The Relationship Between Soil pH and Fungal Biodiversity on the Leatherstocking Trail

Brittney Benjamin, Jacob Egelberg, Lusangelis Ramos, Rachel Rivera

New Rochelle High School, New Rochelle NY

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Abstract

Approximately 2.82 million tons of sulfur dioxide (SO_2) is emitted annually into the atmosphere by the United States alone. At low temperatures, SO_2 condenses in the atmosphere and reacts to produce acid rain, which results in soil acidification and ion depletion. Previous research investigating the influence of acid rain and acidified soil on fungal biodiversity is conflicting. The current study contributes to this body of scientific knowledge by establishing the correlation between fungal biodiversity and soil pH levels on the Leatherstocking Trail in New Rochelle (NY). Five locations along the trail were randomly selected, at each of which soil pH was measured and fungi were sampled. To confirm the species of each fungal sample, tissue underwent DNA isolation, PCR amplification with the Internal Transcription Spacer (ITS1F and ITS4) primers, and DNA Subway barcoding analysis. Following species identification, soil pH was correlated to fungal diversity indices representing biodiversity. The resulting coefficient of determination (R^2=0.03) demonstrated that there is a negligible association between fungal biodiversity and soil pH levels.

Introduction

Existing literature is conflicted regarding the association between fungal biodiversity and soil pH (Rousk et al., 2009; Rousk et al., 2010). The current study aimed to address this conflict by determining the correlation between soil pH and fungal diversity on the Leatherstocking Trail in New Rochelle (NY). Considering the vital ecological role fungi play as environmental decomposers, fungal biodiversity is a valuable metric of ecosystem stability. Understanding the impact of acidified soil on fungal diversity is an important step towards understanding the impact of acid rain on long-term ecological homeostasis.

Primary Question

Is there a significant association between soil pH and fungal biodiversity on the Leatherstocking Trail?

Hypothesis

Soil with lower pH values will show decreased fungal biodiversity due to depleted soil ion concentrations.

Materials & Methods

Five locations along the local Leatherstocking Trail were randomly selected (Figure 1) using a random number generator and soil pH measurements and fungal samples were collected (Figure 2) at each location. Each sample was assigned an identification number, classified with a taxonomic key, and stored for genetic analysis (Figure 3). DNA was isolated from each fungal sample using the Cold Spring Harbor Laboratory Rapid DNA Isolation protocol (DNA Learning Center Barcoding 101) before storage at -20°C. Polymerase chain reactions (PCR) involving primers ITS1F and ITS4 was used to amplify the ITS gene from each fungal sample's DNA and gel electrophoresis confirmed successful amplification (Figure 4). Successfully amplified DNA samples were digitally documented and correlated to specimen pictures on the DNA Learning Center Barcode Sample Database prior to being sent to GeneWiz for sequencing. GeneWiz uploaded the DNA sequences to DNA Subway and the specific genus and species of each sample was identified with nucleotide BLAST searches and bit score comparisons (Figure 5). Fungal diversity at each pH level was quantified using a Diversity Index (Figure 6). Regression analysis was performed to correlate each Diversity Index with the pH of its corresponding soil (Figure 6). A graph was created to show the relationship between soil pH and diversity indexes, and R^2 was used to assess the significance of the association between soil pH fungal biodiversity.

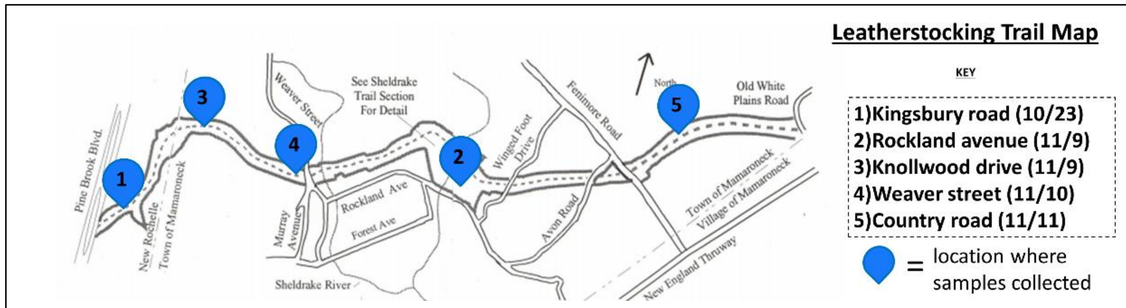

Figure 1. Five locations along the Leatherstocking Trail were randomly selected for sampling.

Figure 2. Example fungi sample.

Sample #	Location	Sample Tag	Soil pH
1	Kingsbury	KDY-001	6
2	Kingsbury	KDY-002	6
3	Kingsbury	KDY-003	6
4	Kingsbury	KDY-004	6
5	Kingsbury	KDY-005	6
6	Kingsbury	KDY-006	6
7	Kingsbury	KDY-007	6
8	Rockland	KDY-008	5
9	Rockland	KDY-009	5
10	Rockland	KDY-010	5
11	Rockland	KDY-011	5
12	Rockland	KDY-012	5
13	Rockland	KDY-013	5
14	Rockland	KDY-014	5
15	Rockland	KDY-015	5
16	Knollwood	KDY-016	6
17	Knollwood	KDY-017	6
18	Knollwood	KDY-018	6
19	Knollwood	KDY-019	6
20	Weaver	KDY-020	7
21	Weaver	KDY-021	7
22	Weaver	KDY-022	7
23	Weaver	KDY-023	7
24	Country	KDY-024	5
25	Country	KDY-025	5
26	Country	KDY-026	5

Figure 3. Sample identification numbers.

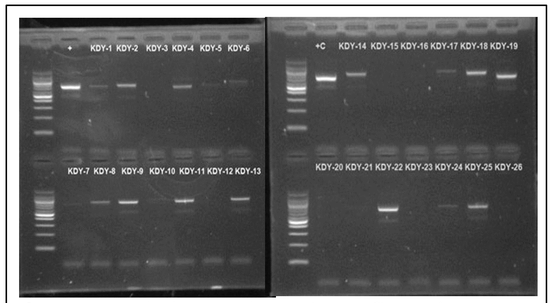

Figure 4. Gel electrophoresis confirmed successful amplification.

Results

$$Diversity\ Index = \frac{\#\ of\ Unique\ Fungal\ Species}{Total\ \#\ of\ Fungal\ Species}$$

pH	# of Samples	# Unique	Diversity Index
5	7	5	0.714285714
6	9	5	0.555555556
7	4	3	0.75
ACTUAL DATA			

Figure 6. Diversity Indices for each pH.

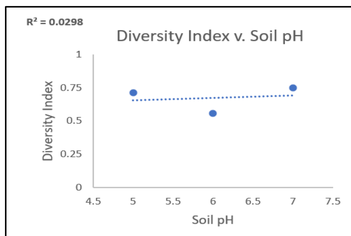

Figure 7. Regression analysis.

Soil pH	Location	Date	Sample Tag	Predicted Species	PCR Success	Species	Bit Score
5	Rockland	11/9	KDY-008	*Tylopilus felleus*	TRUE	*Russula atropurpurea*	1247
5	Rockland	11/9	KDY-009	*Marasmius ?*	TRUE	*Mycena filopes*	1305
5	Rockland	11/9	KDY-010	*Marasmius ?*	FALSE		
5	Rockland	11/9	KDY-011	*Marasmius ?*	TRUE	*Mycena arcangeliana*	1131
5	Rockland	11/9	KDY-012	*Marasmius ?*	FALSE		
5	Rockland	11/9	KDY-013	*Boletus longicurvipes*	TRUE	*Mycena arcangeliana*	1131
5	Rockland	11/9	KDY-014	*Marasmius ?*	TRUE	*Mycena filopes*	1305
5	Rockland	11/9	KDY-015	*Marasmius ?*	FALSE		
5	Country	11/11	KDY-024	*Leptonia basidissima*	TRUE	*Entoloma infula*	1032
5	Country	11/11	KDY-025	*Inocybe tubarioides*	TRUE	*Hypholoma fasciculare*	1254
5	Country	11/11	KDY-026	*Entoloma subgenus Leptonia*	FALSE		
6	Kingsbury	10/23	KDY-001	*Boletus edulis*	TRUE	*Sistotrema brinkmannii*	1131
6	Kingsbury	10/23	KDY-002	*Agrocybe pediades*	TRUE	*Psathyrella prona*	1236
6	Kingsbury	10/23	KDY-003	*Morchella esculenta*	FALSE		
6	Kingsbury	10/23	KDY-004	unknown 1	TRUE	*Sistotrema brinkmannii*	1135
6	Kingsbury	10/23	KDY-005	*Amanita brunnescens*	TRUE	*Sistotrema brinkmannii*	1135
6	Kingsbury	10/23	KDY-006	*Amanita brunnescens*	TRUE	*Leucoagaricus griseodiscus*	1063
6	Kingsbury	10/23	KDY-007	*Clitocybe nuda*	TRUE	*Sistotrema brinkmannii*	1103
6	Knollwood	11/10	KDY-016	*Clitocybe nuda*	FALSE		
6	Knollwood	11/10	KDY-017	*Marasmius ?*	TRUE	*Mycena rebaudengoi*	812
6	Knollwood	11/10	KDY-018	*Marasmius ?*	TRUE	*Mycena rebaudengoi*	812
6	Knollwood	11/10	KDY-019	unknown 2	TRUE	*Coprinellus micaceus*	1232
7	Weaver	11/10	KDY-020	*Marasmius ?*	FALSE	*Marasmius ?*	N/A
7	Weaver	11/10	KDY-021	unknown 3	FALSE	unknown 3	N/A
7	Weaver	11/10	KDY-022	unknown 4	TRUE	*Coprinellus micaceus*	1236
7	Weaver	11/10	KDY-023	*Marasmius ?*	FALSE	*Marasmius ?*	N/A

Figure 5. The identification of fungal species with sample data stratified by pH.

Discussion

Regression analysis yielded an R^2 value of 0.03, which suggests that only 3% of the variance in the species diversity indexes is explainable by soil pH. Correlations are considered strong when R^2 falls between 0.5 and 1, and weak to negligible when below 0.5 (DePaul University, N.D.). The findings of this study indicate that the association between soil pH and fungal biodiversity is negligible. Our finding that only 3% of variation in fungal diversity is explainable by soil pH encourages optimism about the impact of acid rain on ecological homeostasis. While plant and animal survivability may be significantly disrupted by acid rain, the capability of fungal communities to withstand changes in soil pH provide environments the ability to recover from ecological disruption. Future research should seek to further investigate the ability of ecological communities to rebound from exposure to acid rain, and the role fungi likely play in this process.

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