Microbial symbionts buffer hosts from the demographic costs of environmental stochasticity

Author List:

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General Comments

Dear Dr. Han,

Thank you for the opportunity to submit a revision of our manuscript for your consideration. Thanks also for the extra time we were given to execute the revisions requested by reviewers. We have made the following major changes in response to the feedback we received:

1. Several reviewers commented on the adequacy of model fit for certain species and vital rates. In response, we have re-analysed the data with more flexible models and added new figures of posterior predictive checks demonstrating goodness of fit.
2. Following suggestions from reviewers, we performed a new vital rate decomposition analysis revealing that contributions made by mean effects and variance buffering arise primarily from host survival and growth.
3. In accordance with the reviewers' suggestions, we have added a new conceptual figure illustrating our central hypotheses.
4. We have developed new material for an expanded Discussion section that addresses the evolutionary implications of host-symbiont interactions in variable environments.
5. We have reformatted our Manuscript to align with Ecology Letters formatting specification, most notably adding separate Results and Discussion sections.

We describe these major changes and more minor revisions in greater detail below, where we provide responses to the Reviewers' point-by-point comments . All of these changes can also be found in the "track changes" version of our re-submitted manuscript and supplemental material. We feel that the review process has greatly strengthened our manuscript. We hope you agree.

On behalf of myself and my coauthors,

Joshua Fowler

RESPONSE TO THE EDITOR:

COMMENT 1

"The paper has received four reviews that each provide substantive comments for improving the manuscript, which was generally very positively received. I hope the authors find these comments to be constructive for improving the manuscript. I'm looking forward to seeing a revised version.

> **Response:**
>
> **We appreciate the constructive feedback and thoughtful attention of the editors and reviewers of our manuscript. Incorporating their recommendations has strengthened the presentation and clarity of our ideas.**

RESPONSE TO REVIEWER 1

COMMENT 1

"It was a real pleasure to read this document. The analysis of the importance of the effects of variance on population dynamics and its relative role in relation to mean effects is critical and innovative. I am positive that this type of approach will influence future analysis in demographic studies. The document is extremely well written, the study design is robust, and the analysis carefully explained. While the level of involvedness of the modeling and statistical approaches is impressive, the details of the procedures are easy to follow and convincing. This is an extremely valuable document because provides a superb example of the value of long-term data from a well-designed work to address significant academic questions."

> **Response:**
>
> **We are glad for Reviewer 1's support for the importance of our work, and we appreciate the constructive feedback.**

COMMENT 2

"I have only two minor concerns. The first is a suggestion to temper their conclusions because the study still does not address the role of spatial variation and habitat heterogeneity, which may change these results in unpredictable ways. The study was done in relatively controlled (fenced, similar soils in a research station) and homogeneous environments in a very small portion of the distribution of the study species."

> **Response:**
>
> **We agree that spatial heterogeneity is an important consideration . We have added material to our new Discussion section (line 437) which addresses the importance that other dimensions of variation may play in symbiont-mediated variance buffering. The reviewer is correct that we average over spatial variation, and set up our experiment to minimize spatial heterogeneity, for the purpose of zeroing in on the temporal variance buffering mechanism. As we now acknowledge in the Discussion (line 518), we see many ways in which other sources of demographic variability could be incorporated in future investigations.**

COMMENT 3

"The second is an observation that the model of P. sylvestris of Spikes/Infl. in page 25 does not fit well the data in the left portion of the abscissa range. This is particularly concerning because while it has the larger sample size it appears the be the farther from its model distribution."

>   Response:
>
>   We thank this reviewer for their careful attention to our supplemental figures. Reviewer 2 makes a similar point in their comment 10 below, as does Reviewer 4 in their comment 13. These comments prompted us to re-evaluate more thoroughly the fit of our models to the data for each species. We agree that here and elsewhere in the vital rate figures, the visual correspondence of points and lines was a little dissatisfying for certain species and vital rates. We first discuss our evaluation of the fit of our models to the data, and also discuss features of the (lack of) correspondence that are due simply to data visualization within the vital rate figures (Figure S1-S10 in our original submission).
>
>   We evaluated model fit by generating graphical posterior predictive checks for each vital rate, specific to each species, demonstrating whether or not simulated data from our models adequately reproduce the mean, variance, skewness and kurtosis of the observed vital rate data across across the size distribution of host plants for each species. The previous version of this figure (Fig. S19 in our original submission) showed these moments for vital rate across the multi-species dataset.
>
>   Following this evaluation, we have re-run our analysis with two major changes. First, we now include parameters describing potential non-linear effects of size on host vital rates. Second, we now model differences between "original" plants (those that were transplanted into the plots) and "recruit" plants (those that recruited naturally) more flexibly, allowing for species- and size-specific differences between originals and recruits. These new vital rate models improved the fit for certain species, although the previous and new models had similar goodness-of-fit as judged by WAIC across the multi-species dataset. This new analysis improved the fit of our vital rate models to size structure within the data, and estimated effects of endophyte symbiosis on the mean and variance of vital rates changed very little. We re-ran our matrix analyses using these new models and have included these updated methods (Eqn. S1; Line 871) and results throughout the manuscript accordingly. In the end, the results are qualitatively similar between linear and non-linear models, and we appreciate that the reviewer's comments prompted us to more accurately capture nuance of size-structure in the data.
>
>   To provide support for the adequacy of our vital rate models as providing good parameter estimates for the MPM, we have added supplemental figures showing these graphical posterior predictive checks for each vital rate, specific to each species (new Figures S30 - S68). We think the new plots make a convincing case for goodness of fit. Additionally, we have added re-drawn vital rate figures to our supplement showing the size-dependent vital rate regressions splitting out the data and the model fit for data including either only recruit plants or plots showing only original plants (Fig. S2-S21).

Finally, as we now describe in the manuscript (Fig. 2 Caption Fig. S2-21 Caption, Line 950), there are two data visualization features of the vital rate plots that readers should keep in mind when comparing points and lines. First, the points are not the data to which the models were fit; instead they are means of the raw data, discretized and binned over arbitrary intervals of the size distribution. Changing the bin interval can make the plots look better or worse, even though nothing about the data or the model is actually changing. Second, the binned means are raw means, over many plants, years, and plots, while the lines show the expected value from a mixed model that includes year and plot as random effects. Years or plots that strongly influence the raw means are "shrunk" toward their expected value by the mixed model, which can contribute to the apparent mismatch.

Line 950 <*We visualized the interactions between plant size, origin status, and endophyte status for both the interannual mean expected value for each vital rate (averaging over year and plot variance) (Fig. S2 - S11) and for the expected vital rate values specific to each year (averaging over plot variance) (Fig. S12 -S21). We graphically checked vital rate model fit with posterior predictive checks comparing simulated and observed data (Fig. S30-S68). Initial analyses including only linear effects of size produced estimates of endophytes' effects on vital rate means and inter-annual variances that were similar to those from the more flexible quadratic models, but provided worse fit to size-structure in the data in some cases. We therefore proceeded with the more flexible quadratic models. Results from subsequent matrix model analyses were qualitatively similar regardless of this choice.*>

RESPONSE TO REVIEWER 2

COMMENT 1

"The authors use long-term demographic data on seven grass species to investigate the role of microbial symbionts on host performance. As endophytes were removed in half of the plants, the authors could test for the effects of endophytes on various vital rates, by fitting generalized linear mixed models. The authors specifically tested the hypothesis that the symbionts buffer against inter-annual variability in demographic rates, benefiting long-term host fitness. To do so, they use the vital rate models to construct stochastic matrix population models, and decompose the endophyte effect into contributions from effects on the means, variances and their interaction. In general, I enjoyed reading this manuscript."

> **Response:**
>
> **We are grateful for Reviewer 2's detailed attention to our whole manuscript and appreciate their thoughtful comments which helped us develop the broader context for our results.**

COMMENT 2

"The idea that a symbiont may buffer against fitness variance across years is interesting, and the authors use a great dataset to test this. When reading the manuscript, I kept on pondering about how this idea relates to the idea that microbes may sometimes also benefit hosts by increasing phenotypic variance, for example enabling hosts to faster explore the fitness landscape (Henry et al., Nat. Comm., 2021) or conferring phenotypic plasticity (Lange et al., ISME, 2023). In this study, does the symbiont affect the within-year variance?

> **Response:**
>
> **We appreciate the reviewer raising these interesting connections to our research from an evolutionary perspective. We have not examined yet the question of whether symbionts affect within-year phenotypic variance or plasticity in traits. However we are currently working on a separate manuscript investigating the effects of endophytes on host genetic diversity, based on SNP data from our long-term plots. Symbiont-mediated increases in host genetic diversity could also increase phenotypic variance through greater genotypic variance. As we detail further in response to the next comment, we have added material to our discussion (Line 513) detailing the broad evolutionary implications of our results. Because we have another manuscript focused on genetic variation in the works, we do not delve deeply here into the implications of endophyte symbiosis for host genetic or phenotypic diversity.**

> **Line 513 <*A further step could incorporate diverse symbiont partners (e.g. different strains of Epichloë fungi or multiple species within the microbiome) to understand how microbial diversity contributes to host genotypic and phenotypic variance (Henry et al. 2021).*>**

COMMENT 3

Also, associating with variable microbes may help hosts to express a diversified bet hedging strategy (Bruijning et al., Nat. Ecol. Evol., 2021), maximizing the chances that some hosts can cope with any environment they may encounter, also reducing fitness variance across generations. Further, demographic buffering may be seen as a form of conservative bet hedging, if the decrease in variance comes at the cost of arithmetic mean fitness (Childs et al., Proc. Roy. Soc. B., 2010). This doesn't seem to be the case in this study, as the presence of the symbiont not only decreases the variance, but at the same time increases the expected fitness for the majority of species. I am missing some discussion on such points, that put the results of this study in a broader context and that include other recent ideas on how symbionts may benefit hosts under changing environments. In general, I find that there is little discussion of the results in a broader (evolutionary) context. I hope that the authors can add such as discussion, reflecting for instance on the above points."

> **Response:**
>
> **We hope that our work spurs readers to think about the broader implications of our results in a similar way to Reviewer 2. We have expanded our discussion section and interpret our results through the lens of bet-hedging theory (line 500), as this reviewer suggests. We have incorporated the recommended literature and related papers demonstrating how environmental stochasticity can favor imperfect symbiont transmission as a bet-hedging strategy.**

Line 500<*Demographic buffering may be considered a bet-hedging strategy if reduced temporal variance comes at the cost of arithmetic mean fitness (Childs et al 2010). This may be unlikely in this system, where most host species exhibited both reduced variance and elevated mean fitness through symbiosis (Fig. 3C). However, the context-dependent fitness effects that underlie demographic buffering may favor other forms of evolutionary bet-hedging. Theory suggests that imperfect transmission (the production of S- offspring from an S+ maternal individual) may be an adaptive host strategy in spatially or temporally varying environments when the fitness effects of symbionts are environment-dependent, because it extends phenotypic variance of offspring and improves the odds of some having the symbiont status that is optimal for their environment (Brown et al. 2019, Bruijning et al 2023, Lange et al. 2023). Imperfect vertical transmission is well-documented in grass-endophyte symbioses (Afkhami and Rudgers 2008), including our focal taxa (Table S2), and could be incorporated into our modeling framework by dynamically linking S+ and S- populations (Yule et al. 2013, Chung et al. 2015).***>

COMMENT 4

" Further, the authors mention that they test for a context-dependency in the benefits of the symbionts. I am not convinced that this is what is actually tested (but maybe I'm missing something). It seems that the endophyte generally has a positive effect on host performance across years, so I don't understand how these results indicate a context-dependence, where the symbiont is beneficial in some environments, but harmful in others? Even in the absence of a context-dependence, the symbiont could still benefit hosts both by effects through the mean and variance, correct? Could the authors elaborate a bit more on this in the text? Related to this, it would be helpful to illustrate their hypothesis (L134) in a conceptual figure early on in the manuscript, and perhaps even adding a schematic overview of the study design and approach."

> **Response:**
>
> **We appreciate the opportunity to clarify connections between context-dependence and variance buffering. As suggested, we have added a new conceptual figure that illustrates these connections (new Fig. 1). Consistent with prior work, we define context-dependence to be not only variation in interaction sign (i.e. switching from mutualism to parasitism) but also variation in magnitude (more or less beneficial) depending on conditions (following Chamberlain et al. 2014 and Catford et al. 2021). As we now clarify in the manuscript (line 136), we view context-dependence as the key ingredient that gives rise to the pattern of variance buffering, such that an S+ fitness advantage that strengthens in harsh years dampens inter-annual fluctuations relative to S- hosts (new Figure 1). This figure also allows us to visually communicate competing hypotheses regarding symbiont effects on mean and variance. We are glad to have been nudged in this direction.**

Line 136 <*We hypothesized that context-dependent benefits from symbionts may buffer host populations against variability through strong benefits during harsh periods and neutral or even costly outcomes during benign periods, reducing the impacts of host exposure to extremes and dampening inter-annual variance relative to non-symbiotic hosts (Fig. 1A). Variance buffering is a previously unexplored mechanism by which symbionts may benefit their hosts instead of or in addition to elevating average fitness (Fig. 1C), the focus of most previous research.*>

COMMENT 5

"I have no major comments on the methodology and the presentation (I really like the figures!). My main suggestion here is to improve clarity, as I found it not always easy to follow the text, in particular the link between the methods and results sections. If I understand it correctly, sigma_{tau_{e,h}} is one of the core parameters, quantifying the inter-annual variance, estimated per endophyte treatment, per host species and per vital rate. Then, beta_{2h} is a second crucial parameter, denoting the mean effect of the endophyte, for each host-vital rate combination. This is what is shown in Fig. 1 (after standardization), correct? It took me a while to get this, and I suggest to make this clearer throughout the text (for instance adding to the legend of Fig. 1 how these are calculated in terms of tau etc, explicitly linking Eq. 2 to the presented results)."

> **Response:**
>
> **Thank you for the opportunity to clarify our methods. Reviewer 2's interpretation of our results and the associated parameters from our models is correct. We revised the referenced figure (new Figure 2) to more clearly show the connection between our regression modeling and the standardized effect sizes. We have also revised the text to clarify the connections between model parameters and core hypotheses (lines 224), as this reviewer highlights.**

line 224 *<All size-structured models included the same linear predictor, including two key parameters for each species: one which described the effect of endophyte symbiosis on the mean of that vital rate, and another which described the inter-annual variance in the vital rate for symbiotic and symbiont-free plants, estimated using random year effects specific to each species and endophyte status. This species- and endophyte status- specific random year effect allowed us to quantify the effect of endophytes on inter-annual variance for each vital rate. Other parameters accounted for size structure in the data (defined as the number of tillers) as well as the difference between originally transplanted plants (started in a greenhouse) and those which recruited naturally into the plots.>*

COMMENT 6

"I believe that the coefficient estimates are nowhere provided, except for the appendix figures showing the posterior distributions for one parameter. It would be useful to compare different effects, for instance the effect of the symbiont compared to the 'origin' parameter. I would also like to see the how the inter-annual variance compares to e.g., the plot variance or residual variance. Please provide all coefficients, credible intervals, and variance components (either in the main text or in an appendix). Similarly, I can only find the differences in mean lambda between symbiont + and – host populations. But what are the actual lambda estimates? (are they anywhere realistic?)"

> **Response:**
>
> **We appreciate the value of showing fitted parameter estimates. In response to this comment, we have added new supplemental figures showing parameter posteriors for all parameters of the vital rate models (Figs. S69-S77). We have also added a new figure showing absolute lambda values (Fig. S78), which generally indicated declining population growth rates (lambda < 1), consistent with recent population trends in our experimental plots.**

COMMENT 7

" I appreciate the attempt to show the amount of variation between years in Fig. 1D-E, but these graphs are difficult to interpret (the same applies to the similar appendix figures). Would there be another way to visualize the effects of the endophyte presence on the inter-annual variance? "

> **Response:**
>
> **We agree that our original attempt to show inter-annual variation in vital rates was difficult to interpret. In our revision we provide a new, simpler visualization (new Figure 2) that shows the posterior estimates of interannual standard deviation for S+ and S- populations.**

COMMENT 8

" I really like Fig. 2C. Is the bottom left corner where the symbionts lead to conservative bet hedging?"

> **Response:**
>
> **We appreciate the reviewer's enthusiasm! Whether or not this region of parameter space qualifies as adaptive bet hedging depends on whether the reduction in variance is sufficiently strong to outweigh the cost to mean fitness, hence "mutualism possible". We now indicate this possibility more clearly in the text (line 500).**

Line 500 <*Demographic buffering may be considered a bet-hedging strategy if reduced temporal variance comes at the cost of arithmetic mean fitness (Childs et al. 2010). This may be unlikely in this system, where most host species exhibited both reduced variance and elevated mean fitness through symbiosis (Fig. 3C). However, the context-dependent fitness effects that underlie demographic buffering may favor other forms of evolutionary bet-hedging…*>

COMMENT 9

" Fig. 4 shows the results of a decomposition analysis, quantifying the effects of the symbiont on long-term fitness through its effects on the mean and/or variance. Could this analysis be extended, further decomposing it into the contributions through each of the different vital rates? This would highlight through which vital rates the symbiont affects fitness the most, whether this is through effects on the mean of variance, and how the importance of different vital rates may change under future (more extreme) climates."

> **Response:**
>
> **Thank you for this great suggestion. As the reviewer suggested, we performed a new analysis decomposing symbionts effects through mean and variance into contributions that come from reproductive vital rates vs those that come through survival and growth. We found strong evidence that changes in variance buffering are largely driven by variance buffering in survival and growth rather than in reproduction. The results of this decomposition are shown in the new Fig. S72 and we have added interpretation of these results into our Results (line 409) and Discussion (line 526).**

Line 409 <*Decomposing this result further into contributions through endophytes effects on different vital rates demonstrated that demographic buffering arose primarily from symbionts' effects on host survival and growth, rather than from effects on reproduction (Fig. S72).*>

Line 526 <*We identified damping variance in survival and growth as the key avenue by which symbionts' variance effects contributed to host fitness (Fig. S82). Elasticity analyses could explore the selection that drives diverse symbiont effects across host vital rates.*>

COMMENT 10

"For the fitted vital rates, there are a few cases where the mean predictions seem to be quite off, not following the data so well, even though my understanding from Eq. 2 is that most parameters are estimated per host species, so they should be flexible enough to capture the observations (indeed, in most other cases, mean predictions do match the averaged observations nicely). For instance, in the case of adult growth of P. sylvestris and P. alsodes (Fig. S2), or the flowering probability of L. arundinacaea (Fig. S2). What is causing this? "

> **Response:**
>
> **We appreciate the reviewer's attention to our supplemental figures. Please see our response to Reviewer 1's Comment 3. We believe that response, and the changes described therein, also address this reviewer's comment.**

COMMENT 11

"L382: How strong was the phylogenetic signal in each of the life history traits?"

> **Response:**
>
> In response to this comment, we have added calculations of Pagel's λ to Table S2, and added text to our results section describing the strength of phylogenetic signal in our phylogenetic regressions (line 387). Because we performed life history analysis for both host and symbiont phylogenies, we have two sets of Pagel's λ values. We found that Pagel's λ consistently averaged around 0.22 for analyses using the host phylogeny and averaged around 0.56 for analyses using symbiont phylogeny.

Line 387 <*Models indicate moderate phylogenetic signal in the effect of variance buffering (average Pagel's λ of 0.22 (90\% CI: 0-0.8) and of 0.56 (90\% CI: 0-0.9) from models including host and symbiont phylogenetic covariance respectively (Table S2)).*>

COMMENT 12

"L374: Should this be Table S2? "

> **Response:**
>
> We have corrected this error. Thank you.

RESPONSE TO REVIEWER 3

COMMENT 1

"Here, the authors use an experimental set-up combined with long-term demographic data to test for biotic mechanisms of demographic buffering. Overall, the manuscript is well written. Furthermore, the manuscript makes a timely contribution to the area of stochastic demography and includes 14 years of hard-fought demographic data across multiple grass species (very impressive).

I found the paper very interesting as, admittedly, this is an area of the literature in which I am deeply interested. However, there are a couple of points that I think would be beneficial to address to improve clarity (especially around the methods)."

> **Response:**
>
> **We thank Reviewer 3 for the positive, constructive comments, and we are glad they recognize the timeliness of our work.**

COMMENT 2

"Keywords: I would recommend using keywords not already in the title."

> **Response:**
>
> **Thank you for the suggestion. In response to this comment, we have broadened our set of keywords to include: "stochastic demography, plant-microbe interactions, environmental variability, symbiosis, mutualism, long-term data, life history, Epichloë, Poaceae"**

COMMENT 3

" L96: GCC is not increasing environmental variability everywhere. See Bathiany et al. (2018) Science Advances. You could approach it from the point of view that it is increasing in areas hosting the highest biodiversity. "

> **Response:**
>
> **We appreciate this comment and agree it is important to communicate nuance about forecasted global change. In response to this comment, we have edited our introductory sentence to acknowledge the heterogeneous nature of changes in climate variability (line 94). As described in our response to Reviewer 1 Comment 2, we have added to our new discussion consideration of the ways that spatial variation in environmental variability may intersect with symbiont-mediated variance buffering (line 519).**

Line 94 <*Global climate change involves heterogenous changes in environmental variability, including increases in the frequency of extreme weather events and of ``whiplash events'' that alternate between climate extremes (Seneviratne et al 2012, Bathiany et al 2018, Swain et al. 2018, IPCC 2021).*>

Line 519 <*Endophytes may dampen spatial heterogeneity in host fitness in ways that parallel their effects on temporal variance, and this hypothesis could be explored by leveraging the plot replication of our experimental design. At larger spatial scales, buffering effects of symbionts may vary across the broad geographic distributions of these eastern North American grass species, especially since*>

historical and projected trends in climate variability are geographically heterogeneous (Bathiany et al. 2018).>

COMMENT 4

" L98: This final sentence needs some citations for this sort of claim. I'd recommend citing the initial Tuljapurkar papers as well as Doak et al (2005) American Naturalist.

> **Response:**
>
> **We have added a citation to support our claim here (line 100).**

COMMENT 5

Equation 1: Right now, log is being rendered looking like three separate variables. Perhaps remove the italics and make normal text. "

> **Response:**
>
> **We appreciate the attention to readability and interpretation of equations. We have removed the italics as suggested (Eqn 1).**

COMMENT 6

" L116: Negative correlations among vital rates "

> **Response:**
>
> **Done.**

COMMENT 7

" L123: The shift to this paragraph is quite abrupt. The introduction starts with a very concise journey through the fundamental ideas of stochastic demography that support the paper, and then, out of nowhere, a first-time reader is told about the roles of symbiotic microbes. I think this could be made a bit simpler and easier for the reader. I would recommend starting the paragraph talking about a variety of biological factors that can act as a buffering mechanism in a variable environment (e.g., maternal effects (bird literature), social network connectivity (deer), etc.). And then introduce the idea of symbiotic microbes. Currently the introduction is slightly on the lean side (especially with regard to the stochastic demography literature) – a little more fleshing and smoothing out would very much help the pitch. "

>   **Response:**
>
>   **We appreciate these suggestions. In response to this comment, we have revised the preceding paragraph to better foreshadow the introduction of biotic interactions as a topic of primary focus in the manuscript (line 114).**

Line 114<*Both inherent characteristics of species and the environments they experience can buffer demographic fluctuations. Inherent characteristics include life history traits (Pfister 1998), trade-offs among vital rates (Compagnoni et al. 2016), and transient shifts in population structure (Ellis et al. 2013). For example, theory predicts long-lived species, those on the slow end of the slow-fast life history continuum, to be less sensitive to environmental variability than short-lived species (Murphy 1968), a pattern with empirical support across plants (Davison et al. 2019, Compagnoni et al. 2021) and animals (Le et al. 2022, Morris et al. 2008). Demographic variance is also determined by external abiotic factors, such as the magnitude of environmental variability (Rodriguez et al. 2021) or environmental autocorrelation (Tuljapurkar 1980, Fieberg et al. 2001). The complex interplay of these factors determines populations risks of extinction (Menges 2000) and underlies management strategies promoting ecosystem resilience (Kuparinen et al 2016). Yet, little is known about how inter-specific interactions contribute to demographic buffering (Hilde et al. 2020).***>**

COMMENT 8

" L118: Just a heads up, throughout the introduction, many terms such as variance and stochasticity have been stated. I would highly recommend adding the word temporal before quite a few of them. As you will likely know, there are quite a lot of people from the stochastic demography side that will read "environmental stochasticity" and think about it in terms of temporal stochasticity (as I do). However, there are a lot of people in meta-population theory, community ecology and anthropogenic stressors researchers who may think about this in terms of "spatial stochasticity." The one word that tipped me off to write this comment was "autocorrelation" for which there is a vast literature in both camps. This comment is really because the paper is being pitched for a general audience – the clarification is important. "

> **Response:**
>
> **This is a great suggestion. We now clarify our focus on temporal variance in several places in the paper (lines 110, 136, 144).**

COMMENT 9

" L135: This ("buffer hosts against variability") is too vague to the non-buffering indoctrinated reader. I would recommend being explicit. What is buffered? Is this an attribute attributed to an individual or a population (host seems to implicate individual – heads up)? What is the variability? I apologize for being pedantic about definitions and language, but this is quite important. "

> **Response:**
>
> **We agree about the importance of clear and accessible definitions. We now provide more explicit definitions (line 111) of our central hypotheses along with a new conceptual figure 1, and we replaced several uses of "variance buffering" with language that more directly describes reduced inter-annual variance in vital rates or fitness (line 136).**

Line 111 <*There are accordingly two pathways to increase population viability in variable environments: increase the arithmetic mean growth rate and/or dampen temporal fluctuation in growth rates, also called ``demographic buffering''..*>

Line 136 <*We hypothesized that context-dependent benefits from symbionts may buffer host populations against variability through strong benefits during harsh periods and neutral or even costly outcomes during benign periods, reducing the impacts of host exposure to extremes and dampening inter-annual variance relative to non-symbiotic hosts (Fig. 1A).*>

COMMENT 10

" L141: Inverting this sentence: "To test the hypothesis that ….., we used a combination…." Would make the transition to the new paragraph a little smoother. "

>   **Response:**

>   **We have edited this sentence following this suggestion for readability (line 144).**

Line 144 <*To test the hypothesis that context-dependent benefits of symbiosis dampen interannual variance in host fitness, we used a combination of long-term field experiments and stochastic demographic modeling.*>

COMMENT 11

" L157: Throughout the introduction, "variance buffering" has been used. This is the first introduction of "demographic buffering." Personally, I was happy to see it, I would recommend standardizing across the manuscript. "

>   **Response:**

>   **In line with our responses to Comments 8 and 9, we have edited to more consistently refer to demographic buffering in several places throughout the paper (eg. Line 111, Line 136)**

COMMENT 12

" L194: Any estimate of the size? "

>   **Response:**

>   **Size at first planting varied by species between 1 tiller plants and 6 tiller plants. We have added this information to our supplemental methods (line 847).**

Line 847 <*We established experimental populations with vegetatively propagated clones of similar sizes (ranging from one to six tillers).*>

COMMENT 13

" L329: Apologies if I am misreading the methods, but (as I understand it) isn't the model a generalized integral projection model with two states? If this is the case, I don't see why this is not stated. The entire methods section reads like the methods of an IPM without ever saying it. Honestly, a little puzzled.

> **Response:**
>
> **Thank you for the opportunity to clarify our methods. We understand the reviewer's confusion (we actually get this comment a lot). This is not an integral projection model because there is nothing to integrate. Size is a discrete state variable (tiller number) and size transitions follow discrete probability distributions. However, the reviewer is correct to recognize that we parameterize the matrix model with statistical sub-models, an approach more commonly associated with IPMs but equally applicable to MPMs. For example, we modeled growth using Poisson-Inverse Gaussian regression, providing a flexible, discrete distribution that predicts the probability that, for example, a three-tiller plant will shrink or grow to size 1,2,3,4,...N conditional on initial size, endophyte status, and other covariates and random effects. In response to this comment, we have revised our methods to more explicitly acknowledge the IPM-like parameterization of our MPM (lines 239).**

Line 239 <*We parameterized the models using the fitted statistical vital rate models in a manner similar to continuous IPM models (Ellner 2016), while accounting for the discrete data representing our focal species' growth (Ellner et al. 2022).*>

COMMENT 14

Slightly separate to this point……. I think the methods would greatly benefit from a life cycle graph. Even if this is only added in the supplementary materials, a life cycle graph would greatly aid the reader in how the model is constructed. There are plenty of examples of how to do this for IPMs (simple example of pre-breeding census in Ellner IPM textbook). "

> **Response:**
>
> **This is a great suggestion. We have added a generalized life cycle graph to the supplement (Fig. S1) to aid readers in their interpretation of our model.**

COMMENT 15

"More about the methods: From my reading, it seems as though the authors use a matrix/kernel stochastic approach by selecting certain matrices for their variance treatments. Not saying that this is the wrong way to do it (as there is no right way), I think this needs more support. Is there are reason the methods includes a matrix stochastic approach instead of parameter stochastic approach (i.e., terms of regressions for example)?

> **Response:**
>
> **We appreciate the opportunity to clarify the rationale behind our modeling choices. In this case, matrix selection was a natural way to preserve vital rate correlations which, as emphasized by Reviewer 4, can be an important element of population dynamics in stochastic environments. By selecting transition matrices corresponding to observed years, we can implicitly account for correlated vital rate responses (e.g., years that are good for reproduction are bad for growth) without having to estimate the correlations. The matrix selection approach has precedent in the literature and has been shown to perform similarly to parameter selection approaches (see Metcalf et al. 2015). We now provide this context for readers (line 286).**

Line 286 <*Sampling observed transition matrices (rather than independently sampling regression coefficients) produces models that realistically capture inter-annual variation by preserving correlations between vital rates (Metcalf et al. 2015).*>

COMMENT 16

Also, I do not know why lambda-s has not been standardized across variance treatments. This is very easy to do and seems like a more elegant way to mitigate the issue of the expected asymptotic properties of the treatments. This may not make it to the hall-of-fame that is the manuscript but I think is worth consideration. "

> **Response:**
>
> **We appreciate the suggestion but unfortunately we are unclear what Reviewer 3 means by standardization in the context of our variance treatments. We compute the stochastic growth rate through simulations that select annual transition matrices. We are happy to revisit this idea if the reviewer would like to clarify their suggestion and perhaps provide some references.**

COMMENT 17

"L.377: What is meant by reference condition?"

> **Response:**
>
> **Because life history traits are estimated from the transition matrices, we must choose the S+ or S- matrix (or average them) to designate species-level traits. We have edited this sentence (line 301) to clarify that our calculations of life history metrics come from the symbiont-free population matrix. This allows us to test our hypothesis that a longer-lived species may not benefit as much from endophyte-mediated buffering because it is more inherently buffered in the endophyte-free (reference) state.**

Line 301 <*... we calculated generation time, longevity, net reproductive rate R_0, Keyfitz entropy (describing survivorship across lifespan), and Demetrius entropy (describing reproduction across lifespan) from the mean transition matrix for symbiont-free populations.*>

COMMENT 18

" L.439: Saying average is a bit loaded, lambda-s is also an average. I would recommend thinking of standardizing terminology to the arithmetic and geometric means. That way there is no confusion. "

> **Response:**
>
> **Thank you. We have edited our references to averages throughout the manuscript in an attempt to improve clarity (e.g. lines 111, 326).**

COMMENT 19

" L.443: This is a very tricky sentence to read and understand. "

> **Response:**
>
> **We have edited this sentence for clarity (line 332).**

Line 332 <*We simulated scenarios of increased variance relative to that observed during our study by sampling subsets of the 13 observed annual transition matrices. We created two scenarios of increased environmental variance by sampling the transition matrices associated with the set of either six or two most extreme λ_{t} values for S- populations. These extreme lambda_{t} values represent the best and worst years experienced by symbiont-free populations.*>

COMMENT 20

" Fig 3: Why isn't longevity used as it is throughout the manuscript? Also, longevity is just one part of the pace-of-life syndrome. To show the impact of life history, I would recommend showing the impacts of other components of POLS (e.g., generation time, Keyfitz entropy). Furthermore, to show POLS is the axis of interest and not reproductive schedule, I would highly recommend also running this analysis for R0 and Demetrius' entropy. Following your writing, you would hypothesize positive relationship for POLS components and minimal covariance with reproductive schedule (although see Tuljapurkar et al. 2009 Proc B and their description of demographic dispersion in variable environments). Right now, seven points is not the most convincing…… broadening the pool of life history traits may aid with this. "

> **Response:**
>
> **We have added a new figure (Figure S83-S85) with the expanded set of life history traits including Keyfitz entropy and Demetrius' entropy, as the reviewer suggests, and clarified our predictions related to life history pace of life in our introduction (lines 116). We appreciate the suggestion to include further components of POLS, although regardless of which life history traits we consider we will be limited to seven points (species).**

Line 116 <*For example, theory predicts that long-lived species, those on the slow end of the slow-fast life history continuum, will be less sensitive to environmental variability than short-lived species (Murphy 2968).*>

COMMENT 21

" Fig 4: I am a big fan of this plot. Thank you. Out of curiosity though, does the increased positive effect of symbiosis on lambda-s rescue the populations? Buffering has a positive effect but has its limits. You cite Rodriguez-Caro et al 2021 which discusses this with tortoises…….. it may be worth a look to show how meaningful buffering across the scenarios. Do they all persist or do some of them more than others? "

> **Response:**
>
> **In response to this comment, we have added a new supplemental figure showing the computed stochastic growth rates (Fig. S80). While endophytes generally improve population growth, both S+ and S- populations have population growth rates <1 for most species. This reflects the trajectories of the experimental plots which have generally declined across the 14 year study. Extrapolating from our experimental plots to forecasts for natural populations across the ranges of these grasses is a major challenge, which we have added to our Discussion (line 476).**

RESPONSE TO REVIEWER 4

COMMENT 1

"This paper highlights the important issue of demographic rate variance and its effects on long-term stochastic population growth rates. Because variance in vital rates decreases population growth, all else equal, incorporating stochasticity into demographic analyses provides a more accurate representation of population dynamics. The authors use a multi-species dataset to address the potential for variance-buffering effects of endosymbionts by modeling long-term dynamics estimated from data collected from an 14-year symbiont removal experiment to compare the effects of variance-buffering relative to those of improved mean vital rates, among populations with endosymbionts removed vs. those with endosymbionts present.

The authors provide simulations that demonstrate what has long been known – that decreasing variance in vital rates (and thus in annual population growth rates) increases long-term stochastic population growth rates. Their endosymbiont removal experiment provides relevant data and demonstrates not just that lower variance increases population growth, but that protecting or increasing the prevalence of endemic endosymbionts would likely improve population performance, which could have valuable applications in conservation settings."

> **Response:**
>
> **We thank Reviewer 4 for thoughtfully reviewing our work and for recognizing the importance of building understanding of stochastic demography in the context of host-microbe interactions.**

COMMENT 2

" A few important issues were overlooked, however. One important caveat is that long term population growth rates depend not just on the variability (or noise) in population vital rates, but also upon the correlations between these vital rates and upon the importance of the vital rates for population growth (reflected in vital rate sensitivities or elasticities). While Eq(1) uses a version of the stochastic growth rate (λS) that depreciates the mean population growth rate by the scaled variance in annual population growth rates, there is a more accurate formula that also incorporates the effects of vital rate correlations and elasticities - the small noise approximation of the stochastic population growth rate (Tuljapurkar 2013).

> **Response:**
>
> **We completely agree with this reviewer's concern that a complete presentation of the stochastic growth rate for structured populations should incorporate vital rate correlations and elasticities. However, such a presentation is not our aim in the second paragraph of the Introduction. We intentionally chose the classic (and simple) Lewontin and Cohen result for unstructured populations as a "cartoon" to illustrate, for non-specialist readers, the conceptual idea that variability can reduce long-term population growth, which then sets up the hypothesis that symbionts can elevate fitness by dampening variability. In our revision, we are more explicit in acknowledging the additional complexity that comes into play for structured populations with fluctuating vital rates (lines 110, 536). While we do not represent**

vital rate correlations in this simple notation, we have edited the text to clarify that correlations and vital rate elasticities are accounted for in our matrix selection approach (see Comment 15 from Reviewer 3).

Line 110 <*Populations structured by size or stage experience similar costs of temporal variability (Cohen 1979, Tuljapurkar 2013).*>

Line 536 <*Our ``matrix sampling'' approach accounted for vital rate correlations implicitly (Metcalf et al. 2015) but exploring whether and how endophyte symbiosis alters the correlation structure of host vital rates could add nuance to our understanding of how symbionts contribute to variance buffering.*>

COMMENT 3

Positive correlations compound the negative effects of variance, whereas negative vital rate correlations can buffer long term population growth rates against fluctuations in annual growth rates. Considering that the authors are interested in buffering effects, these negative (compensatory) correlations and positive correlations (knock-on effects) could be important for their analysis and they would provide a more complete and nuanced view of the role that endosymbionts play in buffering population growth rates against annual fluctuations in vital rates. In particular, it would be interesting to know if endosymbionts promote more negative correlations (which would further buffer against stochastic fluctuations) or whether they increase positive correlations (improving multiple vital rates simultaneously but likewise hurting multiple rates during "bad" years). Although it could be merely noted (with some preliminary predictions about the effects of correlations), addressing the full range of stochastic effects would make their findings more robust and allow for more context in interpreting their findings. "

> **Response:**
>
> **We appreciate these thoughtful suggestions, and we agree that it would be fascinating to explore whether symbionts modify vital rate correlations in ways that dampen the negative effects of environmental fluctuations. Doing this rigorously would require a different modeling approach that estimates the variance-covariance matrix of vital rates for S+ and S-hosts. Having done such analyses before (Compagnoni et al. 2016, *Ecological Monographs*), we know this is a serious undertaking. We chose a simpler strategy that implicitly accounts for vital rate correlations through the sampling of year-specific transition matrices. We now incorporate the reviewer's very good ideas in a new discussion section (lines 535) that addresses the potential for variance buffering effects to emerge from changes in the correlation structure of vital rates.**

Line 535 <*Similarly, correlated responses of multiple vital rates could amplify or dampen demographic variance (Tuljapurkar 2013, Davison et al. 2013, Compagnoni et al. 2016).*>

COMMENT 4

" These observations are included in comments annotating the draft pdf, which also include some questions about the methodology. In all, I think this paper uses an appropriate study system and relevant experimental approach to study the variance-buffering potential of endosymbionts, and could be a welcome contribution to our understanding of stochastic effects that could help advance theoretical understanding of population dynamics and provide the motivation for further studies highlighting the conservation potential of symbiotic relationships. However, they overlook other important elements of stochastic population dynamics in addition to variance-buffering and could benefit from additional analysis that examined the effect of endosymbionts on vital rate correlations and population-level differences in the force of selection that could be identified through differences in vital rate sensitivities or elasticities. "

> **Response:**
>
> **We appreciate this reviewer's expertise in stochastic population dynamics and their excellent suggestions for new directions of our analyses. We hope to reassure the reviewer that nuanced elements of the stochastic growth rate were not "overlooked" but rather acknowledged and embedded into our analyses implicitly, such that we can better center the manuscript on the biology and demography of host-symbiont interactions. We also hope that the new decomposition analyses provided in response to Reviewer 2 - Comment 9 provides some of the nuance in vital rate sensitivities and contributions that this reviewer was looking for.**

COMMENT 5

"Eqn 1 : This formulation iignores correlations and differences in elasticities (Tuljapurkar 2013), which can be important (Davison et al. 2013).

Tuljapurkar, S. (2013). Population dynamics in variable environments (Vol. 85). Springer Science & Business Media.

Davison, R., Nicole, F., Jacquemyn, H., & Tuljapurkar, S. (2013). Contributions of covariance: decomposing the components of stochastic population growth in Cypripedium calceolus. The American Naturalist, 181(3), 410-420."

> **Response:**
>
> **We believe our response to Reviewer 4 Comment 1 adequately describes our reasoning for presenting this equation, and for using matrix selection methods that preserve observed correlations and differences in elasticities. We thank this reviewer for sharing these references related to decomposing stochastic growth rates and have added citations where appropriate (line 288, 536).**

COMMENT 6

"L120 : good point - the math is much better understood than the ecology"

> Response:

> We agree with this reviewer's comment, and we hope that our paper does some work to fill in understanding about the ecology of stochastic populations. We have edited this paragraph in response to this comment and to comment 7 by reviewer 3 to clarify the different environmental and biotic factors that can influence stochastic growth rates (line 114).

COMMENT 7

"L 444 : Would it be a more elegant solution to use the small noise approximation (Tuljapurkar 2013) to estimate the effect of variance by toggling variance directly instead of re-sampling extreme years?"

> Response:

> This is an intriguing question. More elegant? Probably. But using Tuljapurkar's small noise approximation would require estimation of the variance-covariance matrix for all vital rates. As we detail in response to this reviewer's comment 3, we had the more modest goal of adequately accounting for vital rate correlations without having to explicitly model them.

COMMENT 8

"L482: Not sure what this "declined by as much as 170%" means. Wouldn't a decline by 100% reach zero?"

> Response:

> Thank you for pointing out this error. We have edited these results for clarity (line 370).

Line 370 <*For some host species, the CV of λ declined by more than 62% (\P. alsodes, F. subverticillata), while for others, endophyte effects on variance were substantially smaller (5% lower for E. villosus, 13% lower for A. perennans), or even positive (37% increase for E. virginicus).*>

COMMENT 9

"L534 : Relative contributions of variance vs. mean rates to differences in population growth rates differ widely across plants and effects of variance are sometimes larger than effects of mean vital rates (Davison et al. 2019)

Davison, R., Stadman, M., & Jongejans, E. (2019). Stochastic effects contribute to population fitness differences. Ecological Modelling, 408, 108760."

> **Response:**

> **Thank you for sharing these additional relevant references. We have added citations where appropriate (line 536).**

COMMENT 10

"L559 : This is fairly obvious - that the effects of variance-buffering will be larger when variances are larger"

> **Response:**

> **We agree with the reviewer that there is a straightforward logic to this result. Our primary aim in this analysis is to illustrate the potential importance of variance buffering as a novel mechanism through which symbiosis can act on host fitness. In our new discussion section, we acknowledge ways in which our simulation is not intended as a forecast, but how we can work towards useful forecasts of host-symbiont dynamics that include the magnitude of change in climate drivers and in the sensitivities of host vital rates to changes in variance (line 476) and added consideration of potentially more realistic scenarios of variability under future climates (line 531).**

line 476 <*While our results highlight symbiont-mediated demographic buffering as a potential source of resilience against increased environmental stochasticity, much work remains to connect symbiont effects on mean and variance to quantitative forecasts of host-symbiont dynamics under global change. Like most temporally stochastic population projection models, our approach quantified demographic variance across years (and simulated increasing variance) without attributing its cause(s). Realistic forecasts for host-symbiont dynamics under environmental change will require explicit connections between driver variables and demographic responses.*>

line 531 <*Further, our simulations assumed an independently distributed environmental distribution through time, but auto-correlation of the environment can be an important component of stochastic population projections (Tuljapurkar et al. 2006) and might modify the fitness consequences of symbiont-mediated variance buffering.*>

COMMENT 11

"L601 : Do you expect that under global climate change the likelihood of losing endosymbionts would increase? If so, this would be important to note - that global climate change could both increase variances and reduce the variance-buffering effects of endosymbionts."

> **Response:**
>
> **This reviewer raises an important point that symbiont-mediated variance buffering would have little effect if we expect symbionts to be less common under future climates. We have incorporated this idea into our new discussion section. It is not well known how climate change will alter the transmission of *Epichloë* symbionts. Of the few studies that have investigated this, they have generally found that transmission rates are not influenced by climate drivers (David et al. 2019), however rates of vertical transmission can vary strongly across populations and species (Sneck et al. 2017). In our new discussion section, we outline the implications that imperfect transmission has for our model predictions (line 548).**

COMMENT 12

"Fig. 1: So, in some species (e.g., L. arundinaceae and P. alsodes), the largest single effects were from variance-buffering as opposed to higher mean rates? If this is true, it deserves greater note, since it is strong support for your hypothesis that endosymbiotes benefit plants through variance-reduction."

Response:

> **We appreciate the reviewer catching these interesting species-specific results. In response to this comment, we have edited our results to highlight cases where vital rate effects rival mean effects (line 351).**

Line 351 <*The magnitude of mean and variance effects differed among host and vital rates. Symbiont effects on vital rate variance were as large and even exceeded effects on vital rate means for certain species. For example, endophytes modestly increased mean adult survival (Fig. 2C) and strongly reduced variance in survival (Fig. 2D) for Festuca subverticillata, while for Poa alsodes, variance buffering was more apparent in seedling growth and inflorescence production (Fig. 2E).*>

COMMENT 13

"Fig S3: I'm not sure how you modeled the regression here, but the curve for A. perennans (Fig. S3A) looks like it does not accommodate the down-tick in flowering probability observed for larger plants. Is there some reason to believe that size- (or age-) related declines in flowering is an artifact? If not, why do you model it as a monotonic increase with size?"

> **Response:**
>
> **This is an interesting observation that relates to potentially important effects of endophyte symbiosis on host longevity. We believe our response to Reviewer 1 Comment 3 which details our assessment of vital rate fit to the data addresses this question. The new analysis provides more flexibility to capture nuance in size effects. In brief, we estimated size effects of originally transplanted and naturally recruited plants separately, and included parameters for a potentially quadratic relationship with size. The decline in flowering probability is more apparent in the originally transplanted plants (we now show the regressions and data for recruit and original plants separately (Fig. S2-S11). The inclusion of quadratic terms did improve model fit including for the example of *A. perennans* flowering (Fig S6 - S7), however the model does not predict a strong decline in size for either recruit or original plants. It is also important to note the difference in bin size across these plots. In certain cases, recruits or original plants make up relatively more or less of the dataset, which may pull the expected vital rate curves to be more or less similar to either status. It would be very interesting to model age-structure more explicitly to investigate the effects of endophytes across host lifespan.**

COMMENT 14

"Fig S14: So E. villosus has higher Interannual SD in Seedling Growth *without* endosymbionts? Since it goes counter to your hypothesis, and counter to the bulk of your findings for other vital rates and other species, this discrepancy should be noted and, if possible, interpreted in the context of the variance-buffering effects of endosymbionts."

> **Response:**
>
> **In response to this comment, we have edited our results to more clearly highlight these examples (line 358). We have also added to our discussion to highlight the important consideration that this could be a case of demographic lability, where high variance strategies in less sensitive vital rates may be adaptive (line 522).**

Line 358 <*Additionally, some vital rates showed costs of endophyte symbiosis. Symbiotic individuals of A. perennans grew larger than those without endophytes (Fig. 2B), yet endophytes also reduced this species' mean recruitment rates (Fig. 2A). Similarly, endophytes increased variance for certain species' vital rates, such as in seedling growth for Elymus villosus and Festuca subverticillata (Fig. 2A).*>

Line 522 <*Finally, our demographic modeling framework could be further ``unpacked'' to explore other elements of fitness in stochastic environments. We identified damping variance in survival and growth as the key avenue by which symbionts' variance effects contributed to host fitness (Fig. S82). Elasticity analyses could explore the selection that drives diverse symbiont effects across host vital rates. Small changes in variance of vital rates that are highly important to population growth (i.e. those with high elasticities) may be more strongly selected for than larger changes in less important vital rates (Doak et al. 2005), and symbionts may even provide an adaptive advantage by increasing temporal variance in certain vital rates (i.e. demographic lability (Koons 2009).*>