Summer 8-2-2023

The Impact of Soil Disturbance on Soil Bacterial Community Composition

Marie A. Rodriguez
Southern Adventist University, mariearodriguez@southern.edu

MarK Peach
Southern Adventist University

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

The Impact of Soil Disturbance on Soil Bacterial Community Composition

Marie A. Rodriguez*, Mark Peach, and Timothy D. Trott

Abstract

Soil bacterial communities are an important part of terrestrial ecosystems due to their roles in biogeochemical cycling processes. Consequently, understanding how soil disturbance affects the soil bacterial diversity is vital to understanding the entire ecosystem. In this study we examined the effects of soil disturbance (by mining) on the soil bacterial community composition from three sites on Bauxite Ridge in Southeast Tennessee compared to three undisturbed sites in a nearby location. The soil bacterial community was analyzed by 16S rDNA amplicon sequencing of total DNA extracted from the soil samples collected from each of the six sites. Characterization of the bacterial community of these six sites showed that soil disturbance does not appear to be correlated with the differences in the diversity of the bacterial community, though this does not correlate with previous research.

Introduction

Soil microbes are an unseen majority of life whose roles in the catabolic pathways and biogeochemical cycling are essential for the functioning of terrestrial ecosystems (Whitman et al. 1998; van der Heijden et al. 2008). Because of their importance to the optimal functioning of plants and animals who share these ecosystems, the study of soil bacterial composition allows a new understanding into the interrelationships within ecosystems. Although soil structure and nutrient composition are also important to the biome, the diversity of the bacterial community is possibly of equal or greater importance (Van der Heijden et. al, 2012). It is also important to note that the myriad uses of land have an impact on soil properties, nutrient availability, and the

composition of soil microbial communities (Frąc et. al, 2020). In this report, total DNA extraction was performed on soils from either disturbed or undisturbed areas, and subsequent 16S metagenomic sequencing was used to determine the impact of past large scale mining disturbances have had on the soil bacterial communities.

DNA analysis has become the most common method used to differentiate between species when examining the microbiota community and composition in soil samples. One of the complications of this analysis is the rapid natural degradation of the DNA itself that occurs at ambient temperatures and variability. These factors lead to an accumulation of highly fragmented, single stranded, and undifferentiated DNA which must be targeted for extraction (Patzold, 2020). However, using the small subunit RNA (rRNA) for targeted fingerprinting helps circumvent this complication since it is highly conserved and can be readily identified by polymerase chain reaction (PCR) primers and subsequent bulk sequencing known as amplicon sequencing (Hugerth & Andersson, 2017). Operational taxonomic units (OTUs), often used equivalently with the term Amplicon Sequencing Variants (ASVs), of PCR reads are then clustered based on similarity and used in this process to help determine the species of microbes (Hugerth & Andersson, 2017).

Previous research has indicated that disturbances of soil structure significantly reduces the bacterial diversity and nutrient availability of those soil (Lei et. al, 2020). This is supported by research done on mining sites. In these sites, bacterial diversity was analyzed using the 16S rRNA subunit. The resulting sequences were then grouped by taxa and analyzed. It was concluded that mining decreased bacterial diversity, suggesting that continued disturbance has a negative effect on bacterial diversity (Fernandez et al, 2018). Also it has previously been demonstrated that the stoichiometric ratios of carbon, nitrogen, and phosphorus affect the

nutritional status of soil which in turn affect bacterial community growth and diversity. These nutrients in the soil are gathered from plants and other macro-organisms that interact within the ecosystem. Disturbed soils create disturbances in the plant communities they support, which leads to a reduction in available nutrients, which in turn can lead to reductions and changes in the soil bacterial community (Tian et. al, 2020).

The study area for this project is Bauxite Ridge, a section of which is protected on the property of Southern Adventist University, in Southeast Tennessee. It is in the Appalachian Valley, in the southern part of Hamilton County. The Bauxite Ridge's maximum topographic relief is about 300 feet. It is composed of non-pisolitic baukite that ranges in color based on the amount of iron it contains. Baukite is composed of 95.84% silicon dioxide, 1.51% aluminum oxide, 1.33% titanium dioxide, 0.71% iron (iii) oxide, 0.40% magnesium oxide, 0.15% calcium oxide, and 0.06% carbon dioxide (Davison, 1927). The geology of Bauxite Ridge is also characterized by underlain shale, limestone and dolomite in the floors of the valleys, both of which have a low chert content while the ridges are formed by very chert carbonate rocks or sandstone (Dunlap, 1965).

Although the misleading name of Bauxite Ridge suggests that the land is made of actual bauxite, in reality it is composed of the impure sandstone called "baukite", which is a siliceous refractory material which occurs in stratified formations. This misunderstanding of the name of Bauxite Ridge and the discovery of abandoned quarries lead to the creation of local lores about "bauxite mines," however, the facility is more accurately termed a quarry rather than a mine. The quarries operated under the American Baukite Company (later known as Baukite Refractories Inc.) from 1924 till circa 1955 for extracting baukite, which was used as heat resistant material to line kilns and furnaces (Mills, 2021).

In the late 1970's, the McKee family purchased 200 acres of Bauxite Ridge and began development of the lower ridge. Ellsworth Mckee's (the former chairman of Mckee Food corps.) son, Rusty Mckee, began the process of protecting the upper ridge from development for the enjoyment of the community by suggesting conservation of the land. Later on due to the unfortunate circumstances of his father's business partner, development of the lower ridge came to a halt and now Bauxite Ridge is a popular destination due to its well maintained walking and mountain-biking trails.

Overall, disturbances of soil structure significantly reduce the bacterial diversity and nutrient availability of the soil (Lei et. al, 2020). This leads to the hypothesis that the disturbed soils of Bauxite Ridge will be composed of less bacterial diversity than the undisturbed soils. Using six soil sample sites located on Bauxite Ridge and 16S amplicon sequencing to determine the soil bacterial diversity, we were able to elucidate if there was a correlation between soil disturbance and the soil bacterial community composition.

Methods and Materials

Establishment and Description of Sample Sites

Six sites located near the Bauxite Ridge Trail system next to Southern Adventist University in the mined (disturbed, D) and unmined (undisturbed, UD) ecosystems of Southeastern Tennessee were established and used as soil sample collection sites.

History of mining activities was obtained from Mills McAuthur and Rusty Mckee. The six sites were then established with three of them being previously mined/heavily disturbed and three were (supposedly) unmined/undisturbed. The sites were of similar habitat, type of soil horizons, elevation, and organic and inorganic material present. The GPS coordinates were then

recorded for each site. This process was repeated for all six sites. The first UD sample was collected at the coordinates 35°1’28”N 85°210”W. The second UD sample was collected 35°1’28”N 85°2’7”W. The final UD sample was collected 35°1’32”N 85°2’15”W. The first D sample was collected at the coordinates 35°1’28”N 85°2’11”W. The second D sample was collected 35°1’19”N 85°2’18”W. The final D sample was collected 35°1’19”N 85°2’20”W.

Soil Sample Collection

Soil core samples were collected using a Varomorus Compact Soil Sampler Probe 12" Stainless Steel (Varomorus, Florida, USA) which was driven to a depth of 2 to 3 cm (3inches) into the soil with a mallet. Samples were taken from each site to examine the bacterial community composition. The Soil Sampler Probes were autoclaved and washed with an ethyl alcohol solution using the procedure which Wu, Wu, Zhang, & Cheng demonstrated reduced the DNA contamination in amplicon sequencing (2018). A total of six soil samples were taken, one form each of the mined and unmined sites. Before soil samples were collected the leaf-litter was removed from the portion of the sites from which the samples were collected. The samples were placed into separate sterile plastic bags, and then they were stored on ice until transferred to the lab where each soil core was stored at -20℃ until DNA extraction was performed.

Total DNA Extraction

Total DNA was extracted from 0.25 g of soil from each site 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, 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). 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 from each soil sample was shipped to an external lab (LC Sciences, Houston, TX) for 16S/18S/ITS rDNA amplicon sequencing. For this work we chose to focus primarily on the 16S data. 16S rDNA amplicon sequencing identifies bacteria, 18S rDNA amplicon sequencing is for eukaryotic microorganisms identification, and ITS rRNA amplicon sequencing is for fungi.

Results

The six soil sites located near the Bauxite Ridge Trail system consisted of three disturbed (D) and three undisturbed (UD) sites. Disturbed sites were identified along the trail where previous indications of bauxite mining operations can be found, including discarded cabling and equipment. Undisturbed sites were established some distance off the trails where no signs of mining were visually detectable.

16S amplicon sequencing allowed the characterization of the bacterial soil community composition of each of the six sites. An analysis of the total number and diversity of bacterial

Operational Taxonomic Units (OTUs), often used equivalently with the term Amplicon Sequencing Variants (ASVs), was provided as part of this data. In this paper, the term ASV will be used as a functional analogue for bacterial species. ASV sequences are key for downstream analysis, including diversity, taxonomy, and differential analysis because they are functional as species identification. In prokaryotes, the 16S rRNA transcript is composed of nine variables (V1 to V9) and ten conserved regions. For taxonomic purposes, the variable regions shown to be highly diverse among species were used.

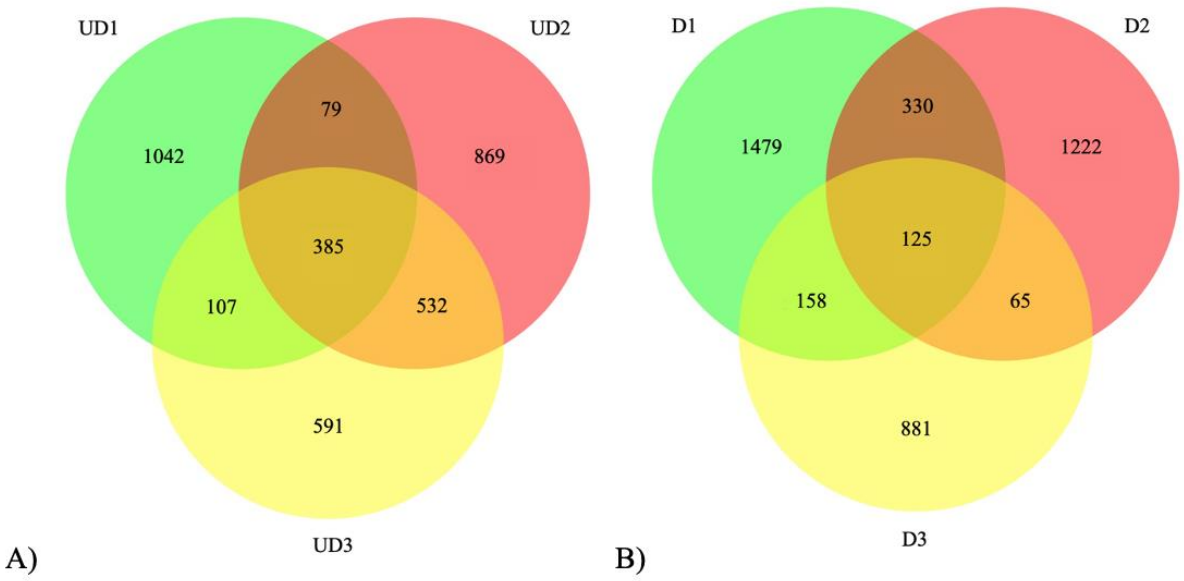


Figure 1. Venn diagram comparing the ASV's of: A) All three undisturbed (UD) plots. B) All three disturbed (D) plots. Each individual circle represents the amount of unique ASVs of their respective plots. Overlap of the circles signifies which ASVs were found in common between two compared plots, or all three plots.

As shown in Figure 1, a high degree of ASV variation can be found between each of the three undisturbed plots and between each of the three disturbed plots. Only 385 species were

found in common between all of the UD plots, and only 125 species were found in common between all of the D plots.

In the venn diagram of Figure 1A, UD3 has 591 unique species, UD2 has 869 unique species, and UD1 has 1042 unique species. Overall, UD3 has 1,615 species in total, UD2 has 1,865 species in total, and UD1 has 1,613 species in total. Based on this information, out of the total of all UD3 species only 37% are unique species, only 47% are unique for UD2, and only 65% are unique for UD1.

Figure 1B shows that D3 holds 881 unique species, D2 holds 1222 unique species, and D1 holds 1479 distinct species. Overall, D3 has 1,229 species in total, D2 has 1,742 species in total, and D1 has 2,092 species in total. That means that out of all the D3 species, only 72% are unique, of all the D2 species only 70% are unique, and of all the D1 species only 71% are unique.

The ASV comparison between the three UD plots shows that for each of the UD plots the bacterial communities have great distinction–meaning that the undisturbed sites were more distinct than similar between one another. The same is true for the D plots, respectively.

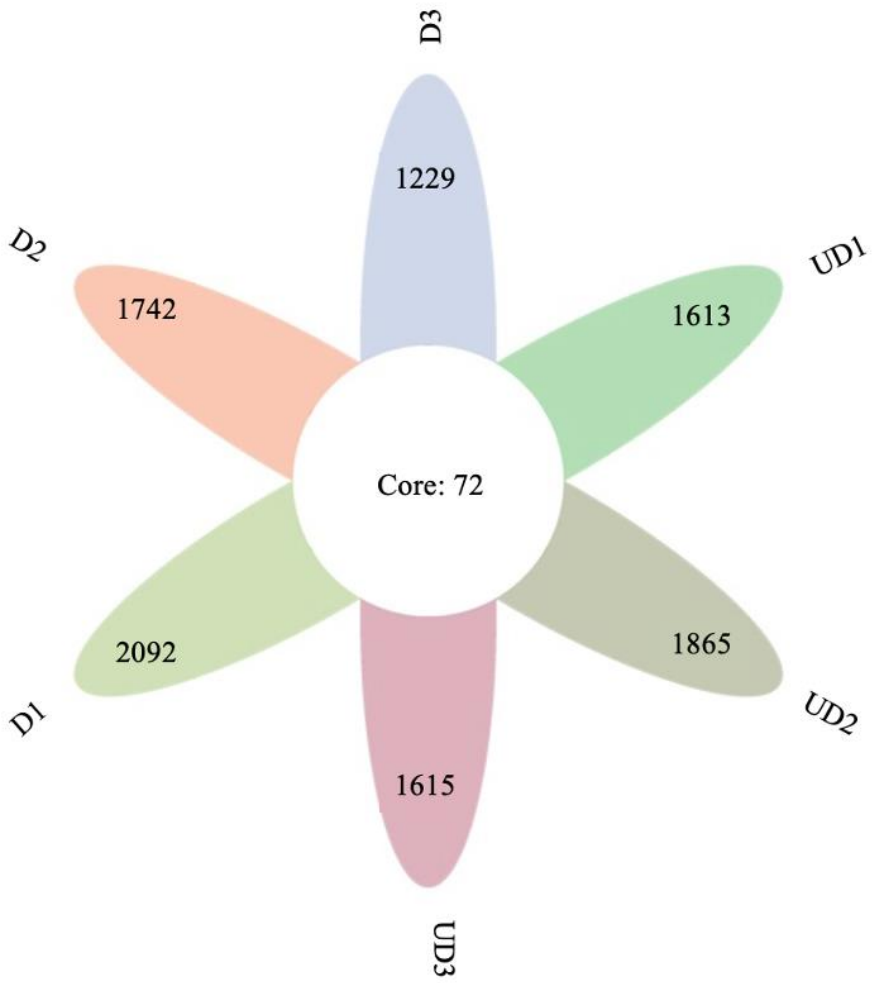


Figure 2. Venn diagram comparing the ASVs of all six undisturbed (UD) and disturbed (D) plots.

ASV comparisons between all six plots was conducted and aims to show if there is similarity between them. Figure 2 shows that very few species are common between each of the six soil plots–even between the supposedly disturbed and undisturbed plots. This result further fortifies the findings from Figure 1. Of all 10,228 species represented from Figure 2, only 0.7% of those species were found to be shared between all of the six plots.

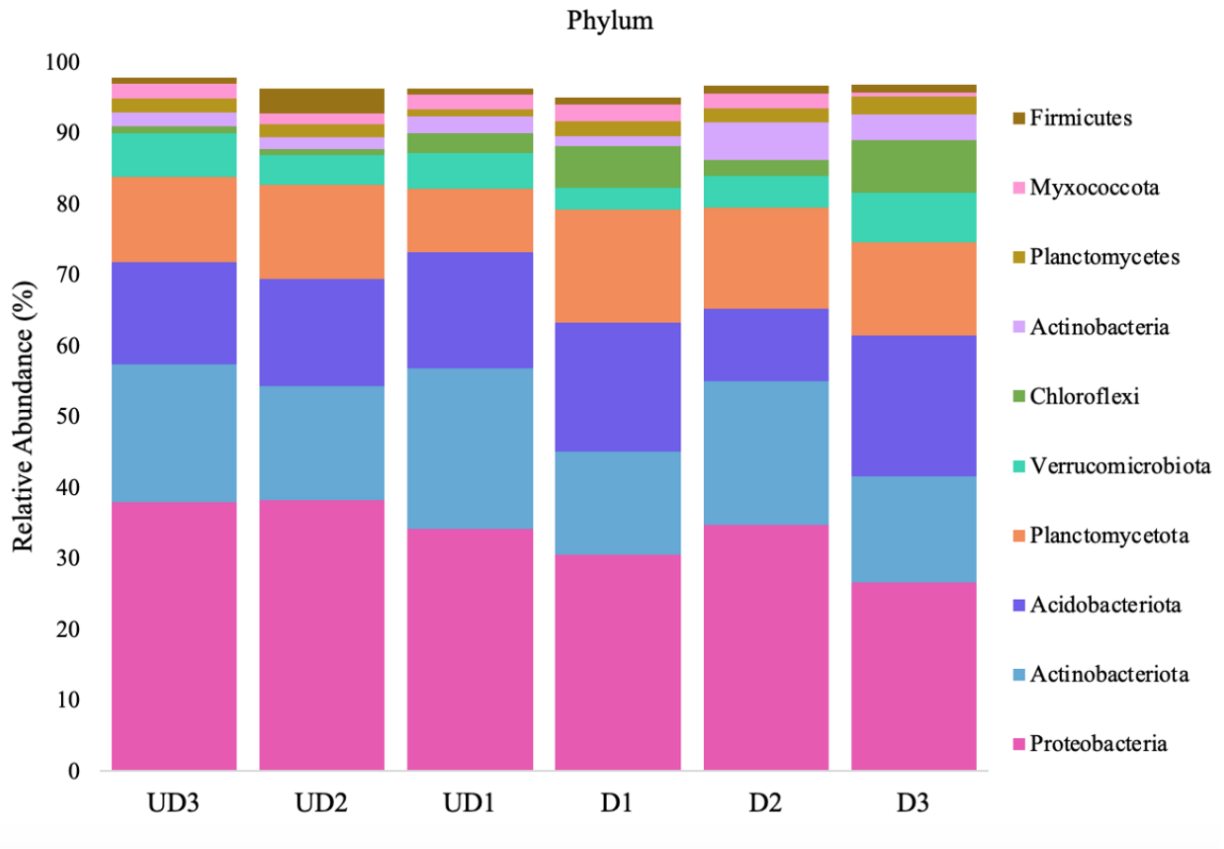


Figure 3. Stacked bar-graph comparing ASV abundances of the phyla of all six plots. The horizontal axis contains the names of the plot each sample was from, while the vertical axis represents the relative abundance of a given taxonomy classification. The top 10 phyla with the highest abundance shown.

Comparing the phylums of all six sites assists the determination of phylum abundances and identifying the structure of the bacterial communities in each site. The stacked bar graph of Figure 3 allows the visual comparison of the abundances of the 6 sites by phyla. This same type of analysis was performed for higher taxonomic levels (class, order, family), however, these did not indicate any discernible trends. All sites show similar trends in abundance particularly noted in the *Proteobacteria, Actinobacteriota, Acidobacteriota, Plactomycetota, and Verrucomicrobiota* .

Discussion

As indicated in the results above, the trends in bacterial species appear largely similar across disturbed and undisturbed plots. Figure 1A shows that there is minor similarity in species (ASVs) between the samples of the undisturbed sites. Figure 1B shows that there is minor similarity in species (ASVs) between the samples of the disturbed sites. From the results, more species were found to be unique between each undisturbed sample and disturbed samples. This major finding is surprising since it was hypothesized that the soil community diversity and abundance metrics would be distinct between disturbed and undisturbed sites but similar between the disturbed sites and all the undisturbed sites. Figure 3 shows that at the phyla level, there is no abundance difference between all six sites. From previous studies, it should be expected that there is more microbial abundance in UD soil since higher concentrations of oxygen and nitrogen (the main energy sources of many bacteria) are found in UD soil (Park, 2020).

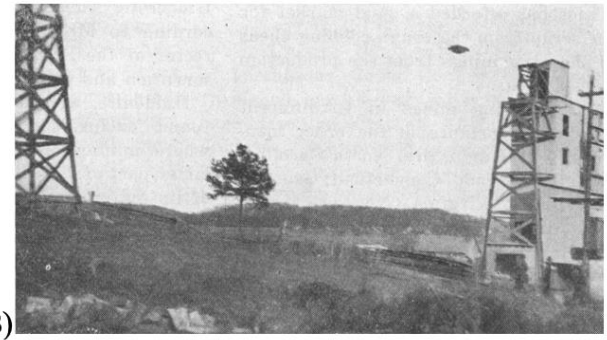
A)  B) 

Figure 4. A) Picture depicting the Baukite quarry at Apison, Tennessee. B) Picture depicting the mill's end of tram line.

One possible explanation for this observation of lack of distinction between D and UD sites is that all areas of Bauxite Ridge have been heavily mined since 1927 and microbial communities have yet to reestablish their niches (McArthur, 2021). Figure 4 depicts Bauxite

Ridge in 1927, and Figure 4B specifically shows that hills of Bauxite ridge were left clear cut which indicates uniform disturbance (McArthur, 2021). Further research must be conducted to verify the hypothesis about reestablishment.

Mining practices are one of the most severe human activities as it alters the chemical, physical, and biological properties of the soil environment (Fernandez et al, 2018). In previous studies, intense mining done in the historical area of Iron Quadrangle, Minas Gerias, Brazil revealed that their top 5 phyla in the disturbed soil to be *Proteobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes,* and *Bacteroidetes*. Besides *Bacteroidetes*, all other phyla are also found in the top 10 phylum in this study as shown in Figure 3. In fact, this study and the study previously mentioned both have the same most common phylum, which is *Proteobacteria*.

Proteobacteria abundance is consistent with other studies where the most dominant phylum was also *Proteobacteria* (Janssen, 2006). From identifying the dominant soil bacterial taxa in libraries of 16S rRNA of different soil types, the dominant phyla in the libraries are *Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes,* and *Firmicutes* (Janssen, 2006). The members of these nine phyla make up an average of 92% of most soil libraries. From comparing this information to the data in Figure 3, six out of these nine most common phyla are found in this study's soil samples.

Furthermore, in a study conducted in China concerning the reclamation of soil by microbes, it was discovered that regardless of reclamation ages *Proteobacteria* was the most abundant and most ubiquitous and common group in soil (Li, 2014). Phylum-level profiling also showed that soil bacteria were mainly composed of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes Planctomycetes* and *Proteobacteria* regardless of

soil type and environment (Li, 2014). The findings of the study previously mentioned and Janssen's study illustrate that some phyla are common throughout most soils and should not be used to represent the impact of soil disturbance on soil bacterial community composition.

Comparison of the abundances of these common phyla mentioned above and total biodiversity should be emphasized when comparing the disturbed and undisturbed sites. The study in China showed that much higher diversity levels of bacterial communities belong to older reclaimed soils and undisturbed soils in comparison to recently disturbed soil (Li, 2014). Likewise, variation in the abundances of each group of the most common phyla were observed between reclamation times and the undisturbed soil control (Li, 2014).

From the hypothesis and in accordance with the previously mentioned study, it was expected that there would be more diversity in undisturbed sites. However, this present study does not show more diversity between D and UD sites. From analyzing Figures 1 and 2, all sites have common ASV (diversity) quantities. This goes against the hypothesis that was established. Likewise, the abundances of the phyla between all disturbed and undisturbed sites were also relatively the same.

The microbial community plays an important role in the soil system. This study provides important insights into the structure of the prokaryotic community of a mining, and surrounding, area under regeneration. Visibility of plants is usually termed as recovered land, yet microorganism communities play a vital role in the ecology of land (Sanda, 2017). Analysis like those used in this study can be used to give a detailed picture of the structure and diversity within the soil microbial communities.

In future studies, comparisons of 18S and ITS genomic sequencing can also be analyzed. 18S data indicates eukaryotic microbial diversity and ITS described fungal microbial diversity.

Data of 18S and ITS genomic sequencing was obtained for this study but not included in the analysis presented. Analysis of these sequences could lead to a better understanding of complete microbial biodiversity in disturbed and undisturbed soils in contrast to just knowing terrestrial plant biodiversity and abundance. Also, repeated measurements at more soil sites could make this study's data more accurate by increasing the sample size.

Works Cited

Fernandez, C. C., Kishi, L. T., Lopes, E. M., & Omori, W. P. (2018, February 13). Bacterial communities in mining soils and surrounding areas under regeneration process in a former ore mine. *Brazilian Journal of Microbiology*. Retrieved April 6, 2023, from https://www.sciencedirect.com/science/article/pii/S1517838217305890

Frąc, M., Lipiec, J., Usowicz, B., Oszust, K., & Brzezińska, M. (2020). Structural and functional microbial diversity of sandy soil under cropland and grassland. *PeerJ, 8*. doi:10.7717/peerj.9501

Gremion, F., Harms, H., & Chatzinotas, A. (2003). Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil. *Scopus*. Retrieved April 6, 2023, from https://www.scopus.com/inward/record.uri?eid=2-s2.0-

84884128066&doi=10.1016%2fj.compositesb.2013.08.027&partnerID=40&md5=773de57e5bc002be5d3997399f31667e

Hugerth, L. W., & Andersson, A. F. (2017). Analyzing microbial community composition through amplicon sequencing: From sampling to hypothesis testing. *Frontiers in Microbiology*, 8. https://doi.org/10.3389/fmicb.2017.01561

Janssen, P. H. (2006). Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. *American Society for Microbiology*. Retrieved April 6, 2023, from https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/referencespapers.aspx?referenceid=1901938

Lei, H., Liu, A., Hou, Q., Zhao, Q., Guo, J., & Wang, Z. (2020). Diversity patterns of soil microbial communities in the Sophora flavescens rhizosphere in response to continuous monocropping. *BMC Microbiology*, 20(1), NA. https://link.gale.com/apps/doc/A635041301/AONE?u=tel_a_sau&sid=AONE&xid=2967b318

Li, Y., Wen, H., Chen, L., & Yin, T. (2014). Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and revegetation on coal mine spoils in China. *PLOS ONE*. Retrieved April 6, 2023, from https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0115024#pone.0115024-Rastogi1

McArthur, M, personal communication, April 12, 2021.

Park, J. (2020, August 22). Undisturbed vs. disturbed: Soil microbial diversity. *CURE*. Retrieved April 6, 2023, from https://sites.brown.edu/cure/2020/07/29/undisturbed-vs-disturbed-soil-microbial-diversity/

Patzold, F., Zilli, A., & Hundsdoerfer, A. K. (2020). Advantages of an easy-to-use DNA extraction method for minimal-destructive analysis of collection specimens. *PLOS ONE*, 15(7), 1–24. https://doi.org/10.1371/journal.pone.0235222

Tian, Y., Qiao, D., Xu, S., & Wang, N. (2020). Effects of tree species and topography on soil and microbial biomass stoichiometry in Funiu Mountain, China. *BMC Ecology*, 20(1), NA. https://link.gale.com/apps/doc/A650613053/PPGS?u=tel_a_sau&sid=PPGS&xid=74ab306f

Van der Heijden M.G.A., Bardgett R.D., Van Straalen N.M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11:296-310

Van der Heijden, M. G., & Wagg, C. (2012). Soil microbial diversity and agro-ecosystem functioning. *Plant and Soil,* 363(1-2), 1-5. doi:10.1007/s11104-012-1545-4

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. *PNAS* 95:6578-6583