Spring 2017

Analysis of Microbial Diversity in Disturbed Soil

Tyler G. Sanda
University of Akron, tgs19@zips.uakron.edu

Analysis of Microbial Diversity in Disturbed Soils

Senior Honors Project in Biology

Tyler Sanda
Spring 2017

Introduction

Surface mining can result in the disturbance of ecological communities throughout the world. Extracting valuable resources through methods such as strip mining can cause devastating effects on the ecosystem. Strip mining is a process in which land is excavated to reach a coal seam. After extraction of coal, the crushed and homogenized overburden is then replaced and covered by topsoil. This leads to decreases in both plant and microbial mass (Poncelet et al., 2013). Until recently, analysis of the land mass recovery and reclamation has been limited to surface examinations which often lead to false conclusion due to the eventual recovery of plant mass at these locations. These studies however do not characterize the possible devastating effect to the subsoil (Mummey et al., 2002). This approach concludes that visibility of plant communities at the surface is recovered land, but this approach often pays little to no attention to the microorganisms. These microorganism communities play a vital role in the ecology of the land mass. They contribute to pedogenesis, which could cause vast changes in the underlying chemistry of the soil (Poncelet et al., 2013). It has been shown that microorganisms may excrete acids which contribute to the chelation involved in rock weathering and pedogenesis (Shatz, 1963). Also in karst regions, autotrophic microorganisms living on rocks can fix nitrogen and carbon from the air and become the main producers of primary products on the rocks. These microorganisms can also capture dust and soil particles brought in by wind and rain, which is then used to produce more soil materials (Lian B, et al., 2010).

Soil microorganisms are sensitive to environmental change, such as the aforementioned strip mining (Coleman et al., 1993). These communities can experience significant degradation in biomass as well as species composition following a disturbance (Harris et al., 2003). It is proposed that analysis of microbial communities associated with disturbed land masses may serve as a better microbial indicator of recovery post land mass disturbance (Poncelet et al., 2013).

To further examine reclamation efforts and disturbed land recovery, we analyzed the microbial distributions in disturbed soil alongside their depth-dependent distribution in undisturbed soil. We tested for appearance of microbial species as well as the relative abundance. This may allow for a more precise characterization of ecosystem recovery. Further analysis on other metrics will be needed to have conclusive evidence.

Materials and Methods

Site description and sampling approach

Soil was collected from a strip-mined area in the Huff Run Watershed location located in the Appalachian coal basin near Mineral City, OH. This is located near Mineral City, OH. The watershed consists of shale of the Pennsylvanian Allegheny group and contains siltstone, sandstone, and limestone (Lamborn, 1956). The headwaters portion of the watershed was developed for agriculture and is not yet disturbed by mining; however, the downstream portion has experience multiple disturbances including deep mining, soil mining, and surface mining (“Huff Run Watershed” 2000*)*.

Samples were taken from soil/overburden samples (MT) and undisturbed soil (HW) with a depth of approximately 120 cm. Soil was collected at depth intervals of 10 cm, 40 cm, 80 cm, and 120 cm using a flame-sterilized hand agar. DNA was extracted from soil using MoBio (Carlsbad, CA) Power Biofilm DNA isolation kit recovered from extractions were quantified using a Nano-drop ND-1000 spectrophotometer (Thermo Scientific)

DNA Sequencing

DNA was sequenced by Illumina at Molecular Research LLC (Shallowater, TX). The 16S rRNA gene V4 variable region PCR primers 515/806 were used in a single step 30 cycle PCR using the HotStarTaq Plus Master Kit under the following conditions: 94^{o} C for 3 minutes, followed by 28 cycles of 94^{o} C for 30 seconds, 53^{o} C for 40 seconds and 72^{o} C for 1 minute, after which a final elongation step at 72^{o} C for 5 minutes was performed. Sequences were then depleted of barcodes and primers, then sequences <150 bp were removed. The sequences were then denoised, OTUs generates chimeras removed. Operational taxonomic units (OTUs) were defined with 97% similarity. Finally, OTUs were then taxonomically classified using BLASTn against a database derived from RDPII (Wang et al., 2007) and NCBI (NCBI, 2017).

MacQIIIME

Quantitative Insights In Microbial Ecology or QIIME, is a software application that performs microbial community analysis. It is used to analyze and interpret nucleic acid sequence from microbial 16S rRNA gene sequences (Kuczynski et al., 2011). To evaluate the bacterial diversity from disturbed and undisturbed samples, rarefaction curves, OTU distribution, and weighted/unweighted UniFrac principle coordinate analysis (PCoA) charts were generated. Sequences were separated into OTUs based on 97% sequence identity.

Results and discussion:

OTU Distribution – Characterization of the Sample Sets

Figure 1 shows the phylum-level distributions of OTUs recovered from the MT and HW soils. Differences between the distribution of each OTU in the separate sample sets gives insight into possible changes shown in the PCoA plots. There is more variability in distinct OTUs in the disturbed territory compared to the undisturbed territory. When examining a specific OTU such as Chloroflexi across all depths, the MT has a much larger range when compared to the HW (Table 1). This may lead to further discussion on the type of chemical environment in the disturbed area as well as the amount of recovery.

Comparisons at each depth for the HW territory against its counterpart MT allows for characterization of the microbial communities. Outliers can be easily viewed when compared to their counterparts. Specific outliers of note are seen in the MT depth 40. The Chloroflexi and Acidobacteria show different relative abundances when compared against the HW location sample set depths as well as the other depths in the MT. These may be attributed to poor sampling, but also can be attributed to a distinctly new microbial community dynamic. Other measurements are needed in order to justify whether this area has recovered to levels of

undisturbed land or if this is a new dynamic of the community. Measurements of microbial community DNA could be taken as the site continues to age. If the percentages of OTU distribution begin to show similar levels as undisturbed lands at later dates it might be concluded that this site has not yet reached full recovery at this specific time or there was may have been an error while collecting the sample and/or during DNA extraction. Either one of these errors may give false measurements of microbial abundances, which may alter current conclusions drawn on soil recovery.

Alpha Rarefaction Metrics

Alpha rarefaction curves show species (i.e. OTU) richness, which is the number of different species represented in each sample. However, species richness does not take into account species abundance, it only takes a count of each species found. Each data point represents the amount of new sequences per sampling. Each depths sampling success can be characterized by a slope that steadily decreases. As the slope of each depth lowers with more sequences sampled, it is concluded that depth was sampled to saturation. Examining Figure 2, as the sequences per sample increases there is a steady decrease in slope of the data points. The ideal sampling would contain a slope that reaches an asymptote. In this case, it can be assumed that if more sequences were sampled the data would eventually reach an asymptote.

Weighted and Unweighted UniFrac

UniFrac is a beta-diversity measure that uses phylogenetic information to compare environmental samples (Lozupone et al., 2010). Figure 3 displays the weighted UniFrac, which is a quantitative measure, while Figure 4 displays the unweighted UniFrac, a qualitative metric. The unweighted graphing relies on the presence or absence of OTUs to compare community composition. The weighted UniFrac takes the relative abundance of each type of organism into account. Weighted UniFrac is very important because the relative abundance of a given microbial OTU can be vital for describing community changes as well as similarities amongst sample sets. Weighted and unweighted UniFrac comparisons can often display vastly different information that points to different conclusions about certain relationships amongst samples. Using both metrics can provide a more holistic analysis of the data. However, one may show a stronger relationship than the other (Lozupone et al., 2010). Analyzing the data points relative to one another can give insight on the differences in diversity among each sample depth as well as between the sampling territories. This may allow the formation of possible conclusions for soil recovery that has occurred. Clustered data points indicate similar microbial communities. If these clusters contain points that have similar depths but different territories, it may be a possible indicator of soil recovery.

Examining the weighted UniFrac PCoA plots, distinct clustering is observed between sample sets. Clustering can be observed between the sample sets MT80, MT120, and HW120. Clustering can also be shown near MT10, which shows proximity to both HW10 and HW40. This weighted analysis suggests a relationship between similar depths of soil between territories. This information provides the possibility that the disturbed land territory shows signs of recovery to microbial levels of undisturbed territories. However, to determine if the recovery is not just limited to the microbiota of each territory, other measurements such as pH, electrical conductivity, and mineral composition must also be characterized.

The unweighted UniFrac points to a different pattern. Upon examining the graph, the territories are clustered with emphasis on location rather than depth. The HW territories are clustered together and separate from the MT. This information points to less soil recovery, which is attributed to differences in the microbial communities at each territory. In this setting, relative abundance of OTUs is necessary to properly determine soil recovery. Therefore, while the unweighted metric provides an interesting correlation between samplings, the weighted metric provides a more thorough conclusion in concordance with soil recovery. It also indicates that while topsoil microbial communities are similar between the HW and MT, the subsoils differ.

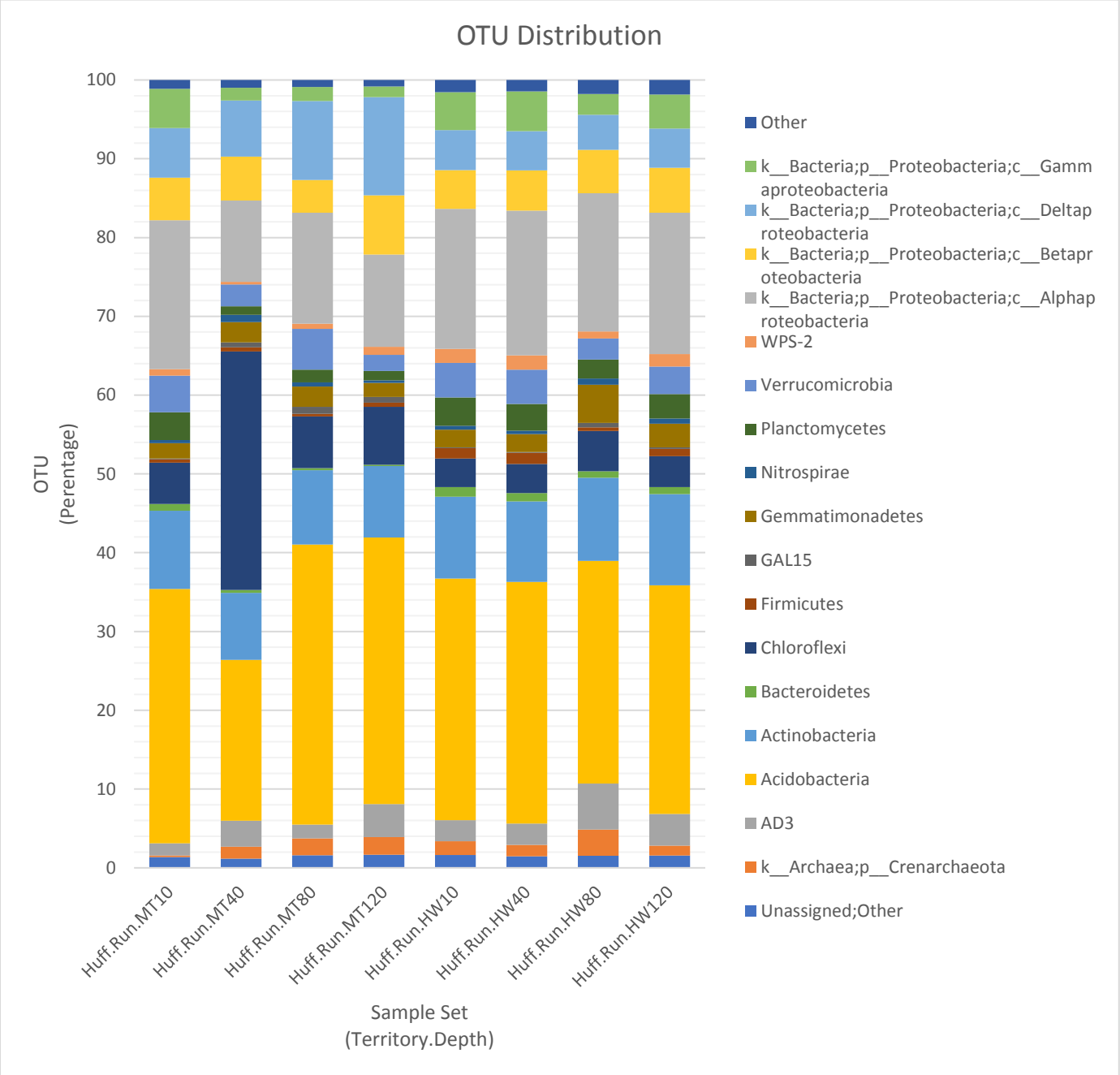

Figure 1. Phylum-level OTU percentages at both sample sites as well as every depth.

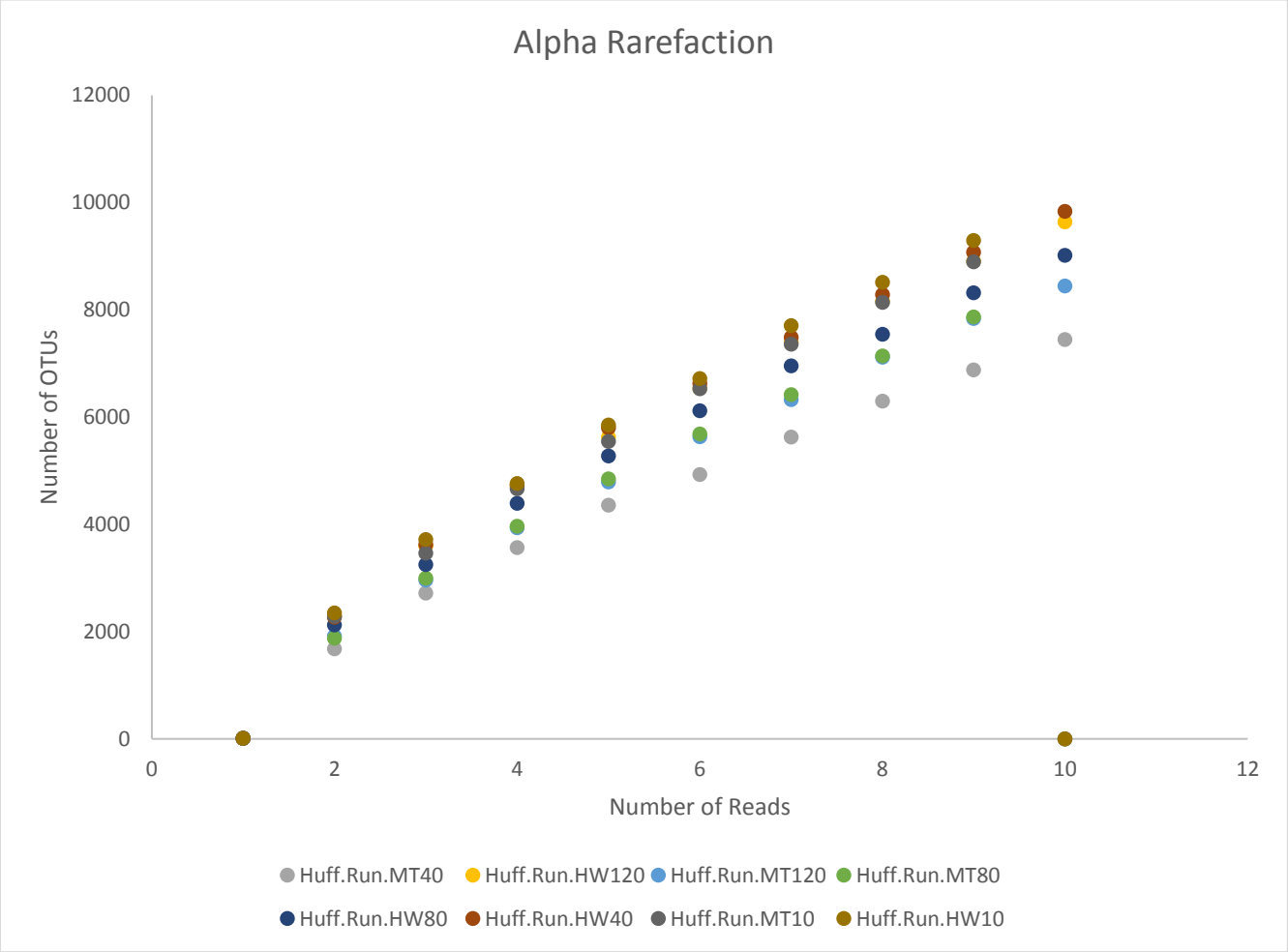

Figure 2. Rarefaction curves of sequence libraries recovered from Huff Run soils.

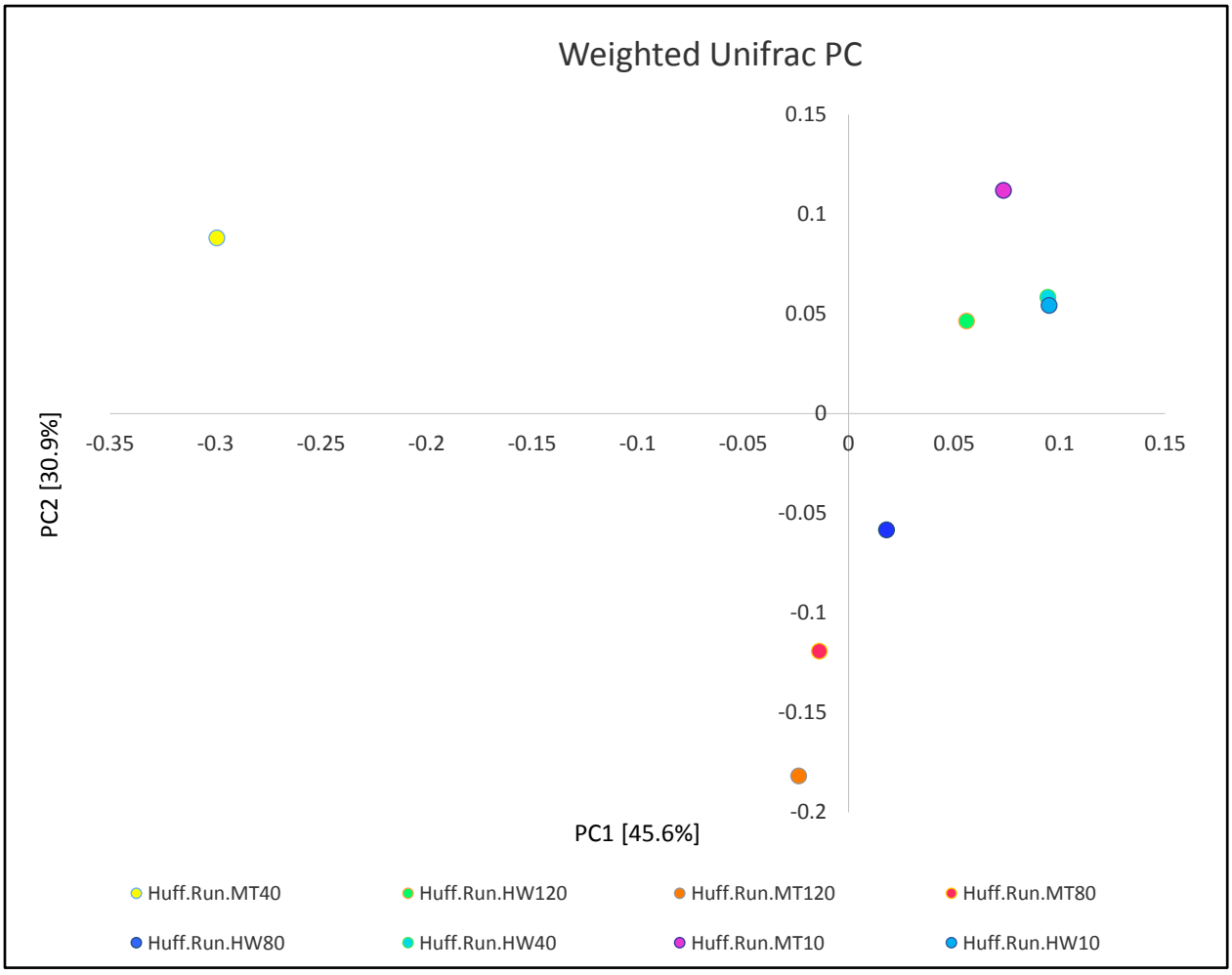

Figure 3 PCoA plot comparing soil associated microbial communities using the weighted UniFrac metric. Values in parentheses indicate percentage of variation explained on the respective axes.

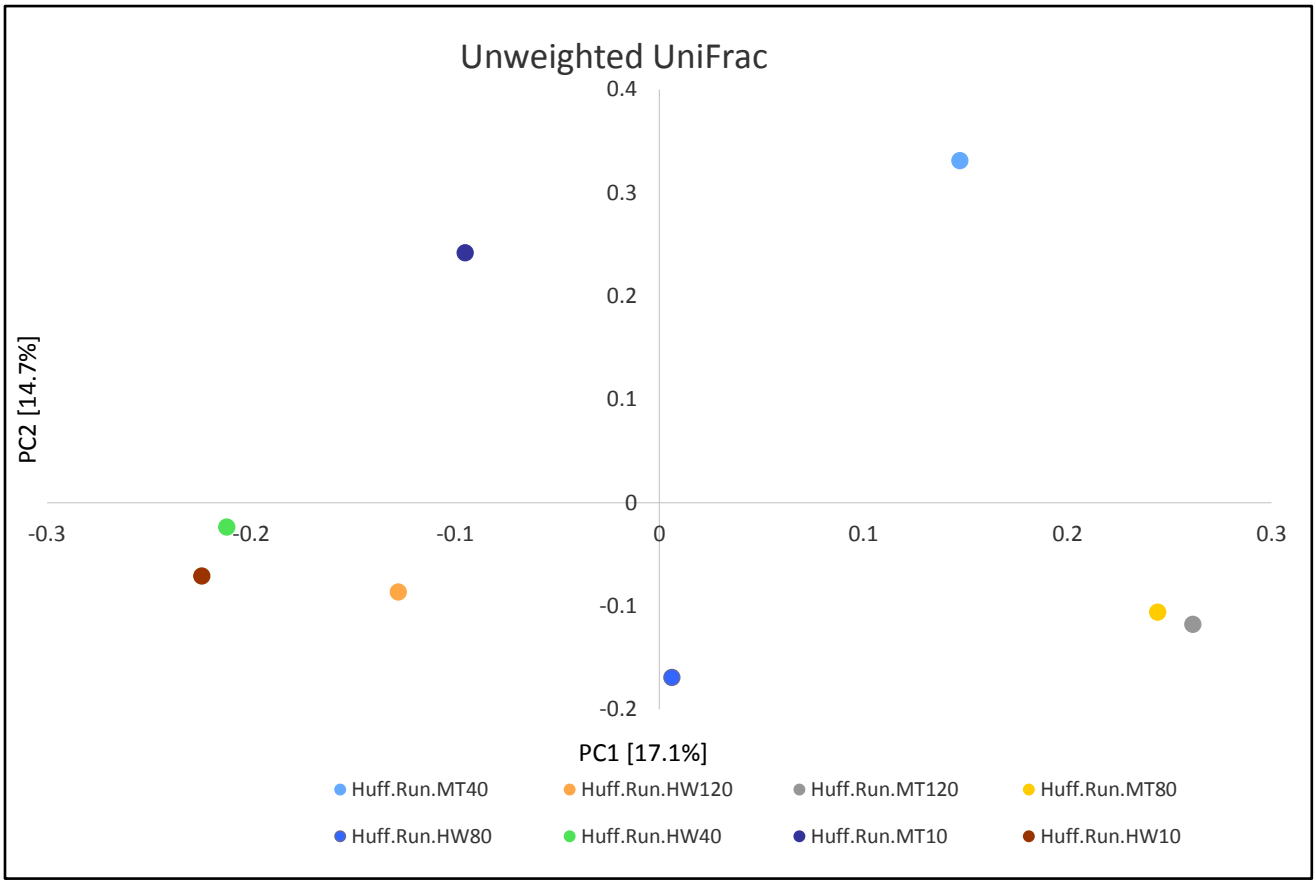

Figure 4. PCoA plot comparing soil associated microbial communities using the weighted UniFrac metric. Values in parentheses indicate percentage of variation explained on the respective axes.

Conclusions:

This experiment allowed the characterization of microbial communities in the disturbed and undisturbed soils. Examining distribution alongside the weighted Unifrac graph displays that both the MT and HW territories share similar microbial communities. Future studies may examine the site as it continues to develop. Continued microbial testing in the future may lend insight into the dynamics of recovery. While the data indicates recovery of the microbial community, may not depict recovery the entire ecosystem. Measurements of organic carbon, manganese, microbial respiration rates, and plant activity alongside microbial community quantification may depict a fuller picture of soil recovery.

References

Doran, J., Coleman, D., Bezdicek, D., Stewart, B., Turco, R. F., Kennedy, A. C., & Jawson, M. D. (1994). Microbial Indicators of Soil Quality. SSSA Special Publication Defining Soil Quality for a Sustainable Environment. doi:10.2136/sssaspecpub35.c5

Harris, J. A., Bentham, H., & Birch, P. (2003). Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science,54(4), 801-808. doi:10.1046/j.1351-0754.2003.0559.x

Huff Run Watershed Acid Mine Drainage Abatement and Treatment Plan (2000) Prepared for Ohio DNR by Gannett Fleming

Kuczynski, J., Stombaugh, J., Walters, W. A., González, A., Caporaso, J. G., & Knight, R. (2011). Using QIIME to Analyze 16S rRNA Gene Sequences from Microbial Communities. Current Protocols in Bioinformatics. doi:10.1002/0471250953.bi1007s36

Lamborn, R. E., Geology of Tuscarawas County. Division of Geological Survey: 1956; Vol. 55.

Lian, B., Chen, Y., & Tang, Y. (2010). Microbes on carbonate rocks and pedogenesis in karst regions. *Journal of Earth Science, 21*(S1), 293-296. doi:10.1007/s12583-010-0240-8

Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., & Knight, R. (2010). UniFrac: an effective distance metric for microbial community comparison. The ISME Journal,5(2), 169-172. doi:10.1038/ismej.2010.133

Mummey, D. L., Stahl, P. D., & Buyer, J. S. (2002). Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Applied Soil Ecology,21(3), 251-259. doi:10.1016/s0929-1393(02)00090-2

National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [2017]. Available from: https://www.ncbi.nlm.nih.gov/

Poncelet, D. M., Cavender, N., Cutright, T. J., & Senko, J. M. (2013). An assessment of microbial communities associated with surface mining-disturbed overburden. Environmental Monitoring and Assessment,186(3), 1917-1929. doi:10.1007/s10661-013-3505-8

Schatz, A. (1963). Chelation in Nutrition, Soil Microorganisms and Soil Chelation. The Pedogenic Action of Lichens and Lichen Acids. *Journal of Agricultural and Food Chemistry, 11*(2), 112-118. doi:10.1021/jf60126a004

Wang, Q, G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microbiol.* 73(16):5261-5267; doi: 10.1128/AEM.00062-07