Summary Comments - 14133

As a friendly reviewer, I find this an interesting proposal that proposes to investigate a scientific area that seems mostly unexplored. It is well organized and seems well thought out. Congrats! Overall, a key suggestion is related to improving the impact of the writing through word choice, a simpler, streamlined writing style, and statements highlighting the significance of the proposal at strategic locations, especially at the end. The other focus of suggestions is clarifying ambiguities in the text with the objective of anticipating reviewer questions and addressing points of potential confusion. In addition to this summary, you will find line-by-line edits and comments in the margins of the Word document. I hope you find the friendly review and edits helpful.

1. I compacted the text as much as possible because, as written, the proposal exceeds the 15-page ISF limit. Please read carefully to be sure I have preserved the intent of your text throughout. I have noted sentences that I rearranged or reworded more extensively. After editing, you should be below the 15-page limit after figure and format adjustments.

2. A minor point, perhaps, but I suggest that “microbes” and “microorganisms” are synonymous. Given the number of instances of microorganisms, a significant amount of space could be saved by using microbes throughout.

3. Another small point, perhaps. Although in common usage in scientific writing, “suggests” or “may suggest” (which is redundant) is associated with human behavior. People suggest; data indicates or may indicate.

4. Generally, the Background should be an introduction based on established and mostly published research from which you derive your proposal. I suggest avoiding references to upcoming sections, if possible. For example, “see Preliminary Results” in the Background section. Thinking as a friendly reviewer, referencing forward can detract from the reading flow of the proposal by encouraging reviewers to look ahead in the proposal for a section not yet covered. However, I understand the dilemma of wanting Figure 1, a preliminary result to support your hypotheses. Referring to earlier sections is generally acceptable because it is a reminder for reviewers. I hope this helps!

5. Line 111. I tried to make your hypotheses more explicit. Because proposals are hypothesis-driven, I suggest stating explicitly reviewers whenever a hypothesis is presented. You may even wish to highlight these hypotheses for impact. As a friendly reviewer, the hypotheses, research goals, and experimental aims to achieve the goals should be as explicit as possible. I would look at those items as a reviewer to orient my reading.

6. For figure legends, I suggest using a different 11-point font to distinguish the legends from the main text.

7. Fig. 1 (line 108). I suggest indicting the statistics used for the error bars and the number N of repetitions. Since this is discussed in the Background, it should be well-supported data. The proposal depends upon this data for your hypotheses and objectives.

8. line 145. Considering reviewer impressions, I presume this proposal will be the work of multiple people, plus collaborators, if any. Thus, although PI formulates the hypotheses, I suggest that “we” is the more accepted way of referring to the research. As a friendly reviewer, it is clear that the ideas are from the PI, and generosity is rarely criticized!

9. Figure 2. Outlining the proposal is great. Reviewers will appreciate it as a reference.

10. Where possible, I edited the writing from the third person to the first person, which is the accepted style of science writing these days.

11. Lines 159 and 177. For a non-expert reviewer, are you investigating transfer from adults to larvae or larvae to adults? This may lead to confusion because you are feeding fungi from adult guts to larvae, then examining if the fungi are present in the resulting adults. This is a complicated concept. Perhaps you can add a statement for reviewers explicitly stating why you are looking at adults to larvae transfer if that is the case.

12. Line 182. As a friendly reviewer, I suggest clarifying the effect desired. Optimum growth? How will this be tested beforehand? What are the criteria needed to achieve the effect?

13. At first mention (line 81), P. tropicalis is named as the species. However, throughout the text, C. tropicalis is used.

14. Line 198. I suggest that the point of the objective is to make conclusions rather than acquire data. As a friendly reviewer, I suggest that this is an important distinction. I hope this makes sense.

15. The word ‘tested” seems overused. This can be monotonous to readers, so I suggested alternatives throughout.

16. Generally, throughout the text, it is not always clear which yeast or yeasts are being referred to. I made comments in the margin to indicate locations.

17. I suggest removing the automatic lists feature in Word so that all headings can be aligned with the left margin for a more organized appearance. The extra indents don’t seem necessary because there are already well-organized numberings and headings. As written, the different indents seem more of a distraction than an organizational aid.

18. Line 61. “good’ seems ambiguous. I suggest being more specific. What is a good model? Do you mean a generalized model for all insect-microbiome interactions or all mycobiome-insect interactions? A model for fly species or, more generally, for all microbial-insect interactions? I suggest defining what you propose to model rather than leaving it to reviewers. A reviewer may interpret the statement too broadly, criticizing the lack of evidence of a broad mycobiome-insect mechanism in invasive insects. This would argue against the significance of the proposal. Of course, the proposal's objective is to gain such knowledge, but we want to answer any reviewer criticisms proactively*.* If there is no knowledge about how widespread mycobiome-insect interactions are in invasiveness, then understanding how general the phenomenon is may be a future objective beyond the scope of this proposal. This can be explicitly stated. I hope this is a useful point.

19. Throughout the proposal, there are references to “the yeast”. For example, at line 295, I suggest being explicit about which or how many fungi are being referred to. I also suggest being specific about the species or number of yeast throughout to keep reviewers organized. As written, it seems unclear in places which species are being referred to. I made comments in the margin to indicate some other specific locations, but I suggest trying to clarify throughout.

20. Similarly, “microorganisms” is often used to describe fungi. While this is correct, the more specific terms, such as yeast, fungi, or the species name, make it clear that you are focused on specific fungi throughout the proposal. This extends to “microbiome” vs. “mycobiome”. I suggest using the most specific terms throughout. As a friendly reviewer, I would prefer some redundancy for clarity.

21. Lines 298 - 305. If I understand correctly, even if BSF contributes to fungal colonization non-specifically, this seems like a valuable contribution and not necessarily a pitfall. If you agree, I suggest stating this more positively as a last statement at line 304. This statement seems to make sense after the paragraph confirming that the preference is non-specific. The inserted in the text is only a hypothetical example to demonstrate the concept. The text is for you to decide.

22. Line 317. “Effects” is used throughout the proposal. As a friendly reviewer, at each instance, I find myself thinking about what the effects are being referred to, which can interrupt the reading flow if this makes sense. Where possible, to add clarity for reviewers, I suggest stating what the effects are when feasible. In this case, is the effect tracking larval preference? Larval weight?

23. Line 344, I suggest explaining why diet stress may reveal differences. Perhaps it more closely resembles a native environment where competition is more significant. I suggest proactively addressing reviewer questions where possible.

24. Lines 154, 161, 197, 201, 208, 224, 233, 242, 253, 258, 281, 294, 307, 312, 322, 340, 350, 357, 369, 296, 413, 418, 427, and 436. I suggest these types of subheadings (C.2.1, C2.3, etc.) be in plain text (not underlined) to distinguish them from the objectives (C.1, C.2, C.3, etc.) which are underlined. As noted previously, I would remove automatic indents and align all headings to the left margin.

25. Line 364. I suggest clarifying for reviewers whether you are adding the three pathogens to the substrate in combination or separately, which will require three different substrate-pathogen substrates. The latter would triple the experimental samples.

26. Line 397. I suggest an explanation for why a stress-inducing diet is biologically relevant. For example, the sentence might end with the phrase, “which may more accurately reflect natural environments.” or something similar. Of course, the text content is for the PI to determine.

27. Line 403. I suggest stating for reviewers what effect is expected. Do you expect enhanced body size and survival relative to control? The concept is to provide reviewers with the most precise description of the anticipated effect to avoid any possible confusion. The concept of removing ambiguities applies throughout the proposal, as noted here, for example, in comments 12, 16,18, 19, 20, and 22.

28. Lines 405-406. I suggest clarification as to the number of species. Are BSF larvae fed with each fungal species separately or the four species combined? Because there are many experiments involving multiple Diptera and fungi in the experimental plan, readers may become confused or forget what species and their numbers are being proposed. Thus, readers may need regular reminders, such as the number of species, combined or alone, and the list of fungal and Diptera species.

29. Line 413. The reference to previous sections seems a bit confusing. Is the machine learning based on the results of the previous objectives? If I understand correctly, experiments are also proposed in section C.7.3 for incorporation into the model. Are you proposing to use the experimental conditions (rather than results) established in Objectives 1 to 5 or other more specific sections?

30. Figure 3, line 453). The figure shows data from species not discussed in the text. I suggest clarifying this point in the text or figure legends. I also suggest indicating the statistics used in calculating the error bars in the legend.

31. Line 479. At the end of the Preliminary Results, I strongly suggest stating explicitly the significance of the Preliminary Results for the proposal and its success. I would also suggest an ending paragraph restating the impact of the proposal on the field and the broader scientific community. As written, reviewers are left to draw their conclusions, which may differ from those most supportive of the proposal. It is typically better to lead reviewers by stating your conclusions, which they can agree or disagree with. I hope this is helpful.

32. I do not recall in the proposal where Illumina or nanopore sequencing is introduced or required. Only qPCR and Sanger sequencing are described. I suggest clarifying this point for reviewers.

33. Please remember that you will need to add a timeline within the 15-page limit. Also, section C.7.4 may need text as well.