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"Examining Continent-scale Variation in Aerial Arbuscular Mycorrhizal Fungi Communities Using eDNA

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1. Abstract

Arbuscular mycorrhizal (AM) fungi form an important symbiosis with plants that make these fungi critical for plant health and ecosystem functioning. Although previous studies have shown that AM fungi disperse through the air, few studies have examined which environmental factors may be impacting their dispersal and aerial community composition, especially at large geographical scales. The goal of this study was to determine which environmental factors influenced air-dispersed AM fungal species composition across five different research sites in the Midwest and Western regions of the United States. By collecting dust at three different heights in the air, using modified Wilson and Cooke (MWAC) dust collectors, aerial AM fungal eDNA was extracted. Species were identified and then analyzed by looking at which biotic and abiotic environmental factors explained variations in aerial AM fungal species richness between the five research sites. Data trends showed that sites at higher elevation levels had greater species richness than lower elevation sites. The results suggest that elevation is a predictive environmental variable of AM fungal species richness. This could have important implications for land management as climate change and human activities continue to threaten vulnerable high-elevation ecosystems.

2. Introduction

2.1 AM Fungi and Their Relationship with Plants

Arbuscular mycorrhizal (AM) fungi are a below-ground group of species that form relationships with plants from all terrestrial ecosystems. These fungi form mutualistic relationships with plants in which the plants receive better access to soil nutrients and the AM fungi obtain fixed carbon (Johnson et al. 2006; Smith & Read 2008). As a result of this symbiotic relationship, AM fungal species are critical for the establishment, functioning, and survival of plant species across a variety of ecosystems (Camargo-Ricalde 2002).

Analyses of well-preserved fossilized plants provide evidence that ancestral AM fungi likely evolved with some of the first terrestrial land plants (Rosendahl 2008). Current evidence suggests that the earliest AM fungal species arose approximately 450 million years ago (Lewis 2016). This mutualism continues in extant plant species that have evolved to conserve the ability to form this plant-fungi symbiosis (Gianinazzi-Pearson 1996). This ancient yet enduring relationship is important to the success of plant communities and entire ecosystems. Therefore, it is critical to understand how these fungal communities are dispersing and establishing in various environments. This will help ensure that plant communities can thrive in natural, built, and agricultural environments.

2.2 AM Fungi Dispersal

Movement ecology is an important field of study related to the change in an organism's spatial location. This movement related framework, while widely applied to many taxa, has just recently been applied to fungi (Bielčik et al. 2019). To better understand the movement of AM fungi within an ecosystem, it is important to consider mechanisms of dispersal. AM fungi are known to disperse in the air as well as well as through animal vectors (Allen et al. 1989; Camargo-Ricalde 2002; Paz et al. 2020). This study focuses specifically on the aerial dispersal of AM fungi. Many microorganisms including bacteria, protists, as well as fungi are known to be transferred in the air (de Groot et al. 2021), and fungi have even been found to move across especially large distances (Golan & Pringle 2017).

However, there is still a lack of information regarding mechanisms of AM fungal dispersal, which is critical for understanding how these species spread and establish in a variety of different ecosystems (Allen et al. 1989; Chaudhary et al. 2020; Egan et al. 2014). Paz and colleagues (2020) found that there has also been unequal attention on small mammals as dispersal vectors, with abiotic factors like air and water being underrepresented in research related to AM fungal dispersal.

Additionally, variations in AM fungal dispersal and community composition are not well studied over large geographical scales. Analyzing AM fungal species across larger scales is especially important as Chaudhary and colleagues (2014) noted that AM fungi appear more variable over larger geographical scales as compared to smaller ones. Different insights about AM fungal dispersal ecology might be gained when analyzing these fungal species at larger scales. Analyzing AM fungi beyond regional levels might also help researchers gain important information on global differences between AM fungal species' ranges and functionality differences between ecoregions.

2.3 Environmental Factors and How They Affect AM Fungi Community Composition

There is a lack of information regarding what environmental factors are influencing the dispersal and establishment of AM communities. Previous studies have primary concentrated on how abiotic and biotic factors might change AM fungal community composition persistence. That is, most studies focus on how environmental factors impact already established communities. These studies examined how precipitation (Gao et al. 2016; House & Bever 2018; Wang et al. 2021; Zhang et al. 2016), soil properties (Alguacil et al. 2015; Oehl et al. 2010), elevation (Gai et al. 2012), and land usage (Alguacil et al. 2008; Soka & Ritchie 2018; Vályi et al. 2015) changed AM fungal community persistence. Few studies have analyzed aerial spores; however, Chaudhary and colleagues (2020) did examine dispersing AM fungal spores in relation to wind speed and temporal seasonal changes.

2.4 The Use of eDNA in Fungal Research and Restoration Techniques

Since AM fungi are a below-ground, microscopic species, they are difficult to observe in natural settings. One emerging technique for analyzing these fungal species is utilizing metabarcoding of eDNA. Samples of eDNA are genetic material gathered directly from environmental sources such as soil, water, dust, and sediment (Thomsen & Willerslev 2015). Using next generational sequencing (NGS) and metabarcoding allows for accurate, cost-effective identification of fungal communities from eDNA (Fernandez Nuñez et al. 2021; Yan et al. 2018). Using eDNA is beneficial to research replicability, as collecting environmental samples can be easily standardized, making it possible for researchers to obtain similar results regardless of their taxonomic knowledge. Additionally, gathering eDNA represents a non-invasive method of sample collection that does not damage species of interest or the surrounding environment (Thomsen & Willerslev 2015). This study uses environmental DNA (eDNA) to observe the diversity of AM fungal species dispersing in the air at each of the five research sites.

2.5 Using AM Fungi in Land Management Strategies

Traditionally, below-ground organisms have been ignored when analyzing restoration success or conservation monitoring. Analyzing eDNA using metabarcoding represents a cost-effective strategy to survey a wide range of microscopic, underground species (Fernandez Nuñez et al. 2021; Yan et al. 2018). This provides the opportunity to better understand how microorganisms such as fungi impact restoration success, as well as how diversity and community composition may be changing throughout the progression of a restoration project.

Given how recently eDNA has been utilized to examine AM fungi in restoration projects, the best methods for restoring and promoting the health of native AM fungal communities in degraded environments remains unknown (Chaudhary et al. 2020). A better understanding on how AM fungal communities disperse and establish will help to inform better land management practices that will support native AM fungal communities and the plants that rely on their mutualistic relationship.

One major potential source of managing AM fungal communities involves inoculum. Over the past few decades, AM fungal inoculum products have been produced and distributed in greater quantities (IJdo et al. 2011). While there is a potential for benefiting plant growth and success, there are many risks. Non-native AM fungi are already being introduced via human involvement as non-native plants are transported and planted in new areas. Inoculum has the potential to increase the amount of non-native AM fungal communities (Pringle et al. 2009). The implications of this are still unknown. Is there a possibility that these introduced species could outcompete native varieties? Additionally, given the current procedures available for growing inoculum,

there is no way to completely ensure non-contamination by other microorganism that will be included in any inoculum used (IJdo et al. 2011). This could include anything from benign bacteria to incredibly detrimental invasives. Until these potential risks are addressed, AM fungal inoculum should be considered very cautiously. It may be more beneficial to better understand the current native AM fungal community and consider how to increase the health and abundance of the communities that are already present. This strategy would help to ensure that the fungi being managed provide some benefit to the desired native plant communities(Hart et al. 2018; Koziol et al. 2018).

Native AM fungal communities have been found to have an important role in promoting the success of agricultural crops as well. Specifically, AM fungi can reduce the need of commercial fertilizers, increase crop yield, reduce stress and pathogen impacts, and bolster plant health and survivability (Benami et al. 2020; Ortaş et al. 2017; Zhang et al. 2019). Despite the benefits that AM fungi provide for plant and soil health, many current agricultural techniques – tilling, fertilizer use, monocropping - can harm AM fungal diversity and abundance (Benami et al. 2020). As human populations increase, environmental challenges due to climate change and growing food insecurity are heightened. Understanding and utilizing AM fungi to create more successful native ecosystems and sustainable agricultural systems is critical to maintaining human health.

2.6 Project Goals, Guiding Research Questions, and Hypotheses

This study aims to improve the understanding of AM fungal species air dispersal. Specifically, this research examines how different biotic and abiotic factors affect aerial AM fungal community dispersal and establishment. Previous studies have examined a variety of environmental factors being analyzed in this project however, many of these studies restricted their environmental factors to just a few parameters for each study and most of the research was limited to regional scales.

By analyzing several environmental factors in conjunction with aerial AM fungal species data across a large geographic scale, this study will create a better understanding of AM fungal community composition and dispersal in the continental United States. The results of this study may be important when considering new land management practices, especially as climate change continues to alter many environmental factors that may be shaping AM fungal communities worldwide. Changes in AM fungal community composition and functioning could have negative effects on natural plant communities, as well as agricultural crops, making understanding changing AM fungal communities of paramount importance for both environmental and human health.

Below are the guiding research questions and hypotheses for this project:

1. Is there variation in aerial AM fungal communities between the five research sites?
    a. Differences in AM fungal species identity
    b. Differences in the number of AM fungal species present
    c. Differences in number or identity of species found at different heights in the air
2. If there is variation in aerial AM fungal communities, what environmental factors are influencing those differences?
3. I hypothesize that there will be variation in aerial AM fungal communities between the five sites
    a. Some environmental factors will be more predictive than others in determining AM fungal community composition
4. I hypothesize that the strongest predictors of aerial AM fungal communities will be precipitation, elevation, and mean canopy height.
5. I hypothesize that the weakest predictors of aerial AM fungal communities will be wind speed and wind direction. This hypothesis aligns with findings by Chaudhary and colleagues (2020), that found wind speed did not have strong impacts on AM fungal species composition.

3. Materials and Methods

3.1 Research Sites

The research sites used for this study are located at terrestrial field stations that are part of the National Ecological Observation Network (NEON), which is funded by the National Science Foundation (NSF). NEON is a continent-scale observation project that plans to collect long-term ecological data in the United States. NEON sites are separated into 20 different Domains, each of which contain various sites – often both terrestrial and aquatic (Figure 1). Domain locations were chosen by statistically separating the continental United States, as well as Alaska, Hawaii, and Puerto Rico into different ecoclimate regions. Domains vary in terms of key vegetation types, dominant landscapes, and ecosystem dynamics. The following sites were used for this study and are denoted below with the site name, site type, state where the site is located, as well as the Domain number and name:

1. University of Notre Dame Environmental Research Center (UNDE)
   Core Terrestrial, MI, D05: Great Lakes

2. Konza Prairie Biological Station (KONZ)
   Core Terrestrial, KS, D06: Prairie Peninsula
3. Chase Lake National Wildlife Refuge (WOOD)
   Core Terrestrial, ND, D09: Northern Plains
4. Central Plains Experimental Range (CPER)
   Core Terrestrial, CO, D10: Central Plains
5. Niwot Ridge (NIWO)
   Core Terrestrial, CO, D13: Southern Rockies & Colorado Plateau

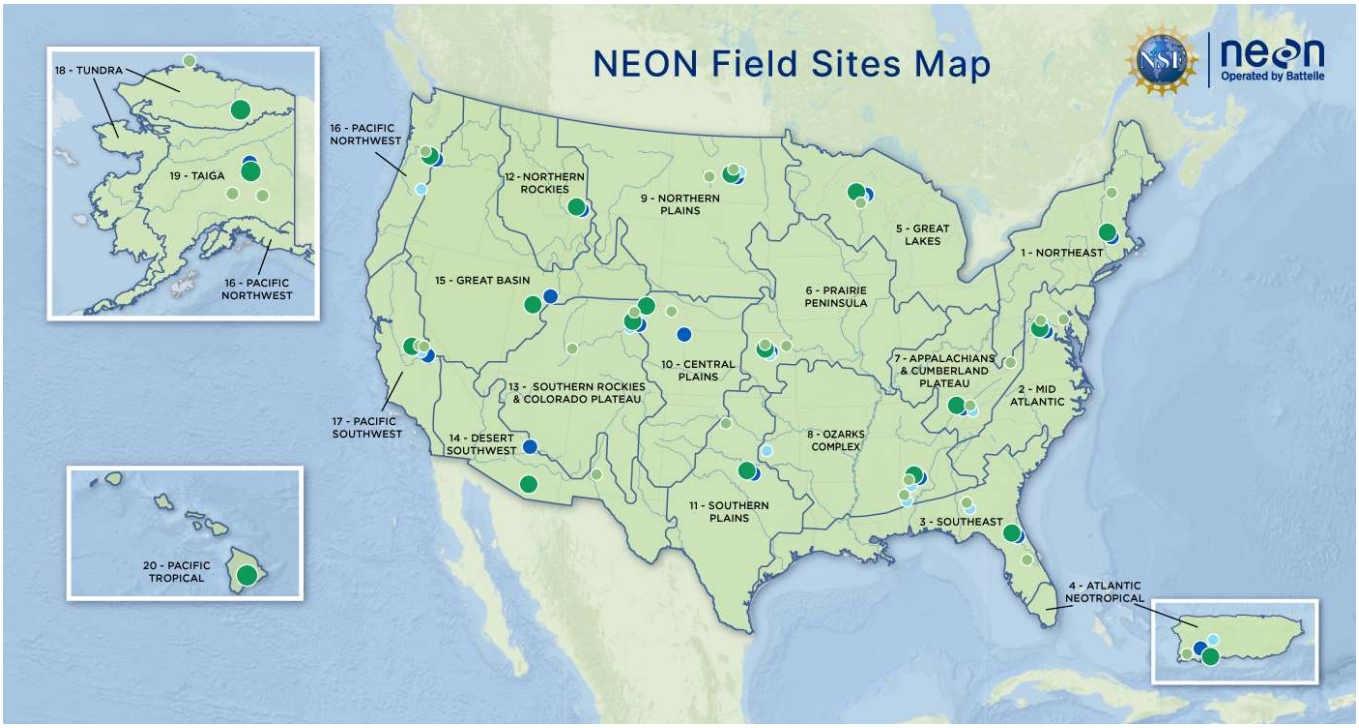

Figure 1: Map of NEON Domains (1-20). The blue outlines on the map indicate the border lines between separate domains. Retrieved from https://www.neonscience.org/field-sites/about-field-sites.

3.2 AM Fungal Sample Collection from the Air

To collect aerial dust samples containing AM fungi, five modified Wilson and Cooke (MWAC) dust collectors (Wilson & Cooke 1980) were set up (Figure 2)at one plot at each of the five research sites. At each 20 x 20 m NEON plot being used for this study, MWAC's were set up on two sides of the 40 x 40 m buffer zone surrounding the plot. Small adjustments were made at some setup locations to avoid rocks or other obstacles. The MWAC dust collectors passively collected particles from the air using the three collection chambers that were mounted on the mast. There was a wind vane that oriented the MWACs to face the wind. Each MWAC was mounted and secured in a way

that allowed free rotation in the wind. Each of the sampling canisters had hose clamps to account for the decline of particle transportation with height. Each canister lid had two tubes where air flows into the longer tube and exits the shorter tube. Particles flowed into and were captured at the bottom of the canisters.

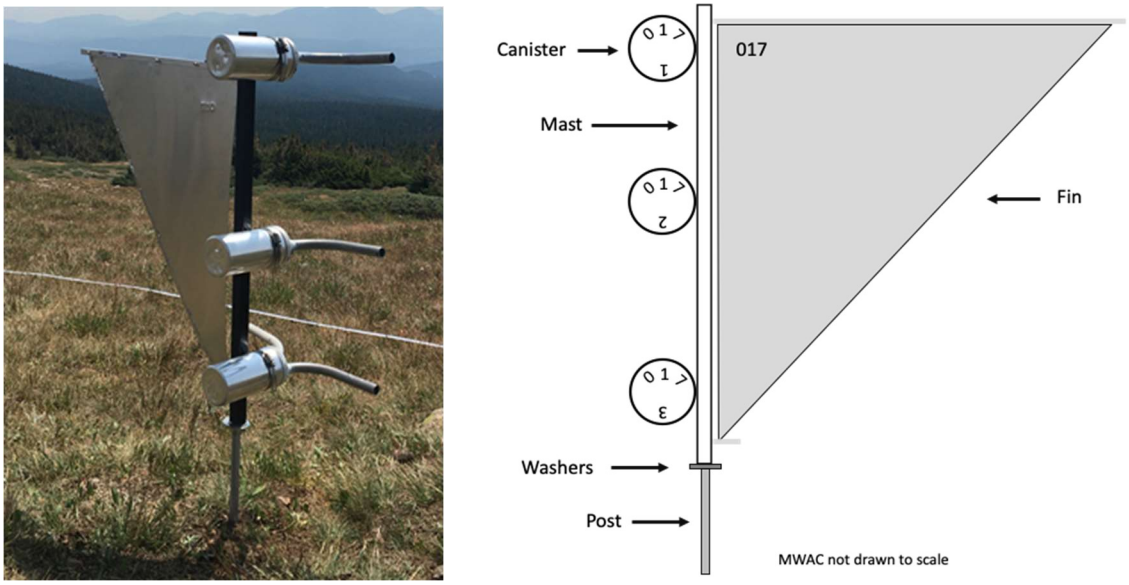

Figure 2: MWAC dust collector in the field (left) and an MWAC model outlining its components (right). Picture taken by Dr. Bala Chaudhary and diagram created by Paul Metzler.

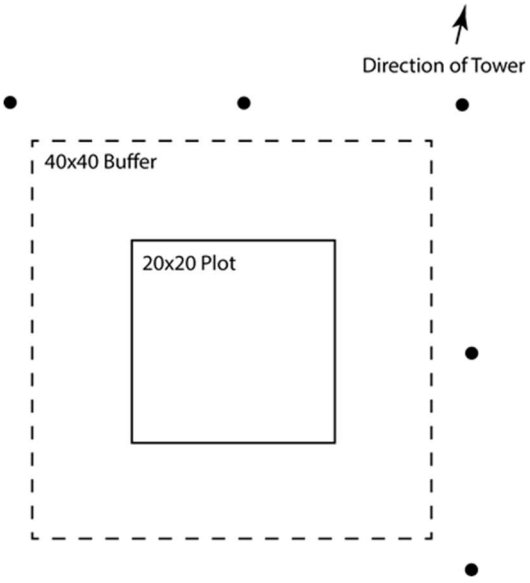

Figure 3: Example diagram outlining a NEON tower in the N/NE direction, so the north and east sides were chosen for MWAC installation. The black dots indicate the locations where each of the dust collectors were set up. This image is not to scale, but the dust collectors were set up approximately one meter outside of the 40 X 40 m buffer zone around the plot. Diagram created by Paul Metzler.

3.3 Dust Sample Collection and Processing:

The dust collectors were originally planned to be left for six months and then samples were to be collected by NEON staff members. Due to the COVID-19 pandemic, the field set-up for the MWAC's was delayed. As a result, the dust collectors were left for approximately 3-4 months. Appropriate meta-data such as canister height from the ground, any canister or MWAC damage, and obstruction by vegetation was noted by NEON staff on the appropriate data sheet. Samples were refrigerated until they could be shipped overnight to the Chaudhary Lab. Once at the lab, samples were again refrigerated until they could be processed. To process the samples, vacuum filtration was used to collect the fungal spores onto filter paper. The filter paper was then separated into three sections to apply to future downward applications: DNA extraction, spore trait analyses, and vital staining. For this project, only the third of the filter paper reserved for DNA isolation was utilized. This section was rolled up or cut into strips and placed into 750 µL tubes that were stored at -20°C until DNA extraction could be performed.

3.4 DNA Extraction:

DNA was extracted using QIAGEN DNEasy Powersoil DNA extraction kits. The extraction procedure involved multiple solutions, centrifuging, and vertexing steps that resulted in the isolation of DNA at the end of the process (Figure 4)

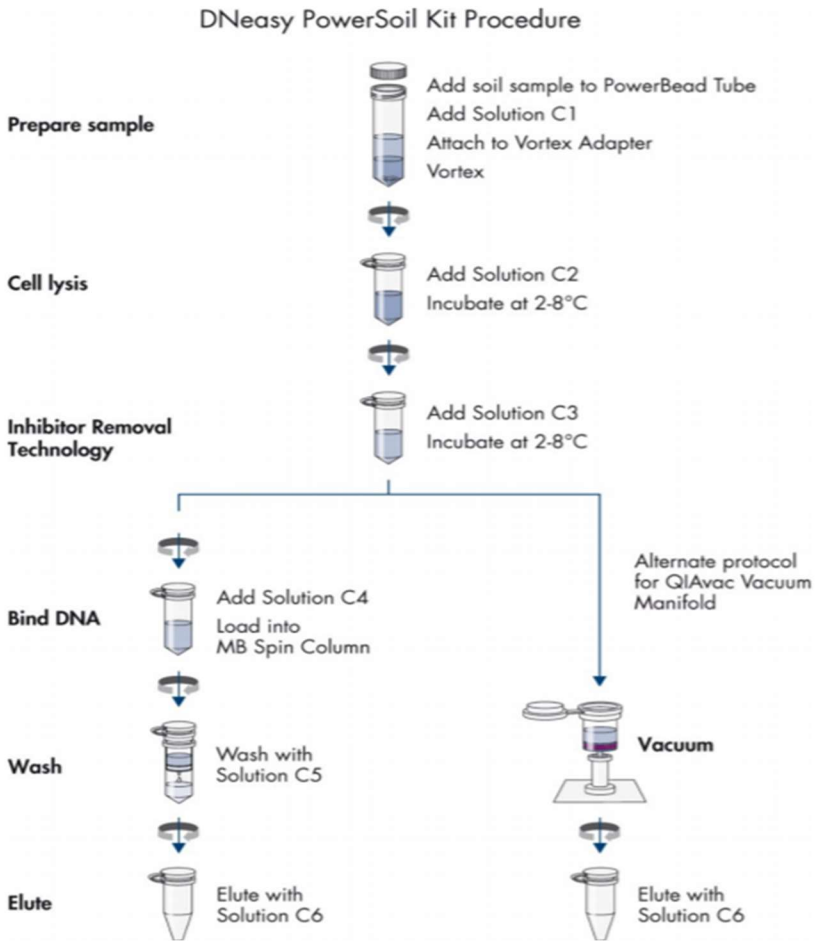

Figure 4: Process of DNA extraction using the DNeasy PowerSoil Kit by Qiagen. Retrieved from https://www.qiagen.com/us/resources/resourcedetail?id=5a0517a7-711d-4085-8a28-2bb25fab828a&lang=en

3.5 DNA Concentration Determination:

For each sample, the DNA concentration was determined in ng/mL using a Qubit 4 fluorometer. Samples were prepped by creating a working solution of 199 µL of Qubit buffer solution and 1 µL of Qubit reagent solution per sample quantified. Next, 199 µL of working solution and 1µL of DNA from each sample was added to a new tube and vortexed for 2-3 seconds. Each vortexed tube was then left to sit at room temperature for 2 minutes before concentrations were taken. Concentrations were run three times for each sample, and then averaged and recorded. In between each Qubit run, samples were allowed to sit for 30 seconds before another reading was made ("Quibit 4 Flourometer" Quibit 4 User Product Manual).

3.6 DNA Sequencing

Once DNA extraction and concentration determinization was complete, samples were prepped by removing 20 µL of DNA from the 100 µL samples. The 20 µL was placed into a 96-well full-skirted PCR plate and frozen until dry ice could be obtained. The plate was placed inside a Styrofoam container and surrounded by dry ice to ensure the samples remained frozen during transportation. The samples were then brought to the Argonne National Laboratory in Lemont, IL. The staff at Argonne National Laboratory ran polymerase chain reactions (PCR) using small subunit rRNA and NS31/AML2 primers. This PCR method and the two primers used have been found to provide accurate amplification of AM fungal species (Morgan & Egerton-Warburton 2017). Once PCR was completed, the Argonne National Laboratory staff sent back DNA sequences in the form of demultiplexed FASTQ files. These FASTQ files were then downloaded and transformed into an Amplicon Sequence Variant (ASV) table that included the raw DNA sequences and counts for each sample (Appendix 9.1).

3.7 Fungal Species Identification:

Once DNA sequences were isolated for each sample, the MaarjAM database (https://maarjam.ut.ee) was used to assign species identity to each fungal DNA sequence. The MaarjAM database is an online collection of AM fungal species and their identifying DNA sequences. The two values used for species identification on the MaarjAM database are sequence similarity and query coverage. Both are represented as a percentage. A 97% sequence similarity and 97% query coverage is a cutoff that has been shown in previous studies to provide the most accurate representation of AM fungal species identity (Morgan & Egerton-Warburton 2017). Ninety-five percent and 90% have also been used as sequence similarity cutoffs in prior work (Bell-Dereske et al. 2017; Boeraeve et al. 2019; Morgan & Egerton-Warburton 2017; Öpik et al. 2009). Query coverage cutoffs for previous studies vary from 97%, 95%, to 75% (Bell-Dereske et al. 2017; Boeraeve et al. 2019; Mahmoudi et al. 2019; Morgan & Egerton-Warburton 2017; Öpik et al. 2009). Species data were collected at three different cutoff intervals for sequence similarity and query coverage: 97% sequence similarity and 97% query coverage, 95% sequence similarity and 95% query coverage, and 90% sequence similarity and 97% query coverage. At sequence similarity and query coverage percentages less than 97%, the associated DNA sequences were run through the National Central Biotechnology Information (NCBI) DNA database to determine if there was an organism that had a better match than the AM fungal species being obtained by running the DNA sequence through the MaarjAM database.

For this study, the DNA forward and reverse reads were initially merged, but due to the poorer quality in the reverse reads and the joining region, most of the sequences did not successfully merge. So, only forward reads were analyzed in this study. Previous research has found analyzing only forward reads to be a successful way to determine AM fungal species compositions (House & Bever 2018; Stevens et al. 2020; Vasar et al. 2017)

3.8 Data Collection: Environmental Factors

To examine variations in aerial fungal communities in conjunction with environmental factors, climate and geography data were obtained. Data was collected for the following environmental factors:

- Climate – average temperature, average precipitation, average wind speed, wind direction
- Geography – elevation, vegetation types, average canopy height, and soil properties
- Height of dust container – travel height in the air

Many factors being analyzed in this study were already collected and recorded by NEON in conjunction with some of their data collection projects. So, much of the needed environmental data was able to be downloaded and recorded directly from NEON. Average windspeed, wind direction, landscape features, elevation, vegetation types, and average canopy height were recorded from NEON and were specific to the plot where the dust collectors were setup. The soil properties were recorded from NEON as well but were NEON site specific as opposed to plot specific. For average temperature and precipitation, the Parameter-elevation Regressions on Independent Slopes Model (PRISM) database was used. The PRISM database allows for searches of temperature and precipitation data by year and geographical coordinates. Temperature and precipitation values were recorded over the last thirty years (1991-2020) as a historical average. Values for temperature and precipitation only during the time of sampling was also recorded. The height of each dust container was documented by NEON staff members and sent back to the Chaudhary Lab for future analyses.

3.9 Statistical Analyses

All analyses were performed using R version 4.1.2 (R Core Team 2021) and the RStudio interface (RStudio Team 2021).

3.9.1 DNA Concentration by Site and by Height

After DNA concentrations were determined for all the samples available for each of the sites, an ANOVA test was performed to determine if there were variations in DNA concentration by site. Additionally, an ANOVA test was done to determine if there were variations in DNA concentration by the height of the sampling jars. The ANOVA test for concentration by site was followed up by a Tukey HSD test to determine which of the sites had variations in mean DNA concentrations. The *ggplot2* (Wickham 2016) and *ggthemes* (Arnold 2021) packages were used to created boxplots to visualize the variations in DNA concentrations by site and by height.

3.9.2 Species Richness and Environmental Data Table

To begin analyses examining AM fungal species along with environmental data, species richness was determined for each of the five sites and recorded in a data set along with all the environmental variables being analyzed.

3.9.3 Determining Correlation Values for Variables Being Analyzed

Before any analyses could be run using the environmental variables, a Spearman's correlation matrix (Appendix 9.2) was created and exported from RStudio into an Excel file. The matrix was used to test for multicollinearity between the explanatory environmental variables. Any variable that had a correlation value of +/- 0.7 was excluded, as it was considered highly correlated. Using the R package *ggpubr* (Kassambara 2020) allowed for individual correlations to be visualized for a better understanding of the relationship between different environmental variables of interest.

3.9.4 Examining AM Fungal Communities Using Richness

To analyze variations between the fungal communities at each of the five research sites, species richness was used to determine if differences in

environmental conditions at each of these sites impacted the number of species found at each site.

Using the R packages *fitdistrplus* (Delignette-Muller & Dutang 2015) and *logspline* (Kooperberg 2020), various distribution models were run to determine the most accurate model to analyze this data set. The data was best represented using a generalized linear model (GLM) with a Poisson distribution. Using a GLM, environmental factors that significantly influenced AM fungal species richness were determined. Results were considered significant if the GLM's had p-values <0.05 and moderately significant if they had p-values <0.1.

4. Results

4.1 DNA Concentration Results

The CPER and NIWO sites had significantly higher mean aerial DNA concentrations than the KONZ and UNDE sites. The WOOD site had a higher mean concentration than the UNDE site, but the mean was not significantly different from the CPER, NIWO, or KONZ site means. Finally, UNDE had the lowest mean DNA concentration which differed significantly from all other sites.

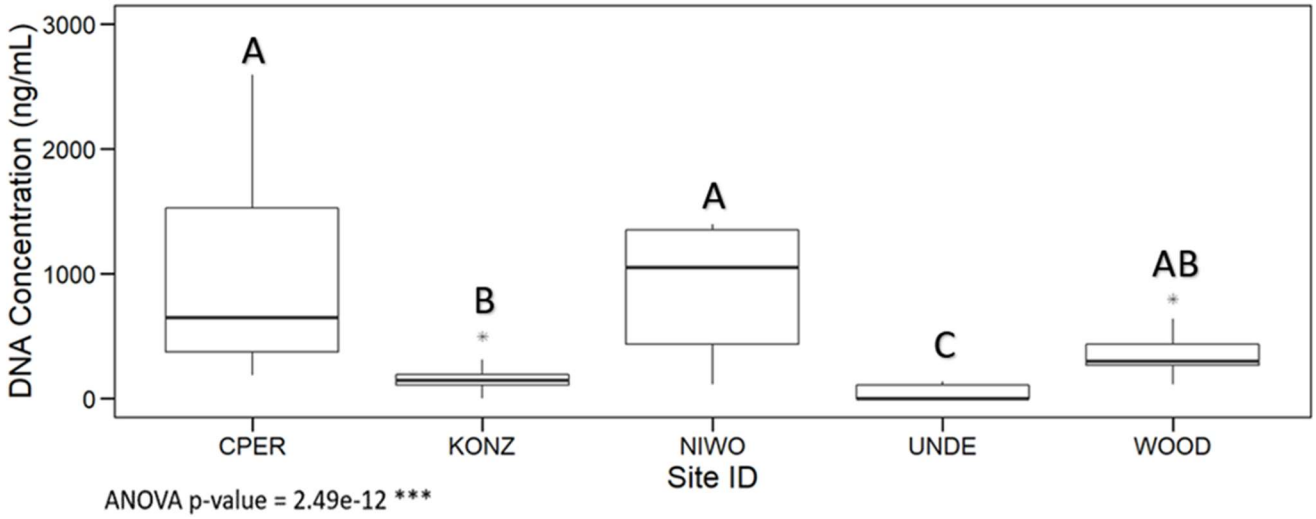

Figure 5: DNA concentrations for each of the five sites: CPER, KONZ, NIWO, UNDE, WOOD were found using a Qubit 4 fluorometer. The results of the ANOVA test yielded a significant p-vale of $2.49e^{-12}$. The letters above the boxplots indicate significant differences in the means as determined by a Tukey's HSD test. Outliers are indicated by dots above the boxplots. The y-axis limit was changed to 3000 ng/mL to get a better view of the site concentrations. Before changing the y-axis limit, some extreme outliers were causing distortions in the graph. As a result, a few outliers are not shown on this boxplot.

4.2 DNA Concentration by Sample Height

When DNA concentrations across all five sites were combined, DNA concentration was not found to significantly vary by sample height.

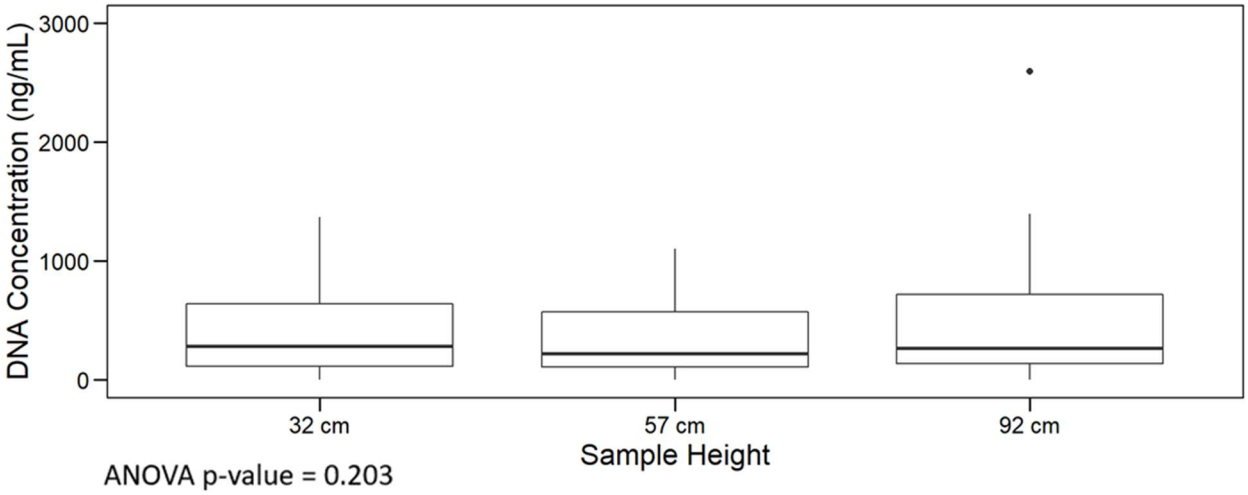

Figure 6: Samples were taken at three different heights within the air: ~ 32 cm, 57 cm, and 92cm. The ANOVA test performed resulted in a p-value of 0.203. This indicates that there is not a significant difference in the mean DNA concentrations found at each of the three sample heights.

4.3 DNA Concentration by Site ID and Sample Height

When DNA concentration and sample height were analyzed between each of the five sites, DNA concentration by site did not vary significantly by sample height.

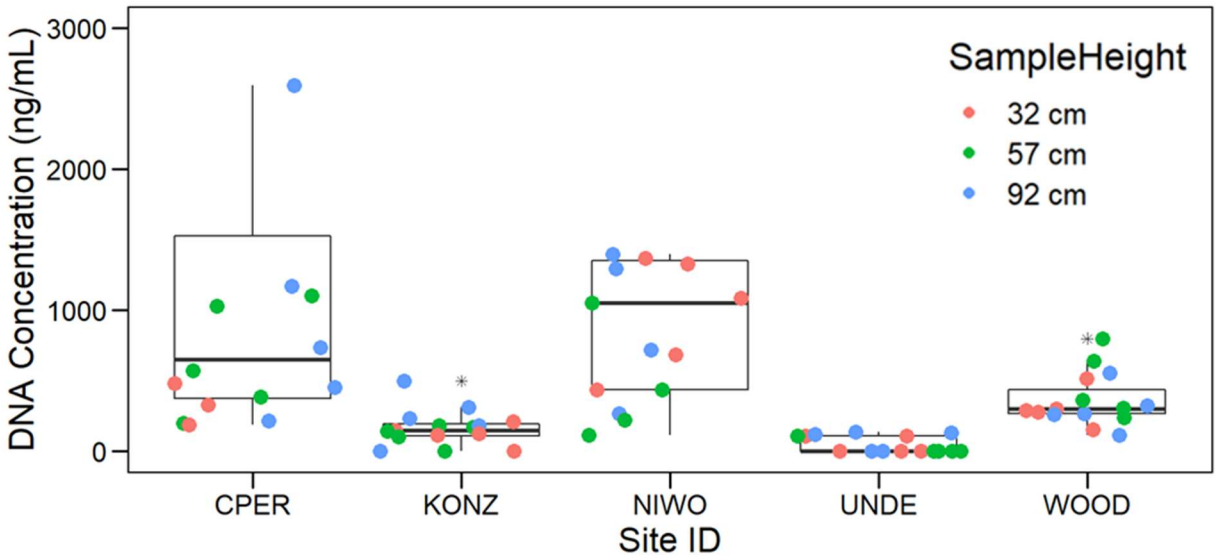

Figure 7: DNA concentration by site location – CPER, KONZ, NIWO, UNDE, WOOD - depicted as boxplots. The sample height for each sample is overlayed and represented by points. The red dots indicated

samples taken at 32 cm. The green dots represent samples taken at ~57 cm. The blue dots indicate samples taken at ~92 cm

4.4 AM Fungal Species Present at Three Different Max Identity and Query Coverage Values

During species identification using the MaarjAM database, three different max identity and query coverage cutoffs were used for classification. Each cutoff yielded different AM fungal results at the genera level (Table 1).

Table 1: List of AM fungal genera present at each of the five research sites. Genera are organized by three different levels of max identity and query coverage values as reported on the MaarjAM database. Genera were included based on unique VTX numbers.

Site ID	Max Identity (97%); Query Coverage (97%)	Max Identity (95%); Query Coverage (95%)	Max Identity (90%); Query Coverage (97%)
UNDE	-	-	Paraglomeraceae Paraglomus IH1 VTX00444
WOOD	-	-	Paraglomeraceae Paraglomus IH1 VTX00444
CPER	Diversisporaceae Diversispora sp. VTX00062	Diversisporaceae Diversispora sp. VTX00062	Diversisporaceae Diversispora sp. VTX00062
	Archaeosporaceae Archaeospora sp. VTX00245	Archaeosporaceae Archaeospora sp. VTX00245	Archaeosporaceae Archaeospora sp. VTX00245
		Paraglomeraceae Paraglomus IH1 VTX00444	Paraglomeraceae Paraglomus IH1 VTX00444
NIWO	Gigasporaceae Scutellospora sp. VTX00049	Gigasporaceae Scutellospora sp. VTX00049	Gigasporaceae Scutellospora sp. VTX00049
	Archaeosporaceae Archaeospora sp. VTX00005	Archaeosporaceae Archaeospora sp. VTX00005	Archaeosporaceae Archaeospora sp. VTX00005
	Archaeosporaceae Archaeospora sp. VTX00456	Archaeosporaceae Archaeospora sp. VTX00456	Archaeosporaceae Archaeospora sp. VTX00456
	Ambisporaceae Ambispora sp. VTX00283	Ambisporaceae Ambispora sp. VTX00283	Ambisporaceae Ambispora sp. VTX00283
	Paraglomeraceae Paraglomus IH1 VTX00444	Paraglomeraceae Paraglomus IH1 VTX00444	Paraglomeraceae Paraglomus IH1 VTX00444

KONZ	Paraglomeraceae Paraglomus sp. VTX00239	Paraglomeraceae Paraglomus sp. VTX00239	Paraglomeraceae Paraglomus sp. VTX00239
	Paraglomeraceae Paraglomus IH1 VTX00444	Paraglomeraceae Paraglomus IH1 VTX00444	Paraglomeraceae Paraglomus IH1 VTX00444

4.5 Determining Correlations Between Environmental Variables

Before analyzing multiple environmental factors together along with richness, a Spearman correlation was performed to determine which environmental factors were highly correlated at values of +/- 0.7 or higher and should not be analyzed together in relation to predicting richness (Appendix 9.2).

The results of the Spearman correlation test found high correlations (value ≥ 0.7) between several of the environmental factors being analyzed, including several factors I had hoped to analyze including elevation and mean canopy height, as well as elevation and precipitation. This limited the number of factors that could be analyzed together in the final generalized linear model.

4.6 Using Multiple Environmental Factors and AIC Values to Get a Final Poisson Regression Model

After eliminating highly correlated environmental factors, generalized linear models were run that analyzed richness individually with each of the other environmental factors. When the model was ran looking at each environmental factor individually it was found that average elevation, Al, K, P, Si, and Sr concentrations were significantly related to richness. Al, P, Si, and Sr were all highly correlated (ranging from 0.7 – 0.9) with each other and none of these variables fit the model as well as average elevation or K in terms of explaining variations in richness. Elevation was determined to be the most biologically relevant in terms of AM fungal dispersal, so all the final models included average elevation and any other environmental variables that resulted in a more accurate model looking at how well environmental variables were explaining species richness at the study sites. The final models for each of the three cutoff intervals includes the variables that resulted in the lowest AIC values.

Species richness increased by elevation at all three cutoff points in the generalized linear models with only elevation. Models that included elevation and another explanatory variable were significantly better based on ANOVA comparisons and AIC values at the 97% max identity 97% query coverage and the 95% max identity 95% query coverage cutoffs, though elevation did not remain significant in explaining differences in species

richness when more than one explanatory variable was included. At the 90% max identity and 97% query coverage cutoff elevation remained significant when another explanatory value was added, however based on the ANOVA comparison and AIC value the model with just elevation was significantly better at this cutoff interval.

4.6.1 Generalized linear models with Poisson distributions at max identity (97%) and query coverage (97%)

Max Identity (97%); Query Coverage (97%) GLM with Poisson Distribution

	Estimate	Standard Error	z Value	p Value
(Intercept)	-1.0273447	0.8472911	-1.213	0.2253
Average Elevation	0.0007181	0.0003026	2.373	***0.0177***

Null deviance: 9.1523 on 4 df
Residual deviance: 3.0555 on 3 df

AIC: 14.935

Table 2: Results of a GLM looking at how well variation in average site elevation explains differences in species richness by site. The model resulted in a p-value of 0.0177, which is significant at a p-value < 0.05 significance level.

Max Identity (97%); Query Coverage (97%) GLM with Poisson Distribution

	Estimate	Standard Error	z Value	p Value
(Intercept)	-9.754449	9.408587	-1.037	0.300
Temperature	0.536679	0.526506	1.019	0.308
Average Elevation	0.002430	0.001952	1.245	0.213

Null deviance: 9.15233 on 4 df
Residual deviance: 0.28375 on 2 df

AIC: 14.163

Table 3: Results of a GLM at how well variations in average sample site elevation and average temperature during the sample collection explain differences in species richness by site. The model did not have significant p-value (s).

4.6.2 ANOVA test Between max identity (97%) and query coverage (97%) Models

When elevation was analyzed with temperature, there was not a significant relationship. There is a moderate correlation between elevation and temperature so that could explain why the significance of elevation is lost when analyzing this variable alongside temperature. The ANOVA test with a Chi-squared comparison resulted in a p-value of 0.09594 which is moderately significant. This indicates that the models are significantly different from one another. So, analyzing elevation and temperature together represents a better fit model than just analyzing elevation alone.

4.6.3 Species Richness by Elevation at max identity (97%) and query coverage (97%)

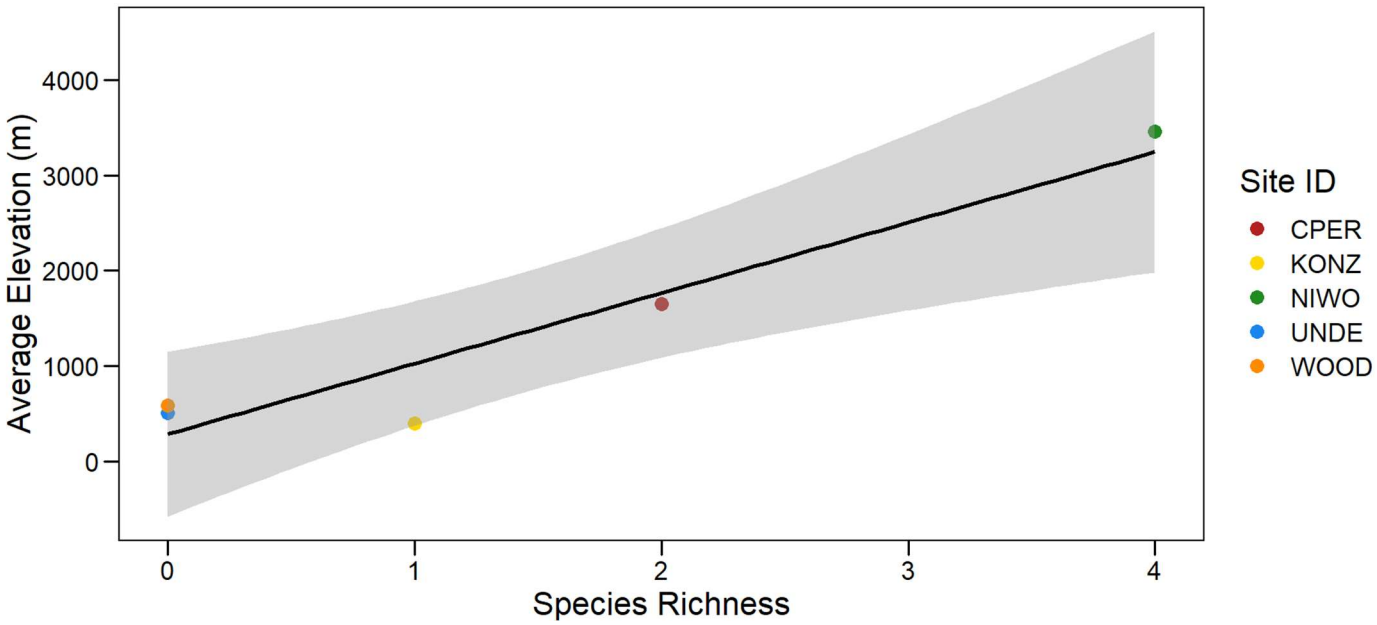

Figure 8: Graph showing the relationship between species richness and average elevation at the max identity (97%) and query coverage (97%) level. Points on the graph correspond with each of the five research sites. A best-fit line was added to highlight the trend of increasing richness with increasing elevation

4.6.4 Generalized linear models with Poisson distributions at max identity (95%) and query coverage (95%)

Max Identity (95%); Query Coverage (95%) GLM with Poisson Distribution

	Estimate	Standard Error	z Value	p Value
(Intercept)	-8.776485	6.682823	-1.313	0.189
Temperature	0.002224	0.001380	1.612	0.107
Average Elevation	0.522759	0.377167	1.386	0.166

Null deviance: 11.59570 on 4 df
Residual deviance: 0.65705 on 2 df

AIC: 15.743

Table 4: Results of a GLM looking at how well variations in average sample site elevation and average temperature during the sample collection explain differences in species richness by site. The model did not have significant p-value (s).

Max Identity (95%); Query Coverage (95%) GLM with Poisson Distribution

	Estimate	Standard Error	z Value	p Value
(Intercept)	-0.3753417	0.6427172	-0.584	0.5592
Average Elevation	0.0005954	0.0002427	2.453	***0.0142***

Null deviance: 11.5957 on 4 df
Residual deviance 5.5152 on 3 df

AIC: 18.601

Table 5: Results of a GLM model looking at how well variation in average site elevation explains differences in species richness by site. The model resulted in a p-value of 0.0142, which is significant at a p-value < 0.05 significance level.

4.6.5 ANOVA test Between max identity (95%) and query coverage (95%) Models

Analyzing the results at the 95% species identity and 95% query coverage cutoff showed that when elevation was analyzed individually it yielded a p-value of 0.0142. This means that sample site elevation significantly explained the variation in species richness at a p-value < 0.05 significance level. However, the model with temperature and elevation was not significant at a p-value < .05 significance level.

When comparing the two models using an ANOVA test with a Chi-squared comparison, the X2 p-value = .02752, which is significant at the p-value < 0.05 significance level. Therefore, the model with elevation and temperature is a better fit model than elevation individually. However, as noted in the previous section, the moderate correlation between elevation and temperature could explain the lack of significance in elevation predicting richness in the model that included both elevation and temperature.

4.6.6 Species Richness by Elevation at max identity (95%) and query coverage (95%)

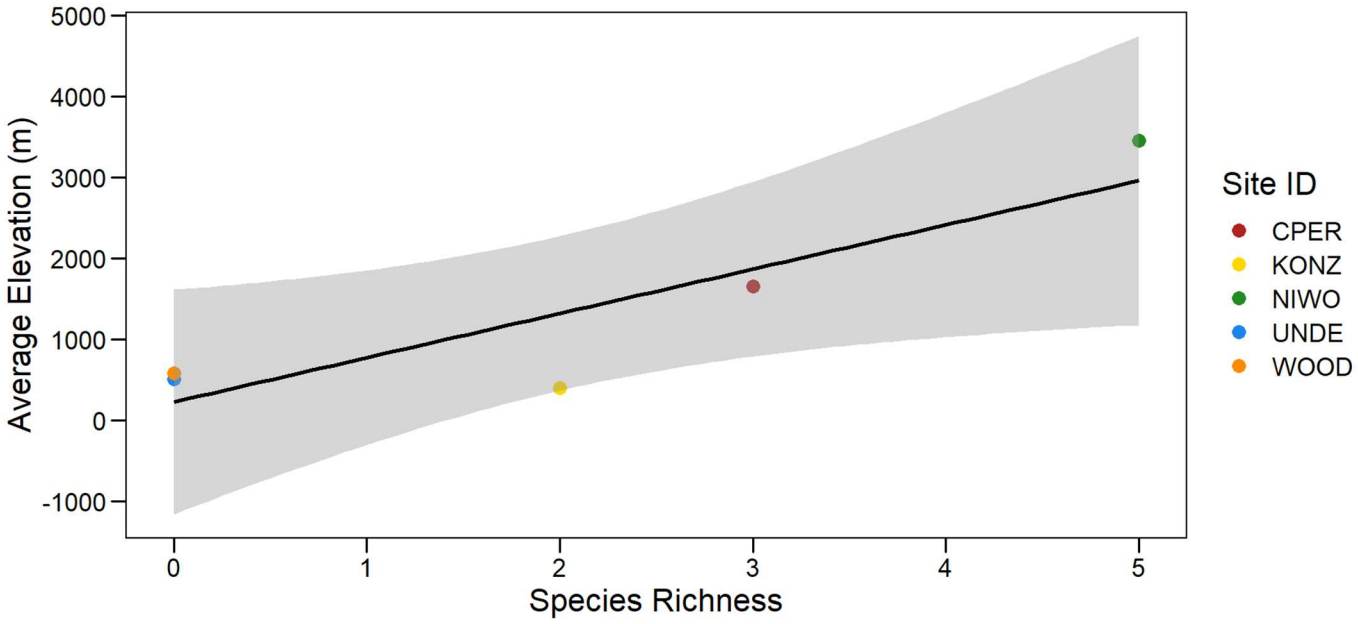

Figure 9: Graph showing the relationship between species richness and average elevation at the max identity (95%) and query coverage (95%) level. Points on the graph correspond with each of the five research sites. A best-fit line was added to highlight the trend of increasing richness with increasing elevation

4.6.7 Generalized linear models with Poisson distributions at max identity (90%) and query coverage (97%)

Max Identity (90%); Query Coverage (97%) GLM with Poisson distribution

	Estimate	Standard Error	z Value	p Value
(Intercept)	0.1692689	0.5234821	0.323	0.7464
Average Elevation	0.0004276	0.0002170	1.971	***0.0488***

Null deviance: 4.44739 on 4 df
Residual deviance: 0.74196 on 3 df

AIC: 17.828

Table 6: Results of a GLM looking at how well variation in average site elevation explains differences in species richness by site. The model resulted in a p-value of 0.0488, which is significant at a p-value < 0.05 significance level.

Max Identity (90%); Query Coverage (97%) GLM with Poisson Distribution

	Estimate	Standard Error	z Value	p Value
(Intercept)	-1.3591162	1.9992073	-0.680	0.4966
Average Elevation	0.0006965	0.0004142	1.682	***0.0926***
Temperature	0.1093757	0.1316690	0.831	0.4062

Null deviance: 4.447392 on 4 df
Residual deviance: 0.019831 on 2 df

AIC: 19.106

Table 7: Results of a GLM looking at how well variations in average sample site elevation and average temperature during the sample collection explain differences in species richness by site. The model resulted in a p-value of 0.0926, which is not significant at a p-value <0.05 significance level but could be considered moderately significant if considering p-values of <0.1.

4.6.8 ANOVA test Between max identity (90%) and query coverage (97%) Models

The GLM examining elevation has a p-value = 0.0488, which is significant at a p-value < 0.05 significance level. This result shows that sample site elevation significantly explained the difference in species richness between research sites. Analyzing this cutoff level using both temperature and elevation in the Poisson regression model gave a p-value for elevation = 0.0926, which is not significant at a 0.05 significance level but could be considered moderately significant when looking at values < 0.1.

When comparing the two models utilizing an ANOVA test with a Chi-squared comparison, the X2 p-value = 0.08145 which is moderately significant. However, unlike the previous two max identity and query coverage cutoff intervals, in the case of the data for 90% max identity and 97% query coverage the best fit model is the one that just analyzes elevation.

4.6.9 Species Richness by Elevation at max identity (90%) and query coverage (97%)

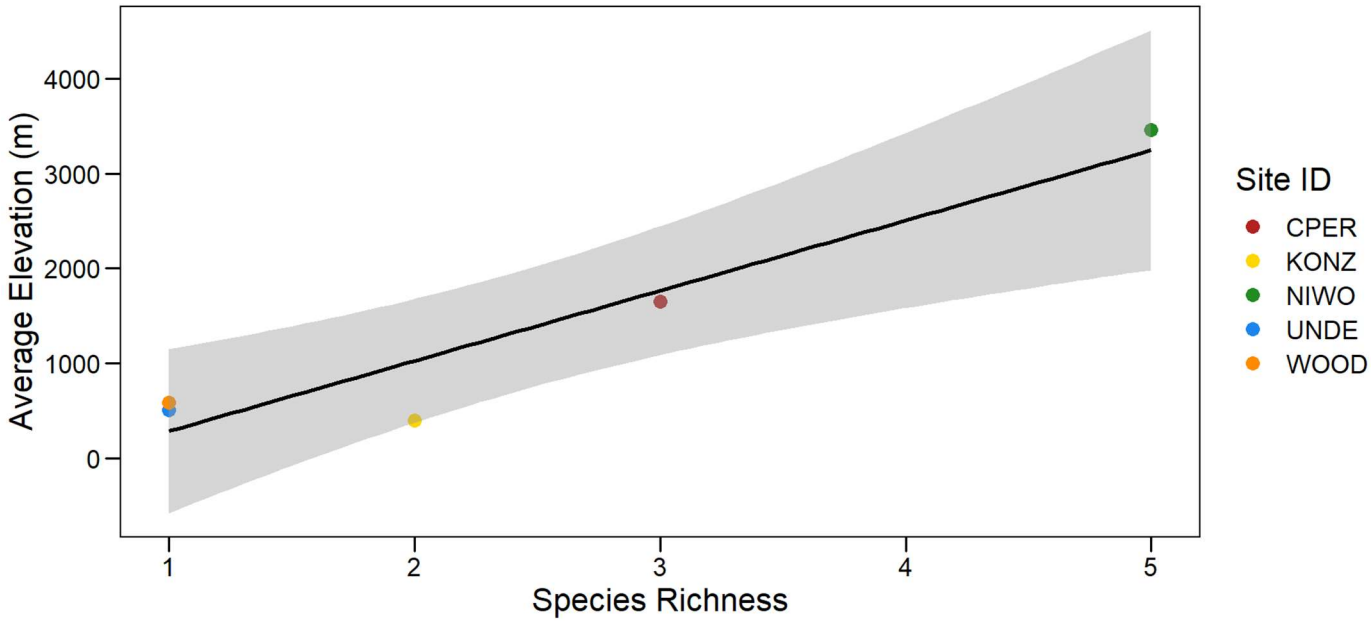

Figure 10: Graph showing the relationship between species richness and average elevation at the max identity (90%) and query coverage (97%) level. Points on the graph correspond with each of the five research sites. A best-fit line was added to highlight the trend of increasing richness with increasing elevation

5. Discussion

5.1 Elevation and Species Richness

Elevation was found to be a significant predictor of species richness at all three of the cutoff intervals analyzed in this study. This indicates that elevation may be an important component of predicting AM fungal species richness across a variety of ecosystems in the continental United States. However, more research is needed to verify this relationship between elevation and species richness. This study only examined five different sites, which vary in many aspects other than elevation. It is possible that the

variation in richness could be attributed to other site differences. When a relationship between species richness and elevation is found, it is important to analyze whether this is a direct relationship or if this result is the outcome of interactions from several other factors (Rahbek 1995). High elevation sites vary in terms of steepness, temperature and precipitation patterns, air pressure, vegetation gradients, and many other factors that could be influencing this species richness and elevation relationship. Additionally, it is important not to overstate the results with such a small sample size, and with a study that used many highly-moderately correlated explanatory environmental variables. However, the possibility of higher species richness at locations of higher elevation could have important implications for AM fungal species composition when considering climate change and human alterations to the natural landscape.

Current research relating fungal species richness and elevation is limited. Some studies have found high species richness at high elevation locations (Liu et al. 2011). However, some studies have found that species richness actually decreases along elevation gradients (Gai et al. 2012). Another study found a unimodal distribution with peak richness falling somewhere around 4000 m (Kotilínek et al. 2017), which somewhat supports the data in this study where the highest richness was found at an elevation around 3500 m. Future research with more high elevation sites would help to confirm if the results in this study match the unimodal richness distribution that was found by Kotilínek and colleagues (2017). One study found that host plant identity and distribution may be more important when considering AM fungal community composition than elevation (Kivlin et al. 2022). Previous work has found a positive relationship between AM fungal richness and plant species richness in greenhouse/field experiments (Van Der Heijden et al. 1998; Vogelsang et al. 2006) and natural settings (Hiiesalu et al. 2014). Additionally, plant species richness has been shown to follow a unimodal distribution with peak plant species richness falling around 3000 m (Bryant et al. 2008). As previously discussed, a unimodal pattern for elevation and aerial AM fungal richness may fit with the results of this study, and a unimodal distribution on host-plant species richness could explain this pattern.

Some of the aforementioned studies that analyzed elevation, broke down richness by different fungal groups. Some of these studies found different results for AM fungi, ectomycorrhizal fungi, fungal leaf endophytes, fungal root endophytes, and saprotrophic fungi (Kazenel et al. 2019; Veach et al. 2018). There were also differences found when looking at different genera or species within fungal groups.

It is important to note that all the studies discussed above were analyzing fungal communities that were already established and thus, fungal samples were taken from soil or leaves – depending on the fungal group. None of these studies were analyzing

these species dispersing in the air. Most studies that have analyzed fungal spore movement in the air were looking at pathogen species such as molds. These pathogen studies were also focused on spore concentrations rather than on species richness. Despite this lack of information regarding elevation and aerial AM fungal species richness in the air, there have been studies examining dust travel in the air at higher elevations. These studies have found that dust travels over large geographic distances and that large amounts of this dust settle in high-elevation regions (Dong et al. 2020; Sarangi et al. 2020). Although the aerial movement of dust on the planet can be a result of natural desert systems, aerial dust levels have increased as a result of human activities that disturb the soil such as agriculture, industry, and building development (Neff et al. 2008). Whether it is natural and anthropogenic sources of dust, it is possible that aerial AM fungal species follow these same trends seen in previous research related to dust movement. This could explain higher aerial AM fungal species richness at higher elevations.

5.2 Comparing Species Presence Between this Project and Previous Work

Using the MaarjAM database eight different aerial AM fungal species were identified by unique virtual taxa numbers (VTX). In Table 8, using VTX numbers the aerial AM fungal species found in this study are compared to where the species were found and cataloged in the MaarjAM database. Other locations in which these VTX were found were also recorded by searching each VTX number in Google Scholar.

Table 8: MaarjAM and other research location results by country/territory where each species (VTX) was found

Species Identity	**MaarjAM Results**	**Previous Research**
Paraglomeraceae Paraglomus IH1 VTX00444	Estonia	No additional countries/territories found
Diversisporaceae Diversispora sp. VTX00062	Estonia, China, England, Germany, Sweden, United States, Italy, Ethiopia, South Korea, Spain, Iceland, Scotland, Serbia, Austria, Canada, Croatia, India, Poland, Slovakia, Australia, Georgia, Mongolia, Argentina, Chile, Finland, South Africa	No additional countries/territories found.
Archaeosporaceae Archaeospora sp. VTX00245	Estonia, China, England, United States, Namibia, Australia, Austria, Colombia, Venezuela, Georgia, Cape Verde, Norway, Argentina, Spain, New Zealand, Canada, Slovenia, Croatia, India, France, Tanzania,	No additional countries/territories found

	Mongolia, Chile, Russia, Gabon, France, Israel, South Africa, French Polynesia,	
Gigasporaceae Scutellospora sp. VTX00049	Philippines, England, United States, Italy, Portugal, Spain, Estonia, France, China, Georgia, Russia, Tanzania, Thailand, Canada, Western Cape, Sweden, French Polynesia, New Caledonia, Lithuania	Tibet (Chai et al. 2018)
Archaeosporaceae Archaeospora sp. VTX00005	England, United States, Russia, Thailand, China, Estonia, Canada, New Zealand, Scotland, France, Australia, Senegal, Georgia, Argentina, Chile, Gabon, Finland, India, South Africa, Germany, Western Cape, Sweden, Cape Verde, Iceland	Qatar (Adenan et al. 2020)
Archaeosporaceae Archaeospora sp. VTX00456	Estonia	No additional countries/territories found
Ambisporaceae Ambispora sp. VTX00283	Finland, Spain, Ethiopia, South Korea, China, Russia, United States, Australia, Georgia, Mongolia, Argentina, Western Cape, French Polynesia, Sweden, Iceland, Lithuania, Greece,	No additional countries/territories found
Paraglomeraceae Paraglomus sp. VTX00239	Brazil, South Korea, Venezuela, United States, India, China, Georgia, Chile, Estonia, Iceland, Sweden, Australia, Guadeloupe, French Guiana	Japan (Berruti et al. 2017)

Most of the species found in this study appear to be present at many different locations around the globe. This could indicate that these AM fungal species are generalist and able to survive and thrive in many various environments. However, more research is needed into what the climate and other environmental conditions are at each of these locations to determine how different the ecosystems are where these species are found.

Paraglomeraceae Paraglomus IH1 VTX00444 and Archaeosporaceae Archaeospora sp. VTX00456 have only be found in Estonia previously. Archaeosporaceae Archaeospora sp. VTX00456 was only found at one location in this study, so perhaps this species is more specialized to a particular plant host or climate conditions. Paraglomeraceae Paraglomus IH1 VTX00444 on the other hand, was found at all five research sites. This is surprising given it is only recorded in Estonia on the MaarjAM database. This species was mostly recorded at the lowest confidence cutoff interval, so it is possible that the

DNA found at these research sites might not actually be Paraglomeraceae Paraglomus IH1 VTX00444. More research will be needed to determine the presence of this species at these sites, as well as other locations.

5.3 DNA Concentration Variations by Site and Species Richness

DNA extraction yielded many samples that were low in concentration. Most samples had a concentration lower than what was ideal for PCR amplification. Since the samples were being collected from dust, lower concentrations were somewhat expected. The low concentrations could be due to collecting DNA from dust, the lack of aerial spores present, or a result of the shortened sampling time due to the COVID-19 pandemic. Overall, species with higher DNA concentration also showed more species richness, which was expected. The exception was the WOOD site, where DNA concentration was higher than UNDE, and not significantly different from the other sites, but had lower species richness.

5.4 Height, DNA Concentration, and Species Richness

Dust samples were collected at three different heights within the air: ~ 92 cm, 57 cm, and 32 cm. This was done to determine if there were differences in DNA concentration and species richness at different heights in the air. The results from this study did not find significant variation in DNA concentration or species richness by sample height. However, further research is needed as the result could change when analyzing DNA concentrations and species richness at different locations, different sampling heights, or over a longer sampling time.

AM fungal species vary in terms of spore sizes (Aguilar-Trigueros et al. 2019). Smaller spores may be able to travel higher and farther in the air than larger spores. Other physical spore traits such as surface ornamentation or spore color might influence how AM fungal spores move through the air (Chaudhary et al. 2020). Spore traits were not considered as part of this study but may provide an avenue for future research.

5.5 Potential Errors Related to Species Identity and Query Coverage Values Less than 97%

Any species examined at less than a 97% species identity and 97% query coverage value was ran through the NCBI database. There were a few matches for other non-AM fungal species that were a similar or closer match. For that reason, it is possible that some of

the species at the 95% and 90% species identity level may not be AM fungal species. However, the significant relationship between elevation and species richness was consistent at all three cutoff intervals.

5.6 Correlation of Environmental Variables

Many of the environmental variables that were examined in this study were found to have a high-moderate correlation with each other. It is possible that all these variables are correlated with each other. However, this correlation could also be a result of the limited sample size. Analyzing more sites may allow for more environmental factors to be modelled together if the correlations between these factors are lessened by including more data values.

5.7 Implications for Ecosystem Health, Land Management, and Restoration

The results of this study showed that increased aerial AM fungal species richness was found at sites with higher elevations. This information could have potential implications for ecosystem heath and management strategies in high elevation areas, especially in the wake of climate change and increased human alternations to the environment. High elevation ecosystems – specifically mountain regions – have been found to be both biologically diverse and sensitive to environmental change. Mountain ecosystems are vulnerable to natural disasters, land alteration, and the degradation of glacial and permafrost integrity (Diaz et al. 2003). Around 25% of the Earth's land surface is covered by mountains. Mountains provide important habitat for plants, wildlife, and many humans also inhabit mountainous regions. Additionally, mountains provide critical freshwater resources – up to 90-100% of the freshwater in arid or semi-arid regions (Meybeck et al. 2001). Therefore, it is crucial that high elevation, mountainous areas are protected.

Understanding the richness of AM fungal species in high elevation areas could help inform land management or restoration strategies. High elevation sites are considered environmentally stressed and some of the most abiotically severe environments. For this reason, fungal-plant mutualisms should be especially beneficially in these ecosystems. There are changes in plant and fungal responses based on the species that make up the symbiosis. (Bever 2002). This can impact the growth rate and robustness of plant species. Therefore, it is crucial that AM species diversity is maintained at these sites to promote healthy plants and high-functioning ecosystems. If certain areas are found to be lacking in AM fungal diversity, perhaps there are ways to manage or restore the land to promote an ecosystem that would support a vigorous and diverse AM fungal

network. Keeping AM fungal communities healthy will help strengthen plant communities and thus, entire ecosystems. Healthier ecosystems will in turn be more resilient to degradation from threats such as erosion. However, more informed management strategies must be coupled with a global response to minimize the effects of climate change to affectively address the threats to high elevation ecosystems.

5.10 Future Research and Remaining Questions

This study is part of a larger project that will examine AM fungal species composition across a total of twenty different sites: the five sites analyzed in this study as well as fifteen additional sites. When thinking towards future research on this project, several questions arose related to how DNA concentration and richness results may change when analyzing a larger, more variable data set (Table 9). These questions provide an avenue to expand upon this study moving forward through the duration of the larger project. These questions will help to guide future analyses and ensure that the results of this study can be reexamined and possibly reevaluated once more data becomes available.

Table 9: List of questions that arose at the conclusion of this study.

Questions For Future Research
1. Will DNA concentrations at these study sites increase when left for closer to six months?
2. Does sampling height become a significant factor in determining species richness when analyzing data from more sites?
3. When all 20 sites are analyzed, will elevation continue to be a significant predictor of species richness?
4. Are there any other environmental factors that will be significant predictors of species richness when analyzing data across the 20 research sites?
5. Do the high-to-moderate correlations between environmental variables decrease when data is analyzed across 20 sites as opposed to the 5 analyzed in this study?
6. Spore traits were not analyzed for this study, but if spore traits are documented in future research, do spore traits impact species richness by site or by sampling height?

6. Conclusion

The purpose of this study was to gain new insights on how AM fungal species are dispersing in the air, by examining if different biotic and abiotic environmental factors affect species dispersal and establishment. The results of this research show that elevation was a predictor of species richness at the five different study sites, with higher elevation sites having increased levels of species richness. Further research is needed to determine if this trend is present in other ecosystems. Next steps for continuing this study include adding more study sites to determine if elevation remains a predictor of species richness, as well as if additional sites and species data will add new significant environmental predictors for species richness. As this thesis study is part of a larger, on-going project, these steps are already in process.

The results shown in this study have important implications for land management strategies, especially at high elevation locations. These sites show high species richness that may be vulnerable as climate change and human activities threaten these fragile ecosystems. Plant-AM fungi symbiotic relationships change with certain species or genera of AM fungal species (Bever 2002). This can impact plant growth, health, and survivability. It is important that AM species diversity is maintained at these sites to promote the diversity of plant life and overall ecosystem health and functioning of these systems.

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9. Appendices

9.1 Getting Fastq Files into an ASV Table

Getting an ASV Table from FASTQ Files

Prepared by: Raechel Hearth

Adapted from: “DADA2 Pipeline Tutorial (1.16)”

https://benjjneb.github.io/dada2/tutorial.html

Notes Before Starting:

- You have Illumina-sequenced paired-end fastq files
- Make sure files are demultiplexed
- Barcodes and adaptors removed
- Reliable-fast internet connection – downloading the fastq files can take a long time depending on number of samples. Many of the steps in R take a long time to run.

Instructions:

1. Install “dada2” package

devtools::install_github("benjjneb/dada2", ref="v1.16")

*** There are other methods of installation if this does not work for you see instructions on https://benjjneb.github.io/dada2/dada-installation.html ***

2. Bring data into R – DADA2 Pipeline Tutorial suggests naming the data "path"

```
path <- "C:/~YOUR_DATA"
```

3. Separate forward and reverse reads

Forward and reverse fastq filenames have format: SAMPLENAME_R1_001.fastq and SAMPLENAME_R2_001.fastq

```
fnFs <- sort(list.files(path, pattern="_R1_001.fastq", full.names = TRUE))
fnRs <- sort(list.files(path, pattern="_R2_001.fastq", full.names = TRUE))
```

4. Extract sample names, assuming filenames have format: SAMPLENAME_XXX.fastq

```
sample.names <- sapply(strsplit(basename(fnFs), "_"), `[`, 1)
```

5. Plot and analyze the quality of the reads:

```
plotQualityProfile(fnFs[1:2])
plotQualityProfile(fnRs[1:2])
```

6. Create new names and file locations for filtered reads

```
filtFs <- file.path(path, "filtered", paste0(sample.names, "_F_filt.fastq.gz"))
filtRs <- file.path(path, "filtered", paste0(sample.names, "_R_filt.fastq.gz"))
names(filtFs) <- sample.names
names(filtRs) <- sample.names
```

7. Filter and trim the reads

```
out <- filterAndTrim(fnFs, filtFs, fnRs, filtRs, truncLen=c(300,300),
                     maxN=0, maxEE=c(2,5), truncQ=2, rm.phix=TRUE,
                     compress=TRUE, multithread=FALSE, matchIDs = TRUE)
```

*** These are the parameters used to filter and trim the data I used for a specific project – all parameters could be altered depending on your data ***

*** The original DADA2 Pipeline Tutorial would not filter and trim my data until the matchIDs command was added – this may or may not be necessary with your data ***

8. Merge your forward and reverse reads

   mergers <- mergePairs(dadaFs, filtFs, dadaRs, filtRs, verbose=TRUE)

9. Make a Sequence Table

   seqtab <- makeSequenceTable(mergers)

10. Export the ASV File

    write.csv(seqtab,"C:~YOUR_FILE_PATH")

    *** You can also create an ASV file using only forward reads by following these steps after filtering and trimming: ***

    dadaFs <- dada(filtFs, err=errF, multithread=TRUE)

    seqtab <- makeSequenceTable(dadaFs)

    write.csv(seqtab,"C:~YOUR_FILE_PATH_FORWARD_READS")

11. Your ASV file can be opened in Excel or any other similar program. The ASV file will give a DNA sequence by sample name matrix. Any value other than 0 for a DNA sequence indicates it is present within the corresponding sample.
12. DNA sequences can then be run through the desired database to determine species identity.

9.2 Correlation Matrix

	AVG_	AVG_PRE	AVG_TEN	PREC	TEMP	C_TOT	N_TOT	S_TOT	OC	Al	Ca	Fe	K	Mg	Mn	Na	P	Si	Sr	Ti	Zr	pH_CaCl	pH_H2O	EC	SAND	SILT	CLAY	BULK_DE	AVG_WII	AVG_CAN
AVG_ELE	1	0	-0.6	-0.6	-0.6	0	-0.3	-0.2	0	0.3	-0.5	0.1	0.6	0.1	-0.2	0.2	0.5	-0.3	0.8	-0.5	-0.8	-0.1	-0.1	-0.5	0.3	-0.7	-0.3	0.5	-0.6	-0.9
AVG_PRE	0	1	-0.4	0.6	-0.4	1	0.8	-0.8	1	0.7	-0.5	0.9	0.4	0.4	-0.3	0.3	0.5	-0.7	0.2	0.5	-0.2	-0.9	-0.9	-0.5	-0.8	0.7	0.3	-0.5	0.1	-0.1
AVG_TEN	-0.6	-0.4	1	-0.2	1	-0.4	0.1	0.6	-0.4	-0.1	0.7	-0.3	-0.2	-0.3	0.6	-0.6	-0.3	0.5	-0.4	-0.1	0.4	0.7	0.7	0.7	-0.1	0.1	0.5	-0.3	0.2	0.3
PREC	-0.6	0.6	-0.2	1	-0.2	0.6	0.4	-0.6	0.6	-0.1	-0.3	0.3	-0.2	0	-0.5	0.1	-0.3	-0.1	-0.6	0.9	0.6	-0.7	-0.7	-0.3	-0.4	0.9	-0.1	-0.3	0.3	0.7
TEMP	-0.6	-0.4	1	-0.2	1	-0.4	0.1	0.6	-0.4	-0.1	0.7	-0.3	-0.2	-0.3	0.6	-0.6	-0.3	0.5	-0.4	-0.1	0.4	0.7	0.7	0.7	-0.1	0.1	0.5	-0.3	0.2	0.3
C_TOT	0	1	-0.4	0.6	-0.4	1	0.8	-0.8	1	0.7	-0.5	0.9	0.4	0.4	-0.3	0.3	0.5	-0.7	0.2	0.5	-0.2	-0.9	-0.9	-0.5	-0.8	0.7	0.3	-0.5	0.1	-0.1
N_TOT	-0.3	0.8	0.1	0.4	0.1	0.8	1	-0.3	0.8	0.8	0.1	0.9	0.1	0.6	0.3	0.3	0.6	-0.7	0.2	0.2	-0.2	-0.5	-0.5	0.1	-1	0.7	0.8	-0.9	0.5	0
S_TOT	-0.2	-0.8	0.6	-0.6	0.6	-0.8	-0.3	1	-0.8	-0.3	0.9	-0.5	-0.6	0.1	0.8	0	-0.1	0.3	0	-0.7	0	0.9	0.9	0.9	0.3	-0.5	0.3	-0.1	0.4	0.1
OC	0	1	-0.4	0.6	-0.4	1	0.8	-0.8	1	0.7	-0.5	0.9	0.4	0.4	-0.3	0.3	0.5	-0.7	0.2	0.5	-0.2	-0.9	-0.9	-0.5	-0.8	0.7	0.3	-0.5	0.1	-0.1
Al	0.3	0.7	-0.1	-0.1	-0.1	0.7	0.8	-0.3	0.7	1	-0.1	0.9	0.5	0.6	0.3	0.3	0.9	-0.8	0.7	-0.2	-0.7	-0.4	-0.4	-0.1	-0.8	0.2	0.7	-0.6	0.1	-0.6
Ca	-0.5	-0.5	0.7	-0.3	0.7	-0.5	0.1	0.9	-0.5	-0.1	1	-0.2	-0.7	0.3	0.9	0.1	0	0.1	-0.1	-0.5	0.1	0.7	0.7	1	-0.1	-0.1	0.6	-0.5	0.7	0.3
Fe	0.1	0.9	-0.3	0.3	-0.3	0.9	0.9	-0.5	0.9	0.9	-0.2	1	0.3	0.7	0.1	0.5	0.8	-0.9	0.5	0.1	-0.5	-0.7	-0.7	-0.2	-0.9	0.5	0.6	-0.7	0.3	-0.3
K	0.6	0.4	-0.2	-0.2	-0.2	0.4	0.1	-0.6	0.4	0.5	-0.7	0.3	1	-0.3	-0.4	-0.4	0.3	-0.1	0.4	0.1	-0.4	-0.3	-0.3	-0.7	-0.1	-0.1	-0.1	0.3	-0.8	-0.7
Mg	0.1	0.4	-0.3	0	-0.3	0.4	0.6	0.1	0.4	0.6	0.3	0.7	-0.3	1	0.5	0.9	0.8	-0.9	0.6	-0.4	-0.6	-0.3	-0.3	0.3	-0.6	0.1	0.6	-0.7	0.7	-0.2
Mn	-0.2	-0.3	0.6	-0.5	0.6	-0.3	0.3	0.8	-0.3	0.3	0.9	0.1	-0.4	0.5	1	0.2	0.4	-0.2	0.3	-0.7	-0.3	0.6	0.6	0.9	-0.3	-0.2	0.8	-0.6	0.6	-0.1
Na	0.2	0.3	-0.6	0.1	-0.6	0.3	0.3	0	0.3	0.3	0.1	0.5	-0.4	0.9	0.2	1	0.6	-0.8	0.5	-0.3	-0.5	-0.4	-0.4	0.1	-0.3	0	0.2	-0.4	0.6	-0.1
P	0.5	0.5	-0.3	-0.3	-0.3	0.5	0.6	-0.1	0.5	0.9	0	0.8	0.3	0.8	0.4	0.6	1	-0.9	0.9	-0.5	-0.9	-0.3	-0.3	0	-0.6	-0.1	0.6	-0.5	0.2	-0.7
Si	-0.3	-0.7	0.5	-0.1	0.5	-0.7	-0.7	0.3	-0.7	-0.8	0.1	-0.9	-0.1	-0.9	-0.2	-0.8	-0.9	1	-0.7	0.2	0.7	0.6	0.6	0.1	0.7	-0.2	-0.5	0.6	-0.4	0.4
Sr	0.8	0.2	-0.4	-0.6	-0.4	0.2	0.2	0	0.2	0.7	-0.1	0.5	0.4	0.6	0.3	0.5	0.9	-0.7	1	-0.7	-1	-0.1	-0.1	-0.1	-0.2	-0.5	0.3	-0.1	-0.1	-0.9
Ti	-0.5	0.5	-0.1	0.9	-0.1	0.5	0.2	-0.7	0.5	-0.2	-0.5	0.1	0.1	-0.4	-0.7	-0.3	-0.5	0.2	-0.7	1	0.7	-0.6	-0.6	-0.5	-0.2	0.8	-0.3	0	-0.1	0.6
Zr	-0.8	-0.2	0.4	0.6	0.4	-0.2	-0.2	0	-0.2	-0.7	0.1	-0.5	-0.4	-0.6	-0.3	-0.5	-0.9	0.7	-1	0.7	1	0.1	0.1	0.1	0.2	0.5	-0.3	0.1	0.1	0.9
pH_CaCl	-0.1	-0.9	0.7	-0.7	0.7	-0.9	-0.5	0.9	-0.9	-0.4	0.7	-0.7	-0.3	-0.3	0.6	-0.4	-0.3	0.6	-0.1	-0.6	0.1	1	1	0.7	0.5	-0.6	0.1	0.2	0	0
pH_H2O	-0.1	-0.9	0.7	-0.7	0.7	-0.9	-0.5	0.9	-0.9	-0.4	0.7	-0.7	-0.3	-0.3	0.6	-0.4	-0.3	0.6	-0.1	-0.6	0.1	1	1	0.7	0.5	-0.6	0.1	0.2	0	0
EC	-0.5	-0.5	0.7	-0.3	0.7	-0.5	0.1	0.9	-0.5	-0.1	1	-0.2	-0.7	0.3	0.9	0.1	0	0.1	-0.1	-0.5	0.1	0.7	0.7	1	-0.1	-0.1	0.6	-0.5	0.7	0.3
SAND	0.3	-0.8	-0.1	-0.4	-0.1	-0.8	-1	0.3	-0.8	-0.8	-0.1	-0.9	-0.1	-0.6	-0.3	-0.3	-0.6	0.7	-0.2	-0.2	0.2	0.5	0.5	-0.1	1	-0.7	-0.8	0.9	-0.5	0
SILT	-0.7	0.7	0.1	0.9	0.1	0.7	0.7	-0.5	0.7	0.2	-0.1	0.5	-0.1	0.1	-0.2	0	-0.1	-0.2	-0.5	0.8	0.5	-0.6	-0.6	-0.1	-0.7	1	0.3	-0.6	0.4	0.6
CLAY	-0.3	0.3	0.5	-0.1	0.5	0.3	0.8	0.3	0.3	0.7	0.6	0.6	-0.1	0.6	0.8	0.2	0.6	-0.5	0.3	-0.3	-0.3	0.1	0.1	0.6	-0.8	0.3	1	-0.9	0.6	-0.1
BULK_DE	0.5	-0.5	-0.3	-0.3	-0.3	-0.5	-0.9	-0.1	-0.5	-0.6	-0.5	-0.7	0.3	-0.7	-0.6	-0.4	-0.5	0.6	-0.1	0	0.1	0.2	0.2	-0.5	0.9	-0.6	-0.9	1	-0.8	-0.2
AVG_WII	-0.6	0.1	0.2	0.3	0.2	0.1	0.5	0.4	0.1	0.1	0.7	0.3	-0.8	0.7	0.6	0.6	0.2	-0.4	-0.1	-0.1	0.1	0	0	0.7	-0.5	0.4	0.6	-0.8	1	0.5
AVG_CAN	-0.9	-0.1	0.3	0.7	0.3	-0.1	0	0.1	-0.1	-0.6	0.3	-0.3	-0.7	-0.2	-0.1	-0.1	-0.7	0.4	-0.9	0.6	0.9	0	0	0.3	0	0.6	-0.1	-0.2	0.5	1

Also viewable through this link:
https://docs.google.com/spreadsheets/d/1KV69g9nDMs9ThgyR9AvWwxw5Ca9sNB8BiqDopFt9WI4/edit?usp=sharing

9.3 *Raw Study Data*

Viewable through this link:

https://docs.google.com/spreadsheets/d/1tTwYGOKLUGbXRQB9FlSrewmLuk9Pga-h/edit?usp=sharing&ouid=110569072861672657729&rtpof=true&sd=true