Systemic Primer Bias in Studying Soil Fungi in an Artificial Rainforest

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Central question: Is there systemic bias in the groups of soil fungi targeted by ITS2 reverse primers in Biosphere 2 rainforests?

Background

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Biosphere 2 is a research facility located north of Tucson, AZ. It is the largest enclosed ecosystem ever created, and presents a valuable opportunity for studying the workings of ecosystems at a much finer level of detail and with greater control than is possible in the field. However, the diversity of soil flora in the ecosystems here was not specifically engineered, and is not well understood.

Biosphere 2's soil microbiota were introduced incidentally through flora, soil, and phoresy. This means that there could be key differences between the fungal diversity in this soil and the diversity present in habitats Biosphere 2 is designed to mimic.

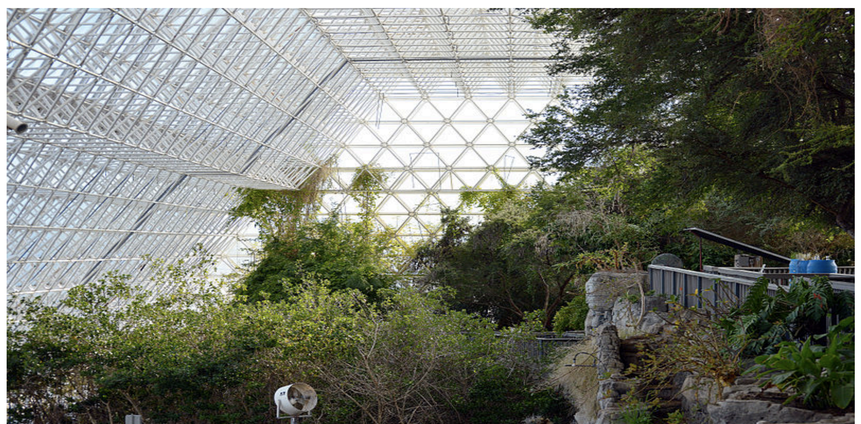
In order to serve as an effective model for rainforest ecosystems, every dimension of the diversity and ecology of the soil microbiota must be understood. Soil flora are the primary decomposers in the food chain, and and involved with many symbiotic ecosystem processes. Thus, this study aims to rectify this lack of understand to aid future research.

Project Goals

The aim of this study is to reveal the diversity of soil fungi that are present in the soil of the rainforest biome of Biosphere 2 using two different ITS primer sets, while additionally documenting any primer bias present between the primer sets.

Previous studies have aimed to examine Biosphere 2's fungal diversity, but the lack of consistency in the results of primers has been an issue. As most fungal processes are essentially invisible to the naked eye, DNA primers that target Internal Transcribed Spacer (ITS) regions of fungal DNA are important for understanding fungal diversity. These primers make copies of the selected regions, allowing researchers to identify and quantify the species present in an ecosystem.

In order to keep data standard and interchangeable, it is important that research be conducted with primers that are as species-inclusive, accurate, and comprehensive as possible. If significant differences are found, researchers must document those differences, so future efforts at primer creation can succeed.



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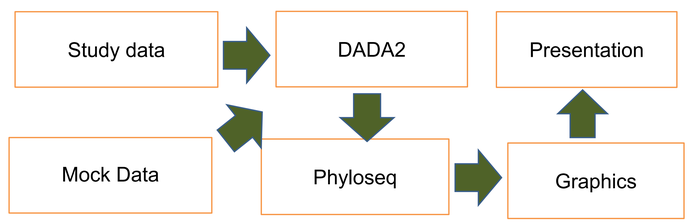
Data sourcing and processing

ITS data collected from the Biosphere 2 rainforest environment during the dry season by Young *et al.* 2019 will be used for this analysis.

In order to serve as a control, an artificially created mock fungal database of known species created for testing primer effectiveness will be sourced from U'Ren *et al.* 2019. This mock community was sequenced using both primer sets, the standardized ITS2 reverse primer, along with a recently-made ITS2 primer created by the Dept. of Energy Joint Genome Institute.

Taxonomic assignment will be made by processing the raw data using the DADA2 and phyloseq data packages in R. The sequences will be matched to existing fungal databases like UNITE and GenBank via BLAST to illustrate the differences in real vs. detected diversity between the primer sets.

Analysis Workflow



Citations

U'Ren, J. M., Lutzoni, F., Miadlikowska, J., Zimmerman, N. B., Carbone, I., May, G., & Arnold, A. E. (2019). *Host availability drives distributions of fungal endophytes in the imperilled boreal realm. Nature Ecology & Evolution.* doi:10.1038/s41559-019-0975-2

Young, Juliana, Sengupta, Aditi, U'ren, Jana, van Haren, Joost L. M., & Meredith, Laura K. (2019). *Microbial drivers of spatial heterogeneity of nitrous oxide pulse dynamics following drought in an experimental tropical forest. UA Honors College.*