Winter 4-21-2023

Examining the Different Soil Horizons of White Oak Mountain, TN and their Effect on the Soil Microbial Community

Ji Won Moon
Southern Adventist University

Timothy D. Trott
Southern Adventist University, timtrott@southern.edu

Examining the Different Soil Horizons of White Oak Mountain, TN and their Effect on the Soil Microbial Community

Ji Won Moon and Timothy Trott, Ph.D.
Hickman Science Center, Southern Adventist University, Collegedale, TN

SOUTHERN ADVENTIST UNIVERSITY
Biology Department

Background

Soil environment is one of the key living systems that consist of the biosphere, and the microbiome that habits the soil plays an important role in soil multifunctionality. Soil microorganisms are also identified to yield a greater total biomass production than those of plants or animals when it comes to supplying essential nutrients (Islam et al., 2020). Furthermore, the soil microbial community contributes to the diverse interaction of different ecosystems, which can consequently affect human and animal populations (Bender et al., 2016).

Among the various edaphic factors, this research study will focus on investigating the different soil types and horizons, and how they affect soil microbial diversity. Previous studies show how soil depth and horizons impact the microbial community composition of the soil. It is observed that the soil microbial community composition is significantly affected by the soil depth as analyzed from phospholipid fatty acid analysis. (Fierer, Schimel, & Holden, 2003). Soil displays diverse physical, chemical, and biological characteristics the deeper it goes, further changing the texture and structure (Lennon, 2020).

This research study examined the effect of soil depth and horizon on the soil microbial community composition of White Oak Mountain, aiming to establish a baseline of the representative soil microbial diversity of White Oak Mountain and provide data to be used as a reference for future studies.

Methods and Materials

Description of Sample Sites

Ten previously selected 8 ft by 4 ft quadrants located on the property of Southern Adventist University on the eastern slope of White Oak Mountain in the ridge and valley ecosystem of Southeastern Tennessee were used as soil sample collection sites.

Soil Sample Collection

Soil core samples were collected using a Varomorus Compact Soil Sampler Probe 12" Stainless Steel (Varomorus, Florida, USA) One sample was taken from each soil horizon from each quadrant, with a total of six soil samples. which was driven to a depth of 2 to 3 cm into the soil with a mallet. Samples were taken from each quadrant from September through October. Before soil samples were collected, the leaf-litter was removed from sample collection sites. The samples were placed into separate sterile plastic bags, and then they were transferred to the lab where each soil core was stored at -20℃ until DNA extraction was performed. Half of a gram of soil was collected from each of the horizons, and this process was repeated for each of the three quadrants.

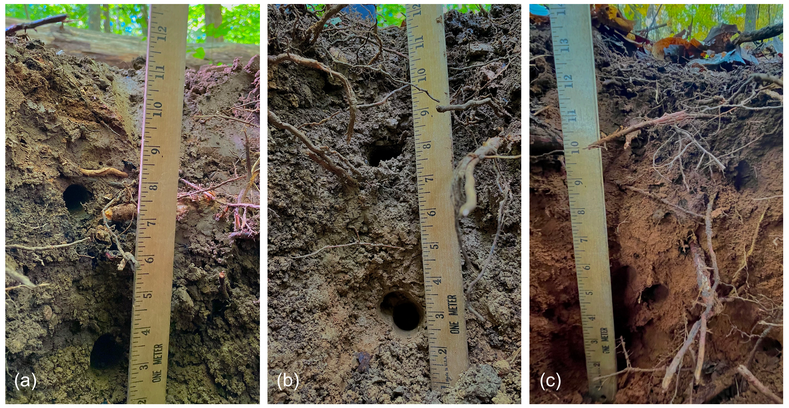

Figure 1. Soil sample collection from the upper and lower soil horizon from each study site quadrant: (a) Quadrant 2, (b) Quadrant 4, and (c) Quadrant 9.

Soil DNA Extraction

Total DNA was extracted from 0.25 g of soil from each horizon of each quadrant following the established protocol in the DNeasy® PowerSoil® Pro Kit (Qiagen, Hilden, DE) for extracting total DNA from soil samples. To quantify the purity of the extracted DNA samples spectral analysis was performed using a Thermoscientific NanoDrop Onec spectrophotometer. 2 ul of each DNA extract was measured, and 1x TAE Buffer was used for the blank. Absorbance was measured at 230nm, 260nm, and 280nm, and the ratio of 260/230 and 260/280 were determined. Absorbance ratios were compared with standard purity values for analysis; standard values for the 260/230 ratio are 2.0-2.2, while the 260/280 ratio approximately be 1.8 (Barta et al 2017; Cummins, & Trott, 2019; Echevarria-Zomeno, 2012; ThermoScientific, 2011). The total volume of each sample was calculated by pipetting. DNA concentrations were then used to calculate total DNA yield (ng) for further processing and sequencing.

Amplicon Sequencing

Extracted DNA was sent to an external lab (LC Sciences, Houston, TX) for 16S/18S/ITS rDNA amplicon sequencing with DADA2 (Divisive Amplicon Denoising Algorithm).

Results

Soil Horizon Identification

Table 1. Soil depth description of sample soil horizons. Sample was collected only from the first two horizons identified for each study site.

Quadrant	Soil Map Unit (Map Unit Composition)	Soil Horizon	Depth (in)	Soil Horizon Composition
2	MoE (Montevallo Shaly Silt Loam)	H1	0 to 6	Channery Silt Loam
		H2	6 to 18	Very Channery Silt Loam
4	uMvC (Minvale Gravelly Silt Loam)	A	0 to 6	Gravelly Silt Loam
		Bt1	6 to 18	Gravelly Silt Loam
9	HcE (Hanceville Loam)	H1	0 to 6	Loam
		H2	6 to 36	Clay

16S/18S/ITS rDNA Amplicon Sequencing Data Analysis

Figure 2. Flower plot presenting the number of unique ASV (Amplicon Sequence Variants) tags identified for each sample and the ASV tags shared by all samples.

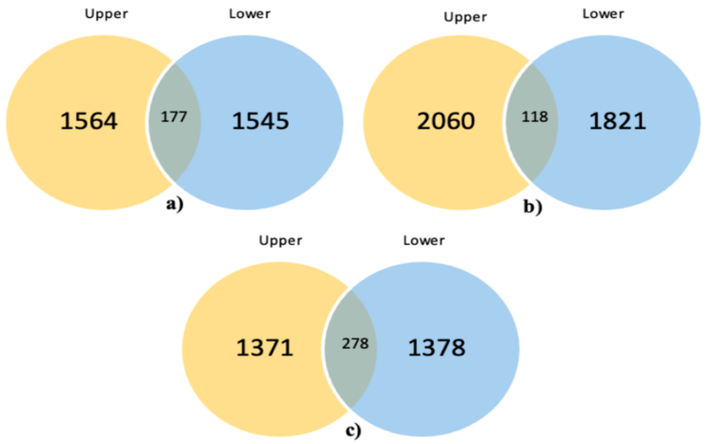

Figure 3. Venn diagrams presenting the number of ASV tags in the upper and lower soil horizons of a) Q2, b) Q4, and c) Q9 study sites and their respective shared number of ASV tags. The Shannon index of the upper layers of study sites Q2 and Q4 are higher than their lower counterparts, indicating higher microbial diversity.

Results (cont.)

- Overall trend shows a greater abundance of microbial communities in the upper soil layers in all study sites, whereas a significant difference can be observed for certain phyla, such as Chloroflexi and Firmicutes.
- No difference was found in the phylum rank of microbiome composition between the different soil types.

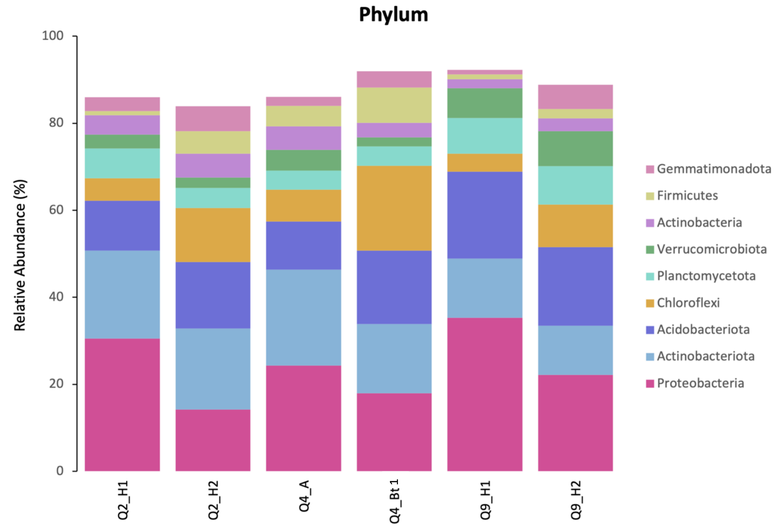

Figure 4. Stacked bar graph presenting the relative abundance of the top 10 phyla found in each sample based on ASV abundance.

Conclusion

- Microbial diversity is seen to decrease with increasing soil depth due to uneven dispersal of nutrients and plant roots in the soil (Eilers et al., 2012).
- Chloroflexi and Firmicutes phyla are found in higher abundance in lower soil horizons, due to increasing soil pH with increasing soil depth, history of agriculture use, and lower oxygen level.
- Future research can examine an edaphic factor and its influence on the relative abundance of a single phylum.

Acknowledgements

- Southern Adventist University Academic Research Committee (ARC) Research Sustainability Grant
- Biology / Allied Health Department of Southern Adventist University

References

- Barta, E. C., Bolander, B., Bilby, R. S., Brown, H. J., Brown, N. R., Duryee, M. A., … Prawitz, A. R. (2017). In Situ Dark Adaptation Enhances the Efficiency of DNA Extraction from Mature Pin Oak (Quercus palustris) Leaves, Facilitating the Identification of Partial Sequences of the 18S rRNA and Isoprene Synthase (IspS) Genes. *Plants*. https://doi.org/10.3390/plants6040052.
- Bender SF, Wagg C, van der Heijden MGA (2016). An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol 31:440-452. https://doi.org/ 10.1016/j.tree.2016.02.016
- Cummins, D. and Trott, T. D., (2019). "Comparative Analysis of Four DNA Preparation Kits for Quercus falcata, palustris, and rubra DNA Extractions and Subsequent DNA Sequencing". *Research in Biology*. 12. https://knowledge.e.southern.edu/research_bio/12
- Echevarría-Zomeño, S., Abril, N., Ruiz-Laguna, J., Jorrín-Novo, J., & Maldonado-Alconada, A. M. (2012). Simple, rapid and reliable methods to obtain high quality RNA and genomic DNA from Quercus ilex L. leaves suitable for molecular biology studies. *Acta Physiologiae Plantarum*, *34*(2), 793–805. https://doi.org/10.1007/s11738-011-0880-z
- Eilers, K. G., Debenport, S., Anderson, S., & Fierer, N. (2012). Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry, 50*, 58-65. https://doi.org/10.1016/j.soilbio.2012.03.011
- Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, *35*(1), 167–176. https://doi.org/10.1016/s0038-0717(02)00251-1
- Islam, W., Noman, A., Naveed, H. *et al.* Role of environmental factors in shaping the soil microbiome. *Environ Sci Pollut Res* 27, 41225–41247 (2020). https://doi.org/10.1007/s11356-020-10471-2
- Lennon, J. T. (2020b). Microbial Life Deep Underfoot. *MBio*, *11*(1), 1–3. https://doi.org/10.1128/mbio.03201-19
- ThermoScientific. (2011). NanoDrop: Assessment of Nucleic Acid Purity-asbsortion test. *Protocols and Product Manuals*, 1–2. https://doi.org/10.7860/JCDR/2015/11821.5896.