

Microbial Community Mapping of Long Island's Pine Barren Forest Soil

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August 14, 2008

Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Faculty and Student Team (FaST) Program under the direction of Dr. Timothy Green in the Environmental and Waste Management Division at Brookhaven National Laboratory.

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ABSTRACT

Microbial Community Mapping from Long Island's Pine Barren Forest Soil. NYESHA SMITH, JEFFERY AMBROSE, AND MURTY S. KAMBHAMPATI (Southern University at New Orleans, LA 70126) VISHAL SHAH AND FRED RISPOLI (Dowling College, Oakdale, NY 11769) TIMOTHY GREEN (Brookhaven National Laboratory, Upton, NY 11973).

Management of any ecosystem requires the information on the flora and fauna present in the environment. The current management plans for terrestrial ecosystems are mainly based on the macrofauna. While microorganisms are very critical for maintaining the balance in an ecosystem, no information is available on the types and behavior of microorganisms in the soil of the Long Island Pine Barren Forest. Thus, the existing management plan for an ecosystem does not consider the influence of the actions on the microbial diversity. In the first study of its kind, we mapped the Long Island Pine Barren Forest (LIPBF) soils based on their microbial, community-level, physiological profile (CLPP). Soil samples were collected from different parts of the forest and upon preparation of the inoculum, BIOLOG EcoPlates were inoculated. The clustering analysis based on color intensities illustrates that the entire LIBPF can be divided into four different clusters at every horizon. However, the physiological response of microbial community at each horizon and cluster was different. No correlation between sampling sites and the physiological profile was obtained based on vegetation or geographical location. In conclusion, comparing the physiological profile of the microbial community from each horizon, one can make a list of substrates that are utilized more throughout the LIPBF. However, further studies need to be carried out to test this hypothesis in the future.

1. INTRODUCTION

Although microorganisms are too small to be seen by the naked eye, their importance cannot be ignored. Microorganisms are the foundation of the biosphere—both from an evolutionary and ecological perspective. Microorganisms, the first organisms on earth, have lived on this planet for a period of at least 3.7 billion years of the 4.6 billion-year existence of the earth. Microorganisms were living inhabitants for more than 3.0 billion years before the appearance of plants and animals. Not only did plants and animals evolve rather recently in earth's history, but they evolved from microbial ancestors [1]. The earth's biosphere is largely shaped by geochemical activities of microorganisms that have provided conditions both for the evolution of plants and animals and for the continuation of all life on earth. Therefore, it is not surprising that the diversity of microorganisms—from genetic, metabolic, and physiological aspects—is far greater than that found in plants and animals [2].

Soil, a dynamic living matrix essential to the terrestrial ecosystem, is a critical resource not only to agricultural production and food security, but also to the maintenance of most life processes. Currently, management plans that have been devised to preserve and maintain various terrestrial ecosystems take into consideration all macro-organisms present including plants, animals, bird, and insects. However, microorganisms, which play various important roles in the ecosystem, are not given the importance that is needed to understand the proper functioning of the ecosystem. Microbes are vital in the process of decomposition and recycling of elements in soil. Therefore, changes in microbial communities are often a precursor to the changes in the health and viability of the environment as a whole. Good soil quality within natural or managed ecosystems is essential to sustain plant and animal production, maintain or enhance water and air quality, and support human health and habitation. Thus, the sustained use

of the earth's land resources and thereby plant, animal, and human health is dependent upon maintaining the health of the microbial biota that provide critical processes and ecosystem services.

However, in contrast to plants and animals, the diversity of the microbial world is largely unknown. The task is complicated for microbiologists since the subjects of the census are not visible to the naked eye or easily differentiated morphologically. Currently, approximately 4,000 bacterial species and 75,000 fungal species have been identified by microbiologists—less than the 1% of the total one million bacterial species and 5% of the total one and a half million fungal species estimated to exist today. These numbers are in sharp contrast to plants and animals where 85% to 90% of the total species have been identified [3]. The recent surge of research in molecular microbial ecology provides compelling evidence supporting the claim of the existence of many unidentified new microorganisms in the environment [4].

Since microorganisms play an essential role in soil geology, hydrology, and ecology, knowledge about microbial community structure and composition is important to improve our conceptual and predictive understanding of soil ecosystem processes, functions, and management in the region. Common soil and aquatic habitats are largely studied for microbial life present as they are essential for human well-being [5]. Efforts are underway in many laboratories across the United States of America, European countries, and other regions of the world to elucidate the microbial flora in agricultural and tropical forest soils and water bodies such as lakes, rivers, and oceans. However, no detailed report exists on understanding the microbial flora present in the soil of Pine Barren Forests in the United States and evaluating their role in ecological cycles.

The vegetation known as the Pine Barrens is scattered throughout northeastern United States and beyond. Compared to other vegetation, the Pine Barrens is a unique region owing to the sandy, acidic, nutrient-poor soil made up largely of coarse sands and gravels deposited by ancient glaciers. The term “barrens” was coined by early settlers who unsuccessfully tried to raise their traditional vegetables and field crops in the sandy, acid soils of these regions [6]. Today, we know these areas are not really barren, for many forms of plant life such as members of the pine family (Jack Pine, Red Pine, Pitch Pine), the beech family (Blackjack Oak and Scrub Oak) and the heath family (huckle berries, blueberries, cranberries) do well in the highly acidic sandy soils [7]. However, these areas are still called barrens, a term that is used consistently in both popular and scientific references to these areas. A few characteristics of Pine Barrens soil are:

- a. The soil of the Pine Barrens is acidic because of microbial activity on the plant litter. Pine and Oak trees drop litter composed primarily of needles. This litter is not readily digested by most microorganisms, decomposes slowly, and accumulates on the soil surface. The primary decomposers of the Pine Barrens litter are fungal organisms. Their decomposition by-products are strongly acidic and this makes the soil of Pine Barrens acidic, ranging from pH 4.0 to 4.5.
- b. Because of the acidic nature, the soil in the Pine Barrens contains high concentration of iron and aluminum. The cation exchange capacities are of extremely low order with a low base saturation [8].
- c. Fires are common in Pine Barrens, which are necessary to maintain these regions as it replenishes the soil with nutrition, helps with the control insect infestation, and dispersal of pine seeds [9].

- d. Water drains rapidly through layers of these porous soils to leave the surface droughty in spite of heavy rainfall in the region.

The Long Island Pine Barrens (LIPB) in New York is the second largest Pine Barrens in the country, next to the Pine Barrens in New Jersey. It contains regionally rare wetland and upland communities including pitch pine-oak-heath woodland and the dwarf pine plains. The soil in the Long Island Pine Barrens has all of the above mentioned characteristics. In addition, Long Island Pine Barren soils are also exposed to the variation in temperature, which is very similar to other coastal areas of the Northeastern United States. It has warm, humid summers and cold winters. Average winter temperature is 0.2°C and the summer average is 22.2°C. Rainfall and snow averages are 42 and 30 inches, respectively.

To elucidate the culturable microbial diversity present in the soil of LIPBF, the first step includes establishing the number of sampling points across the Pine Barrens that would represent the entire region. Types of microorganisms present in one area of the LIPBF could be expected to be totally different from the other. Practically, it would be a huge and daunting task to isolate, purify, and identify all culturable microorganisms from a large number of sampling points. Through this study, we report the use of total community substrate utilization pattern to identify the soils across the LIPBF that differ widely in their microbial community profile.

Microbial communities provide useful data for studying both applied and basic environmental events. This study is based on the hypothesis that the similarity in the substrate utilization pattern displayed by the soil microbial communities obtained from different locations within the LIPBF will depend on the differences in the microbial community. If the populations from different soil samples contain similar types of organisms, their substrate utilization pattern

will be the same. By measuring and comparing the pattern, one can determine if the soil contains similar or diverse population.

2. MATERIALS AND METHODS

i. Sample collection

Soil samples were collected randomly from three horizons of 66 sampling locations (20-40g from each horizon) across the LIPBF as illustrated in Figure 1. Vegetation data, that are predominant in these sampling sites, were presented in Table 1. The plots were selected randomly, ensuring that the locations were spread across the Long Island. The protocols of the safety of data collection were rigorously followed as recommended by the report of the U.S. Fish and Wildlife Services and the Foundation for Ecological Research in the Northeast [10]. The locations of sample collections were confirmed by the use of Thales/Magellan Global Positioning System unit (GPS) MobileMapper CE. At each location, soil samples were collected from three horizons: 0 – 10cm; 11 – 25cm and 26- 40cm.

ii. BIOLOG EcoPlates

For analyzing the total community substrate utilization pattern of the soil, 1g of soil from each horizon was dispersed in 9mL of sterile distilled water and after vortexing the mixture for 5 minutes, the solution was allowed to settle for a minute. Of this soil extract, we measured 150 μ l and added it to 14.85mL of sterile distilled water and the solution was vortexed for 2 minutes. One hundred μ l of the diluted solution was added to each well of the 96-well BIOLOG EcoPlates. The plates were incubated at 30°C for 48h and the color formation in the EcoPlates

were read using TECAN Microplate reader at 590 nm. Also, 12 plates from sampling sites were read at 48, 72 and 96h to standardize the color development as shown in Figure 2.

iii. Statistical Analysis

The obtained data was normalized by the average well color development (AWCD). The normalized absorbance for the well k was calculated as:

$$\bar{A}_k = \frac{A_k - A_0}{\frac{1}{31} \sum_{i=1}^{31} (A_i - A_0)}$$

A_i represents the absorbance reading of well i and A_0 is the absorbance reading of the blank well. The BIOLOG EcoPlate contains 31 of the most useful carbon sources for soil community analysis and each of these 31 carbon sources are repeated 3 times in the 96-well plate. The mean of the triplicate absorbance values was calculated prior to the calculation of the AWCD.

As the goal of the current study was to reorganize the sampling locations into relatively homogenous groups based on their total community substrate utilization pattern, cluster methods were used. Cluster analyses of the data were carried out using STATISTICA (v8.0) software.

The cluster analyses were performed in sequential order as described below:

1. Hierarchical agglomerative clustering: In this clustering method, 30 x 30 similarity matrix was created and sequentially the most similar cases were merged in 29 steps. Ward's method was used as the linkage rule and the similarity distance was measured in Euclidean units.
2. Clustering method is used to find out how many homogenous groups (K) are present in the result of clustering study.

Tree clustering analysis was carried out selecting Ward's method as the amalgamation rule and the distance measured as Euclidean units. Results of the analysis yielded hierarchical tree plots and amalgamation schedule. In a hierarchical analysis, increasingly dissimilar clusters must be merged as the cluster fusion process continues. Consequently, the classification is likely to become increasingly artificial. A graph of the level of similarity at fusion versus the number of clusters may help to recognize the point at which clusters become artificial because there will be a sudden jump in the level of similarity as dissimilar groups are fused. The data were analyzed for similarity in the biochemical fingerprint of soil samples by grouping based on carbon substrate consumption.

The Shannon–Weaver index, H , is one of several biodiversity indices used to measure diversity in categorical data. It is simply the information entropy of the distribution, treating species as symbols and their relative population sizes as the probability. The advantage of this index is that it takes into account the number of species and the evenness of the species. The index is increased either by having more unique species, or by having increased species evenness.

If n_i is the number of individuals in each species (abundance of each species) and S is the number of species (species richness), then the total number of all individuals N , and p_i the relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community are

$$N = \sum_{i=1}^S n_i \quad p_i = n_i / N, \text{ and the Shannon Weaver index is given by } H = -\sum_{i=1}^S p_i \ln p_i$$

The substrate evenness for the microbial composition is given by $E = H'/\log S$. For any given number of species, there is a maximum possible H' , $H'_{max} = -\ln S$ which occurs when all species are present in equal numbers. In our paper for the calculation of the substrate richness a threshold value of 0.25 for the relative absorbance was used.

3. RESULTS AND DISSCUSION

In our functional microbial fingerprints, we observed that majority of the 31 carbon sources were utilized in microbial communities of LIPBF. Current data shows that the following substrates are utilized largely by the soil microbial community: D-Galacturonic Acid, D-Glucosaminic Acid, D-Mannitol, Itaconic Acid, L-Asparagine, L-Phenylalanine, N-Acetyl-D-Glucosamine, Pyruvic Acid Methyl Ester, Tween 40, Tween 80, and γ -Hydroxybutyric Acid. We also noticed that the least consumed carbon sources were i-Erythritol, 2-Hydroxy Benzoic Acid, α -D-Lactose in horizons O and B in all sites, which we are continuing to investigate (Figure 3).

The difference between each of these vegetation types is the type of community, the relative abundance of pitch pines and scrub oaks in the area along with blueberry and huckleberry trees (Table 1; [10]). In our study, results indicated that majority of LIPBF sites are dominated by oak-pine and pine-oak vegetation. K-mean clustering shows the clustering of the data into several clusters and the Euclidian Distance between the clusters, which is the geometric distance in multidimensional space. The clustering analysis indicate that based on CLPP, the entire LIPBF can be subdivided into four different clusters. In each cluster, the number of sites varies by horizon from 3 to 53.

4. CONCLUSIONS AND FUTURE WORK

As there is no correlation between the geographical locations of sampling sites, based on the history of fire or type of vegetation, it could be inferred that the CLPP is influenced by soil chemistry. However, further studies need to be carried out to test this hypothesis in the future.

5. ACKNOWLEDGEMENTS

SUNO-FaST expresses sincere gratitude to the National Science Foundation (HRD-0102620), US Department of Education (MSEIP - P120A050086), and the Department of Energy for the research opportunity, facilities, and financial support to participate in 2008 FaST program at BNL. Our special thanks are due to Noel Blackburn (FaST Program Manager), Tim Green (Mentor at BNL), and Vishal Shah (Mentor at Dowling College) for their continued support in our research endeavors. We appreciate NY DEC, Suffolk County, NY, and NY State Parks for accessibility to field sites, Jennifer Higbie (GIS), Kathy Gurski (BNL-OEP), fellow students and colleagues for assistance.

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FIGURES

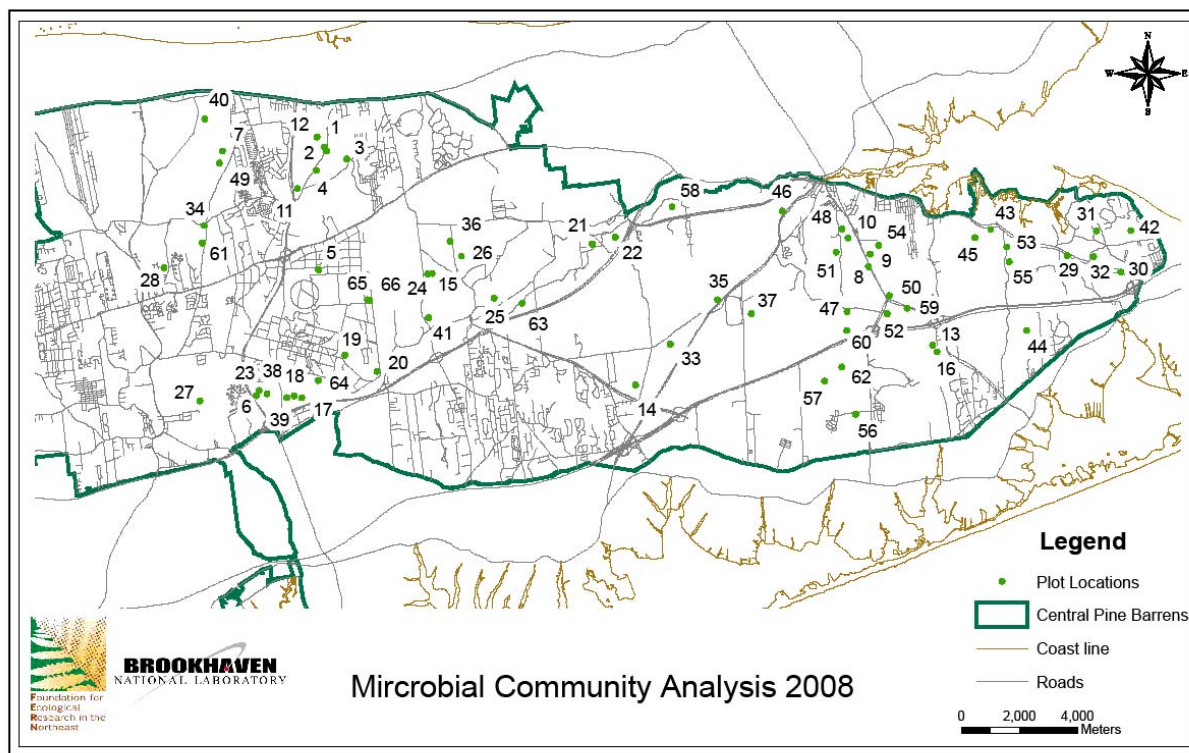


Figure 1: Sampling locations in the Pine Barren Forests of NY.

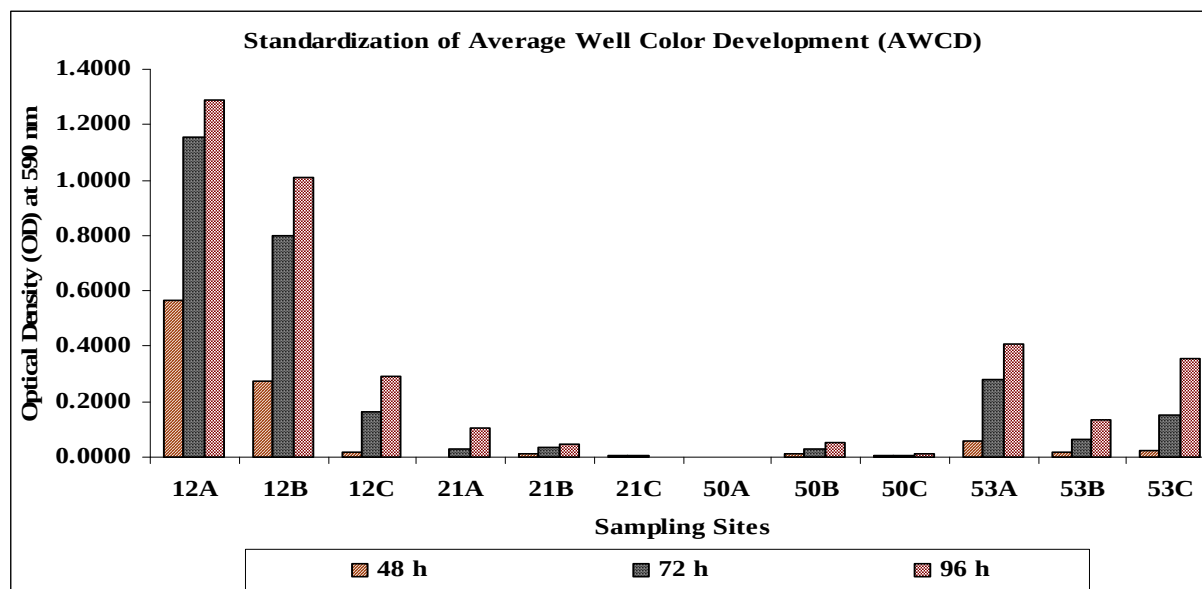


Figure 2: Standardization of Average Well Color Development

α -D-Lactose		D-Galacturonic Acid
2-Hydroxy Benzoic Acid	D-Malic Acid	Tween 80
L-Serine	L-Threonine	Itaconic Acid
D-Cellobiose	4-Hydroxy Benzoic Acid	Tween 40
β -Methyl-DGlucoside	D-Xylose	L-Phenylalanine
i-Erythritol	α -Cyclodextrin	L-Asparagine
Putrescine	α -Ketobutyric Acid	N-Acetyl-D-Glucosamine
Glycyl-Lglutamic Acid	L-Arginine	D-Mannitol
Phenylethylamine	Glycogen	γ -Hydroxybutyric Acid
D,L- α -Glycerol Phosphate	D-Galactonic Acid Y-Lactone	D-Glucosaminic Acid
Glucose-1-Phosphate		Pyruvic Acid Methyl Ester
AbarK < 0.25	.25 < AbarK < 1.0	AbarK > 1.0

Figure 3: Soil metabolic fingerprint of Pine Barren Forest of Long Island as measured using BIOLOG EcoPlate.

TABLES:

Subtarget	Community Type	Canopy Cover	Presence of Pitch Pine	Presence of Scrub Oak	Presence of Blueberry & Huckleberry
Coastal Oak	Forest	60% or more	10% or less	None	Continuous
Oak-Pine	Forest	60% or more	11-49%	Scattered	Continuous
Pine-Oak	Forest	60% or more	50-89%	Scattered	Nearly Continuous
Pitch Pine	Forest	60% or more	90% or more	Continuous	Scattered
Pitch Pine Scrub	Shrub land	59% or less, open	Primarily Pitch Pine, with some Tree Oaks	Continuous	Scattered
Dwarf Pine Plain	Shrub land		Pitch Pine, Dwarf Pine also	Nearly Continuous	

Table 1. Vegetation composition in various areas of Long Island Pi