MICROBIAL COMMUNITIES AS INDICATORS OF ECOSYSTEM FUNCTIONS AT TWO SITES IN GREAT SMOKY MOUNTAINS NATIONAL PARK

A thesis presented to the faculty of the Graduate School of
Western Carolina University in partial fulfillment of the requirements
for the degree of Master of Science in Biology

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ABSTRACT

MICROBIAL COMMUNITIES AS INDICATORS OF ECOSYSTEM FUNCTIONS AT TWO SITES IN GREAT SMOKY MOUNTAINS NATIONAL PARK

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Differences in soil nutrient cycling, including nitrogen cycling, and bio-availability have been linked to the inhabiting microbial and plant community interactions (Powell et al., 2015; Levy-Booth et al., 2014). Understanding the community relationships and their effects may help us describe the role of the microbiome in influencing plant communities and ecosystem functions (Phillips, Brzostek, and Midgley 2013; Isobe et al. 2011; Hacquard and Schadt 2015). Data quality and quantity is needed to describe causal relationships between microbial communities, plant communities, and their distributions in an ecosystem (Mushinski et al. 2017). To address the need for and demonstrate the utility of, functional and process focused data, microbial community analysis was conducted focusing on known bacterial nitrogen cyclers and mycorrhizal fungi. Samples were cored from the mineral soil within the mycorrhizosphere surrounding a Northern Red Oak at both the Purchase Knob and Cataloochee All Taxa Biodiversity Inventory (ATBI) plots (n=12 from each site). Samples were pooled and homogenized by sub site location around the Oak tree at each site (up slope, side slope, down slope; n=3 from each site). DNA extraction and next generation sequencing of the bacterial 16S rRNA and fungal internal transcribed spacer (ITS) rRNA regions were performed by Azenta, Inc. (South Plainfield, NJ). Important bacterial genera

found include the metabolically diverse *Burkholderia*, as well as known participants in soil nitrogen cycling *Nitrospira*, *Bradyrhizobium*, *Rhodoplanes*, and *Pedomicrobium*. Ectomycorrhizal fungi genera that are important plant symbiotes and transporters of nutrients within soil (Allen et al. 2003; Churchland and Grayston 2014) were found, including *Sebacina*, *Russula*, *Elaphomyces* and the potentially ectomycorrhizal *Pseudotricholoma*. Principal components analysis (PCA) of the distributions of sequences showed a clear difference between the Cataloochee and Purchase Knob sites with greater variability in community composition at Cataloochee for both bacteria and fungi. Key and prevalent groups identified are known to be involved in nitrogen cycling, plant associations, and nutrient transport suggesting that their relationships play some role in biogeochemical and nutrient cycling. Future analyses are needed to describe the pathways between microbial and plant, taxa and communities, and the emergent properties relating them to nutrient cycling and ecosystem functions.

BACKGROUND

NITROGEN CYCLING AND CLIMATE CHANGE

Climate change is significantly altering earth's biogeochemical cycles which is affecting ecosystem functions in poorly understood ways. Nitrogen (N) cycling is composed of three main steps where initial atmospheric dinitrogen (N_2) gas is deposited in soil as ammonium (NH_4^+) through the process of nitrogen fixation. Subsequent transformation of NH_4^+ via nitrification into nitrite (NO_2^-) and nitrate (NO_3^-) allows for the third chemical transformation. Denitrification is the final process from which nitrous and nitric oxides (N_2O and NO respectively), and ultimately N_2 can be produced and released into the atmosphere (Levy-Booth, Prescott, and Grayston 2014). N cycling is predominantly mediated by microbes in the soil, with individual processes largely performed by specialized taxonomic groups (Isobe et al. 2011). Soil nitrogen cycling is displayed in a simplified form in Figure 1. Soil nitrification begins with bacterial and archaeal oxidation of ammonia into nitrate using the ammonia monooxygenase (amo) enzyme, encoded partially by *amoA* genes (Horz et al. 2004; Levy-Booth, Prescott, and Grayston 2014). The amo enzyme catalyzes the rate limiting step of ammonia oxidation, which itself is the rate limiting step in nitrification, a process that is essential for increasing N bioavailability in N limited ecosystems (Horz et al. 2004; Isobe et al. 2011; Levy-Booth, Prescott, and Grayston 2014; Norman and Barrett 2014). NH_4^+ and NO_3^- are the main forms by which living organisms obtain N from soil (Isobe et al. 2011; Levy-Booth, Prescott, and Grayston 2014). Nitrification and denitrification dictate N availability and loss in terrestrial systems where N limitations are common (Levy-

Booth, Prescott, and Grayston 2014; Mushinski et al. 2017). Nitrification losses occur when NO_3^- leaches through the soil solution, reducing groundwater and stream quality, causing soil acidification and habitat eutrophication in systems importing the leachate (Isobe et al. 2011; Laverman, Speksnijder, and Braster 2001; Levy-Booth, Prescott, and Grayston 2014). Denitrification losses occur through emission of N_2O and NO gases into the atmosphere, contributing to global climate change by trapping heat, where N_2O has shown to be many times more potent than carbon dioxide over a 100-year period, and has also been linked to ozone depletion (Horz et al. 2004; Isobe et al. 2011; Levy-Booth, Prescott, and Grayston 2014). Increased N compound leaching and emission from forested systems, predominantly through NO and N_2O dissolution into water is largely attributed to increasing N deposition on the system which is caused by industrial processes and fossil fuel combustion (Aber et al. 1991; Galloway et al. 2003; Isobe et al. 2011). Increased N deposition likely alters soil microbiomes through changes in microbial community structure and function, as well as N cycling (Horz et al. 2004; Isobe et al. 2011). Microbial communities may be a mitigating factor regarding climate change, shifting of ecosystems and functions, and may be utilized for forest conservation management purposes if better understood.

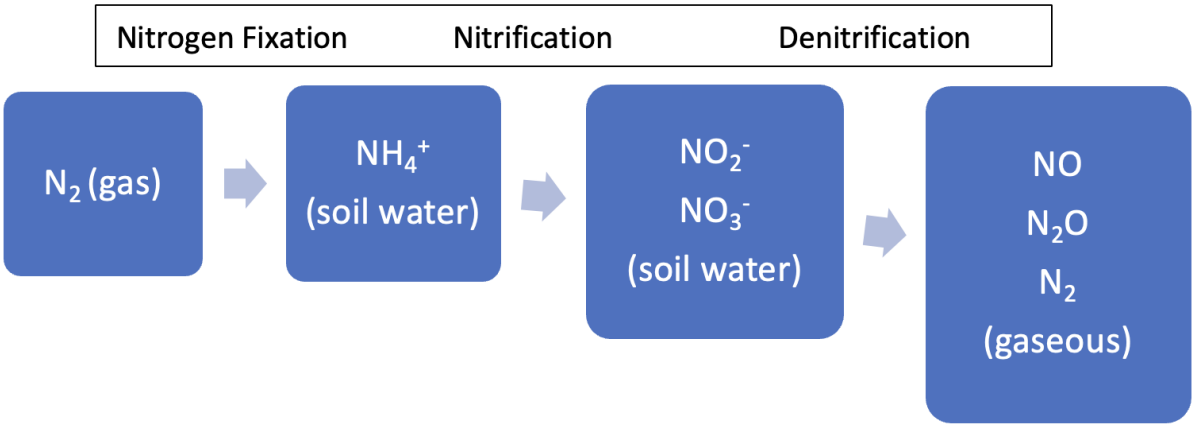

Figure 1: Simplified Nitrogen Cycle

OAK SIGNIFICANCE

It is likely that plants alter microbiomes in the soil they inhabit, and it is thought that the microbial communities within affect the health and fitness of the plant (Hacquard and Schadt 2015; Pinho et al. 2020). The oak genus, *Quercus*, is regarded as a highly ecologically and economically valuable contributor to eastern U.S. forested ecosystems (Conrad et al. 2020; Radcliffe, Hix, and Matthews 2021). Some Oaks are considered keystone species because of their contributions to wildlife food supplies through acorn production, and leaf hosting of highly abundant and diverse insect taxa (Greenberg et al. 2014; Radcliffe, Hix, and Matthews 2021). In this regard oak species diversity may also mitigate food supply shortages through differences in "red" vs "white" subgenus acorn production cycles, and especially after a major frost where acorn production losses are differentially timed between subgenera (Radcliffe, Hix, and Matthews 2021). The oak decline phenomenon, which consists of a complex suite of abiotic and biotic factors whose relationships are little understood, is affecting many oak-dominated forests (Conrad et al. 2020; Radcliffe, Hix, and Matthews 2021). Oak replacement by shade-tolerant mesophytic tree species, and oak regeneration failure have also been observed in several study areas (Radcliffe, Hix, and Matthews 2021). The relationships between soil microbes and plant cohorts may be intrinsic to these phenomena and represent a gap in scientific understanding that could benefit from increased attention.

MYCORRHIZAL FUNGI

Understanding plant-microbe interactions can elucidate the effects of microbiomes

on plant lifestyles and lifecycles (Hacquard and Schadt 2015). These interactions have also been linked to differences in nutrient cycling and availability (Phillips, Brzostek, and Midgley 2013; Isobe et al. 2011). Recent studies have indicated the importance of soil microbiomes in plant health, especially within the rhizosphere (Hacquard and Schadt 2015; Pinho et al. 2020). Mycorrhizal fungi are ubiquitous, contribute to plant growth and nutrient cycling, and have shown to organize around plant functional type (Allen et al. 2003; Phillips, Brzostek, and Midgley 2013; Churchland and Grayston 2014; Terrer et al. 2016). Arbuscular and ectomycorrhizal (AM and EM respectively) fungi are likely associated with most tree species in temperate forests and reflect differences in biogeochemical processes between them (Phillips, Brzostek, and Midgley 2013). AM associated plants tend to have faster decomposing litter and require AM to scavenge soil for nutrients, unlike EM associates that can extract nutrients from organic matter and whose litter tends to decompose more slowly (Phillips, Brzostek, and Midgley 2013; Read and Perez-Moreno 2003). Phillips, Brzostek, and Midgley (2013) found that EM associated trees were well correlated to the ratio of organic N to inorganic N in the upper surface soil and increasing percentages of AM associated trees were correlated to increased N losses in a system. Nutrient cycling and bacterial communities have been linked to processes involving mycorrhizae; this is especially so in soil around the mycorrhizal root system, termed the mycorrhizosphere (Allen et al. 2003; Phillips, Brzostek, and Midgley 2013; Churchland and Grayston 2014; Terrer et al. 2016).

MICROBIAL COMMUNITIES AND ECOSYSTEM FUNCTIONS

Microbial community data is generally regarded as noisy (containing complex background information that can make it hard to interpret trends), sparse (many observations are zero counts), and compositional (community composition is derived from interactions of

the individuals) (Busato et al. 2023; Weiss et al. 2016). These factors make microbial community correlation analysis difficult and lead to loss of aspect specificity in any particular interaction, especially when more than two aspects of a community are involved (Busato et al. 2023; Weiss et al. 2016). Although there has been little progress in relating microbial communities to in situ ecosystem biogeochemical functioning, this is being remedied with advanced molecular techniques and modeling (Graham et al. 2016; Isobe et al. 2011; Powell, Welsh, and Hallin 2015). Microbial gene abundance has been correlated with changes in process rates, and soil characteristics when using quantitative analysis to analyze functional gene sequences (Levy-Booth, Prescott, and Grayston 2014). Also, statistical models have shown to be more accurate and predictive of ecosystem function and soil characteristics when incorporating microbial functional genes (Graham et al. 2016; Powell, Welsh, and Hallin 2015). The spatial patterns of functional communities in soil likely play an important role in determining where specific functions take place and may indicate a spatially specific ability to mitigate changing environmental conditions. Ubiquity and complexity of microorganisms in the soil may preclude the direct application of studies regarding them into issues of resource or conservation management. Finer details may need to be resolved for specific issues and environments, especially when scaling up to the ecosystem level. But these qualities also may present a background on which issues in these areas become apparent or understood more clearly, such as understanding the mycorrhizae plant complex's influence on the distribution of biogeochemically functional microbes within an ecosystem. Knowing how these complex species associations interact in their environment may give us a way to isolate functional species within an ecosystem to a specific niche, allowing us to derive a spatially explicit map of ecosystem functions.

INTRODUCTION

It is known that plants, microbes (including fungi), and soil abiotic factors act on one another dynamically, but the spatial patterns and mechanisms are not always well understood (Horz et al. 2004; Mushinski et al. 2017; Norman and Barrett 2014). Because it is logistically difficult, time consuming, and expensive to probe and analyze soil microbial communities, there is a shortage of research in this area regarding spatial distribution and functional gene assemblages within soils or across landscapes (Levy-Booth, Prescott, and Grayston 2014; Mushinski et al. 2017). The inability to culture many soil bacteria in laboratories likely leads to reservations when deciding on research to be funded and this contributes to a lack of microbial species resolution regarding what species are present and their function in the soil environment, (Horz et al. 2004; Mushinski et al. 2017). To establish causal relationships there is a need for data quantity and quality regarding soil microbial communities and their spatial distribution in ecosystems (Mushinski et al. 2017). Spatial information of these communities allows us to know where they are in soil environments, and when combined with measures of abundance and functional genes, can indicate the ecological importance of what they do in situ. Ammonia oxidation is a critical process in the global nitrogen cycle while mycorrhizal fungi dominate nutrient exchange in forested soil environments and oak species are both economically and ecologically important in their environmental range. The purpose of this research was to investigate the microbial communities within the mycorrhizosphere of Northern Red Oak (*Q. rubra*) in southeastern U.S. hardwood forests of the Appalachian Mountains.

METHODS

Samples were taken at the historic Cataloochee and Purchase Knob ATBI (All Taxa Biodiversity Inventory) sites within Great Smoky Mountains National Park on May 12, 2022 with the help of Paul Super and Sean O'Connell. At each site a mature Northern Red Oak tree was chosen as the sample area from which mineral soil samples would be taken by coring. Four replicates each were gathered from up slope, cross slope and down slope of the main stem for a total of 24 samples; 12 from each site. After removing leaf litter and humus to expose bare soil, samples were taken by using a soil corer to a depth of approximately 12-18 inches. Approximately 75 grams were collected from the lower portion of the corer into WhirlPak bags and flash frozen on dry ice in a cooler. Samples were stored in a -80°C freezer on Western Carolina University's campus.

DNA was extracted using the Qiagen DNeasy® Powersoil® Pro Kit. Following the quick start protocol (May 2019) we substituted step 2 with 30 seconds at 2,500 rpm in a bead beater, then 1 minute on ice, followed by another 30 second bead beating. This was done because the vortex adapter referenced in step 2 has been implicated in the extreme shearing of DNA. DNA extractions were completed June 27, 2022 and stored at -20°C. Agarose gel electrophoresis was performed to test DNA quantity and quality, and polymerase chain reaction (PCR) was performed following to amplify bacterial and Fungal DNA.

Initial primers used include 341F (forward) with a GC clamp and 907R (reverse) for bacterial 16S rRNA region (Laverman, Speksnijder, and Braster 2001; Zhalnina et al. 2014; Ribbons et al. 2016; Pinho et al. 2020), EF4 (forward) and ITS4R (reverse) for the first reaction of a nested PCR method for fungi, ITS1F (forward) with a GC clamp and ITS2R (reverse) for the second reaction(Selosse, Bauer, and Moyersoen 2002; Nguyen, Nickerson, and Seifert 2013; Ribbons et al. 2016; Pinho et al. 2020). To investigate the presence of

the *amoA* sub-unit among bacteria and *amoA* gene among archaea in our samples, primers used included *amoA*1F (forward) and *amoA*2R (reverse) for the bacterial *amoA* sub-unit, and amo111F (forward) and amo643R (reverse) for the archaeal *amoA* gene (Dillow 2009). All PCR products were stored at 4°C and tested by agarose gel electrophoresis for quality and quantity. Gel images are displayed below in Figures 2-6.

Originally, we had planned to use denaturing gradient gel electrophoresis (DGGE) to separate PCR products for community diversity analysis and use resultant bands to obtain DNA sequences identifying the bacterial and fungal species present. Test runs of DGGE were performed using the BioRad D-Code™ Universal Mutation Detection System. While testing the DGGE process it was found that the chemicals and equipment used could not produce the results required for this project. This irreproducibility led to discarding DGGE in favor of another data collection method. An opportunity for next generation sequencing was found and the remaining soil samples, which had been preserved at -20°C due to a malfunction in the ultralow freezer, were combined into 6 total samples, 3 from each site, 1 each from up slope, side slope, and down slope. Approximately 1g of soil was sent to GENEWIZ of Azenta Life Sciences (South Plainfield, NJ) for sequencing and initial statistical analysis. The processes, methods and reports generated by Azenta are included as appendices to this thesis and Raw sequence files will be uploaded to GenBank for access.

The Western Carolina University (WCU) Fall 2022 semester Principles of Microbiology lab (Biol 413/513) taught by Dr. Sean O'Connell used approximately 4 grams of soil from each of the 6 homogenized samples in lab activities. The soil samples were diluted and suspended in solution and the solution was then plated onto reasoners second agar. Thirty-three colonies were isolated to a single species and metabolic capabilities along with microscopic features were tested. The isolated colonies were sent to GENEWIZ of Azenta Life Sciences for sequencing of the 16s rRNA gene and sequence results were analyzed in the Ribosomal Database Project (RDP) to classify the organism (Dunbar et al. 2002). Sequence results were also submitted to the National Center for Biotechnology Information (NCBI)

Basic Local Alignment Search Tool (BLAST) to find the closest relatives to each organism. Sequences for the isolates will be uploaded to GenBank for access.

STATISTICS, VISUALIZATIONS AND REPORT GENERATION

Amplicons from next generation sequencing of bacteria and fungi were grouped into phyla and genera for further analysis. Bacterial data were subset to individuals (Phyla, Genera) with greater than 100 reads for the construction of stacked bar charts. Fungal phyla were not subset for stacked bar chart construction, but fungal genera were found to be the most diverse at lower sequence read values. With many unknown fungal genera leading to increased background noise and making read proportions difficult to interpret. To make the predominant fungal genera more apparent, only genera with sequence reads greater than 1000 were used in stacked bar chart construction.

Next generation sequencing data analysis and stacked bar chart generation were done in R studio (R Core Team 2022) utilizing embedded packages and functions from the tidyverse package (Wickham et al. 2019). Shannon and Simpsons diversity indices were created using the vegan package (Oksanen et al. 2022) and displayed using functions from the tidyverse package. Correlation matrices and analyses were constructed and performed using recommended analysis techniques and thresholds (Alpha = .001) from (Busato et al. 2023; Weiss et al. 2016). Phyla and genera two-sided Pearson correlation (Confidence Interval = .99) matrix construction, analyses and p-value extraction was done using functions from the rstatix package [@-Rstatix]. Principal components analysis (PCA) calculations and visualizations were performed in R-Studio using the FactoMineR (Lê, Josse, and Husson 2008), factoextra (Kassambara and Mundt 2020), and tidyverse (Wickham et al. 2019) packages. PDF document generation was done in R_Studio as well, utilizing the the knitr (Xie 2015),

latex2exp (Meschiari 2022), and tinytex (Xie 2019) packages. R-Studio package bibliography document generation was done using the papaja package (Aust and Barth 2022). The .Rmd file with all code used to produce this report is included as appendices to this thesis.

RESULTS

Figures (2, 3, 4, 5, 6) display the agarose gel electrophoresis and DGGE test results of genomic DNA and targeted PCR products. Figure 2 shows smeared bands indicating the presence of genomic DNA in all samples but does not show control groups. Figure 3 shows smeared bands indicating the success of our PCR protocol on bacterial 16S rRNA. Figure 4 shows a single visible but smeared band in the Cattaloochee down slope lane suggesting the presence of some bacterial species containing the *amoA* subunit in the sample, but the positive controls did not show amplification. Figure 5 shows visible smeared bands in all sample lanes and a faint smeared band in the positive control, with nothing apparent in the negative control, indicating that all samples contained some archaea with the *amoA* gene. Figure 6 shows the DGGE test displaying 16S rRNA PCR products in all sample lanes each with few and unresolved bands insufficient for further analyses.

Figures 7, 9, 8 and 10 , display the proportion of sequence reads from each sample that correspond with the known phyla or genera of each sequence encountered. Figure 7 shows that at both the Cataloochee and Purchase Knob sites, the two most predominant bacterial phyla were Acidobacteria and Proteobacteria, each accounting for approximately 25-50% of sequence reads in all samples. The next most relatively abundant phyla found were Actinobacteria, AD3, Chloroflexi and Nitrospirae, which when combined encompass approximately 25% of sequence reads in each sample. Acidobacteria were found most predominant at Cataloochee down slope (C3) of the site tree, while Proteobacteria were predominant at

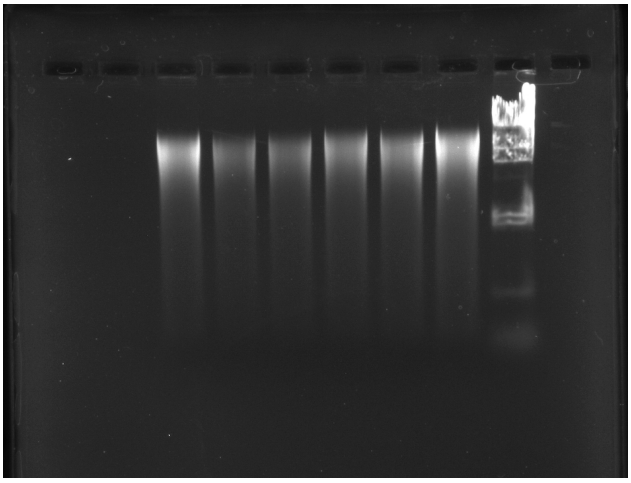

Figure 2: Agarose Gel Results: Bacterial Genomic DNA (DNA ladder far right)

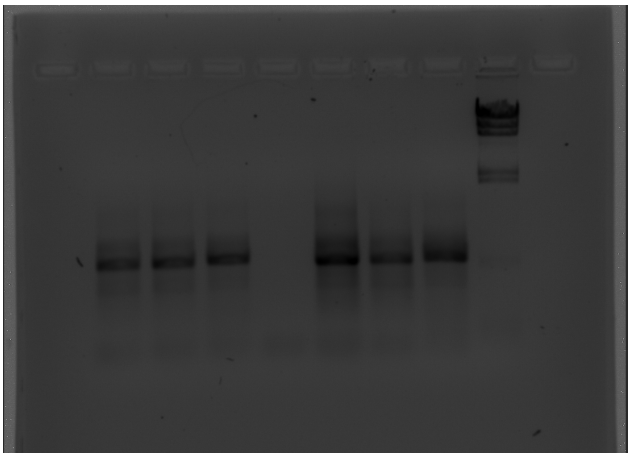

Figure 3: Agarose Gel Results: Bacterial 16S rRNA PCR products (DNA ladder far left)

Cataloochee on the side slope (C2).

The largest proportions of Actinobacteria, AD3, and Chloroflexi were found at Purchase Knob down slope (P3) of the site tree with each AD3 and Chloroflexi almost double the proportion found up slope at Purchase Knob (P1). Nitrospirae were found in similar proportions in all samples, defining an estimated 1-5% of total sequence reads in each sample. The data displayed in Figure 9, show the largest proportions of fungal phyla sequence reads were from the Ascomycota, Basidiomycota, and Mucoromycota phyla. Mucoromycota were found in the highest relative abundance at Cataloochee up slope (C1), and side slope(C2) of the site tree, accounting for an estimated 70% and 37% of total sequence reads in each site

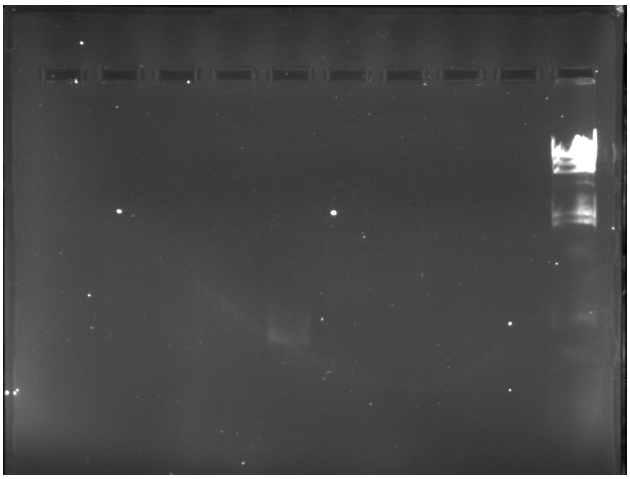

Figure 4: Agarose Gel Results: Bacterial amoA gene PCR products (DNA ladder far right)

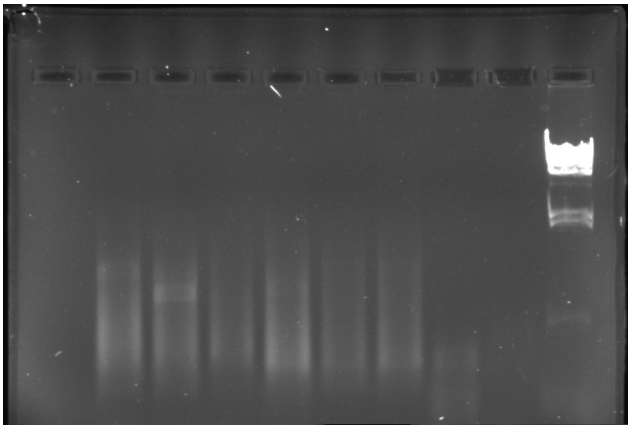

Figure 5: Agarose Gel Results: Archaeal amo gene PCR products (DNA ladder far right)

respectively. The largest proportions of Basidiomycota were found in the Purchase Knob up slope (P1), and down slope(P3) samples, accounting for an estimated 61-63% of total sequence reads in each. Ascomycota accounted for the largest proportion of sequence reads in the Purchase Knob side slope (P2) sample at around 52%.

Figure 8, shows that of the known bacterial genera sequences discovered with sequence counts greater than 100, *Bradyrhizobium* and *Rhodoplanes* were the most abundant in all six samples. *Bradyrhizobium* and *Rhodoplanes* were found to encompass together, roughly 75% of total sequence reads in all samples with *Rhodoplanes* representing the largest proportion of the two genera. On the right in Figure 10, we can see that the fungal genera sequenced were

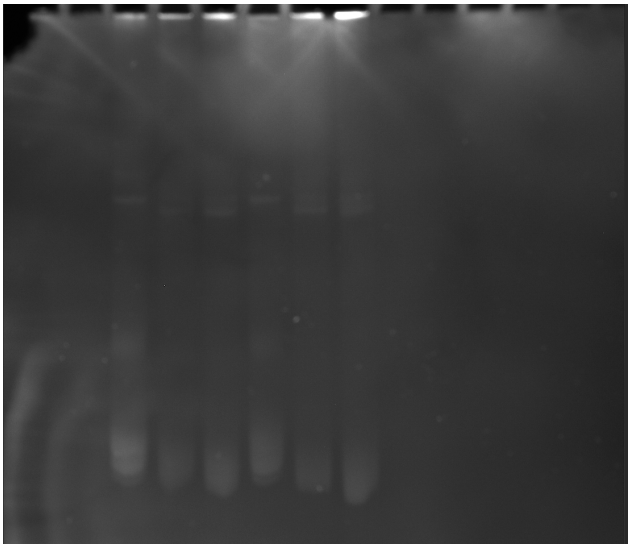

Figure 6: Denaturing Gradient Gel Results: Bacterial 16S rRNA PCR products (No DNA ladder)

considerably more different across sites and slope position than bacterial genera, with some genera only being found in one or two samples. Site C1 is characterized almost completely by a single genera *Mucor* accounting for roughly 80% of total reads, with *Elaphomyces* as the only other visually discernible genera. At C2, *Mucor* and *Pseudotricholoma* were found at roughly 50% each with *Mucor* only slightly more predominant. Site C3 contained predominantly *Russula* and *Geminibasidium*, accounting for approximately 50% and 30% of sequence reads respectively. Site P1 contained *Astreus*, *Elaphomyces* and *Lactarius* at approximately 6%, 13% and 17% respectively, as well as *Saitozyma*, *Geminibasidium*, and *Sebacina* that encompass roughly 25%, 19%, and 21% respectively. Site P2 contained the only high proportion of *Sagenomella* sequence reads at roughly 20%, and a small proportion of *Leohumicola* (~9%). At site P3 we see the largest proportion of *Russula* (~60%) as well as some *Sebacina* (~22%), *Saitozyma* (~8%), and *Leohmicola* (~11%).

Alpha diversity measures are shown in table 3 along with a count of species observed, and the goods coverage calculation for each sample. The goods coverage value reflects the probability that the sequence library included the sequences found in the sample, and higher values indicate lower probability that sequences were not covered in the library. The goods

coverage value for each sample suggests that there is a low probability that the sequences found were not covered in the library used to match sequences. Chao1 and ACE are abundance based species richness estimates, estimating the actual number of species present within each community sample. Both ACE and Chao1 indices for all sites estimate similar numbers of species, and the standard error ranges overlap one another lending increased confidence to the estimates.

Pielou's evenness is a calculation of species abundance and diversity indicating the relative abundance of each species in the sample. Pielou's evenness approaches one when all species observed occur in similar abundances and approaches zero when species abundances vary wildly from one another. The Pielou's evenness values are .50 and greater suggesting moderate community evenness in all samples, with increased evenness in the Purchase Knob samples (.65), and the highest community evenness seen in sample C3 (.74). Shannon H and Simpson's indices are indicators of diversity within samples utilizing species richness and evenness measurements. The calculation of Shannon H weights species richness higher than diversity, and Simpson's calculation weights diversity higher than richness. Higher values for Shannon H indices and Simpson's values approaching 1 indicate larger amounts of diversity within the sample. Correlation analysis of combined fungal and bacterial data sets showed no significant correlation between Phyla, but many significant correlations between Genera (956).

Figures 12, 13, and 14 are plots displaying the results of principal components analysis (PCA) for the bacterial, fungal, and combined data sets respectively. These PCA plots show the relative positional difference between site community members and their abundance values along the two most descriptive calculated dimensions for that data set. The position of points, representing site community diversity, is relative to the dimensions that they are plotted within, and describe a positive or negative relationship with the information contained in the dimension based on that position.

Figure 12 shows the Cataloochee sites differing largely from one another across dimension 1, with the side slope site (C2) differing widely from the up hill (C1), and down hill (C3) sites along dimension 2. This may be due to a difference in the sequence data able to be gathered from the C2 sample, which during extraction at Azenta was reported to have had a much lower yield than the C1, and C3 sites. The purchase knob bacterial communities differed most along dimension two, with side slope (P2), and down slope (P3) sites showing positive relationships, and up slope (P1) showing a negative relationship.

Figure 13 shows that C3 is largely different from all other communities displaying the only positive relationship with dimension one. C1 and C2 show similar negative relationships with dimension one and differing positive relationships with dimension two. The purchase knob site communities share negative relationships with both dimensions, while P1 and P3 are clustered close together, P2 is more negatively related to dimension 2 than either of them.

In the PCA plot shown in figure 14 we see that site C3 is positively correlated with both dimensions, most positively with dimension one. Site C1 is positively correlated to dimension one and negatively with dimension two, and C2 is similarly negatively correlated with both dimensions, but more negatively correlated with dimension 2. Sites P1 and P3 are negatively correlated with dimension 1, while P1 is slightly positively correlated with dimension two, P3 is slightly negatively correlated with dimension two. Site P2 is largely positively correlated with dimension two and somewhat negatively correlated with dimension one.

DISCUSSION

Table 1: Total Bacterial sequence reads, correction factors from normalization, sequences classified to Archaea at Kingdom level, sequences unclassified to Kingdom level, and sequences classified to Genus level (Sequence numbers are totals before normalization; all numbers were rounded to the thousandths place if possible)

	Bacteria Meta Data				
Site	Total Reads	Correction Factor	Archaea	Unclassified to Kingdom	Classified to Genus
C1	87452	6.45688	77	723	9853
C2	13544	1.00000	2	3823	883
C3	85750	6.33122	13	829	9377
P1	95176	7.02717	28	436	17737
P2	81253	5.99919	42	252	14734
P3	84772	6.25901	0	307	10290

Table 2: Total Fungal sequence reads, correction factors from normalization, sequences classified to Plantae at Kingdom level, sequences unclassified to Kingdom level, and sequences classified to Genus level (Sequence numbers are totals before normalization, all numbers were rounded to the thousandths place if possible)

	Fungi Meta Data				
Site	Total Reads	Correction Factor	Plantae	Unclassified to Kingdom	Classified to Genus
C1	28667	1.00000	510	0	23945
C2	111933	3.90459	2077	0	86358
C3	114164	3.98242	6866	20	45336
P1	112282	3.91677	1747	4	81108
P2	104421	3.64255	106	7	71911
P3	113000	3.94181	87	24	84343

Table 3: Alpha Diversity Indices: Values rounded to the nearet digit displayed

		Species estimates & Standard error				Abundance & Diversity estimates			Sequence Confidence
	Species Observed	Chao1	Std.Error Chao1	ACE	Std.Error ACE	Pielou's Evenness	Shannon H	Simpson's	Good's Coverage
C1	1496	2073	69.5	2007	22.9	0.50	3.66	0.81	0.989
C2	1106	1487	55.2	1422	19.0	0.52	3.62	0.89	0.989
C3	1745	2207	55.4	2218	24.0	0.74	5.53	0.99	0.990
P1	1352	1812	58.3	1847	22.7	0.65	4.66	0.97	0.989
P2	1653	2218	63.1	2274	25.0	0.65	4.81	0.97	0.989
P3	1495	2096	72.4	2049	23.6	0.65	4.72	0.95	0.989

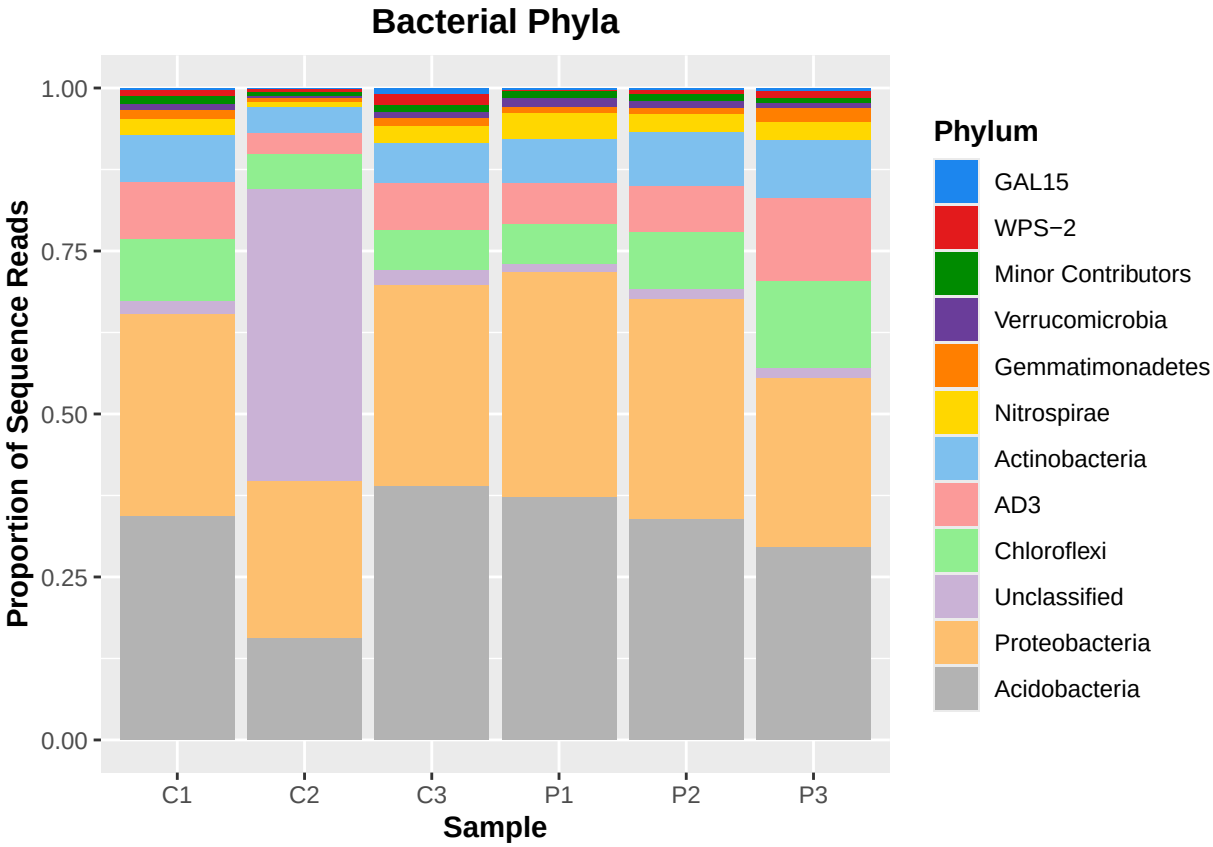

Figure 7: Displaying proportions of bacterial phyla sequence reads by sample; Minor Contributors group includes classified phyla that individually account for 1% or less of sequence reads in all samples; Unclassified group includes all unclassified phyla.

BACTERIA, NITROGEN CYCLING AND ECOSYSTEM FUNCTIONS

Due to the noise, sparsity, and compositionality inherent in the large volume of microbial data gathered, and our current lack of ability to resolve many taxonomic individuals with real confidence, data was subset by known phyla and genera, and these resulting data were subset again by sequence read counts. Sub-setting of data comes with a set of pitfalls including loss of small interaction detection, and likely reduces perceived interaction complexity, but is commonly used to avoid other computational discrepancies, such as artificial indicators of interactions (Busato et al. 2023; Weiss et al. 2016). Within the gram-negative

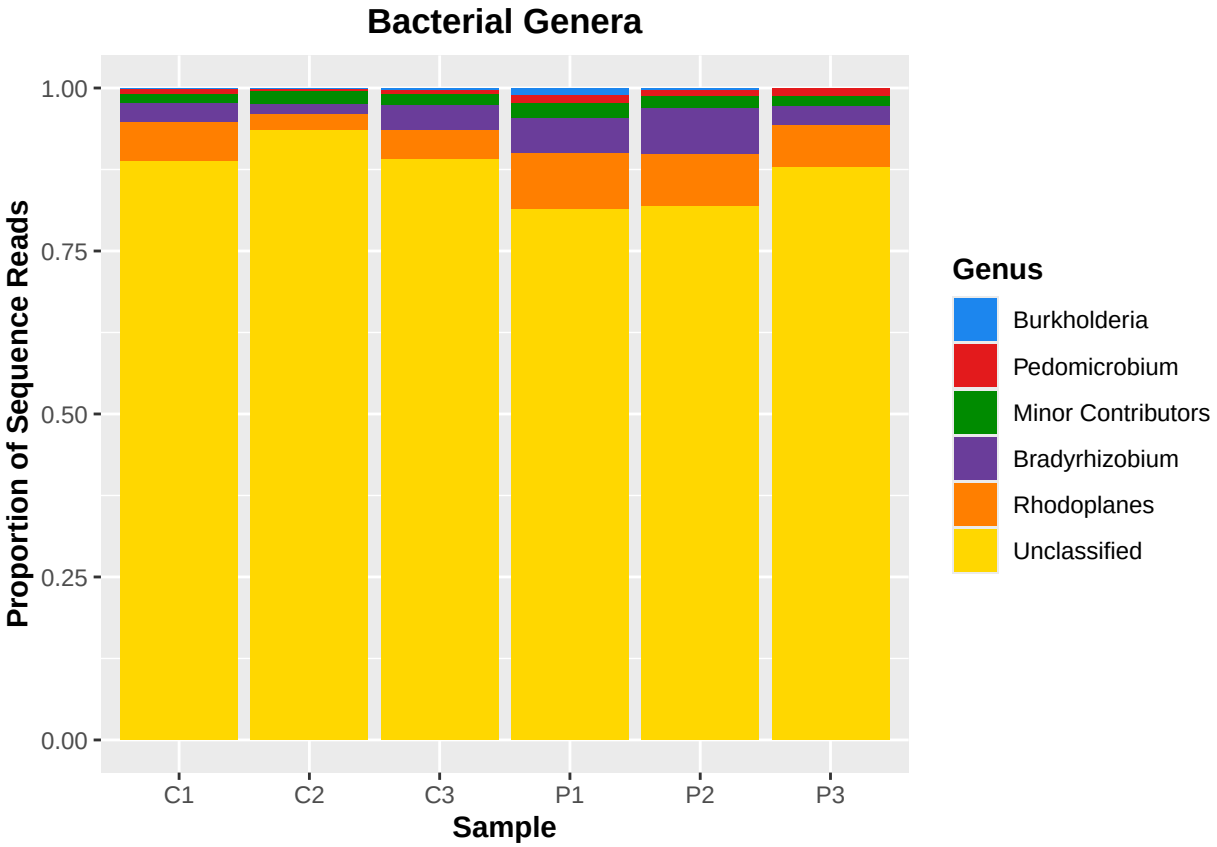

Figure 8: Displaying proportions of bacterial genera sequence reads by sample; Minor Contributors group includes classified genera that individually account for 1% or less of sequence reads in all samples; Unclassified group includes all unclassified genera.

phyla Proteobacteria there are several classes of organisms, such as the Alphaproteobacteria, which encompasses both the *Pedomicrobium*, *Rhodoplanes* and *Bradyrhizobium* (Garrity, Bell, and Lilburn 2015). Many *Pedomicrobium* strains have been shown to reduce nitrate, with some strains able to reduce nitrite and ammonium (NH_4^-) (Hirsch 2015). *Rhodoplanes* are a group of facultative photoorganotrophs that are able to perform nitrogen fixation, gain nitrogen from nitrte and urea (Hiraishi and Imhoff 2021). Many species of *Rhodoplanes* participate in denitrification by utilizing nitrate (NO_3^-) as a terminal electron acceptor for metabolic processes (Hiraishi and Imhoff 2021). The genus *Bradyrhizobium* are chemoorganotrophs, utilizing nitrates and amino-acids as nitrogen sources (Kuykendall 2015). Species within *Bradyrhizobium* are characterized by the ability to participate in nitrogen fixation, predominantly through symbiotic relationships with plants from the family *Leguminousae*

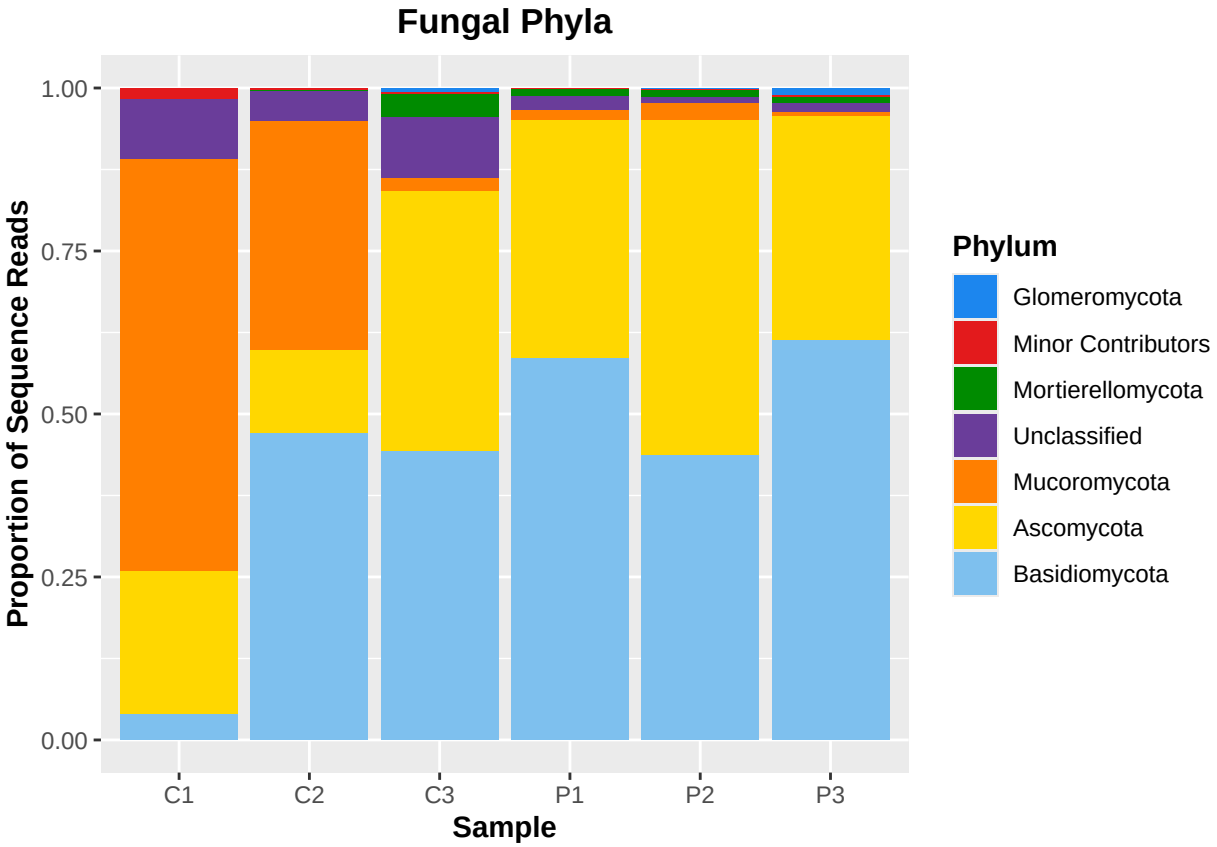

Figure 9: Displaying proportions of fungal phyla sequence reads by sample; Minor Contributors group includes classified non-fungal phyla and classified fungal phyla that individually account for 1% or less of sequence reads in all samples; Unclassified group includes all unclassified phyla.

but some are able to fix nitrogen in a free-living state (Kuykendall 2015). Within recent years the *Rhodoplanes* and *Bradyrhizobium* have been phylogenetically linked much closer than previously thought, with studies calling into question their placement in separate families (Hördt et al. 2020). Another functionally important class within Proteobacteria is the Betaproteobacteria, which contains the genus *Burkholderia* that has constituents known to occupy varied ecological niches through the utilization of an array of metabolic capabilities (Garrity, Bell, and Lilburn 2015; Vandamme and Eberl 2018). *Burkholderia* participate in a wide variety of metabolic processes and are likely also involved in some ecosystem functions outside of carbon cycling.

Genus *DA101* of the phyla Verrucomicrobia have been found in large abundances in

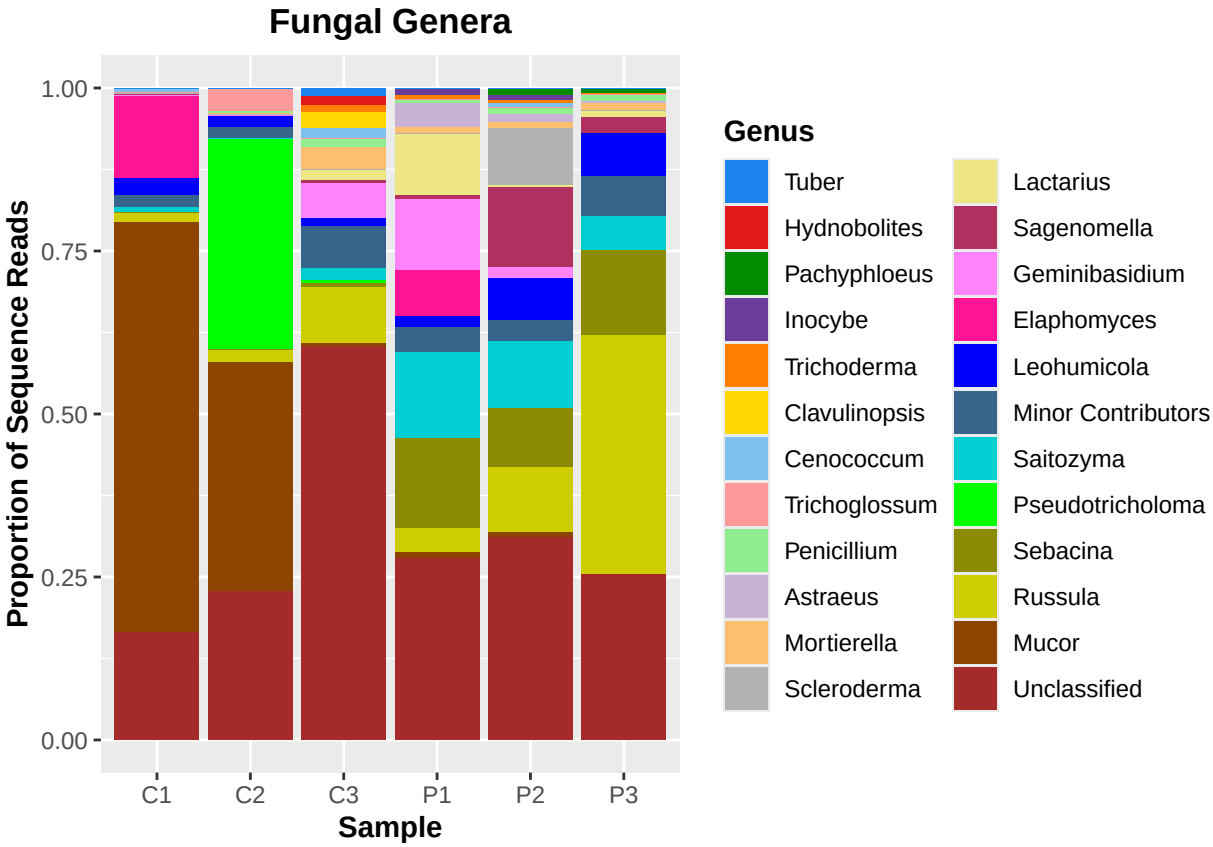

Figure 10: Displaying proportions of fungal genera sequence reads by sample; Minor Contributors group includes all classified genera that individually account for 1% or less of sequence reads in all samples; Unclassified group includes all unclassified genera.

soils, with highest abundances recorded in grass land soils (Brewer et al. 2016). Brewer et al. (2016) suggest that *DA101* prefer soils that do not overlap in range of forest soils dominated by nonsymbiotic *Bradyrhizobium*. The functional capacity of these organisms may be important for both terrestrial and freshwater ecosystems due to their participation in carbon and nitrogen cycling and their occupation of many and varied ecosystems. Of the phyla found, individuals from the gram-negative Acidobacteria, and Proteobacteria phyla can be found in many environments typically making up large proportions of the sequenced microbial community (Thrash and Coates 2015a; Garrity, Bell, and Lilburn 2015). Individual species from these phyla display varied physiological traits, including some species that are known to participate in ecosystem functions (Thrash and Coates 2015a; Garrity, Bell, and Lilburn 2015). Of the six genera within Acidobacteria, *Geothrix* is the only known to

contain species that utilize nitrate (NO_3^-) as an electron acceptor during metabolism (Thrash and Coates 2015b). This reaction is likely part of the larger nitrogen cycle, chemically transforming soil nitrate into nitrite (NO_2^-). Within Proteobacteria, the genus *Rhizobium* are known plant symbiotes, and some species participate in the leguminous form of nitrogen fixation within plant roots (Kuykendall et al. 2015).

SIGNIFICANCE, FUTURE GOALS AND OBJECTIVES

Although many bacteria are unculturable in lab settings and their functional capacities remain unknown, every organism participates in some aspect of ecosystem functions through direct or indirect means. Microbial Ecology harbors a wealth of information, has implications in all environments, and is a field of study where more research is likely required to extrapolate into reliable resources for land management and conservation. To conserve an ecosystem as a functioning entity the actors in the processes creating the function must also be conserved. Under climate change the robustness of landscapes and ecosystems may be mediated by soil communities with the potential to provide benefits including sustainability to landscape level systems and local communities that rely on them. These soil communities also may provide an indication of future landscape changes and be useful as a tool for decision making as they reflect landscape level processes relevant to management goals and strategies. By describing the community relationships of the predominant actors in soils we better understand why and how they occur in the spatial orientation we observe. This may provide a path to developing a spatially explicit method to describe the arrangement of soil microbial communities, and through this describe the spatial qualities of ecosystem functions. Better understanding of how and why the plant, mycorrhizae and bacteria influence one another allows for more informative modelling of biogeochemical cycles, feedback mechanisms, and

responses to changing environmental conditions. Characterizing microbial communities and their contributions to ecosystem form and function through DNA sequencing in concert with biotic, and abiotic environmental measures is the first step in the process of understanding these connections. Ongoing bacterial community surveys may be an effective way of collecting fine scale spatial data while incorporating a community engagement role that allows partners to learn about local landscapes and how they have effects on a global scale.

WORKS CITED

Aber, John D., Jerry M. Melillo, Knute J. Nadelhoffer, John Pastor, and Richard D. Boone. 1991. "Factors Controlling Nitrogen Cycling and Nitrogen Saturation in Northern Temperate Forest Ecosystems." *Ecological Applications* 1 (3): 303–15. https://doi.org/10.2307/1941759.

Allen, M.f., W. Swenson, J.i. Querejeta, L.m. Egerton-Warburton, and K.k. Treseder. 2003. "ECOLOGY OF MYCORRHIZAE: A Conceptual Framework for Complex Interactions Among Plants and Fungi." *Annual Review of Phytopathology* 41 (1): 271–303. https://doi.org/10.1146/annurev.phyto.41.052002.095518.

Aust, Frederik, and Marius Barth. 2022. *papaja: Prepare Reproducible APA Journal Articles with R Markdown*. https://github.com/crsh/papaja.

Brewer, Tess E., Kim M. Handley, Paul Carini, Jack A. Gilbert, and Noah Fierer. 2016. "Genome Reduction in an Abundant and Ubiquitous Soil Bacterium 'Candidatus Udaeobacter Copiosus'." *Nature Microbiology* 2 (2, 2): 1–7. https://doi.org/10.1038/nmicrobiol.2016.198.

Busato, Sebastiano, Max Gordon, Meenal Chaudhari, Ib Jensen, Turgut Akyol, Stig Andersen, and Cranos Williams. 2023. "Compositionality, Sparsity, Spurious Heterogeneity, and Other Data-Driven Challenges for Machine Learning Algorithms Within Plant Microbiome Studies." *Current Opinion in Plant Biology* 71 (February): 102326. https://doi.org/10.1016/j.pbi.2022.102326.

Churchland, Carolyn, and Sue J. Grayston. 2014. "Specificity of Plant-Microbe Interactions in the Tree Mycorrhizosphere Biome and Consequences for Soil C Cycling." *Frontiers in Microbiology* 5: 261. https://doi.org/10.3389/fmicb.2014.00261.

Conrad, Anna O, Ellen V Crocker, Xiaoshu Li, William R Thomas, Thomas O Ochuodho, Thomas P Holmes, and C Dana Nelson. 2020. "Threats to Oaks in the Eastern United States: Perceptions and Expectations of Experts." *Journal of Forestry* 118 (1): 14–27. https://doi.org/10.1093/jofore/fvz056.

Dillow, Carter. 2009. "DIVERSITY PATTERNS OF 16S rDNA OF BACTERIA AND amoA OF ARCHAEA AND BACTERIA FROM EASTERN HEMLOCK (TSUGA CANADENSIS) RHIZOSPHERE SOIL." Western Carolina University.

Dunbar, John, Susan M. Barns, Lawrence O. Ticknor, and Cheryl R. Kuske. 2002. "Empirical and Theoretical Bacterial Diversity in Four Arizona Soils." *Applied and Environmental Microbiology* 68 (6): 3035–45. https://doi.org/10.1128/AEM.68.6.3035-3045.2002.

Galloway, James N., John D. Aber, Jan Willem Erisman, Sybil P. Seitzinger, Robert W. Howarth, Ellis B. Cowling, and B. Jack Cosby. 2003. "The Nitrogen Cascade." *BioScience* 53 (4): 341. https://doi.org/10.1641/0006-3568(2003)053%5B0341:TNC%5D2.0.CO;2.

Garrity, George M., Julia A. Bell, and Timothy Lilburn. 2015. "Proteobacteria Phyl. Nov." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–1. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.pbm00022.

Graham, Emily B., Joseph E. Knelman, Andreas Schindlbacher, Steven Siciliano, Marc Breulmann, Anthony Yannarell, J. M. Beman, et al. 2016. "Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes?" *Frontiers in Microbiology* 7: 214. https://doi.org/10.3389/fmicb.2016.00214.

Greenberg, Cathryn H., Chad E. Keyser, Leah C. Rathbun, Anita K. Rose, Todd M. Fearer, and W. Henry McNab. 2014. "Forecasting Long-Term Acorn Production with and Without Oak Decline Using Forest Inventory Data." *Forest Science* 60 (2): 222–30. https://doi.org/10.5849/forsci.12-106.

Hacquard, Stéphane, and Christopher W. Schadt. 2015. "Towards a Holistic Understanding of the Beneficial Interactions Across the Populus Microbiome." *New Phytologist* 205 (4): 1424–30. https://doi.org/10.1111/nph.13133.

Hiraishi, Akira, and Johannes F. Imhoff. 2021. "Rhodoplanes." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–12. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00826.pub2.

Hirsch, Peter. 2015. "Pedomicrobium." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–18. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00823.

Hördt, Anton, Marina García López, Jan P. Meier-Kolthoff, Marcel Schleuning, Lisa-Maria Weinhold, Brian J. Tindall, Sabine Gronow, Nikos C. Kyrpides, Tanja Woyke, and Markus Göker. 2020. "Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria." *Frontiers in Microbiology* 11. https://www.frontiersin.org/articles/10.3389/fmicb.2020.00468.

Horz, H.-P., A. Barbrook, C. B. Field, and B. J. M. Bohannan. 2004. "Ammonia-Oxidizing Bacteria Respond to Multifactorial Global Change." *Proceedings of the National Academy of Sciences* 101 (42): 15136–41. https://doi.org/10.1073/pnas.0406616101.

Isobe, Kazuo, Keisuke Koba, Shigeto Otsuka, and Keishi Senoo. 2011. "Nitrification and Nitrifying Microbial Communities in Forest Soils." *Journal of Forest Research* 16 (5): 351–62. https://doi.org/http://dx.doi.org/10.1007/s10310-011-0266-5.

Kassambara, Alboukadel, and Fabian Mundt. 2020. *Factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. https://CRAN.R-project.org/package=factoextra.

Kuykendall, L. David. 2015. "Bradyrhizobium." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–11. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00802.

Kuykendall, L. David, John M. Young, Esperanza Martínez-Romero, Allen Kerr, and Hiroyuki Sawada. 2015. "Rhizobium." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–36. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00847.

Laverman, A. M., A. G. C. L. Speksnijder, and M. Braster. 2001. "Spatiotemporal Stability of an Ammonia-Oxidizing Community in a Nitrogen-Saturated Forest Soil." *Microbial Ecology* 42 (1): 35–45. https://login.proxy195.nclive.org/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=bai&AN=506372912&site=ehost-live&scope=site.

Lê, Sébastien, Julie Josse, and François Husson. 2008. "FactoMineR: A Package for Multi-

variate Analysis." *Journal of Statistical Software* 25 (1): 1–18. https://doi.org/10.18637/jss.v025.i01.

Levy-Booth, David J., Cindy E. Prescott, and Susan J. Grayston. 2014. "Microbial Functional Genes Involved in Nitrogen Fixation, Nitrification and Denitrification in Forest Ecosystems." *Soil Biology and Biochemistry* 75 (August): 11–25. https://doi.org/10.1016/j.soilbio.2014.03.021.

Meschiari, Stefano. 2022. *Latex2exp: Use LaTeX Expressions in Plots*. https://CRAN.R-project.org/package=latex2exp.

Mushinski, Ryan M., Terry J. Gentry, Robert J. Dorosky, and Thomas W. Boutton. 2017. "Forest Harvest Intensity and Soil Depth Alter Inorganic Nitrogen Pool Sizes and Ammonia Oxidizer Community Composition." *Soil Biology and Biochemistry* 112 (September): 216–27. https://doi.org/10.1016/j.soilbio.2017.05.015.

Nguyen, Hai D. T., Nancy L. Nickerson, and Keith A. Seifert. 2013. "Basidioascus and Geminibasidium: A New Lineage of Heat-Resistant and Xerotolerant Basidiomycetes." *Mycologia* 105 (5): 1231–50. https://doi.org/10.3852/12-351.

Norman, J. S., and J. E. Barrett. 2014. "Substrate and Nutrient Limitation of Ammonia-Oxidizing Bacteria and Archaea in Temperate Forest Soil." *Soil Biology and Biochemistry* 69 (February): 141–46. https://doi.org/10.1016/j.soilbio.2013.11.003.

Oksanen, Jari, Gavin L. Simpson, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, et al. 2022. *Vegan: Community Ecology Package*. https://CRAN.R-project.org/package=vegan.

Phillips, Richard P., Edward Brzostek, and Meghan G. Midgley. 2013. "The Mycorrhizal-Associated Nutrient Economy: A New Framework for Predicting Carbon–Nutrient Couplings in Temperate Forests." *New Phytologist* 199 (1): 41–51. https://doi.org/10.1111/nph.12221.

Pinho, Diogo, Cristina Barroso, Hugo Froufe, Nathan Brown, Elena Vanguelova, Conceição Egas, and Sandra Denman. 2020. "Linking Tree Health, Rhizosphere Physicochemical Properties, and Microbiome in Acute Oak Decline." *Forests (19994907)* 11 (11): 1153–53. https://doi.org/10.3390/f11111153.

Powell, Jeff R., Allana Welsh, and Sara Hallin. 2015. "Microbial Functional Diversity Enhances Predictive Models Linking Environmental Parameters to Ecosystem Properties." *Ecology* 96 (7): 1985–93. https://doi.org/10.1890/14-1127.1.

R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Radcliffe, Don C., David M. Hix, and Stephen N. Matthews. 2021. "Predisposing Factors' Effects on Mortality of Oak (Quercus) and Hickory (Carya) Species in Mature Forests Undergoing Mesophication in Appalachian Ohio." *Forest Ecosystems* 8 (1): 7. https://doi.org/10.1186/s40663-021-00286-z.

Read, D. J., and J. Perez-Moreno. 2003. "Mycorrhizas and Nutrient Cycling in Ecosystems – a Journey Towards Relevance?" *New Phytologist* 157 (3): 475–92. https://doi.org/10.1046/j.1469-8137.2003.00704.x.

Ribbons, Relena R., David J. Levy-Booth, Jacynthe Masse, Sue J. Grayston, Morag A. McDonald, Lars Vesterdal, and Cindy E. Prescott. 2016. "Linking Microbial Communities, Functional Genes and Nitrogen-Cycling Processes in Forest Floors Under Four Tree Species." *Soil Biology and Biochemistry* 103 (December): 181–91. https://doi.org/10.

1016/j.soilbio.2016.07.024.

Selosse, M.-A., R. Bauer, and B. Moyersoen. 2002. "Basal Hymenomycetes Belonging to the Sebacinaceae Are Ectomycorrhizal on Temperate Deciduous Trees." *New Phytologist* 155 (1): 183–95. https://doi.org/10.1046/j.1469-8137.2002.00442.x.

Terrer, César, Sara Vicca, Bruce A. Hungate, Richard P. Phillips, and I. Colin Prentice. 2016. "Mycorrhizal Association as a Primary Control of the CO2 Fertilization Effect." *Science* 353 (6294): 72–74. https://doi.org/10.1126/science.aaf4610.

Thrash, J. Cameron, and John D. Coates. 2015a. "Acidobacteria Phyl. Nov." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–5. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.pbm00001.

———. 2015b. "Geothrix." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–2. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00005.

Vandamme, Peter, and Leo Eberl. 2018. "Burkholderia." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–45. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00935.pub2.

Weiss, Sophie, Will Van Treuren, Catherine Lozupone, Karoline Faust, Jonathan Friedman, Ye Deng, Li Charlie Xia, et al. 2016. "Correlation Detection Strategies in Microbial Data Sets Vary Widely in Sensitivity and Precision." *The ISME Journal* 10 (7): 1669–81. https://doi.org/10.1038/ismej.2015.235.

Wickham, Hadley, Mara Averick, Jennifer Bryan, Winston Chang, Lucy D'Agostino McGowan, Romain François, Garrett Grolemund, et al. 2019. "Welcome to the tidyverse." *Journal of Open Source Software* 4 (43): 1686. https://doi.org/10.21105/joss.01686.

Xie, Yihui. 2015. *Dynamic Documents with R and Knitr*. 2nd ed. Boca Raton, Florida: Chapman; Hall/CRC. https://yihui.org/knitr/.

———. 2019. "TinyTeX: A Lightweight, Cross-Platform, and Easy-to-Maintain LaTeX Distribution Based on TeX Live." *TUGboat* 40 (1): 30–32. https://tug.org/TUGboat/Contents/contents40-1.html.

Zhalnina, Kateryna V., Raquel Dias, Michael T. Leonard, Patricia Dorr de Quadros, Flavio A. O. Camargo, Jennifer C. Drew, William G. Farmerie, Samira H. Daroub, and Eric W. Triplett. 2014. "Genome Sequence of Candidatus Nitrososphaera Evergladensis from Group I.1b Enriched from Everglades Soil Reveals Novel Genomic Features of the Ammonia-Oxidizing Archaea." *PLoS ONE* 9 (7): e101648. https://doi.org/10.1371/journal.pone.0101648.

APPENDIX

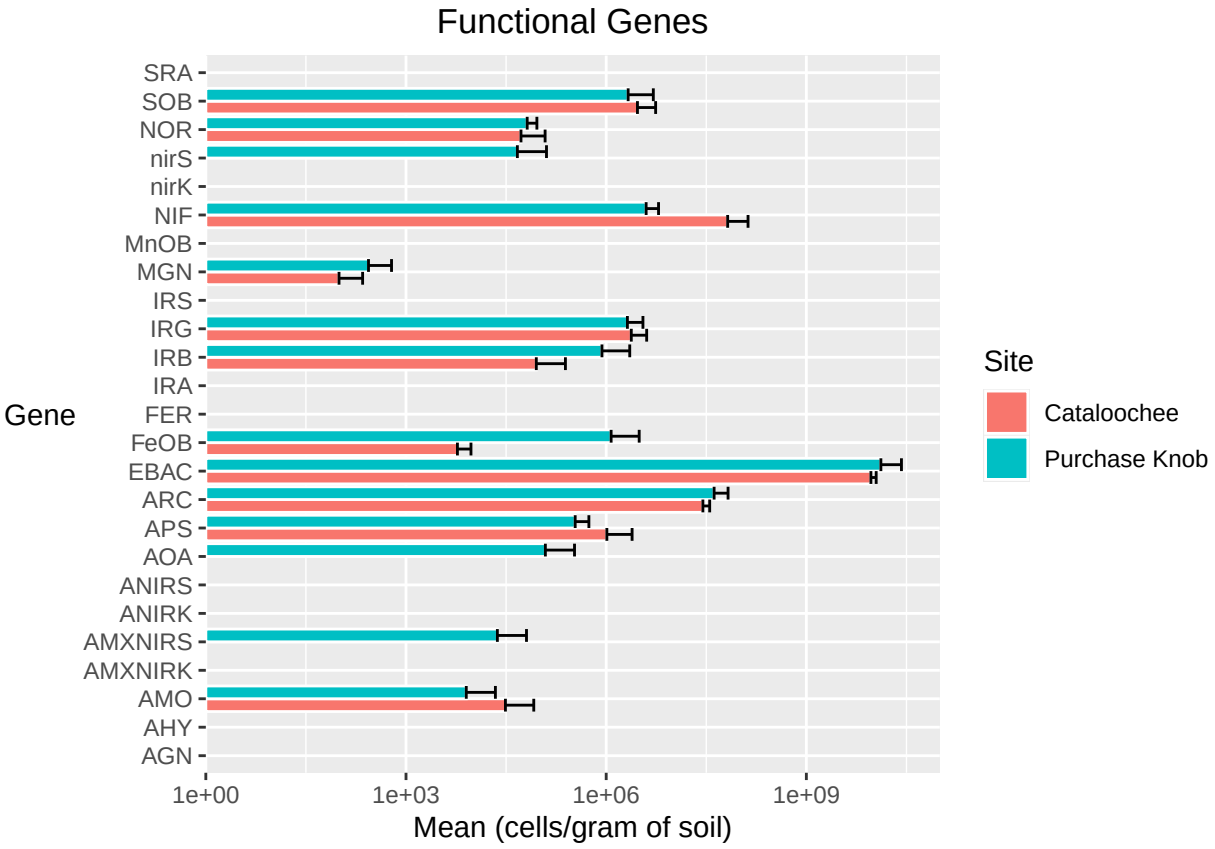

Figure 11: Gene Abundance displayed in Mean (cells/gram) of soil: Error bars display standard deviation above mean: Total Bacteria (EBAC), Total Archaea (ARC), Sulfate Reducing Bacteria (APS), Sulfate Reducing Archaea (SRA), Iron Reducing Archaea (IRA), Iron Reducing Bacteria - Other (IRB), Iron Reducing Geobacter (IRG), Iron Reducing Shewanella (IRS), Iron Oxidizing Bacteria (FeOB), Manganese Oxidizing Bacteria (MnOB), Sulfur Oxidizing Bacteria (SOB), Ammonia Oxidizing Bacteria (AMO), Ammonia Oxidizing Archaea (AOA), Nitrite Oxidizing Bacteria (NOR), Anaerobic Ammonia Oxidizers (AMXNIRK), Anaerobic Ammonia Oxidizers (AMXNIRS), Nitrogn Fixing Bacteria (NIF), Denitrifying Bacteria (nirK), Denitrifying Bacteria (nirS), Denitrifying Archaea (ANIRK), Denitrifying Archaea (ANIRS), Methanogens (MGN), Fermenters (FER), Acetogens (AGN), Acetylene Degraders (AHY)

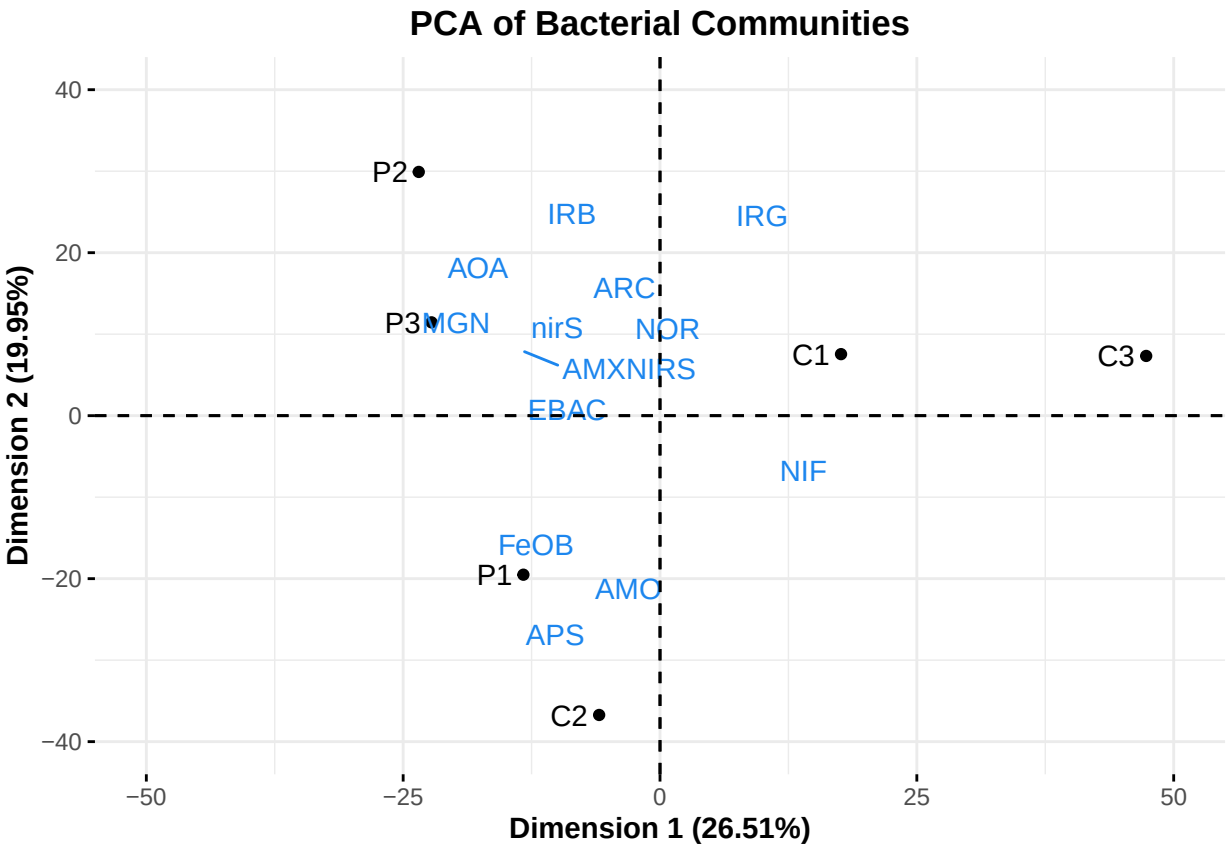

Figure 12: Bacteria: PCA Plot by Site

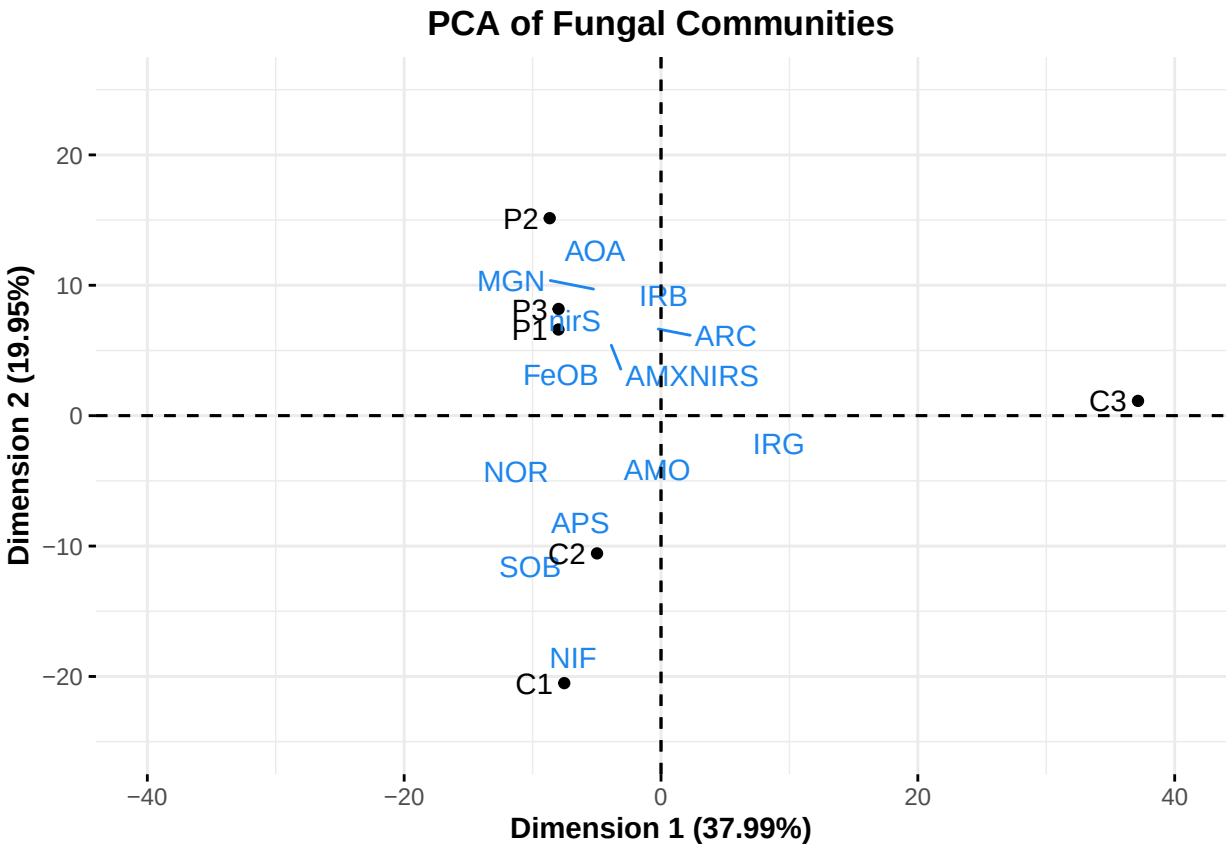

Figure 13: Fungi: PCA Plot by Site

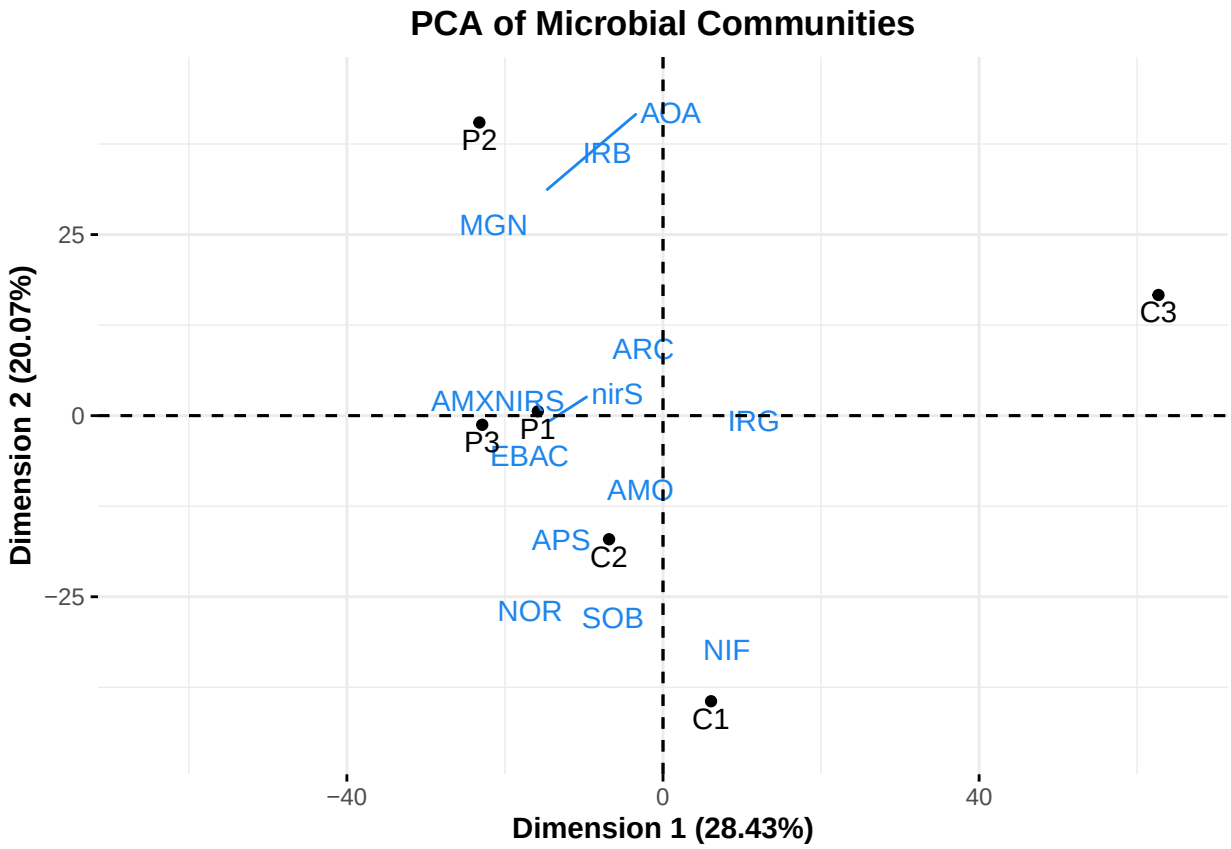

Figure 14: Bacteria and Fungi: PCA Plot by Site