**Report on Research Program proposal (spp. Morchella and symbiotic NFBs in a variety of habitats)**

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**1. Overview**

This is clearly a well-planned program of research assembled by an experienced researcher and accurately situated in the field. The suggestions and comments presented here are by of improving a document that is already strong. With that said, the viewpoint of the proposal is quite close to the research envisaged, and there is thus scope to step back and discuss its significance within the field from a broader perspective, in particular (and as indicated by the stated aims) the wider ecological implications. Also, there are a couple of areas that, while presenting all the relevant information, could perhaps benefit from some revision: for example, the scientific background and the hypothesis discussion. Again, this is looking to further hone an already very solid proposal.

This report will begin by outlining some general observations and suggestions. Then, a section-by-section review of the document is given. Finally, some concluding remarks are offered.

**2. Key observations and suggestions**

***Hypothesis and goals of research***

The hypothesis schema covers a lot of territory but perhaps could be a bit more focused; or if that is inappropriate at this stage, could some indication be offered of ways in which your research could narrow the scope of the hypotheses going forward? As things stand, it could be clearer what would constitute the hypothesis being accepted vs. rejected. A bit of freedom around this area, as it seems is currently offered by the proposal, might be appropriate for an exploratory program like this; on the other hand, a possible lack of investigative focus might be leveled as a criticism. See the analysis of the “**Working hypothesis**” section below for more details.

***Proposed research work planning***

The lab work is comprehensively explained, but one procedural area that is not covered in any detail is the role of the citizen scientist corps. Clearly, this collection phase will be designed for simplicity, but could a protocol be outlined to ensure as much procedural uniformity as possible? Would the citizen scientists perform any additional documentation of the habitats, perhaps by photography, or in regard to variability of sampling location (see first pitfall mentioned in Section VII of the proposal); or would they simply collect the mushrooms? How would the samples be stored and dispatched?

Possible ongoing nature of research: Is the outlined program the complete research plan, or is it envisaged as an initial phase, with further evolution of experiments to depend on initial findings? This would have implications for the planned duration of the research. (Perhaps this distinction is well understood in the research proposal process, and something of a non-issue, but it seems worth raising just in case.)

Is the goal and scope of the research simply to shed light on the role of NFBs throughout the Morchella ecotype/ecosystem range? To put it another way, how do you see your results informing your core question(s) about adaptability?

***Methods***

The proposal shows excellent use of novel methodology and cutting-edge experimental procedures from globally distributed colleagues and institutions.

The general approach is comprehensive and well designed. However, in view of the above observation about ongoing development of the research, perhaps some commentary could be offered for extending the experimental schema in light of results, if appropriate.

***Significance of research***

The connections between anticipated NFB results and ecological flexibility of Morchella could be more thoroughly addressed in several places – see various comments in this report.

Proposal as contribution to literature: Clearly, the study is well positioned in an international network of research into fungi-NFB symbioses. How does the study situate itself in terms of a balance of original discovery vs. supporting existing and ongoing research of others in a replication context? That is, to what extent are you looking to show that the role of Morchella/NFB symbiosis in ecosystems found in Israel fits existing patterns already observed elsewhere, or conversely to leverage the great variability of morels and the significant range of Israeli ecosystems to expand insight into adaptability and perhaps ecosystem evolution in more general contexts?

The bigger picture, both in terms of globally distributed ecosystems and ongoing ecological change, could be explored further, most likely in the scientific background section, especially as harsh and/or disrupted environments are mentioned as a study focus. For example, in Europe, Bonfante (2022; see **5. References**, specific to this report) has addressed the issue of reconnecting mycorrhizal research to natural environments, particularly in view of water scarcity, and has also co-authored a recent research proposal (Eisenhauer et al., 2022; see **5. References**) connecting biodiversity, ecosystem functioning, and biotic interactions.

To extend this example, in terms of fire-stressed environments, Larson et al. (2016; see **5. References**, and also mentioned in Scientific American, September 2, 2016) discussed post-fire morel abundance in Yosemite, California, exploiting morels as a lens for post-wildfire contexts that are becoming more common in western North America, and also Australia. In the latter geographical context, the discovery by Elliott et al. (2014; see **5. References**) of a novel lineage endemic to NSW and Victoria also seems relevant.

***Missing or understated emphases***

Is Morchella just a good experimental subject from a practical standpoint, or could the research extend/point to a more general understanding of fungi-NFB symbioses for other genera? What can be studied in Morchella that generalizes?

Since some of the habitats identified for the study are cultivated, should implications for cultivation techniques be considered? Could something be said about the potential of the study findings to inform ecosystem management generally?

As a general observation, some of the potential gaps identified here might be more appropriately address at the publication stage, once the results are in hand – particularly the ecological aspect; although since this relates closely to the stated aim of the research, it might be given more attention here.

***Suggestions for improvement***

• Scientific background section is strong but perhaps shows a few gaps, such as ecosystem health and resilience – see comments below to that section

• Presentation and discussion of hypothesis – see above general comment as well as comments below to that section

• Aims/goals are stated in a clear but rather spare way – these might benefit from drawing a bit more of a line from the concrete experimental process to leveraging results to inform the stated goal, even if the line is conjectural at this stage. To be more direct: how will the results be used to answer the question posed in the stated study aims?

• Citizen science component – see above comment

• Position research in wider context a bit more thoroughly – see above comments

**3. Specific section-by-section comments**

***I. Scientific background***

Most of the key info is present and all appears accurate, but a “shorthand” quality to delivery perhaps leaves a few gaps in the big picture. For instance:

• Why is “endophytic” an unusual option – are ectophytic Morchella more usual? This may or may not be critical to the adaptability question, but the reader may be left wondering.

• More generally, the background info could be more clearly related to the goal of the research – it is reasonably clear why morels are a good choice in terms of their variability, but how will that translate for instance into leveraging the study results toward ecosystem health? Could more be said about the latter in this section?

• “A more unique phenomenon of N fixation in the fruiting body tissue itself has recently been reported” – how/why is this relevant? Will you be looking at NFB presence in fruiting bodies vs mycelium in the experimental procedure?

• The rationale for using nitrogen-free vs. nitrogen-enriched media would be obvious to anyone acquainted with the field, but for a more general reviewer some words on the mechanics of nitrogen cycling within fungi and over generations might be helpful. For instance, the significance of “[obtaining] successful growth and sclerotia formation of one examined isolate of Morchella sp. grown on agar N-free medium even in the third generation” could be spelled out.

• Would some discussion of epigenetic factors in the context of ecotypes be appropriate? If so, this would seem central to the growth and ecosystem interplay of Morchella ecotypes in different locales.

***II. Research Objectives and Expected Significance***

The early highlighting of adaptation to harsh environments and plasticity to nutritionally different habitats is certainly helpful in contextualizing the proposed research and its significance. Stated objectives 1 through 4 then drop to a very specific view of the research. There is perhaps scope, between these two levels, to expand on how the specific findings could address the overall significance of the project. See also under **Suggestions for improvement** (above).

***III. Detailed Description***

*Working hypothesis*

This section addresses a complex biological/ecological reality – one which the research is intended to explore – and as such it is tricky to frame a conventional hypothesis. The sentence (end of second para) that actually does so, however, could be paraphrased as “We hypothesize that our experiments will yield concrete data about NFBs and their relation to isolate species vs habitat” – to which the naïve answer is, obviously the experiments will accomplish this, if well designed (which they are); but what then? The proposal is perhaps caught in the dilemma of framing open-ended research within a paradigm that has traditionally required closed answers (classically, is the hypothesis rejected or accepted?). And prior to this sentence, at least two alternative ways of viewing the hypothesis are presented, leaving the reader to reconcile these slightly different readings of this aspect of the study.

An alternative approach might be to set up a positive hypothesis that the adaptability of the Morchella genus and the nature of its NFB symbioses allows for a range of adaptability to harsh and/or nutritionally varied environments. While not over-anticipating the specific results, this approach might connect the results in a testable way to the aims of the study, particularly – as stated in the final sentence of this section – the ecological flexibility.

The above commentary and suggestions are not intended as an overhaul of the thinking behind the study, but rather as an option to present its hypothesis in a more structured way. As mentioned, the complex nature of the phenomena studied make this a challenging aspect of the proposal with, perhaps, no one ideal resolution.

*Research design*

This seems clear and is well described. The only comment here is that scope is clearly left to interpret the results in light of the stated study aim – an aspect which is not described here, and is only lightly touched on in the rest of the proposal.

***IV. Methods***

As a general comment, the extent to which the study is making use of globally distributed resources – for instance, INRA in Nancy, France for isotopic determination, genomic sequencing at a plurality of American facilities, a 15N enriched assay technique developed by a Mexican team (Ruiz-Herrera et al., 2015) – is indicative of a truly collaborative approach. While this is now presumably standard for ecologically sited research, it is definitely a plus for proposals of this kind.

That said, a few queries and suggestions do arise. In section 1, how will the citizen-collected fresh morels be integrated into the study? In section 2, the description of 15N:14N ratios and their significance is perhaps a bit cursory – again, more is unnecessary for those already versed in these techniques, but might be helpful for a more general reader. Last, the statistical analysis section is very briefly described and as such perhaps has a “black box” quality – which statistics for the isolates, as derived from the experimental results, are being subjected to ANOVA and the associated Fisher’s test?

***V. Preliminary Results***

No specific comments

***VI. Researcher’s Resources***

No specific comments

***VII. Expected Results and Pitfalls***

The initial statement in this section, on expected results, goes some way toward establishing the linkage (identified as an issue elsewhere in this report) between the experimental results and the ultimate aims of the study. However, it occurs rather late in the proposal and is not provided with much detail or explanation. For the pitfalls, could there be a citizen science solution to the first pitfall identified, regarding mushroom discovery sites vs. nutritional factors used by fungal mycelium? Good practical solutions are offered for pitfalls 2 and 4, and to some extent for pitfall 3, which sounds trickier. Finally, the additional research mentioned in pitfall 5 has been very much left to the future to address: could a couple of specific ideas be mentioned at this stage?

**4. Conclusions**

The main impression is of a proposal that is closely and appropriately focused on the mechanics of the study, while also maintaining a broader view of its significance – specifically, applicability to challenging and disrupted environments in a global context where such environments are undergoing rapid change. Beyond identifying some possible weak points, the general recommendation of this report would be to look at joining some dots and filling in some gaps to establish a better connection between these two levels of focus. Areas in which this could be addressed, as presented in **Suggestions for improvement** above, include adding to the scientific background, revising the hypothesis section, connecting the research design and scope of results more clearly to the overarching aims, clarifying aspects of the citizen science element, and contextualizing the study more fully in the field of research and the ecological concerns which drive it. This might well be unnecessary for the proposal’s approval, but it does manifest as an opportunity to enhance it.

**5. References**

1. Bonfante, P. (2022). How to reconnect mycorrhizal research with natural environments. *Environmental Microbiology*, 2022, 1–5.
2. Eisenhauer, N., Bonfante, P., Buscot, F., & Cesarz, S. (2022). Biotic interactions as mediators of context-dependent biodiversity ecosystem functioning relationships. *Research Ideas and Outcomes*, *8*, e85873.
3. Elliott, T. F., Bougher, N. L., O’Donnell, K., & Trappe, J. M. (2014). Morchella australiana sp. nov., an apparent Australian endemic from New South Wales and Victoria. *Mycologia*, *106*(1), 113–118.
4. Larson, A. J., Cansler, C. A., Cowdery, S. E., Hiebert, S., Furniss, T. J., Swanson, M. E., & Lutz, J. A. (2016). Post-fire morel (Morchella) mushroom abundance, spatial structure, and harvest sustainability. *Forest Ecology and Management*, *377*, 16–25.