes some general remarks on their importance, it would benefit from a few more references and deeper integration of this theme—particularly in the introduction and conclusion. The authors could note that herbaria have traditionally supported taxonomic studies but are now being used in broader fields, including global change biology, biodiversity monitoring, and disease ecology.”*

Response: This is a great suggestion. In response to this comment, we have added references throughout our introduction that touch on these extended uses of herbarium specimens, and have also edited the Discussion and Conclusion sections to highlight the potential role that herbaria can play in global change research.

Line(s): 47 - 54

Line(s): 556 - 558

Line(s): 651 - 654

Comment 11: *“Recent research by Jean Ristaino (on Phytophthora), Charles Davis*

(on plant responses to climate change), and Michael Bradshaw (on the spread of fungal pathogens) provides relevant examples (I noticed a couple references from these authors already). Given that plant diseases represent a form of microbial symbiosis, studies like this one contribute to an already growing body of work demonstrating the potential of herbarium specimens to reveal long-term patterns in host-pathogen dynamics and ecosystem change.”

Response: Thank you for pointing us towards this body of work. We have added relevant citations throughout the introduction and discussion and also expanded the discussion to acknowledge the potential role that herbarium specimens can play in understanding plant-pathogen interactions as a form of symbiosis.

Line(s): 58 - 61

Line(s): 602 - 605