Correlating Fungal Community to Nearby Soil pH in Northern New Jersey

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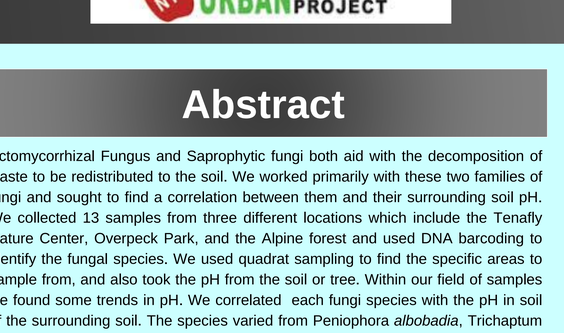
Abstract

Ectomycorrhizal Fungus and Saprophytic fungi both aid with the decomposition of waste to be redistributed to the soil. We worked primarily with these two families of fungi and sought to find a correlation between them and their surrounding soil pH. We collected 13 samples from three different locations which include the Tenafly Nature Center, Overpeck Park, and the Alpine forest and used DNA barcoding to identify the fungal species. We used quadrat sampling to find the specific areas to sample from, and also took the pH from the soil or tree. Within our field of samples we found some trends in pH. We correlated each fungi species with the pH in soil of the surrounding soil. The species varied from Peniophora *albobadia*, Trichaptum *biforme*, Stereum *sp*, Ischnoderma *resinosum*, Ganoderma *applanatum,* and Piptoporus *betulinus*. The streum sp, for example had a consistent surrounding pH of around 6.6 no matter where they were sampled from. The mean surrounding soil pH was 6.87, while the fungi's optimal growth pH is from 5.8 - 8.3. This slight difference in observed pH maybe due to the fact that the samples were taken in the winter when there was an abundant amount of snow on the ground. From this experience we learned the optimal growth condition of fungi and how these results of certain experiments can vary greatly from season and environment.

Introduction

Fungi

- Play a key role in decomposition
- Transform and regenerate most soil nutrients and minerals that limit plant productivity
- **Ectomycorrhizal fungi** form symbiotic relationship with living plants; This type of fungi colonize *on the root system of a host plant,* providing increased water and nutrient absorption while the plant provides the fungi with carbohydrates from photosynthesis.
- **Saprophytic fungi** are the largest group of fungi. They grow *on dead organic matter* (e.g. dead trees), decomposition it and cycling the carbon.



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a) Ectomycorrhizal Fungus | b) Saprophytic Fungus

Figure 1. **Pictures of a) Ectomycorrhizal Fungus and b) Saprophytic Fungus.**

Soil pH = -log [H_3O^+]

- Low pH (more acidic) & High pH (more basic)
- Strongly influences growth of fungi and plants
- Optimal pH for fungi is neutral (around 6 to 8)
- Controls carbon and nutrient availability/ solubility.

Research Goals

Hypothesis: We hypothesize that collecting ectomycorrhizal and saprophytic fungi in the regions of the Tenafly Nature center, Central Park, and Overpeck Park, we could identify the relationship between certain types of fungi to pH levels in the soil within these environments.

Methods

●Sampling

Table 1. **Overview of sampling locations**

Sample numbers	Location
ZBT-001 to ZBT-004	Tenafly Nature Center (40.9246N, 73.945W)
ZBT-005 to ZBT-008	Overpeck Park (40.8654N, 74.00W)
ZBT-009 to ZBT-013	Alpine Forest (40.9559N, 73.9312W)

- We subdivided each location into 20 different divisions of acre plots and randomly selected one of the plots to sample (collection date: Jan 2018).
- We took 4-5 samples of saprophytic and ectomycorrhizal fungi from each location
- After we took each sample we measured the pH of the surrounding soil using a pH meter.
- We placed each sample in a sealed bag and label them.

●DNA Extraction & PCR Amplification

- We extracted 10-20 mg of tissue sample from each specimen and ground them up in 300 mL of lysis solution
- The ground up samples were then incubated and centrifuged
- After the samples were centrifuged, they were put in fresh tubes and mixed with silica resin.
- The samples were then incubated and centrifuged again
- Then, wash buffer was added, samples were centrifuged, and wash buffers were removed.
- We added 100 mL of distilled water to the tubes and mixed them with a pipette. We then incubated it for five minutes.
- We started the PCR by taking 2 mL of the available DNA and putting them in PCR tubes.
- We added fungal *ITS (internal transcribed sequence)* primers to the DNA to reproduce a 600-700 bp section of the DNA strand.

- **Gel Electrophoresis & Gene Sequencing**
  - The DNA strands were then run through the gel electrophoresis to make sure the PCR was successful.
  - The gene sequencing was done by GeneWiz
  - After the sequences were downloaded, the species were determined using a BLAST search and the DNA subway platform.

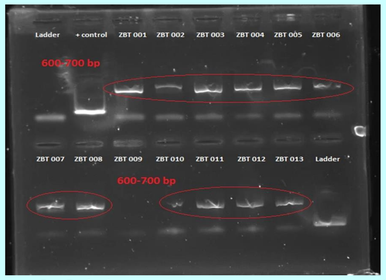

Figure 2. **Gel electrophoresis showing successful PCR amplification of samples ZBT-001 to ZBT-008 and ZBT-010 to ZBT-013.**

Results

- The PCR was successful for twelve of our thirteen samples
- Sample nine was not successfully amplified by the PCR
- Samples two and ten, though present on the gel, were not successfully DNA sequenced.

Table 2. **Summary of species identified in order of increasing pH, with (S) indicating saprophytic fungi & (E) indicating ectomycorrhizal fungi**

pH	Species	Samples	Location
5.24	-	ZBT-002	Tenafly Nature Center
5.82	*Peniophora albobadia* (E)	ZBT-008	Overpeck Park
6.45	*Trichaptum biforme* (S)	ZBT-005	Tenafly Nature Center
6.52	*Stereum sp.* (S)	ZBT-007	Overpeck Park
6.57	*Stereum sp.*(S)	ZBT-004	Tenafly Nature Center
6.61	*Stereum sp.*(S)	ZBT-012	Alpine forest
6.72	-	ZBT-010	Alpine forest
6.98	*Ischnoderma resinosum* (S)	ZBT-011	Alpine forest
7.03	*Ganoderma applanatum* (E)	ZBT-013	Alpine forest
7.25	*Ischnoderma resinosum* (S)	ZBT-003	Tenafly Nature Center
7.26	*Piptoporus betulinus* (E)	ZBT-006	Overpeck Park
8.24	*Stereum sp.*(S)	ZBT-001	Tenafly Nature Center

- The sampled species varied from *Peniophora albobadia, Trichaptum biforme, Stereum sp, Ischnoderma resinosum, Ganoderma applanatum*, and *Piptoporus betulinus*.
- The mean pH of the surrounding soil was 6.87
- The samples containing *Stereum sp* had a consistent surrounding pH of about 6.6 (exception of ZBT-001) even though they were from different sampling locations.

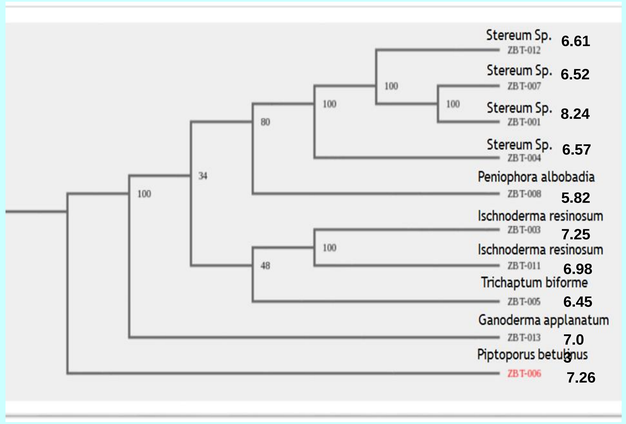

Figure 3. **Phylogenetic tree showing the evolutionary relationship between the species found in each of the samples.**

Discussion

- pH ranges varied for each location:
  - 5.24 – 8.24 (3.00 pH range) for Tenafly NC
  - 5.82 – 7.26 (1.44 pH range) for Overpeck Park
  - 6.61 – 7.03 (0.42 pH range) for Alpine forest
- We believe that the pH reading of the soil for ZBT-001 of 8.24 may have been erroneously high since it is so different from other mean *Stereum sp.* pH
- The phylogenetic tree shows that there does not seem to be any clear relationship between pH and evolutionary relatedness, but each species has an optimal pH range, and our results seem to match what is expected.
  - Ectomycorrhizal fungi have an optimal growth rate at pHs that are close to neutral (6-7.5)
  - Saprophytic fungi tend to prefer pHs that range from acidic to neutral (5-7).
- One limitation of our study was that it was done in the winter after a recent snowfall; the melting snow diluted the soil and brought he pH level closer to 7 (neutral).

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