



MONTCLAIR STATE
UNIVERSITY

Montclair State University
**Montclair State University Digital
Commons**

Theses, Dissertations and Culminating Projects

5-2014

The Relationship Between Ectomycorrhizae and Metal Contamination in an Urban Brownfield

Jessica Marta Evans

Follow this and additional works at: <https://digitalcommons.montclair.edu/etd>



Part of the **Biology Commons**

Abstract

This study examined ectomycorrhizal and plant relationships in contaminated soil *in situ* to determine the interactions between these three factors. Ectomycorrhizal fungi (EMF) were identified by physical morphotyping followed by sequencing of ribosomal DNA. Plant productivity was assessed through Leaf Area Index (LAI) measurements taken from May through August of 2012 and 2013. Changes in EMF community composition and plant productivity were observed based on their position on the metal contamination gradient. While EMF composition changed depending on the level of metal contamination, none of the ectomycorrhizal species consistently outcompeted other species in the highly contaminated environments. *Cenococcum geophilum* was the dominant species in the low contaminated environments. Higher LAI values are seen in environments with higher soil metal loads, however, this could be due to multiple factors such as increased moisture and the dominance of metal-tolerant tree species. The results here highlight the importance of looking at multiple variables to determine the factors that have the greatest relevance in a natural setting.

MONTCLAIR STATE UNIVERSITY

The Relationship Between Ectomycorrhizae and Metal

Contamination in an Urban Brownfield

by

Jessica Evans

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

May 2014

College of Science and Mathematics

Department of Biology

Thesis Committee:

[REDACTED]

Thesis Sponsor Jennifer Adams Krumins

[REDACTED]

Committee Member Dirk Vanderklein

[REDACTED]

Committee Member Greg Pope

[REDACTED]

Committee Member Frank Gallagher

THE RELATIONSHIP BETWEEN ECTOMYCORRHIZAE AND METAL
CONTAMINATION IN AN URBAN BROWNFIELD

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

by

JESSICA MARTA EVANS

Montclair State University

Montclair, NJ

2014

Table of Contents

Abstract	1
Thesis Signature Page	2
Title Page	3
Acknowledgements	5
List of Figures	6
List of Tables	7
List of Photographs	8
Thesis Text	9
Bibliography	29
Figures	33
Tables	36
Photographs	38

Acknowledgements

I would like to thank the funding sources that made this research possible: the National Science Foundation GK-12 fellowship award #0638708, the Margaret and Herman Sokol Fellowship and the NJ Department of Environmental Protection. I would like to thank my advisor Jennifer Krumins and committee members, Frank Gallagher, Dirk Vanderklein and Greg Pope for their tremendous amounts of time, advice and support. I thank Adam Parker, Fatimah Hassan and Lina Halawani for all of their work assisting in the sequencing process. I thank Liljana Kushi for all of her help in the field. Both of my mothers, my fellow lab mates and friends have all provided so much support and feedback that I am very grateful for. I would not have been able to do this without all of you.

List of Figures

Figure 1. The relationship between variance explained by the first two factor scores of the PCA in 2012 (a) and 2013 (b). The factor score from each site is the mean score across the five pins at that site. For each site error bars indicate standard deviation. (MANOVA site effect: $F = 2.43$, $P < 0.03$ and $n = 4$ (a); $F = 4.13$, $P < 0.001$ and $n = 5$ (b)).

Figure 2. The relationship between LAI and Factor 1 of the PCA in 2012 (a) and 2013 (b). The factor score from each site is the mean score across the five pins at that site. For each site error bars indicate standard deviation. (MANOVA LAI effect: $F = 2.43$, $p < 0.03$ and $n = 4$ (a); $F = 4.13$, $p < 0.01$ and $n = 5$ (b)).

Figure 3. A map of the soil metal contamination levels across the interior section of the park (adapted from Gallagher et al. 2008 (1)). The figure was amended to include a diagram of the site locations used in the present study.

Figure 4. Visual representation and comparison of the spread of ectomycorrhizal species across all five sites in 2013: L1 (a), L2 (b), H1 (c), H2 (d) and H3 (e). Percentages shown are calculated from the relative abundance.

Figure 5. A comparison of the results of the Shannon Index for 2012 (a) and 2013 (b). The graphs show the trend across sites. For each site error bars extend from the 75th percentile to the maximum value (upper) and from the 25th percentile to the minimum value (lower).

List of Tables

Table 1. Description of the characteristics of each site, including Leaf Area Index, Shannon Index, Total Metal Load and the concentrations of copper, zinc, arsenic, chromium and lead at each site.

Table 2. Listing of each species that was identified through sequencing, the sites where it was known to be present and the percent accuracy with which it was identified.

List of Photographs

Photograph 1. Taken in 1975, this photograph illustrates the condition of the area before conversion into a park.

Photograph 2. Taken in 2011, this photograph illustrates the present condition of the area.

Introduction:

Ectomycorrhizal fungi (EMF) grow in association with plant roots and form a mutualistic relationship that assists in nutrient acquisition and tolerance to stressful environments (2-4). How much of that assistance is due to the diversity of EMF communities and how much is due to the community composition remains to be determined. If we know which species of EMF are more beneficial to plant tolerance of stressful environments, we can design remediation plans around that information. Improved remediation plans are increasingly important for the growing number of urban brownfields, sites that were previously used for industrial purposes but now lie abandoned and harbor some type of environmental contamination.

Urban brownfields provide a unique place to study EMF and plant relationships because of the stresses placed on both plants and mycorrhizae. Heavy metal contamination, acid rain (5-7), habitat fragmentation (8) and enhanced nitrogen levels (9, 10) all contribute to the stress urban environments place on plants and their mutualistic relationships. EMF are additionally influenced by the reduction in numbers of host plants and the introduction of foreign invertebrates (11, 12). These aspects of contamination, as well as isolation from other natural ecosystems, make urban brownfields unique. In order to re-establish ecosystem function, more research needs to be conducted on the mechanisms which assist the growth and trajectories of plant assemblages in the urban context.

Mycorrhizae have an evident positive effect on plant productivity (13-15), but we know less concerning the extent to which the diversity of mycorrhizal communities contributes to increases in plant productivity (16). There is a strong positive effect of

mycorrhizae in nutrient poor environments where they are necessary in providing limiting nutrients and resistance against disease and drought (15). An increase in phosphorous (P) uptake from mycorrhizae (13, 17) and mycorrhizal release of enzymes that degrade organic matter (15) have been shown to cause an increase in plant productivity. But there are conflicting results as to how much, if any, of this increase is due to diversity. Baxter and Dighton (16) found that mycorrhizal plants were able to take up higher amounts of P under higher mycorrhizal diversity but not when presented with single mycorrhizal species. This suggests that diversity of EMF communities is an important factor to consider in remediation. However, other studies determined that the apparent effect of diversity was actually a sampling effect and that the inclusion of specific species better explained the increase in productivity (18, 19). This indicates that in some settings species composition, and the inclusion of specific species, will have a greater effect on plant productivity. Another factor to consider in the mycorrhizal plant relationship is the distribution of nutrients throughout the soil. Initial nutrient levels may play a role in determining the strength of mycorrhizal associations that will form (16). Studying the relationship in an fluid setting, such as an urban brownfield, where nutrient levels fluctuate and are not homogenous, may provide greater insight to the relationship between diversity and productivity.

Urban brownfields frequently contain metal contamination in soil where it can have a negative impact on mycorrhizal communities and their relationships with host plants (1, 20-22). Ectomycorrhizal species vary in their tolerance of metal contamination, and shifts in community composition will favor species that are more tolerant of contamination (22-24), resulting in a loss of species richness. Several studies

show that there is a decrease in mycorrhizal species richness under high metal loads (11, 21, 25). Additional studies in this area are useful in identifying metal tolerant species, and under what levels of contamination they are able to thrive (5, 20, 26). Furthermore, as many mycorrhizal species are able to reduce metal uptake by host plants, a loss in species richness could decrease this assistance (5). Many mycorrhizae are host-specific, and if those mycorrhizal species are not able to tolerate high metal loads, their associated plant host may not be able to persist without that relationship. This will have an effect on the plant community composition in that environment (27-29). If the composition of EMF communities does have a significant impact on plant productivity, then productivity may be affected as well.

Liberty State Park, located in Jersey City, NJ, is an ideal location to study mycorrhizal and plant relationships in an urban brownfield because it offers a gradient of heavy metal contamination along which changes in plant and EMF community compositions can be seen. This site is unique in that the soil was derived mainly from fill material and was given its own classification, the Ladyliberty series, by the Natural Resource Conservation Service (30). The soil contains high concentrations of copper, zinc, chromium and lead, among other pollutants, as described in Table 1. Despite harboring such toxic materials, a thriving plant community was able to develop on the brownfield site since its purchase by the state in the early 1970's (See Photographs 1 and 2).

This study focused on characterizing the EMF community in association with plant productivity (as measured by Leaf Area Index, LAI) across the contamination gradient. In this environment I have tested the hypothesis that metal contamination in

soil will affect EMF diversity and community composition of bulk soil. I characterized the EMF community composition to identify differences between sites and trends along the metal contamination gradient. I hypothesized that since not all mycorrhizal species are metal tolerant, there would be a negative correlation between diversity and soil metal contamination (11, 21, 25). And finally, I expected that if there were differences in diversity, that the level of diversity would be positively correlated with plant productivity.

Methods:

Study Site

Liberty State Park is located adjacent to the Upper New York Bay in Jersey City, NJ. The area surrounding the park is a densely populated urban environment. Much of the soil in the park is outside fill material made up of debris that was dumped there from New York City. From 1919 to 1967 the area was used as a railroad terminal connecting lines from across New Jersey to the New York harbor and allowing commuters access to ferries for transport to New York City (1). The site was abandoned from 1967 to 1970, at which time the area was deeded to the State of New Jersey for use as a state park. However, there remains approximately 102 hectare within the interior section of the park that remains unmitigated and therefore undeveloped.

Contamination in the soil includes As, Cr, Cu, Hg, Pb, V, Zn and characterized organic pollutants from previous use as a railroad, as described in Table 1. See also (1). The park was colonized by early succession species *Populus tremuloides*, *Betula populifolia*, *Rhus Copilinum* and *Rhus glabra*. The study sites are mainly dominated by *B.*

populifolia. I chose five sites within the interior of the park to be examined because their level of metal contamination lies along a continuum from highly contaminated to relatively less contaminated as determined by Gallagher et al. (1). This difference in metal contamination will allow me to test biotic site characteristics as they relate to the gradient in metal contamination. Here I specifically focus on the response of the ectomycorrhizal community in the bulk soil to metal contamination.

Experimental Design

Four sites at Liberty State Park were chosen to be examined in 2012 and 2013 based on their position along a metal contamination gradient. The sites were grouped into categories of low metal or high metal contamination. Because the differences in EMF community composition and LAI varied so greatly between the two high soil metal load sites in 2012 (See Figures 1a and 2a), I added a fifth site in 2013 (site 25) to better resolve trends at sites with high soil metal contamination. Liberty State Park recognizes the sites as labeled 48, 43, 14/16, 14 and 25. For simplicity, in this study they will be referred to as L1 (48), L2 (43), H1 (14/16), H2 (14) and H3 (25). Sites L1 and L2 have low levels of metal contamination, while sites H1, H2 and H3 have high levels of contamination (See Figure 3). Contamination level was determined by total metal load (TML) as described in Table 1 (1).

The ectomycorrhizal and plant communities were characterized to identify possible relationships between their composition and soil metal load (1). I chose to examine solely ectomycorrhizae because the dominant species at the park form ectomycorrhizal relationships and these were the species for which I measured LAI. To assess the

relationship between EMF and plant productivity I measured mycorrhizal diversity (with morphotyping followed by sequencing) and canopy cover (Leaf Area Index (LAI)). The established total soil metal load index (I) was used as the context for the study. The total soil metal load for each site was developed by performing a rank order transformation on the individual soil metal species concentration as described by Juang et al. (31). The results were then back-transformed using the reverse function of the linear regression, between the original metal data and the ranks (32). The summation of these products produced a total soil metal load ranking (TML), which scaled between 0 and 5. The previous work defined 3 as a critical threshold beyond which productivity, diversity (I , 33) and seed viability (34) were significantly impacted. I present my results for 2012 and 2013 separately, with each year measured independently. Sampling was conducted in replicates of five at each site, where each replicate is marked by a pin. The pins are four meters apart and placed along a straight 16 m transect. All of the data are presented as the mean and standard error of the five pins ($n=5$).

Plant Productivity Measurements

I evaluated plant productivity by taking measurements of overall leaf area coverage in the tree canopy, LAI. This was measured as the meters² of leaf area: meters² of ground area. Measurements were taken using a LI-COR LAI-2200 (Lincoln, NE) once weekly from May through August of 2012 and 2013. This instrument calculates the amount of sunlight coming through the canopy in order to determine the LAI. Five data points were measured and averaged to one value at each of the four sites (five sites in 2013). The data points where the readings were taken aligned with the five pins where soil was sampled for mycorrhizal analysis (see section Mycorrhizae Sampling and Morphotyping).

Readings were collected between 6:30am and 8:30am and included a 'blanking' in a nearby location that did not have any obstruction of the sky for 70 meters in any direction. This allowed the instrument to calculate the amount of sunlight with no leaf cover present to use as a reference.

Mycorrhizae Sampling and Morphotyping

Bulk soil cores were taken at each pin for a total of 5 cores at each site. The cores were 20-25 cm long and 5 cm in diameter. The samples were not separated into soil horizons. Samples were placed in separate Ziploc bags and immediately transferred to a 4°C refrigerator where they were stored prior to analysis. Sampling was conducted once in early June 2012 and once in early July 2013.

Roots were manually separated from each soil sample and gently rinsed with warm water to remove large pieces of soil and debris. They were then placed on a gridded Petri dish for examination. Ectomycorrhizae were characterized following the standards set forth by Agerer (35). Ramification pattern, shape of the unramified end, mantle texture, color, luster and presence of emanating hyphae and/or rhizomorphs were all used to identify tips. Tips that shared the same characteristics were assumed to be the same morphotype. I separated the tips into their respective morphotypes in order to calculate the relative abundance of each tip and overall diversity of the mycorrhizal community (21, 35).

DNA Extraction and Amplification

One of the goals of this study was to identify the species of ectomycorrhizae present at each site. Genetic sequencing of each morphotype sample can be matched to voucher specimens in an online database. Following morphotyping, ectomycorrhizal tips of the

same type were clipped from the root and grouped together into one PCR tube and stored at -20°C for future DNA extraction. An effort was made to keep tips divided by site, but in cases where the sample size of tips was very small they were combined in order to have enough sample to complete an extraction.

I extracted DNA from the root tips using the PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA) following manufacturer's procedures. At the last stage of the extraction, in samples with a small number of mycorrhizal tips (less than 5), 30µl ultra-pure water was used to further concentrate any DNA. Extracted DNA was stored at -20°C.

Extracted DNA was amplified by PCR using ITS4 (TCCTCCGCTTATTGATATGC) and ITS-1F (CTTGGTCATTTAGAGGAAGTAA) primers to target ascomycete and basidiomycete fungal DNA (9, 36, 37). The reactions were run in an Eppendorf Mastercycler Pro thermocycler (Hauppauge, NY) under the following conditions: 94°C initial denaturization for 5 minutes followed by 34 cycles of 30 seconds at 94°C, 2 minutes at 50°C, 3 minutes at 72°C and a final elongation at 72°C for 5 minutes. The PCR product was then stored at -20°C.

Cloning and Sequencing

It is likely that the PCR products contain DNA from more than one type of mycorrhizae due to cross-contamination during physical identification and collection of the tips. In order to obtain pure uncontaminated DNA of just one mycorrhizal species for sequencing, I performed a cloning procedure on the PCR products. PCR products were first ligated and then transformed using the Promega pGEM®-T Vector System (Promega, Madison, WI). The manufacturer's recommended protocol was followed (36).

Two successfully cloned colonies from each sample were chosen at random to be sequenced. The cloned and amplified DNA was prepared for sequencing by the removal of excess nucleotides, salts and amino acids by running through a PERFORMA® Spin Column from EdgeBio manufacturers (Gaithersburg, MD). The samples were then sequenced using an Applied Biosystem Sequencer model 3130 Genetic Analyzer (Grand Island, NY). The Applied Biosystem kit Big Dye version 3.1 was used to prepare the samples according to the manufacturer's recommended protocol. I entered the resultant sequences into the National Center for Biotechnology Information (NCBI) BLAST website (38) which compared the sequence to voucher specimens in order to produce a list of matches. Morphotypes which were not able to be sequenced were labeled as Unknown and numbered 1-9 in order to differentiate them.

Data Analysis

I used the relative abundances of each morphotype in a Principle Components Analysis (PCA) to determine the amount of significant variation in ectomycorrhizal community composition between sites. The relative abundance of EMF morphotypes and change in LAI across sites was used to calculate the amount of variation between sites. PCA was followed with an analysis of variance (ANOVA) of individual factor, a multivariate analysis of variance (MANOVA) considering the first three factors as variables to determine significant differences (Wilks' Lambda), and the Bonnferroni test for means separation. All of the statistical tests were carried out in SAS Version 9.1 (SAS Institute, Inc. Cary, NC).

I used the Shannon Index as a means of calculating the ectomycorrhizal diversity level of each site. The Shannon Index was calculated separately for each pin and then the values were averaged together for each site, resulting in one Shannon Index value for each site.

Results:

Mycorrhizae Sampling:

In 2012, 21 species of ectomycorrhizae were characterized by morphotyping. The factor scores which were generated in the Principle Component Analysis were based on the relative abundances of each morphotype. Factor 1 was positively and significantly correlated to the relative abundance of *Tomentella sublilacina* and *Inocybe lacera*, and negatively and significantly correlated with *Cenococcum geophilum*. Factor 2 was positively and significantly correlated to *Russula illota* or *laurocerasi* (could not be distinguished to the species level by sequencing), and negatively and significantly correlated with Unknown 5 and *Tomentella sublilacina*. The MANOVA revealed a significant effect of site on EMF community composition as determined by morphotyping followed by sequence identification (Figure 1a, Wilks' Lambda $F = 2.43$, $P < 0.03$). The ANOVA of Factor 1 found a significant difference in community composition (Figure 1a, $F = 4.16$, $P < 0.03$), and the posthoc test revealed that the EMF communities of the low metal sites were most significantly similar to each other and site H2. The high metal sites were most significantly similar to each other and site L1 (Figure 1a).

In 2013 the sites were again examined and 22 species were identified. In this year Factor 1 was positively and significantly ($p < 0.05$) correlated to the relative abundance of

Tomentella, and *Inocybe lacera*, and negatively and significantly correlated to *Cenococcum geophilum*. Unknown Species 6 and *Scleroderma bovista* are pulling Factor 1, but not at a significant level ($p = 0.07$). Factor 2 was positively and significantly correlated to *Scleroderma bovista*, *Fusarium oxysporum* and Unknown 9, and negatively and significantly correlated to *Tomentella*, *Inocybe lacera* and Unknown 5. The MANOVA revealed a significant effect of site on EMF community composition as determined by morphotyping followed by sequence identification (Figure 1b, Wilks' Lambda $F = 4.13$, $P < 0.001$). The ANOVA conducted on Factor 1 found a significant difference in community composition (Figure 1b, $F = 5.84$, $P < 0.05$), and the posthoc test revealed that the EMF communities of the low metal sites were most significantly similar to each other and those of the high metal sites were most significantly similar to each other (Figure 1b). There was a tight correlation between the low metal sites, while the high metal sites showed a greater degree of variation and separation. There was no significant difference between sites according to Factor 2.

The Shannon Index of diversity was calculated for each pin and tested for significance using an ANOVA on the values of each pin. The ANOVA showed no statistical difference between sites for 2012 ($F = 0.73$, $P > 0.5$) or 2013 ($F = 0.88$, $P > 0.4$). The trend across sites was the same in 2012 and 2013 (See Figure 4).

Sequencing:

Nineteen species from 13 families and 12 orders were identified by cloning and sequencing the ITS region of ribosomal RNA. Table 2 lists each species that was identified and how closely it was matched to a voucher specimen. There was some

overlap with species occurring at multiple sites. *Cenococcum*, *Inocybe* and *Leptodontidium* were the only genera found in all five sites. The genus *Russula* was seen only at the highly contaminated sites, while the genus *Sebacina* was seen only at the low contaminated sites. Figure 5 provides a visual representation of the variation in relative abundance at each of the sites. On average, I identified ten fungal taxa per site. There were 9 morphotypes that could not be identified due to insufficient physical material or undistinguishable sequencing results. Out of the 22 morphotypes sampled in 2013, 13 were able to be identified by sequencing. Only seven of the 21 morphotypes from 2012 were able to be sequenced due to limited sample size.

Several of the species listed in Table 2 were not counted in the relative abundances at each site. These species were not seen as individual morphotypes, but were identified because they grew on or close to a sampled morphotype. If multiple clones from a single morphotype were identified as different species, then the species that was associated with that morphotype most frequently and with the highest match percentage was identified as the species for that morphotype.

Leaf Area Index

I was interested in the relationship between primary productivity of the plant community and the mycorrhizal community composition of the soil. We analyzed the results of our PCA with respect to the LAI of each site by conducting an ANOVA with LAI as the fixed variable and Factor 1 of our PCA as the response variable. In 2012 there was a significant difference in EMF community composition based on the LAI of that site (Figure 2a, $F = 2.43$, $p < 0.03$). Site H1 had the greatest LAI value and the most different

EMF community composition. The posthoc test confirmed that the LAI values for sites L1, H1 and H2 were significantly similar when compared to Factor 1 (Figure 2a). The LAI values in sites L1, L2 and H2 were also significantly similar. This is the same trend that was seen in the analysis of ectomycorrhizal community by site. The two sites with low metal contamination have LAI values falling in the middle of the range, while site H2 has a very low LAI and site H1 has a very high LAI compared to the other sites.

In 2013 there was a significant difference in EMF community composition based on the LAI of that site (Figure 2b, $F = 4.13$, $p < 0.01$). Site H3 had the greatest LAI value and the most different EMF community composition. The posthoc test revealed that the same trend that was seen in Factor 1 of the mycorrhizae analysis by site was also seen in the analysis of LAI; H1, H2 and H3 were all significantly similar to each other. There was overlap in the groupings with the H1 and H2 sites also being similar to L1 and L2. The highest LAI values are associated with negative Factor 1 scores and high metal contamination (Figure 2b). The lower LAI scores are associated with low metal contamination and positive Factor 1 scores. Site H2 falls outside of this pattern with the lowest LAI value and a neutral Factor 1 score.

Discussion:

The aim of this study was to examine ectomycorrhizal community composition and diversity along a metal contamination gradient in order to gain a better understanding of interactions between soil contamination and mycorrhizal communities. This study describes which species are present at each of the sites, and the differences between sites as it relates to their position on the contamination gradient. I found that there was a

significant difference between ectomycorrhizal community composition at different sites (Figure 1). Specifically, the low metal contamination sites were most similar to one another and, likewise, the high metal contamination sites were most similar to one another. This suggests that different ectomycorrhizal community compositions may be developing on soils with different levels of metal contamination. Similar findings have been reported by Chappelka et al. (25). There was a much greater variation between the high metal sites than there was between the low metal sites. This could be due to variances between sites in concentrations of each of the metal contaminants or the variation in other abiotic factors such as organic content, pH or nutrient availability.

Interestingly, although EMF composition was shown to vary along the metal concentration gradient, there was no significant difference in diversity levels and there was a similar level of species richness at each site. The Shannon Index (Figure 4) showed that sites L1 and H3 had the highest diversity levels, site H1 had the lowest level and sites L2 and H2 were just slightly lower than site L1. Although the diversity levels were slightly lower in 2013 than they were in 2012, the trends between the sites remained the same between years. There was no correlation with metal concentration, disproving the original hypothesis that there would be decreased diversity with increasing metal load. This demonstrates that, at least in this setting, composition of the EMF community, rather than diversity, is more strongly correlated with metal contamination.

Most of the sites had relatively few dominant species and many rare species (Figure 5), similar to studies by Baxter et al. (11) and Regvar et al. (22). This is in contrast to findings by Krpata et al. (21) which observed a much higher species richness and degree of evenness across metal contaminated sites in Austria. The lack of similarity

in diversity trends between field studies in metal contaminated environments supports the hypothesis that there is a highly dynamic relationship between soil conditions and biotic organisms. The differences between studies also suggests that these relationships are highly contextualized since there is a high degree of variability in findings. My findings support this argument since there was no correlation between diversity and level of metal contamination. The lack of difference in diversity levels between sites further suggests that the similarities of these sites had a stronger effect than contamination level on diversity. Since the plant communities were similar at each site I speculate that that may have a strong influence. Bruns (39) suggests that factors such as nutrient availability, competition with other soil organisms and disturbance can have large effects on EMF diversity. Further studies which examine a combination of variables such as nutrient levels, competition, plant species composition and contamination both singularly and in the same treatment may help to resolve which factors have the most influence on EMF diversity.

The species composition between sites showed a large degree of variation. Only three species, *Cenococcum*, *Inocybe* and *Leptodontidium*, were found across all of the study sites. Additionally, there was a large variation in the relative abundance of these species depending on the site (Figure 3). This suggests that although a species may be present at any site, there are other factors controlling how dominant that species is in the community. This may be due to competition, metal contamination, interactions with other soil microbes, plant species composition, soil characteristics or, most likely, a combination of these factors. According to Tedersoo et al. (40), in a study on global patterns of fungal diversity, they found that the phylogenetic family of the host plant was

the largest driver of EMF composition. Additional studies characterizing the plant community composition at each of the sites would determine if that pattern is also seen at this location. However, this study shows that even in sites with the same dominant plant species, considerable variation in EMF community composition can occur.

Similar to other studies (21, 41), *Cenococcum geophilum* was the dominant species across several sites. This species is known to be highly adapted to metal contaminated environments (25). Interestingly, in contrast to the aforementioned studies, this study shows that *C. geophilum* was dominant at the two low metal contaminated sites and only one of the high metal contaminated sites, H2 (Table 2). Site H3 was dominated by *Scleroderma bovista* and *Inocybe lacera* while site H1 was mainly dominated by *Russula illota/laurocerasi* and *Inocybe lacera* (Figure 5). This study was not designed to resolve the cause of this variation, however, perhaps it is related to the health of the plant community. The sites where *C. geophilum* was the most abundant also had the lowest LAI (Table 1). In contrast, the sites with the highest LAI had only 1% and 9% abundance *C. geophilum* (Figure 3). While I speculate that *C. geophilum* may have saprotrophic properties, this has not been tested and there may already be evidence to the contrary as shown by Shaw et al. (42). Their study looked at competition between ectomycorrhizae, including *C. geophilum*, and saprotrophic fungi and found that the *C. geophilum* was suppressed by the saprotrophic species. Bahram et al. (43) and Dickie et al. (44) found that *C. geophilum* was able to colonize a large number of substrates including litter, a fermentation layer, coarse woody debris layer and humus layer. While the mechanism behind this flexibility in niche habitation is unclear, perhaps it points to why *C. geophilum* is so ubiquitous. Our understanding of the ecology of many

ectomycorrhizae is very limited, it is possible we are lacking insight into some of their capabilities.

This study demonstrated that the sites with the lowest levels of metal contamination also had the lowest LAI. The highest contaminated sites, H1 and H3, had the highest LAI (Figure 3). This trend is seen not only in the five sites examined in this study, but across the entire interior section of the park (1). As the most metal tolerant species at the park are tree species, they were the first to colonize the highly contaminated areas and probably remain dominant due to arrested succession. Consequently, these areas have a higher LAI. Furthermore, such a relationship could be facilitated by a diverse EMF community. There was a significant effect of EMF community composition based on site. However, it is still unclear whether this is due to the specific EMF communities associated with high LAI, or another factor such as higher moisture or nutrient levels which have also been shown to have a strong impact on plant productivity (45).

I consider that moisture could be a very important factor in the levels of plant productivity and differences in EMF communities at the study sites. Site H2 was observed to have much drier soil than any of the other sites, had the lowest LAI and showed very different trends in EMF community composition than the other high metal sites. This site is situated at a slightly higher elevation and has a berm that cuts across approximately half of the site. This essentially isolates part of the site and impedes runoff and soil movement. The concentration of metals at site H2 is also different from the other sites (See Table 1). These differences may help to explain why the trends seen there are so different than at the other sites, and may also help to study how multiple

variables interact to shape the community. For example, moisture may be having a large impact on plant productivity and EMF community composition due to the types of metals that have collected there. Studies using additional measures of moisture, plant productivity, further characterization of EMF communities and measurement of nutrient levels will help to clarify the interactions between each of the variables. It would be interesting to see if the same trends occur at other locations with a metal concentration gradient.

There were several species that were identified by sequencing but not recognized during physical morphotyping. The tips of one morphotype were collected, but sequencing showed several species, sometimes from different genera and families, all present in the sample of that one morphotype. This indicates that multiple ectomycorrhizal species may be living on the same section of root. This could be a result of the heterogeneity of resources in the soil matrix (16). Multiple species may be converging in areas where there are abundant resources. *Inocybe* and *Tomentella* were frequently seen together in the same morphotype. The difficulty in differentiating the two species suggests that perhaps one species is growing on top of the other. This could be a type of competition, but the cause of competition is unclear. The relationships between individual species deserves further examination.

Conclusion:

Changes in EMF community composition and plant productivity were observed based on their position on the metal contamination gradient. While EMF composition changed depending on the level of metal contamination, richness remained relatively

constant. *Cenococcum geophilum* was the dominant species in the low contaminated environments. The variation in dominant EMF species at high soil metal concentrations supports the idea that EMF species have different tolerance levels to different metals. This is a significant factor to consider when designing restoration plans for brownfield sites. Restoration initiatives that include inoculation of the soil with metal tolerant ectomycorrhizae will be beneficial to the plant communities. Additional studies are needed to further explore the metal tolerance capabilities and niche variation in EMF species.

The methodology used in this study to separate EMF into morphotypes demonstrated the advantages of using physical features to identify mycorrhizae and determine the relative abundance. However, it also showed the limitations of identifying EMF to a species level and even to a genus level through morphotyping alone. The sequencing data showed that two and sometimes three species were identified from samples that were thought to be the same morphotype. This presents a major drawback to using physical morphotyping as a technique, however, it is a necessary practice in order to count EMF to determine relative abundance. For future studies, I would recommend keeping morphotype tips separated as much as possible to be sure that the counts accurately reflect one species or genus. Additionally, this study showed that an increased amount of sequencing would have led to a higher degree of accuracy in discovering which species were present at which sites. The information gained from sequencing in this study provided valuable insight as to the changes in community composition across sites and further research with additional sequencing will greatly enhance this knowledge.

Higher LAI values were seen in more highly metal contaminated environments, however, this could be due to multiple factors such as increased moisture and the dominance of metal-tolerant species in a situation of arrested succession. Future studies examining moisture and plant composition as well as additional EMF surveys would be beneficial in clarifying the trends that were seen in this study. The results here highlight the importance of looking at multiple variables to determine which factors have the greatest impact in a fluid setting.

Bibliography:

1. F. J. Gallagher, I. Pechmann, J. D. Bogden, J. Grabosky, P. Weis, Soil metal concentrations and vegetative assemblage structure in an urban brownfield. *Environmental Pollution* **153**, 351-361 (2008).
2. P.-E. Courty, M. Buée, A. G. Diedhiou, P. Frey-Klett, F. Le Tacon, F. Rineau, M.-P. Turpault, S. Uroz, J. Garbaye, The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. *Soil Biology and Biochemistry* **42**, 679-698 (2010).
3. M. D. Jones, L. A. Phillips, R. Treu, V. Ward, S. M. Berch, Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) stands in central British Columbia. *Applied Soil Ecology* **60**, 29-40 (2012).
4. L. Karliński, M. Rudawska, T. Leski, The influence of host genotype and soil conditions on ectomycorrhizal community of poplar clones. *European Journal of Soil Biology* **58**, 51-58 (2013).
5. K. Bojarczuk, B. Kieliszewska-Rokicka, Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. *Water, air, and soil pollution* **207**, 227-240 (2010).
6. T. Ochimaru, K. Fukuda, Changes in fungal communities in evergreen broad-leaved forests across a gradient of urban to rural areas in Japan This article is one of a selection of papers published in the Special Forum on Towards Sustainable Forestry-The Living Soil: Soil Biodiversity and Ecosystem Function. *Canadian journal of forest research* **37**, 247-258 (2007).
7. A. Schützendübel, A. Polle, Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of experimental botany* **53**, 1351-1365 (2002).
8. K. G. Peay, M. Garbelotto, T. D. Bruns, Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**, 3631-3640 (2010).
9. J. A. Krumins, J. Dighton, D. Gray, R. B. Franklin, P. J. Morin, M. S. Roberts, Soil microbial community response to nitrogen enrichment in two scrub oak forests. *Forest ecology and management* **258**, 1383-1390 (2009).
10. E. A. Lilleskov, T. J. Fahey, T. R. Horton, G. M. Lovett, Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* **83**, 104-115 (2002).
11. J. W. Baxter, S. T. Pickett, M. M. Carreiro, J. Dighton, Ectomycorrhizal diversity and community structure in oak forest stands exposed to contrasting anthropogenic impacts. *Canadian Journal of Botany* **77**, 771-782 (1999).
12. M. Newbound, M. A. McCarthy, T. Lebel, Fungi and the urban environment: A review. *Landscape and urban planning* **96**, 138-145 (2010).
13. I. Jakobsen, L. Abbott, A. Robson, External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. *New Phytologist* **120**, 371-380 (1992).

14. D. J. Read, J. R. Leake, J. Perez-Moreno, Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany* **82**, 1243-1263 (2004).
15. M. G. Van Der Heijden, R. D. Bardgett, N. M. Van Straalen, The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters* **11**, 296-310 (2008).
16. J. W. Baxter, J. Dighton, Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host-symbiont culture conditions. *New Phytologist* **152**, 139-149 (2001).
17. M. G. van der Heijden, J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, I. R. Sanders, Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**, 69-72 (1998).
18. M. G. Van Der Heijden, R. Streitwolf-Engel, R. Riedl, S. Siegrist, A. Neudecker, K. Ineichen, T. Boller, A. Wiemken, I. R. Sanders, The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist* **172**, 739-752 (2006).
19. K. M. Vogelsang, H. L. Reynolds, J. D. Bever, Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist* **172**, 554-562 (2006).
20. K. Hryniewicz, I. Haug, C. Baum, Ectomycorrhizal community structure under willows at former ore mining sites. *European Journal of Soil Biology* **44**, 37-44 (2008).
21. D. Krpata, U. Peintner, I. Langer, W. J. Fitz, P. Schweiger, Ectomycorrhizal communities associated with *Populus tremula* growing on a heavy metal contaminated site. *Mycological research* **112**, 1069-1079 (2008).
22. M. Regvar, M. Likar, A. Piltaver, N. Kugonič, J. E. Smith, Fungal community structure under goat willows (*Salix caprea* L.) growing at metal polluted site: the potential of screening in a model phytostabilisation study. *Plant and soil* **330**, 345-356 (2010).
23. D. Blaudez, C. Jacob, K. Turnau, J. Colpaert, U. Ahonen-Jonnarth, R. Finlay, B. Botton, M. Chalot, Differential responses of ectomycorrhizal fungi to heavy metals *in vitro*. *Mycological Research* **104**, 1366-1371 (2000).
24. J. Hartley, J. W. Cairney, F. E. Sanders, A. A. Meharg, Toxic interactions of metal ions (Cd^{2+} , Pb^{2+} , Zn^{2+} and Sb^{3-}) on *in vitro* biomass production of ectomycorrhizal fungi. *New phytologist* **137**, 551-562 (1997).
25. A. Chappelka, J. Kush, G. Runion, S. Meier, W. Kelley, Effects of soil-applied lead on seedling growth and ectomycorrhizal colonization of loblolly pine. *Environmental pollution* **72**, 307-316 (1991).
26. T. Leski, M. Rudawska, B. Kieliszewska-Rokicka, Intraspecific aluminium response in *Suillus luteus* (L.) sf gray., an ectomycorrhizal symbiont of scots pine. *Acta Societatis Botanicorum Poloniae* **64**, 97-105 (1995).
27. M. Fomina, I. Alexander, J. Colpaert, G. Gadd, Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biology and Biochemistry* **37**, 851-866 (2005).

28. M. Ledin, Accumulation of metals by microorganisms—processes and importance for soil systems. *Earth-science reviews* **51**, 1-31 (2000).
29. M. Melo, N. Flores, S. Murrieta, A. Tovar, A. Zúñiga, O. Hernández, A. Mendoza, N. Pérez, A. Dorantes, Comparative plant growth promoting traits and distribution of rhizobacteria associated with heavy metals in contaminated soils. *International Journal of Environmental Science & Technology* **8**, 807-816 (2011).
30. Soil Survey Staff, National Resources Conservation Service, United States Department of Agriculture. (National Resources Conservation Service, Somerset, NJ, 2013), vol. 2014.
31. K.-W. Juang, D.-Y. Lee, T. R. Ellsworth, Using rank-order geostatistics for spatial interpolation of highly skewed data in a heavy-metal contaminated site. *Journal of Environmental Quality* **30**, 894-903 (2001).
32. J. Wu, W. Norvell, R. Welch, Kriging on highly skewed data for DTPA-extractable soil Zn with auxiliary information for pH and organic carbon. *Geoderma* **134**, 187-199 (2006).
33. F. J. Gallagher, I. Pechmann, J. D. Bogden, J. Grabosky, P. Weis, Soil metal concentrations and productivity of *Betula populifolia* (gray birch) as measured by field spectrometry and incremental annual growth in an abandoned urban Brownfield in New Jersey. *Environmental pollution* **156**, 699-706 (2008).
34. F. J. Gallagher, I. Pechmann, B. Isaacson, J. Grabosky, Morphological Variation in the Seed of Gray Birch (*Betula populifolia*): The Effects of Soil-Metal Contamination. *Urban Habitats* **6**, (2011).
35. R. Agerer, *Colour atlas of ectomycorrhizae*. (Einhorn-Verlag Eduard Dietenberger GmbH, 1997).
36. R. Landeweert, P. Leeftang, T. W. Kuyper, E. Hoffland, A. Rosling, K. Wernars, E. Smit, Molecular identification of ectomycorrhizal mycelium in soil horizons. *Applied and Environmental Microbiology* **69**, 327-333 (2003).
37. C. Murat, A. Vizzini, P. Bonfante, A. Mello, Morphological and molecular typing of the below-ground fungal community in a natural *Tuber magnatum* truffle-ground. *FEMS microbiology letters* **245**, 307-313 (2005).
38. National Center for Biotechnology Information. (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, 2009).
39. T. D. Bruns, *Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi*. (Springer Netherlands, 1995), vol. 63, pp. 63-73.
40. L. Tedersoo, M. Bahram, M. Toots, A. G. Diedhiou, T. W. Henkel, R. Kjølner, M. H. Morris, K. Nara, E. Nouhra, K. G. Peay, Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* **21**, 4160-4170 (2012).
41. C. L. Cripps, in *Proceedings of the 2003 National meeting of the American Society of Mining and Reclamation and the 9th Billings Land Reclamation Symposium*. American Society for Mining and Reclamation, Lexington, USA. (2003).
42. T. Shaw, J. Dighton, F. Sanders, Interactions between ectomycorrhizal and saprotrophic fungi on agar and in association with seedlings of lodgepole pine (*Pinus contorta*). *Mycological Research* **99**, 159-165 (1995).

43. M. Bahram, S. Põlme, U. Kõljalg, L. Tedersoo, A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS microbiology ecology* **75**, 313-320 (2011).
44. I. A. Dickie, B. Xu, R. T. Koide, Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* **156**, 527-535 (2002).
45. H. P. O. Nagel, The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology* **27**, 1191-1191 (2000)10.1071/PP99173_CO).

Figures

Figure 1:

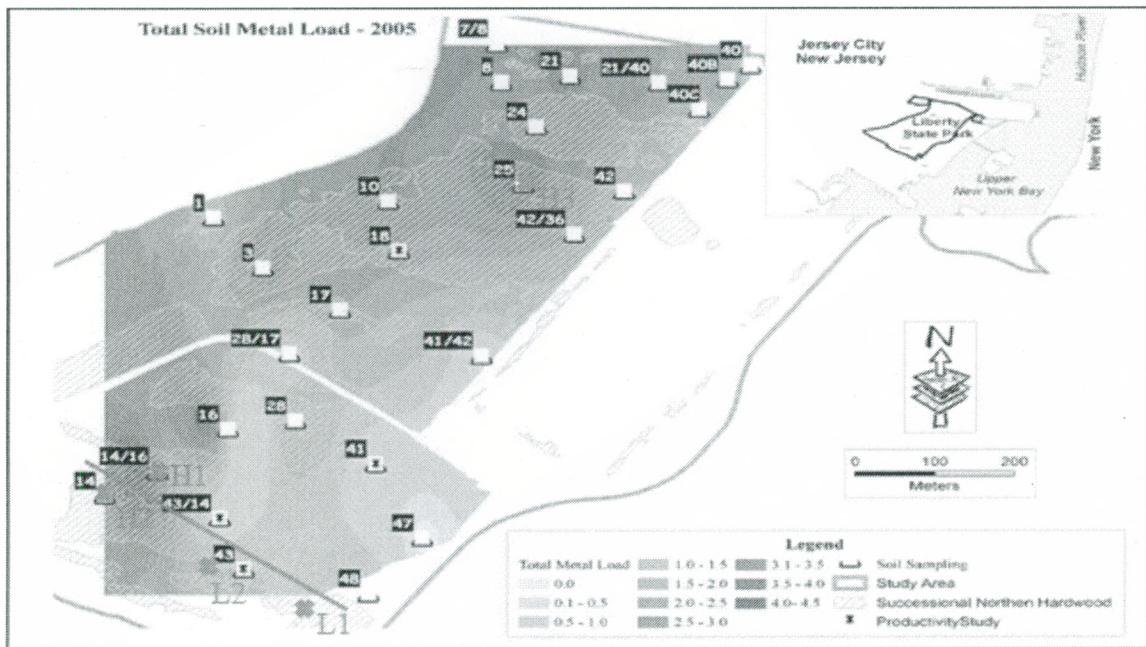


Figure 2:

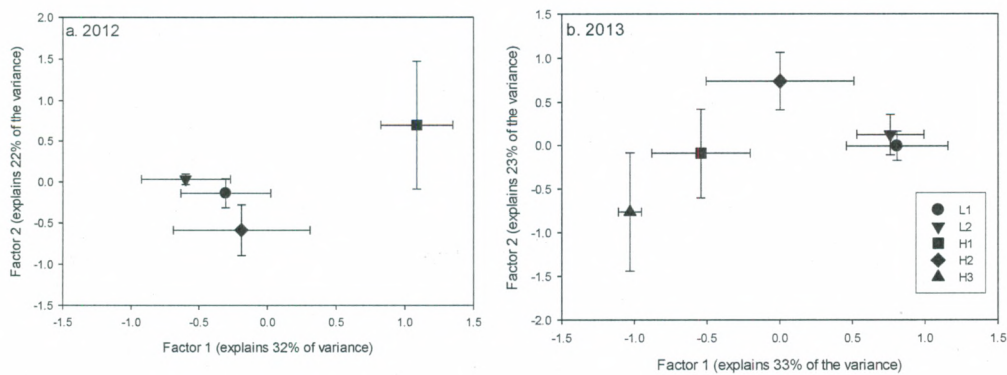


Figure 3:

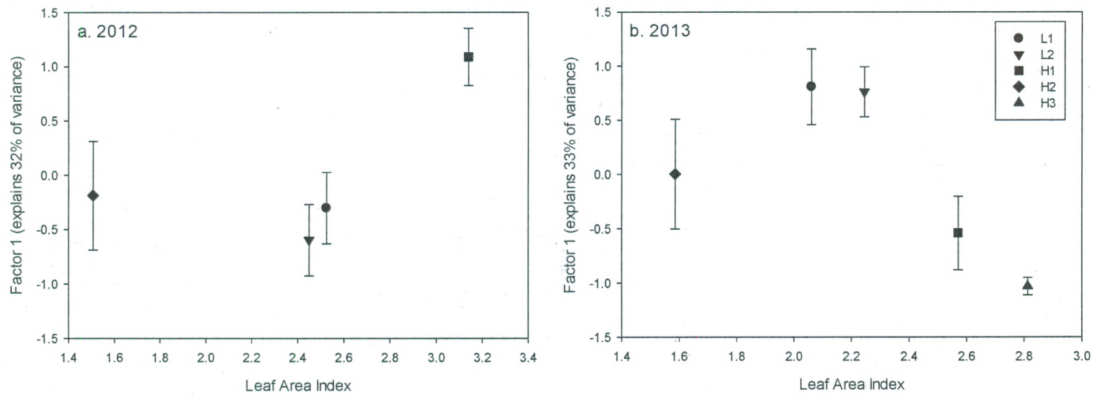


Figure 4:

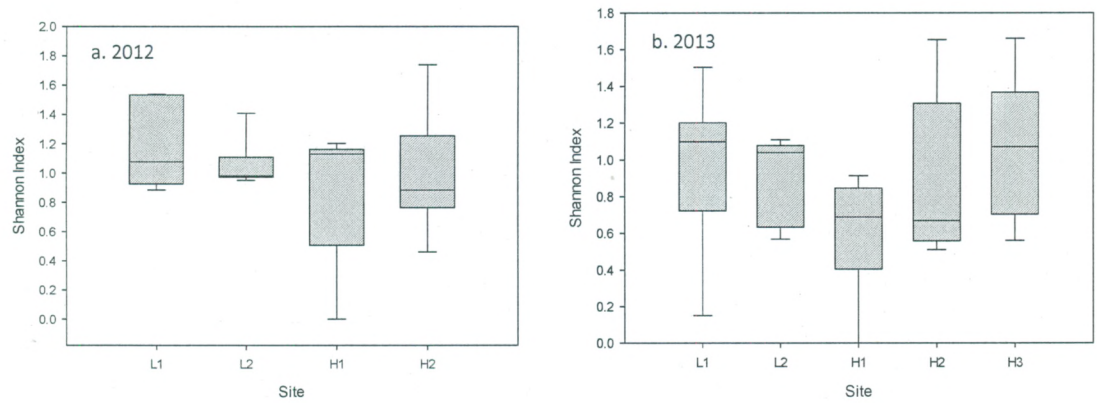
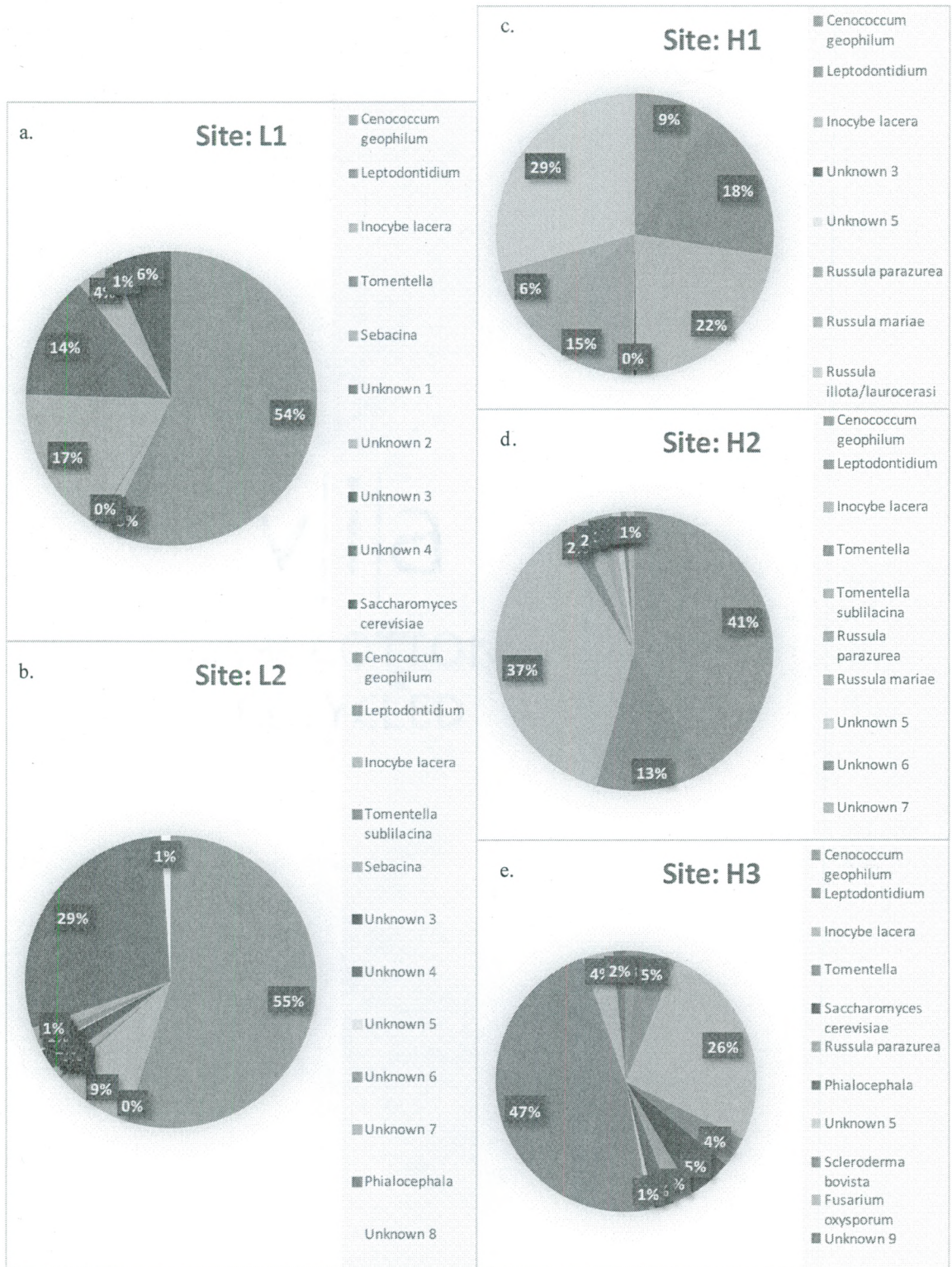


Figure 5:



Tables

Table 1: Site Characteristics

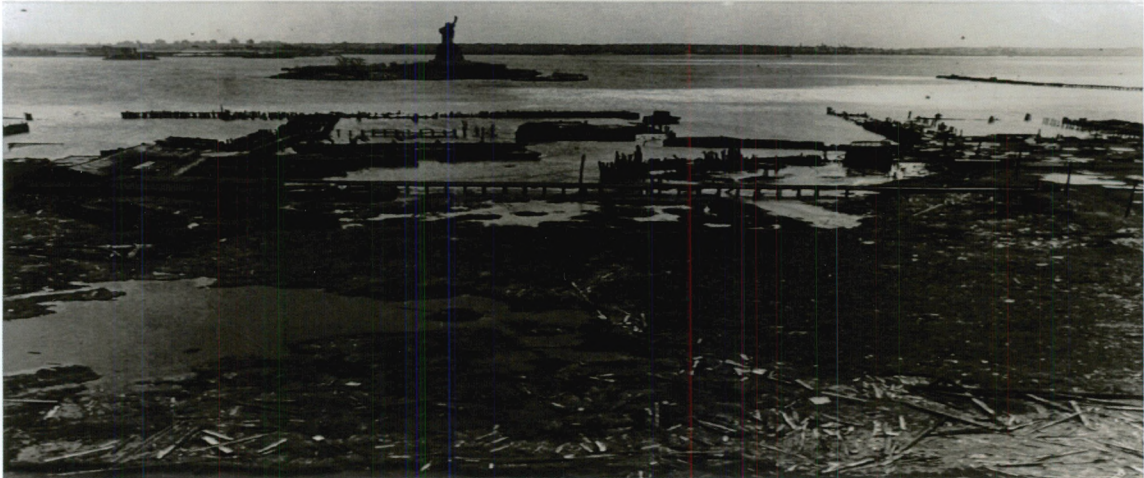
	L1	L2	H1	H2	H3
Avg LAI 2012/2013	2.53/2.06	2.45/2.25	3.14/2.57	1.51/1.59	NA/2.81
◇ Shannon Index 2012/1013	1.19/.953	1.06/.888	0.83/.593	1.01/.92	NA/1.06
*TML	1.56	1.64	3.56	3.08	4.31
*Cu µg g-1	95	230	203	224	2200
*Zn µg g-1	22	89	238	37	2327
*As µg g-1	13	29	68	181	384
*Cr µg g-1	142	19	452	115	66
*Pb µg g-1	245	460	858	926	6673
◇ Describes morphotype diversity by site, as an average of each pin					
*Reproduced from Gallagher et al. 2008					

Table 2: Ectomycorrhizae present by site, as identified by sequencing

Known to be present at sites	Species	Blast Query Cover %	Blast Identity %
L1, L2, H3	<i>Inocybe lacera</i>	97	96
L1	<i>Russula cerolens</i>	97	98
L1	<i>Helotiaceae</i> (family)	94	98
L1	<i>Cenococcum geophilum</i>	98	96
L1	<i>Sebacina</i> sp.	94	97
L2	<i>Rhizoscyphus</i> sp.	96	92
L2, H3	<i>Phialocephala</i> sp.	93	96
H1, H2	<i>Tomentella</i> sp.	97	98
H1, H3	<i>Russula mariae</i>	93	95
H1	<i>Russula parazurea</i>	98	94
H1	<i>Peziza saccardoana</i>	95	94
H1, H3	<i>Leptodontidium</i> sp.	87	99
H2, L2, H1, H3	<i>Helotiales</i> (order)	94	99
H2	<i>Hebeloma mesophaeum</i>	97	99
H3	<i>Cylindrocarpon</i> sp.	90	99
H3	<i>Isaria fumosorosea</i>	97	98
H3	<i>Phialocephala fortinii</i>	90	83
H3	<i>Cadophora</i> sp.	95	97
H3	<i>Cryptococcus terricola</i>	94	99
H3	<i>Meliniomyces</i> sp.	92	97
H3	<i>Sordariomycete</i>	98	97
H3	<i>Scleroderma bovista</i>	98	99
H3	<i>Lecanoromycetidae</i>	95	95
H3	<i>Cylindrocarpon pauciseptatum</i>	96	99
H3	<i>Lactarius glyciosmus</i>	96	98
H3	<i>Fusarium oxysporum</i>	96	99
H3	<i>Saccharomyces cerevisiae</i>	94	99

Photographs

Photograph 1:



Photograph 2:

