Summer 8-2-2023

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

Ji Won Moon
Southern Adventist University, moon@southern.edu

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 D. Trott

Abstract

Soil microorganisms participate in the diverse interaction within virtually all ecosystems, consequently affecting the associated human and animal population. Identifying how edaphic variables alter the functional and taxonomic diversity of the soil microbial community requires an examination of total microbial soil diversity and abundance. This research study examined the effect of soil depth and horizon on the soil microbial community composition of White Oak Mountain. The soil microbial community was characterized by 16S/18S/ITS rDNA amplicon sequencing of the DNA extract of six samples from the three major soil types identified: HcE, MoE, and uMvC. OTU clustering analysis and both alpha and beta diversity were analyzed to determine whether soil depth and horizon correlate with soil microbial community composition. This research project aims 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.

Introduction

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 already known for their participation in the decomposition of organic matter and soil nutrition cycling (Zheng et al., 2019). 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).

Multiple edaphic factors that affect the soil microbial community composition have been identified, such as the soil pH level, soil depth, organic matter composition, porosity, moisture, oxygen and carbon dioxide concentration, soil texture and etc. (Bhattarai, et al., 2015 & Zheng et al., 2019). There are sufficient research studies that determined the effect of such edaphic variables on soil microbial diversity individually, but the incorporation of multiple variables to their multifunctionality effect on soil microbial community composition is now a growing area of study (Zheng et al., 2019). Also, such knowledge has been acquired mostly by focusing on only one type of microorganism in the soil. However, seeing how the edaphic variables alter the functional and taxonomic diversity of the soil microbial community, it is considered effective to refer to the soil microbial diversity to examine the biogeochemistry and functionality of the ecosystem (Uroz et al., 2013 and Islam et al., 2020).

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 based on “lower redox potential, reduced availability of labile carbon, and the leaching of minerals due to the percolation of water through the soil profile” (Lennon, 2020). Followed by a constrained supply of nutrients and energy needed, the present

specific microorganism species are limited in their ability to thrive in such an environment. Consequently, smaller composition microbial communities can be observed in deeper soils relative to the more abundant and diverse pool of species at the surface (Lennon, 2020).

This study will examine the different soil types and horizons of the White Oak Mountain in Collegedale, Tennessee. Three map units were identified within the area, based on the soil information established by the United States Department of Agriculture (USDA): Hanceville loam, Montevallo shaly silt loam, and Minvale gravelly silt loam. With the application of amplicon sequencing, not only the specific microbial community present but also the relative abundance of each species can be determined in operational taxonomic units (OTUs). The following data will be analyzed from the different samples acquired from each soil type and their horizons to investigate the diversity of soil microbial composition present in White Oak Mountain, and its correlation between the soil depth and horizon. This research study aims to identify the soil microbial diversity of White Oak Mountain based on different soil horizons and the relationship between them, which can be applied as a reference for future studies.

Methods and Materials

Description of Sample Sites

Selection of the sample sites referred to Matthew Gano's proposal. 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. The location and descriptive data for each site are listed in Table 1. Site descriptive data was collected in 2014, 2015, and 2019 by Lien Turley, Eli Robinson, and Dr. Benjamin Thornton (Turley, 2015).

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. The Soil Sampler Probes were autoclaved and washed with an ethyl alcohol solution using the procedure that Wu, Wu, Zhang, & Cheng demonstrated reduced the DNA contamination in amplicon sequencing (2018). 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.

Soil DNA Extraction

Soil DNA extraction was conducted with the method previously described in Gano's proposal. 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 One**c** 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.

Results

Soil Horizon Identification

Preliminary study was done with the use of an online web soil survey tool, established by the United States Department of Agriculture (USDA), to find soil information of White Oak Mountain. Three map units were identified within the area of sample sites: HcE, MoE, and uMvC. Detailed descriptions of soil depth are described in the legend (Table 2). The HcE unit consists of Hanceville loam, with a typical soil profile consisting of Horizon H1, H2, H3, and R. The MoE unit consists of Montevallo shaly silt loam, with a typical soil profile consisting of Horizon H1, H2, and Cr. The uMvC unit consists of Minvale gravelly silt loam, with a typical soil profile consisting of Horizon A, Bt1, and Bt2. This research limited the soil horizons within the range of 12 inches, and therefore soil samples were collected from the first two horizons identified for each soil map unit. The samples from the first horizon (0 to 6 inches depth) and the second horizon (6 to 12 inches depth) of each study site will be referred to as the upper and lower layer, respectively, in this research study. One study site was determined for each map unit, with quadrants 2, 4, and 9 selected for the soil map units MoE, uMvC, and HcE respectively. The *Guidelines for Soil Description* (FAO 2006) was referred to in describing each soil horizon sampled.

Table 1. Soil depth description of sample soil horizons

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
		Cr	18 to 28	Bedrock
4	uMvC (Minvale Gravelly Silt Loam)	A	0 to 6	Gravelly Silt Loam
		Bt1	6 to 18	Gravelly Silt Loam
		Bt2	18 to 60	Gravelly Silty Clay Loam
9	HcE (Hanceville Loam)	H1	0 to 6	Loam
		H2	6 to 36	Clay
		H3	36 to 64	Clay Loam
		R	64 to 68	Bedrock

Amplicon Sequencing Data Analysis

The extracted DNA sample sent for 16S/18S/ITS rDNA amplicon sequencing was processed with DADA2 (Divisive Amplicon Denoising Algorithm), further identifying the ASVs (Amplicon Sequence Variants) found in each sample. The flower plot presents the number of unique ASV tags found in each sample, as well as the total number of shared ASV tags found in all six samples (Figure 1).

All study sites show a significantly low number of shared ASV tags between the upper and lower soil horizon compared to the total number of ASV tags identified in each soil horizon (Figure 2). The Shannon index of the upper layers of study sites Q2 and Q4 are higher than their lower counterparts, indicating higher microbial diversity. Study site Q9 presented an opposite

result, however, with a negligible difference. Number of shared ASV tags between soil samples and soil horizons are noticeably small when compared to the number of ASV tags found in each sample and horizons. Accordingly, high microbial diversity is observed in each soil type, and such level of diversity is observed within the same soil type, between the upper and lower soil horizon as well.

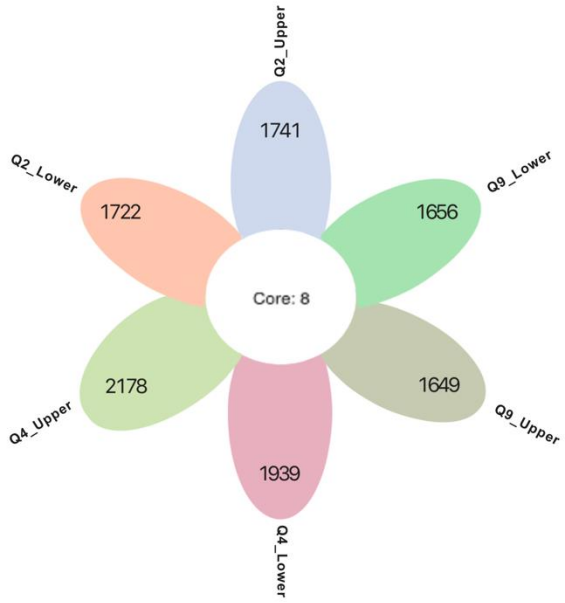

Figure 1. Flower plot presenting the number of unique ASV tags identified for each sample, as well as ASV tags shared by all samples.

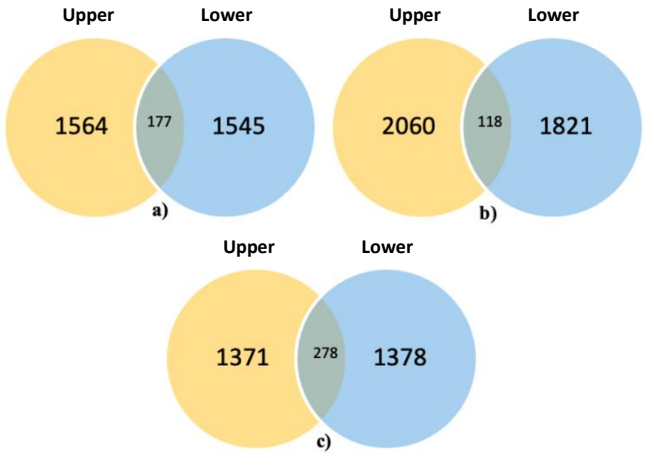

Figure 2. Venn diagrams presenting the number of ASV tags in the upper and lower soil horizons of a) Q2, b) Q4, and c) Q9 study sites and their respective shared number of ASV tags.

Previous study done in Inner Mongolia, China presented a similar result where the soil bacterial community within the 10 cm depth range was significantly different from the 20 cm and 30 cm soil layers, and the diversity and abundance decreased with increasing soil depth (Zhao et al., 2021). Identical result was reported by a study made in Colorado, USA, where bacterial diversity was typically highest in the top 10 cm layer of the soil profile, dropping by around 20-40% to the deepest horizon sampled (Eilers et al., 2012). The 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).

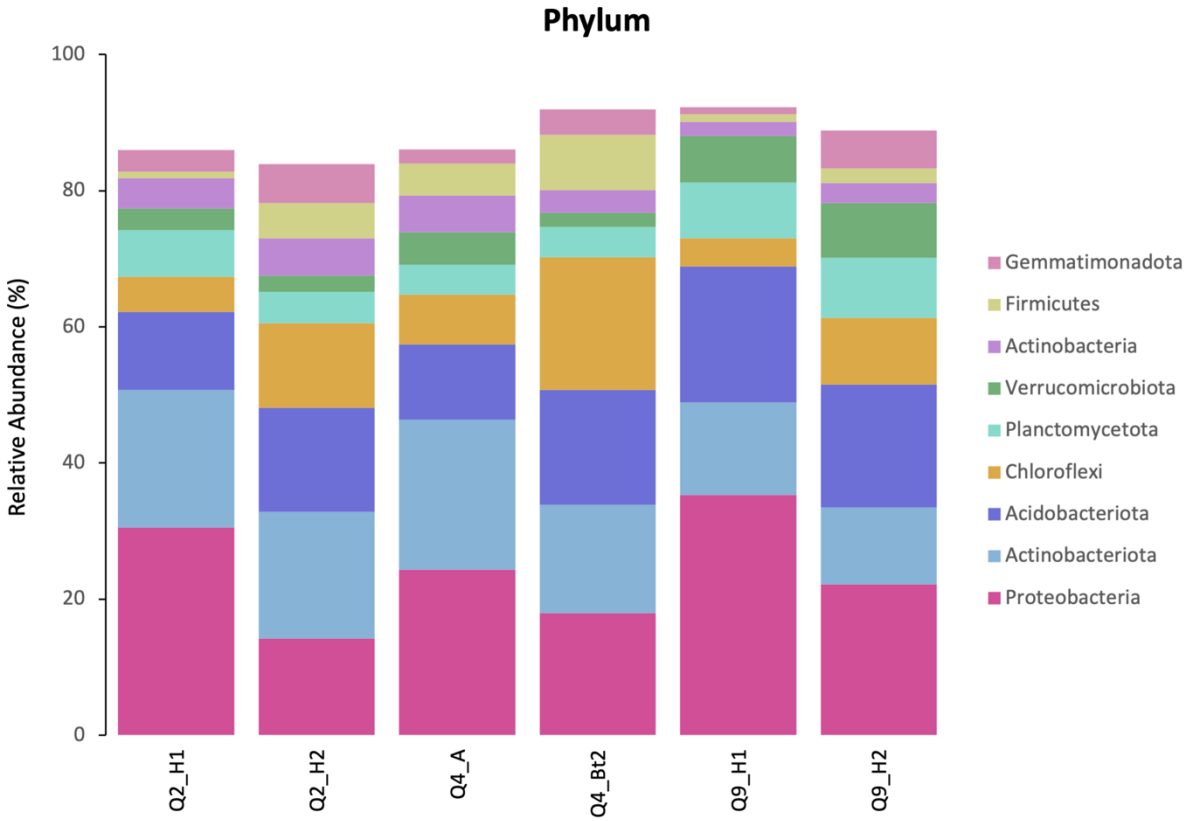

Figure 3. Stacked bar graph presenting the relative abundance of the top 10 phyla found in each sample based on ASV abundance.

The top 10 phyla were identified based on the ASV abundance and the taxonomy annotation, along with the relative abundance as shown in the y-axis of Figure 3. The phyla rank of abundancy is identical between each soil type and soil horizon The 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. Chloroflexi and Firmicutes phyla are known to have high abundance in anaerobic environments,

and Chloroflexi specifically was found abundant in anaerobic digestion systems (Petriglieri et al., 2018). It can be expected that such phyla were discovered in greater abundance in the lower soil horizons due to the lower oxygen level based on the deeper soil depth.

Additionally, it has been observed that there is a higher relative abundance of the phylum Chloroflexi and Firmicutes in agricultural soil when compared to natural soil (Trivedi et al., 2016). As much as the change in land use is a significant factor affecting biodiversity, the chain of effects can consequently modify plant species composition, furthermore the soil microbial community (Sala et al., 2000). With the sample study sites of White Oak Mountain having experienced being used for agricultural purposes, the edaphic factors could have been gradually altered, consequently presenting contrary results to other studies.

One of the other major edaphic factors that influence the soil microbial community is the soil pH level. A lower pH level is typically observed in the surface layer of soil, increasing with soil depth (Zhou et al., 2019). This is due to the accumulation of organic matter and its decomposition in the soil surface layer, which increases organic acid production and results in a decrease in pH level (Hong, Gan & Chen, 2019). Correlation analysis from a previous study reported that phylum Chloroflexi was found to be positively correlated with the soil pH level (Huang et al., 2021). This supports as another possible explanation for the increasing Chloroflexi abundance with increasing soil depth, unlike the majority of phyla.

Conclusion

Soil microorganisms are of significant area of study due to their major participation in soil ecosystem. Examining the soil microbial community and monitoring their activity is crucial in understanding soil functionality. The environment of soil itself is influenced by various

edaphic factors and other components of the ecosystem as well, such as the plants, animal, and human population and their activities. Such edaphic factors and biogeography of macro-organisms can be the controlling variables of the soil microbiome (Fierer & Jackson, 2006). Therefore, examining the effect of the soil depth factor on soil microbial community of White Oak Mountain, TN is necessary for better understanding the local soil functionality. High microbial diversity was observed between different soil types and soil horizons of White Oak Mountain, TN. Among the ten most abundant phyla of the soil samples, phyla Chloroflexi and Firmicutes were found in higher abundance in lower soil horizons compared to their higher counterparts, contrary to general trend. These observations may have been made due to lower oxygen level in deeper soil, history of agricultural use, and increasing soil pH level with deeper soil level. Future research can examine one of the mentioned factors and its influence on the relative abundance of a single phylum to confirm the explanation to contrary results with higher accuracy.

Literature Cited

Bhattarai, B. (2015). Variation of Soil Microbial Population in Different Soil Horizons. *Journal of Microbiology & Experimentation*, *2*(2), 75–78. https://doi.org/10.15406/jmen.2015.02.00044

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

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.

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

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences, 103*(3), 626-631. https://doi.org/10.1073/pnas.0507535103

Hong, S., Gan, P., & Chen, A. (2019). Environmental controls on soil pH in planted forest and its response to nitrogen deposition. *Environmental Research, 172*, 159-165. https://doi.org/10.1016/j.envres.2019.02.020

Huang, T., Liu, W., Long, X., Jia, Y., Wang, X., & Chen, Y. (2021). Different responses of soil bacterial communities to nitrogen addition in moss crust. *Frontiers in Microbiology, 12* https://www.frontiersin.org/articles/10.3389/fmicb.2021.665975

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

Petriglieri, F., Nierychlo, M., Nielsen, P. H., & McIlroy, S. J. (2018). In situ visualisation of the abundant Chloroflexi populations in full-scale anaerobic digesters and the fate of

immigrating species. PloS one, 13(11), e0206255. https://doi.org/10.1371/journal.pone.0206255

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.

Trivedi, P., Delgado-Baquerizo, M., Anderson, I. C., & Singh, B. K. (2016). Response of soil properties and microbial communities to agriculture: Implications for primary productivity and soil health indicators. *Frontiers in Plant Science*, *7* https://www.frontiersin.org/articles/10.3389/fpls.2016.00990

Turley, Lien, (2015). "Global Information Systems Analysis of Changes in Temporal Flowers Distribution, by Species, Across the Biology Trail System of Southern Adventist University in Collegedale, Tennessee". *Senior Research Projects*. 182. https://knowledge.e.southern.edu/senior_research/182.

Uroz S, Ioannidis P, Lengelle J, Ce´bron A, Morin E, et al. (2013) Functional Assays and Metagenomic Analyses Reveals Differences between the Microbial Communities Inhabiting the Soil Horizons of a Norway Spruce Plantation. PLoS ONE 8(2): e55929. doi:10.1371/journal.pone.0055929

Zhao, H., Zheng, W., Zhang, S. *et al.* Soil microbial community variation with time and soil depth in Eurasian Steppe (Inner Mongolia, China). *Ann Microbiol* 71, 21 (2021). https://doi.org/10.1186/s13213-021-01633-9

Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Dietrich, M., Herbold, C. W., Eichorst, S. A., Woebken, D., Richter, A., & Wanek, W. (2019). Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil biology & biochemistry*, *136*, 107521. https://doi.org/10.1016/j.soilbio.2019.107521

Zhou, W., Han, G., Liu, M., & Li, X. (2019). Effects of soil pH and texture on soil carbon and nitrogen in soil profiles under different land uses in mun river basin, northeast thailand. *PeerJ, 7,* e7880. https://doi.org/10.7717/peerj.7880