Quantifying Bacteria in a Mycorrhizal Birth/Death Study using Quantitative PCR

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Introduction

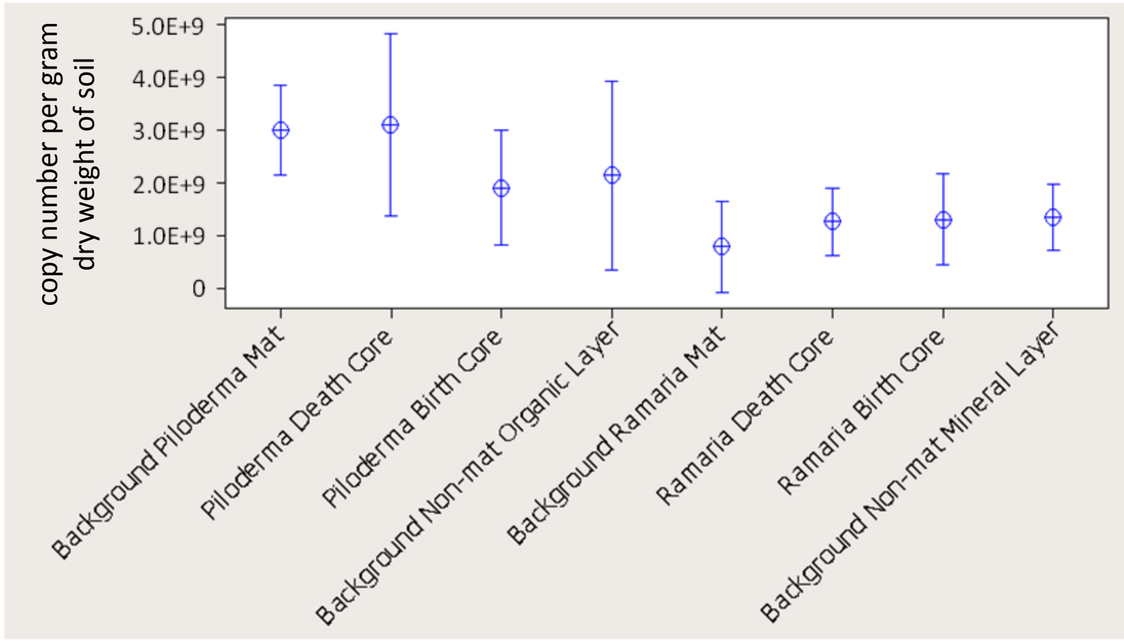
Mycorrhizal fungi form symbiotic relationships with plants. They enhance water and mineral absorption for plants, and in return plants provide photosynthates to the fungi. Mycorrhizae are especially important in forest ecosystems. In fact, many tree species are stunted when grown without mycorrhizae[1]. Some forest trees have mycorrhizal associations with a diverse collection of fungi that span many phyla, especially Douglas-fir.

In the Oregon Cascades, *Piloderma* and *Ramaria* are two important mycorrhizal fungal genera that form hyphal mats in the organic and mineral soil horizons, respectively. In some cases, the fungal mat may be killed due to natural or human causes. It can take a long time for mycorrhizae to reestablish in disturbed soil, and because they are such a crucial part of forest ecosystems, it is important to understand how mycorrhizal fungi recolonize soil after a disturbance. This experiment is part of a larger study that investigates how quickly *Piloderma* and *Ramaria* recolonize disturbed soil, which other fungal species recolonize the soil, and how the soil microbial populations change in response to disturbance over time. This portion of the experiment uses quantitative PCR (qPCR) to show how the number of bacteria in the soil is affected by a disturbance in the fungal mat.

Results

Piloderma

There was no statistical difference between the Background Non-mat treatment or any of the other treatments. This is consistent with a previous study showing that bacterial species were different between *Piloderma* mat and non-mat soils, but that overall bacterial population size was not statistically different[2]. However, there was a potentially significant difference between the *Piloderma* Birth Core and the *Piloderma* Background Mat (P-value = 0.062). Only five of seven replicates are currently complete (three for *Ramaria* Birth Core), so the final data may support or refute a difference. The potential decrease in bacteria could be explained as an artifact of coring disturbance.



Ramaria

There was no statistical difference between any of the *Ramaria* treatments. This indicates that like *Piloderma*, *Ramaria* mats do not affect the bacterial population size.

Piloderma vs. Ramaria

Comparing the Background Piloderma Mat to the Background Ramaria mat showed a significant difference (P-value = .001). This could be because *Piloderma* occurs in the organic layer, where there are more carbon and nutrients available to bacteria. Also, the hydrophobic nature of *Ramaria* mats may reduce available water, making it harder for bacteria to survive[3]. Lastly, *Ramaria* can produce significant amounts of oxalic acid, reducing pH, making it harder for bacteria to survive[4].

Statistically significant differences were defined as having a P-value < 0.05.

qPCR

- DNA amplification is tracked using a dsDNA-specific fluorescent dye (SYBR® Green). Fluorescence is plotted against the number of amplification cycles. (Figure 1)
- Intersection of amplification plots with a fluorescence threshold creates Ct values. Ct values closely correlate to the number of target sequences that were present when the reaction began ('copy number'). Samples with a higher starting copy number amplify more quickly.
- Ct values of known standards are plotted against copy number to create a line of best fit. The Ct values of unknown samples are interpolated to derive absolute quantitation. (Figure 2)
- DNA was extracted using the PowerSoil® DNA Isolation Kit by Mo Bio Laboratories Inc. DNA was amplified using the Brilliant SYBR® Green QPCR Master Mix. An ABI PRISM® Fast Sequence Detection System was used.

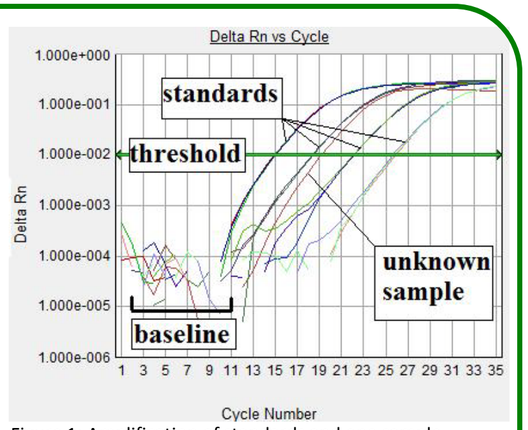


Figure 1: Amplification of standards and one sample

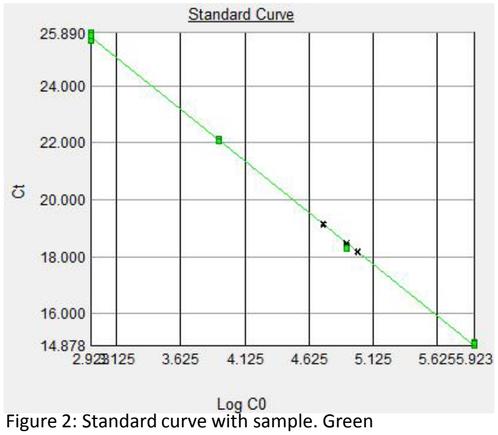


Figure 2: Standard curve with sample. Green squares are standards, black x-marks are samples.

Field Experiment Design

The study took place at various stands of old-growth Douglas Fir in the HJ Andrews Research Forest.

Birth Cores: A soil core was taken from a non-mat area and placed in the middle of a mat area. Over time the mat colonizes the core. This simulates a soil being recolonized after a disturbance.

Death Cores: A soil core was taken from a mat area, and placed in a non mat area, and is permanently encapsulated in a PVC pipe to prevent roots from growing into the core. Starved for host tree photosynthate, the fungus dies.

Control Sections: Undisturbed mat and non-mat areas were also sampled as controls.

Each of these treatment types was performed on *Piloderma* and *Ramaria*, for a total of seven treatments. The experiment was replicated across seven sites.

Birth

Death

References

1. Campbell, Neil A. Biology. Redwood City, CA: Benjamin/Cummings Pub., 1990. Print.
2. Kluber, Laurel A., Jane E. Smith, and David D. Myrold. "Distinctive Fungal and Bacterial Communities Are Associated with Mats Formed by Ectomycorrhizal Fungi." *Soil Biology and Biochemistry* (2011): 1042-050.
3. Myrold, David D. Personal communication. August 29 2011.
4. Kluber, Laurel A. et al. "Ectomycorrhizal Mats Alter Forest Soil Biogeochemistry." *Soil Biology and Biochemistry* (2010): 1607-613.