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Microbial Community, Functioning, and Its Relationship with Heavy Metals in an Urban Brownfield

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MICROBIAL COMMUNITY, FUNCTIONING, AND ITS RELATIONSHIP WITH HEAVY METALS IN AN URBAN BROWNFIELD

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ABSTRACT

MICROBIAL COMMUNITY, FUNCTIONING, AND ITS RELATIONSHIP WITH HEAVY METALS IN AN URBAN BROWNFIELD

by Jay Prakash Singh

The ubiquity of urban brownfields presents a challenge for environmental managers for managing degraded ecosystems that are in close contact with human habitation. Presence of metal contaminants in brownfields further complicate the matters as it cannot be degraded and hence pose a high risk to human and environmental health, and well-being of the local community. However, previous studies indicate that management and restoration of brownfields are possible through the use of low input longer term and sustainable remediation approaches. These sustainable approaches include the use of plants, fungi, and bacteria to increase soil function and mitigate risks from the contaminants. To improve soil function at brownfields, a thorough understanding of their microbial community composition and their responses to metal contamination is required. This understanding is even more critical in brownfields because each brownfield is different from one another. Brownfields differ not only in contaminants but also soil types and climatic context. Therefore, for any restoration and reclamation efforts in brownfields to materialize, it is essential to study the microbial community composition and understand how they respond to contaminants.

Against this backdrop, this dissertation explores the potential of gentle remediation option by understanding the relationship between the microbial community composition and function at Liberty State Park, a unique urban brownfield. LSP is a unique site because of the abundant growth of under-story and over-story vegetation since its abandonment five decades ago. Since vegetation at this site has flourished well, it provides an opportunity to learn more about this

ecosystem, which is in the process of natural restoration. We characterized the microbial community, analyzed phosphatase activity, and quantified the metal contamination. Further, we examined the relationship soil heavy metal concentrations, microbial community, soil organic carbon content, bacterial density, and extracellular phosphatase activity as a proxy of ecosystem functioning.

We also investigated the relative importance of biotic factors (inoculum) and abiotic factors (soil base) on the extracellular enzymatic activities in a reciprocal microbial inoculation experiment. To this end, we cross-inoculated microbial communities between two heavy metal-contaminated soils, with high and low extracellular enzyme activities, respectively. We measured extracellular phosphatase activity, a proxy for soil function, after self- and cross-inoculation of microbial communities into sterilized soils. We also analyzed the microbial community composition and explored its relationship with phosphatase activity.

Finally, we studied the effect of bioaugmentation in chromium spiked, autoclaved soil on plant productivity and soil enzyme function. We also examined the translocation of metal from the soil system to belowground biomass and aboveground biomass. We also analyzed the phosphatase activity to investigate the relationship between soil function and plant-microbe interaction. The dissertation sheds light on the composition and functioning of urban brownfield soils. A deeper understanding of these unique ecosystems can mediate successful remediation, restoration and urban sustainability.

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DEDICATION

To my beloved parents

1 TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

LSP: Liberty State Park

HMF: Hutcheson Memorial Forest

PA: Phosphatase activity

LOI: Loss on Ignition

PC: Principal coordinates

NMDS: Non-metric dimensional scaling

2 INTRODUCTION

2.1 BACKGROUND

Soil is a natural resource developed over geological time scale via weathering of rocks and accumulation of organic matter (Jenny 1994). It is one of the most critical components of the biosphere (Doran and Zeiss 2000) and is a key regulator of global ecology (Coleman et al. 1992). Soil serves as a medium for plant growth, which not only provides food for living being but is also a key sink for carbon sequestration (Schlesinger and Andrews 2000) thereby regulating global climate (Mosier 1998). Soil ecosystem is either an integral part or essential regulator of all global biogeochemical cycles. Therefore, it directly impacts survival and continued sustenance of life on earth.

Soil ecosystem is a complex network of abiotic and biotic constituents that impacts global ecological processes. These abiotic and biotic interactions are intricate and help maintain biological productivity in soil ecosystems, including plant and microbial productivity. Most biotic-abiotic interactions in soil are bidirectional and are often difficult to delineate the relative importance of each component. However, it is well known that microorganisms, an integral element in biotic constituents in soil, play a crucial role in nutrient cycling and maintenance of soil fertility (Fierer 2017), which impacts the health of plants and animals in terrestrial ecosystems. Soil microorganism (also referred to as the soil microbiome) are highly diverse, and their biomass is often comparable to aboveground biomass of plants and animals (Fierer 2017).

Microbes are broadly classified as prokaryotic and eukaryotic. While bacteria and archaebacteria are the most common taxa from the prokaryotes, fungi and microbial metazoans (Caron et al. 2009, Bik 2019) are the most common eukaryotic microorganisms that constitute the microbiome of the soil. Other than the prokaryotes and eukaryotes, viruses are also an integral

part of the soil microbiome. Studying soil microorganisms have traditionally been difficult, but recent advances in sequencing technology have defied Moore's law that led to an exponential decrease in sequencing costs, subsequently boosting microbiome studies. Cheaper sequencing of the targeted genes and whole genomes have enabled us to peek into the soil microbiome and determine their roles in soil. While high-throughput amplicon sequencing targeting bacteria (16S rRNA gene), fungi (ITS), and other eukaryotes (mitochondrial cytochrome oxidase gene) have greatly expanded our understanding of soil microbial diversity, whole genome sequencing have widened our knowledge of biochemical and metabolic pathways in soil ecosystems. The advances in sequencing technology have improved our understanding of microbial diversity and phylogeny in soil, which in turn have revealed meaningful ecological relationships between microbiome and soil ecosystem.

Soil as an ecosystem is exceptionally heterogeneous (Elkateb et al. 2003) with abiotic parameters wildly fluctuating over short distances. Abiotic factors such as – pH (Rousk et al. 2009), organic carbon concentration (Steenwerth and Belina 2008), salinity (Rietz and Haynes 2003), soil texture (Bach et al. 2010), and available nutrient concentration (Torsvik and Øvreås 2002, Torsvik et al. 2002)- impact the community and diversity of soil microbiome, which subsequently affects how soil functions. There is a vast body of literature exploring the relationships of soil function, abiotic parameters, and soil microbiome that have greatly expanded our understanding of soil functioning (Waldrop et al. 2000, Torsvik and Øvreås 2002, Marschner et al. 2003, Zak et al. 2003, Frey et al. 2004, Berg and Smalla 2009). Soil function encompasses sustained biological actions indicated by enzymatic activity (Caldwell 2005), nutrient turnover (Schloter et al. 2003), microbial mass (Waldrop et al. 2000), decomposition (Schneider et al. 2012), microbial diversity (Griffiths et al. 2000), etc. that impacts plant and

animal health (Karlen et al. 1997). These soil function indicators help us determine the health of the soil, which is particularly useful when various issues plague the soil ecosystem. Soil ecosystems around the world are persistently under stress due to deforestation, climate change, degradation, erosion, land use change, and contamination.

One of the massive concerns in the post-industrial revolution age has been contamination of different ecosystems around the planet, and the soil ecosystem has perhaps borne the maximum brunt of it. Soil contamination makes site useless for any public or private use. Moreover, scant attention is paid to such derelict sites (also called brownfields) because of the high cost of remediation and little return on investment. In Europe, there are more than 800,000 brownfields, and a clean-up of the sites is estimated to cost around 115 billion euros (Megharaj and Naidu 2017). In the United States alone, there are more than 450,000 contaminated sites. Most of the sites are contaminated by a suite of heavy metals that pose risks to human and environmental health, and well-being of the local community.

A growing body of literature indicates that management and restoration of these sites is possible through the use of low input longer term and sustainable remediation approaches (ITRC, 2009). These sustainable approaches include the use of plants, fungi, and bacteria to increase soil function and mitigate risks from the contaminants (Cundy et al. 2016, Megharaj and Naidu 2017). These remediation approaches are termed as gentle remediation options (Cundy et al. 2013, Kidd et al. 2015). These approaches can have broad economic, environmental, and societal benefits when they facilitate the restoration of brownfields. Besides being inexpensive, gentle remediation options can increase green spaces in urban areas. Such green space can sometimes become a hotspot of floral and faunal diversity (Goddard et al. 2010). Against this backdrop, this

dissertation explores the potential of gentle remediation option by understanding the relationship between the microbial community composition and function in a contaminated soil ecosystem.

2.2 STUDY SITE

We chose Liberty State Park (LSP), a densely vegetated urban brownfield in New Jersey, as a study site to investigate the relationship between the microbial community and function. LSP is contaminated with a suite of heavy metals such as vanadium, chromium, arsenic, copper, lead, and zinc (Hagmann et al. 2015, Singh et al. 2019). LSP originally was a low-lying marshland, which was filled with construction debris from New York City to build a railway yard in the early 1900s (Gallagher et al. 2008). The railway yard operation began in the 1920s and continued until the late 1960s. The railway yard was used for storage and transportation of several goods, which led to heavy metal contamination of the site (Gallagher et al. 2008). The site was left abandoned after the railway yard ceased its operations in the 1970s. While a section of the site was remediated by soil capping, approximately 100 hectares was fenced off and restricted for public access (Gallagher et al. 2011). The undisturbed and heavy metal contaminated 100 hectares of land showed signs reclamation and a dense early successional vegetation developed at LSP (Gallagher et al. 2011). The early successional vegetation provides a unique opportunity to study soil function at LSP.

2.3 RESEARCH OBJECTIVES

LSP is a unique site because of the abundant growth of under-story and over-story vegetation since its abandonment five decades ago. Public access to this site has been restricted, which provides a remarkable opportunity to study the soil microbial community and function of a unique ecosystem and its response to high concentrations of several heavy metals without the

influence of human intervention. Since vegetation at this site has flourished well, it provides an opportunity to learn more about this ecosystem, which is in the process of natural restoration.

The overarching goal of the dissertation is to disentangle the relationship between the microbial community and extracellular enzymatic activity in a contaminated ecosystem. The research addresses the following three specific objectives:

- Characterize and investigate how the microbial community composition and enzyme response are impacted by heavy metal contamination at LSP.
- Examine whether soil microbial community or edaphic properties are vital in determining the enzyme response
- Explore the effect of bioaugmentation on enzyme response, plant productivity and phytoextraction

To achieve the objectives, we designed three different experiments. In chapter 2, we characterized the microbial community composition at four different sites within LSP and a reference site Hutcheson Memorial Forest (HMF). We also quantified the metal contaminants at these sites to examine the relationship between the microbial community and metal contamination. To understand how soil function responded under different level of metal contamination, we also analyzed phosphatase activity (a proxy for soil function) and explored their relationship.

In chapter 3, we designed an experiment and used reciprocal microbial inoculation to study the effect of bioaugmentation on soil enzyme function (phosphatase activity). We studied the relative importance of microbial inoculation and soil abiotic factors in determining the soil enzymatic function. To delineate the effect of microbial inoculum and soil abiotic properties on

enzyme function, we sterilized the soil by autoclaving and bioaugmented the soil with microbial inoculants from different sources. We surveyed the microbial community composition and measured enzymatic activity at eight different times.

In chapter 4, we examined the soil-plant-microbe interaction by carrying out a mesocosm study where we bioaugmented autoclaved soil that was spiked with chromium. We examined plant-microbe interaction and its impact on plant productivity. We also tracked the translocation of metal from the soil system to belowground biomass and aboveground biomass. Finally, we analyzed the phosphatase activity to investigate the relationship between soil function and plant-microbe interaction.

With the three experiments, we determined the relationship between the microbial community and enzyme function under different conditions. The first experiment helped us characterize the microbial community and function at the study site. The second experiment provided us a better understanding of the relative importance of biotic and abiotic factors in determining soil enzyme function. With the third experiment, we were able to study the effect of plant-microbe interaction on plant productivity and enzyme function. We also explored how plant-microbe interaction impacted the movement of chromium from soil to aboveground and belowground plant biomass.

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3 SOIL MICROBIAL RESPONSE TO METAL CONTAMINATION IN A VEGETATED AND URBAN BROWNFIELD

3.1 INTRODUCTION

Heavy metal contamination is globally prevalent as a result of industrial activity such as mining, extraction, processing of mineral ores, and its accidental release during storage and transportation (Järup 2003). These industrial activities have resulted in contamination of many terrestrial and aquatic ecosystems with significant implications for human health. In the US, contaminated sites are broadly designated as Superfund or brownfield. Superfund sites are highly contaminated sites where the federal government either is or plans to be involved in cleanup efforts. In contrast, the federal government does not get involved in brownfield sites. State government or local stakeholders are involved voluntarily in revitalizing and restoring brownfields. In the US alone, there are more than 500,000 brownfields (Megharaj and Naidu 2017). These brownfields are real properties which cannot be reused because of the presence of contaminants. Due to the lack of active participation by governments, a significant land area remains derelict or underutilized. Therefore, brownfields pose risks to human and environmental health, and well-being of the local community.

A growing body of literature indicates that management and restoration of these sites is possible through the use of low input longer term and sustainable remediation approaches (ITRC, 2009). These sustainable approaches include the use of plants, fungi, and bacteria to increase soil function and mitigate risks from the contaminants (Cundy et al. 2016, Megharaj and Naidu 2017). These remediation approaches are termed as gentle remediation options (Kidd et al., 2015, Cundy et al. 2013). These approaches can have broad economic, environmental, and societal benefits when they facilitate the restoration of brownfields.

One of the main aims of reclaiming land through gentle remediation options is improving soil function (Cundy et al. 2016). Soil is a finite resource and degradation is not recoverable within a human lifespan. Soil degradation can be prevented by improving its function such as improving nutrient cycling, enzymatic activities, and soil structure. Soil function is highly dependent upon microbes, a crucial component of natural and managed ecosystems (Fierer 2017). Therefore, to improve soil function in any soil ecosystem a thorough understanding of microbes and their responses is needed. This understanding is even more critical in brownfields because each brownfield is different from one another. Brownfields differ not only in contaminants but also soil types and climatic context. All these factors affect microbial community composition, diversity and their response (Khan et al. 2018). Therefore, for any restoration and reclamation efforts in brownfields to materialize, it is essential to study the microbial community composition and understand how they respond to contaminants.

In this study, we characterized the microbial community and enzymatic activity of a unique brownfield at Liberty State Park, NJ, USA. The site is unique because of the abundant growth of under-story and over-story vegetation since its abandonment five decades ago. Public access to this site has been restricted, which provides a remarkable opportunity to study the soil microbial community of a unique ecosystem and its response to high concentrations of several heavy metals without the influence of human intervention. Since vegetation at this site has flourished well, it provides an opportunity to learn more about this ecosystem, which is in the process of natural restoration.

The overall objective of the current study is to investigate the relationship between soil microbial community composition and functioning, and the heavy metal concentrations in the soil. We studied the microbial community composition and measured soil extracellular phosphatase

activity, which is often viewed as a fundamental metabolic process that reflects the functioning of active microbial taxa (Nannipieri et al. 1979). Extracellular soil phosphatase activity has been found to correlate with microbial biomass and nitrogen mineralization (Clarholm 1993), therefore it is a good indicator of soil function. We also quantified the metal contaminants at the site and studied their relationship to microbial community composition.

3.2 MATERIALS AND METHODS

3.2.1 STUDY SITE

The case study we present here is on Liberty State Park (LSP), which is a brownfield located in Jersey City, New Jersey, USA and is contaminated with a suite of heavy metals (Gallagher et al. 2008). LSP is located on the west bank of Upper New York Bay, in Jersey City, NJ (400 42'16N, 740 03'06W) and contains metals such as arsenic (As), copper (Cu), chromium (Cr), zinc (Zn), lead (Pb) and vanadium (V). The inorganic pollutants are found in an isolated portion of the park (approx. 100 ha) that is un-remediated and has limited human access (Gallagher et al. 2008). In the latter half of the 19th century, LSP, formerly a salt marsh, was filled with refuse and construction debris from New York City to build a railyard. In 1919, the railyard commenced operations and was active until 1967 (Gallagher et al. 2008). The railyard was used to store and transport a variety of commodities including coal and petroleum products that contaminated the soil with metals and organic contaminants (Gallagher et al. 2008). Since the 1970s, the railyard company ceased its operations, limiting the anthropogenic use of the site and allowing natural establishment and succession of a robust temperate deciduous forest dominated mainly by early successional northern hardwood trees (Gallagher et al. 2011). Both northern hardwood trees like Betula populifolia and Rhus copallinum and herbaceous plant species such as Polygonum cuspidatum and Artemisia vulgaris found at LSP have shown Zn, Cu, Cr and As accumulation in

plant tissues (Qian et al. 2012), suggesting an intricate below-ground and above-ground interaction with the contaminants. It is suspected that ongoing microbial activity in the soil likely facilitates nutrient cycling allowing for plant growth on otherwise restrictive soils (Krumins et al. 2015). The work we present here sheds light on that hypothesis. Recent studies have also shown an elevated level of various enzymatic activities at some sites in LSP compared to uncontaminated sites (Hagmann et al. 2015). Constant exposure to heavy metals and organic contaminants may have exerted a selective pressure on microorganisms and resulted in a community that can survive the stressed environment (Krumins et al. 2015).

3.2.2 SOIL COLLECTION

We collected soil from four sites within the fenced portion of LSP (site labels: 146, 43, 25F and 25R). Site numbers are arbitrary but tied to historical sampling and known metal concentrations across the site (see Table1). We also collected soil from Hutcheson Memorial Forest (HMF), a reference site in central NJ of similar ecological successional age but no history of contamination. While sites 146, 43, 25F and reference site HMF are densely vegetated, site 25R is not. We collected soil samples along three 20 m transects that were five meters apart. From each transect, we collected five soil samples that were four meters apart and mixed them to form one composite sample. We identified the composite soil samples from three transects as the three replicates from each site. We sieved them using a 2 mm sieve to remove large rocks, pebbles, and plant litter.

3.2.3 QUANTIFICATION OF METALS

We quantified the metal concentrations of the soil samples from LSP and HMF using inductively coupled plasma mass spectrometry (ICP-MS) employing the EPA 3050B method. Briefly, we digested 0.5 gm of soil with 10 mL 50 % HNO3 (15.7 M, ACS plus certified) at 95 ± 5 ℃ for 15

minutes and allowed it to cool. Subsequently, we refluxed 5 mL of 15.7 M HNO3 at 95 ± 5 ℃ for 30 minutes. We repeated the steps until the brown fumes were visible. Once the white fumes replaced the browns fumes, we reheated the samples at 95 ± 5 ℃ without boiling until the volume reduced to 5 mL. We allowed the samples to cool down and subsequently added 2 mL of deionized water and 3 mL of H2O2. We reheated the samples at 95 ± 5 ℃ until the effervescence stopped. The samples were then diluted to a final volume of 50 mL and filtered using 0.4 µm filters to remove any soil particles. We further diluted the samples by a factor of 20 with 1% HNO3 and quantified the metals on ICP-MS.

3.2.4 PHOSPHATASE ACTIVITY

We measured the phosphatase activity (PA) in the soil samples fluorometrically in a 96 well plate at 30 ℃ by calculating the rate of product formation. For PA determination, we employed the method from Hagmann et al. (2015). Briefly, we suspended approximately 0.1 gram of soil in 100 mL 0.1M 2-(N-morpholino ethanesulfonic acid (MES)) buffer and sonicated at an output of 25 W for 3 mins. After sonication, we added 160 µl of sample suspension along with 40 µl of the 350 µM fluorescent analog substrate (4-methylumbelliferyl-phosphate) to the 96 well plate. Simultaneously, we generated a standard curve using four different concentrations (0, 500 µM, 1000 µM, 1500 µM, and 2500 µM) of 4- methylumbelliferone (product). Finally, we measured the fluorescence of the samples and the standard curve in a plate reader at 320 nm excitation and 450 nm emission at 30 ℃ with measurements taken every 7.5 minutes over 3 hours. We calculated the PA using the change in product concentration and normalized it over unit dry weight of soil.

3.2.5 ACRIDINE ORANGE DIRECT COUNTS (AODC)

We measured the bacterial cell density using epifluorescence microscopy after staining the soil suspensions with acridine orange. Briefly, we suspended soil samples in phosphate buffered saline (PBS) and fixed them in formalin. The fixed samples were then serially diluted in PBS with a final dilution factor of 10-3. We stained the diluted samples with 0.1% acridine orange and transferred onto a black polycarbonate 0.2 μm IsoporeTM membrane filter (Millipore, Waltham, MA). We then observed and counted the samples under an epifluorescence microscope with a 100 X objective lens (Nikon eclipse Ti-S) (Hobbie et al. 1977, Krumins et al. 2009).

3.2.6 pH MEASUREMENT

We measured the pH of the soil using EPA method 9045D. Briefly, we suspended 20 gm of soil in 20 ml of deionized water in a 50 ml beaker. We continuously stirred the suspension with a magnetic stirrer for 5 mins. Thereafter, we centrifuged the soil suspension at 3000 rpm and measured the pH of the aqueous phase of the soil suspension with a pH meter.

3.2.7 LOSS ON IGNITION (LOI)

We used the gravimetric loss on ignition (LOI) method to estimate organic carbon in soils (Davies 1974). Approximately 2.25 g of oven dried soil was combusted in a muffle furnace at 550 °C for four hours. The percent organic matter was determined gravimetrically.

3.2.8 DNA EXTRACTION AND SEQUENCE ANALYSIS

We studied the microbial community compositions of soils collected from LSP and the reference site HMF through DNA sequencing on Ion Torrent PGM platform. We extracted DNA from the soil samples using the MoBio Power Soil Extraction Kit according to the supplier's manual (MoBIO Laboratories, Carlsbad, CA, USA) in triplicates from each site. The replicates were then consolidated into a single sample and concentrated using vacuum centrifuge for 2 hours.

Following the consolidation of the samples, we quantified the DNA spectrophotometrically. The consolidated samples were then sent to the Molecular Research DNA laboratory (MR DNA, Shallowater, TX) for DNA sequencing. For marker gene sequencing, we chose 515F and 806R primers covering the V4 variable region in bacteria and ITS1 primers for fungi. Following the PCR step, the PCR products were barcoded, and equimolar concentrations of amplicons from all samples were consolidated and sequenced on the Ion Torrent PGM system using the manufacturer's guidelines at MR DNA. The sequences were processed using MR DNA's proprietary pipeline. Briefly, the sequences were demultiplexed, and the barcodes and primer sequences were removed. Sequences with ambiguous base calls and with homopolymer runs exceeding 6 bp and sequences < 150 bp were also removed. Sequences were then denoised, and operational taxonomic units (OTUs) were defined by clustering at 3 % divergence (97 % similarity) followed by removal of singleton sequences and chimeras. Final OTUs were taxonomically classified using BLASTN against a curated database derived from GreenGenes, RDPII, and NCBI (DeSantis et al. 2006).

3.3 DATA ANALYSIS

We used a one-way ANOVA model to statistically determine if the response variables: phosphatase activity, bacterial cell density, loss on ignition and pH varied with the study sites (LSP and HMF) as the independent variables. We also carried out a Tukey's-HSD posthoc test for pairwise comparison among the sites. To investigate the association with phosphatase activity, bacterial cell density, loss on ignition and pH, we used Pearson correlation. We performed principal coordinate analysis (PCoA) on Bray-Curtis dissimilarity to analyze the microbial community composition and performed Pearson correlation analysis between principal coordinates and heavy metal contaminant concentrations to find associations between them. We

conducted all statistical analyses on R (version 3.4) (R core team). We used the tidyverse package by Hadley Wickham (2017) for data structuring and visualization and the ggcorrplot package by Alboukadel Kassambara (2016) for plotting a correlation matrix.

3.4 RESULTS

We measured the phosphatase activity of the soils collected from the five sites (four from LSP and one reference site HMF). The one-way ANOVA on the phosphatase activity showed significant differences among sites (Figure 1, $F = 11.156$, $p<0.005$). A post-hoc Tukey-Kramer HSD test showed significantly higher phosphatase activity at LSP 146 than HMF ($p<0.005$), LSP 43 ($p<0.01$), and LSP 25R ($p<0.005$) (Figure 1). No significant difference was found between phosphatase activities of LSP 146 and LSP 25F.

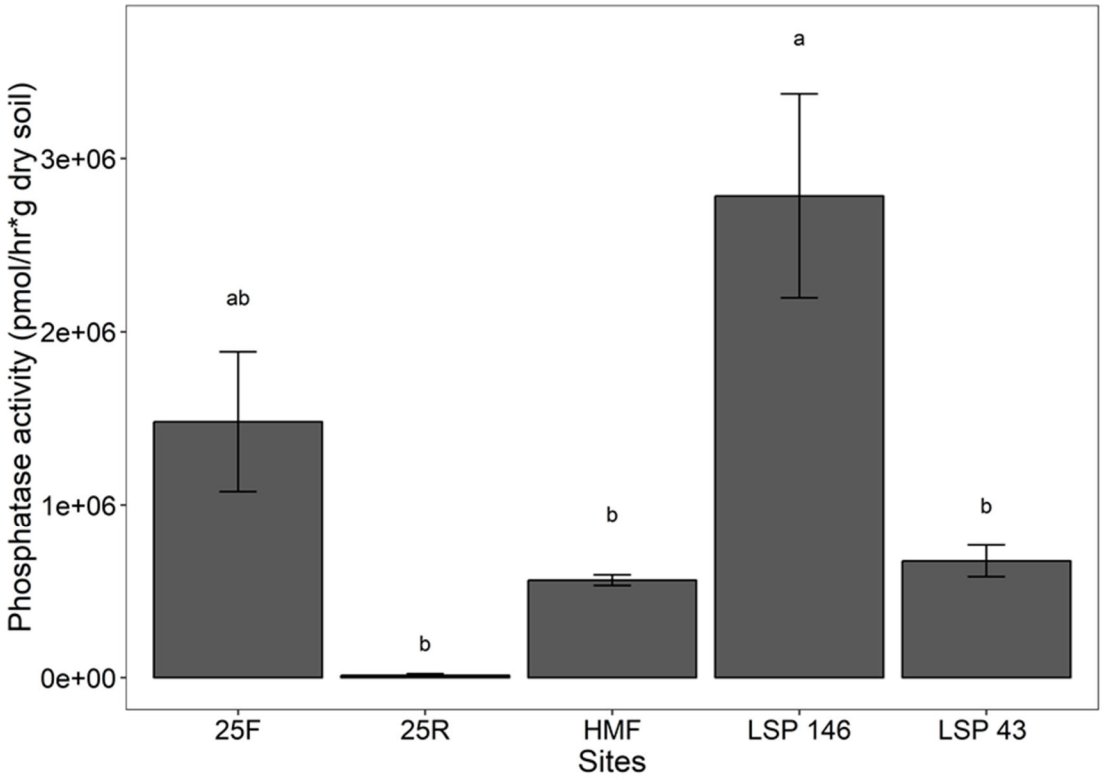

Figure 3-1:Phosphatase activity across LSP 43, 146, 25R 25F and the reference site HMF.

We also measured the bacterial density of the soils and found that it varied significantly among the sites (Figure 2, $F = 8.16$, $p < 0.005$). Similar to the phosphatase activity, bacterial density was significantly higher in LSP 146 than LSP 43 ($p<0.05$) and LSP 25R ($p<0.005$) (Figure 2). We also found that LOI significantly varied among sites (Figure 3, $F = 47.75 p<0.0001$). While the LOI at LSP 146 was significantly higher than at LSP 43 ($p<0.05$), HMF ($p<0.0001$) and LSP 25R ($p<0.0001$), it was not significantly different from LSP 25F ($p>0.05$) (Figure 3). Soil pH also varied significantly between the five sites (Figure 4, $F = 59.37$, $p<0.0001$); LSP 25R had the highest pH and LSP 146 the lowest. The pH at LSP 25R was significantly higher ($p<0.005$) than the pH at the rest of the sites (Figure 4).

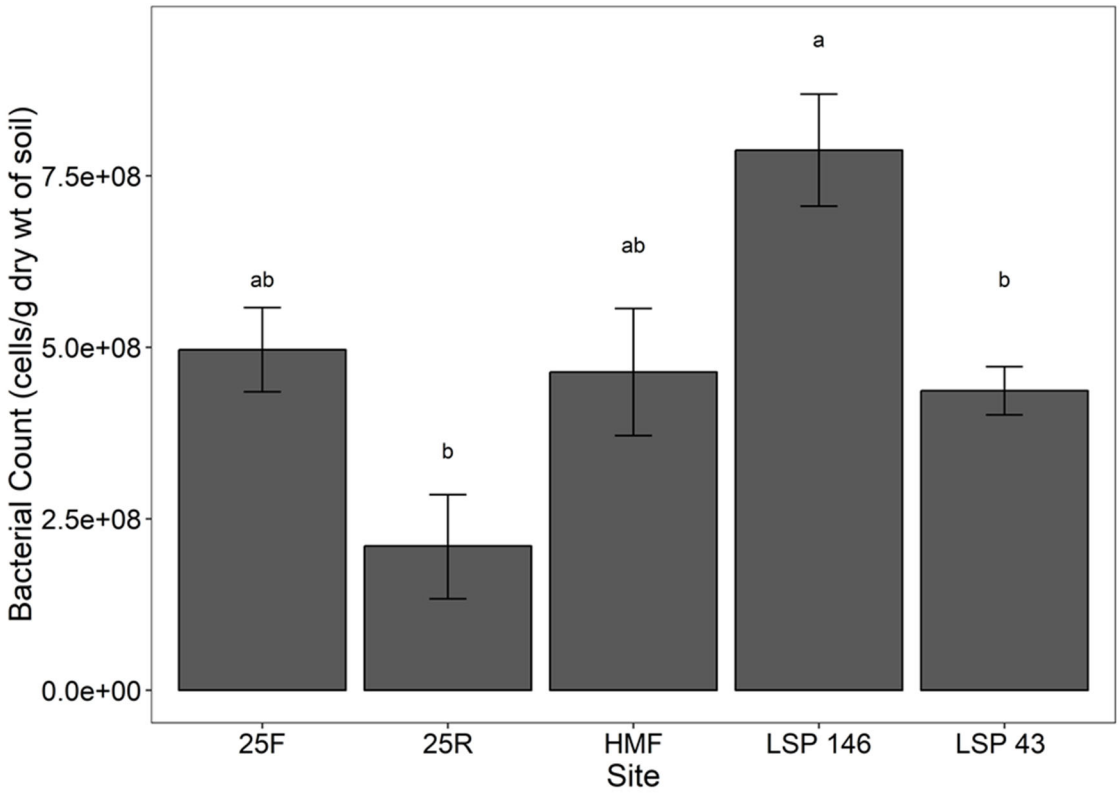

Figure 3-2: Bacterial density across LSP 43, 146, 25R 25F and the reference site HMF.

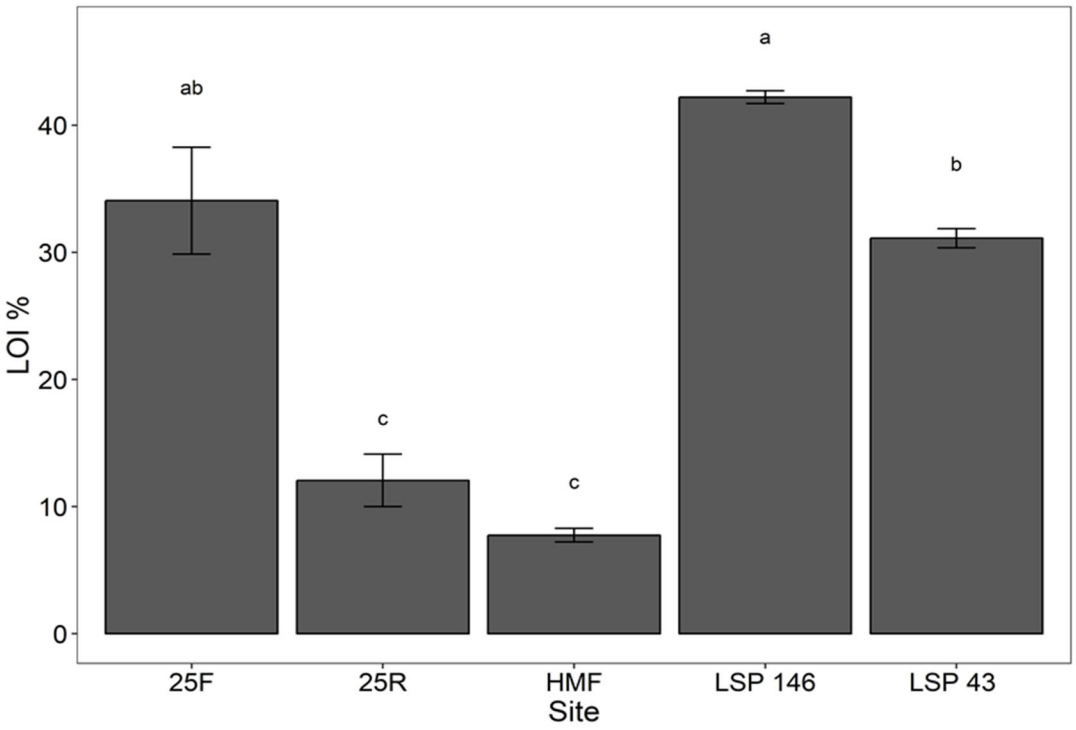

Figure 3-3: Estimate of soil organic carbon via loss on ignition (LOI) of the study sites.

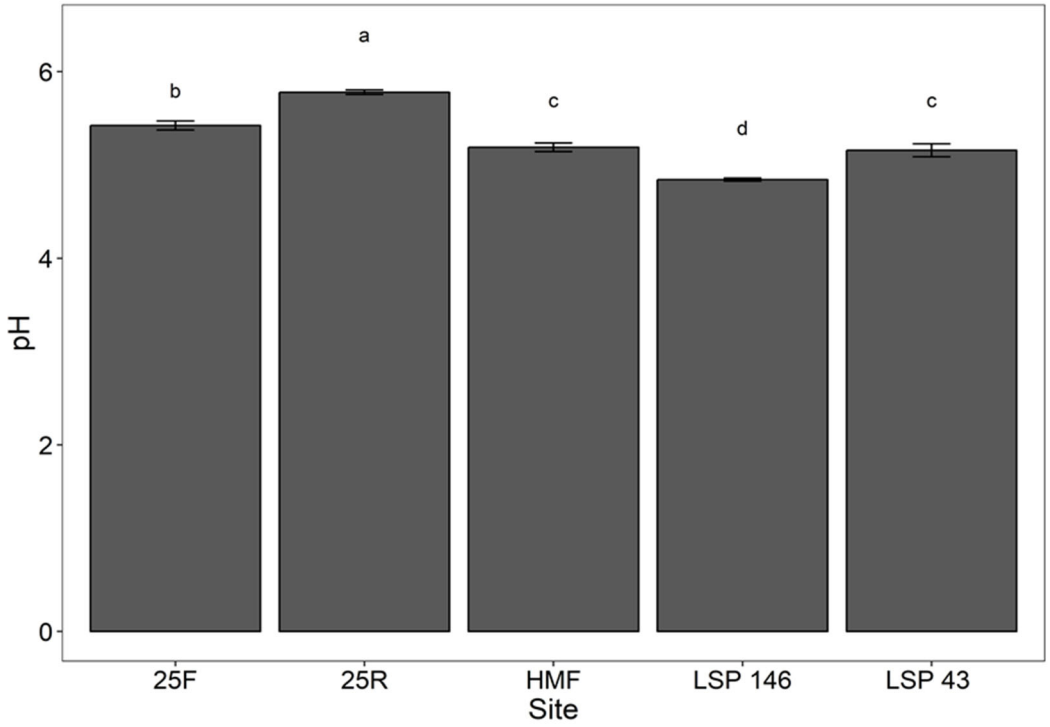

Figure 3-4: pH values of the four sites.

We used Pearson correlation to investigate the relationships between pH, phosphatase activity, LOI, and bacterial density. We found that pH was negatively correlated with phosphatase activity ($r = -0.66$, $p < 0.01$), bacterial density ($r = -0.78$, $p < 0.001$) and LOI ($r=-0.53$, $p<0.05$) (Figure 5). We saw a significant positive correlation between LOI and phosphatase activity ($r=0.73$, $p<0.005$) (Figure 5). Phosphatase activity also showed a positive correlation with bacterial density ($r=0.65$, $p<0.01$) (Figure 5) and with LOI ($r=0.64$, $p<0.01$) (Figure 5).

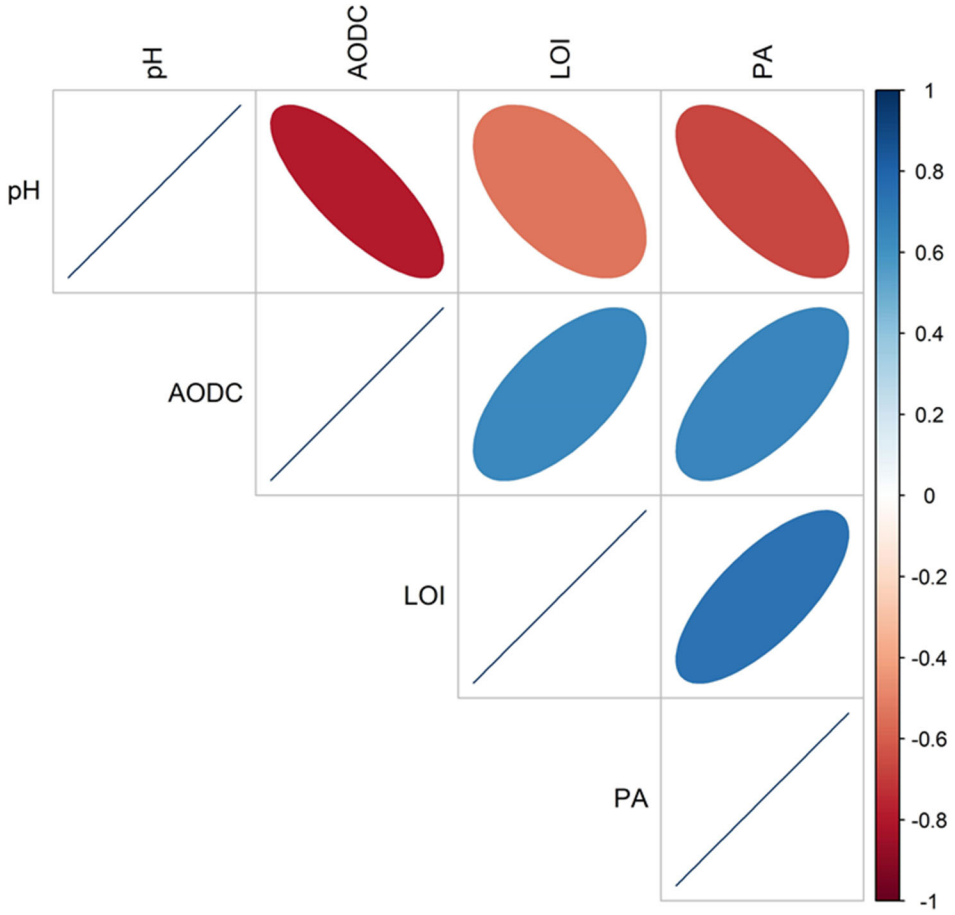

Figure 3-5: Correlation matrix between pH, loss on ignition (LOI), phosphatase activity (PA), and bacterial count per gram of dry weight soil (AODC: Acridine orange direct count).

We analyzed the bacterial communities of the study sites using high-throughput sequencing after amplifying the V4 hypervariable region. We used Bray-Curtis dissimilarity for the PCoA analysis. Our PCoA analysis appears to show that the microbial community at LSP 25R is different from the other sites (LSP 146, LSP 43, LSP 25F and HMF) (Figure 6A). We found that the relative abundance of alphaproteobacterial taxa to be lower at 25R (14.8 %) compared to the other sites, where it ranged from 21 to 25 %. In contrast, the relative abundance of betaproteobacteria was found to be higher at 25R (15.6 %) than the rest of the sites where it ranged from 8 to 10 %. Ktedonobacteria class was also found to be at higher abundance at 25R (7.5 %) compared to the other sites where the relative abundance of Ktedonobacteria was less than 0.5 % of the taxa.

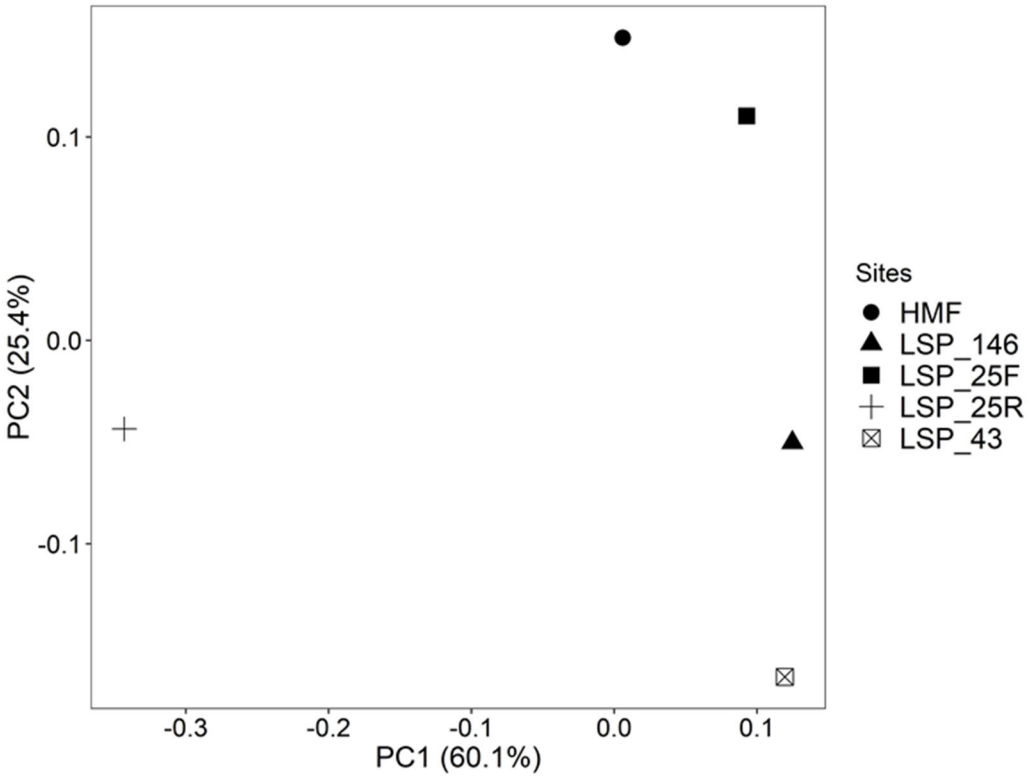

Figure 3-6: PCoA representing the differences in bacterial community composition among the sites.

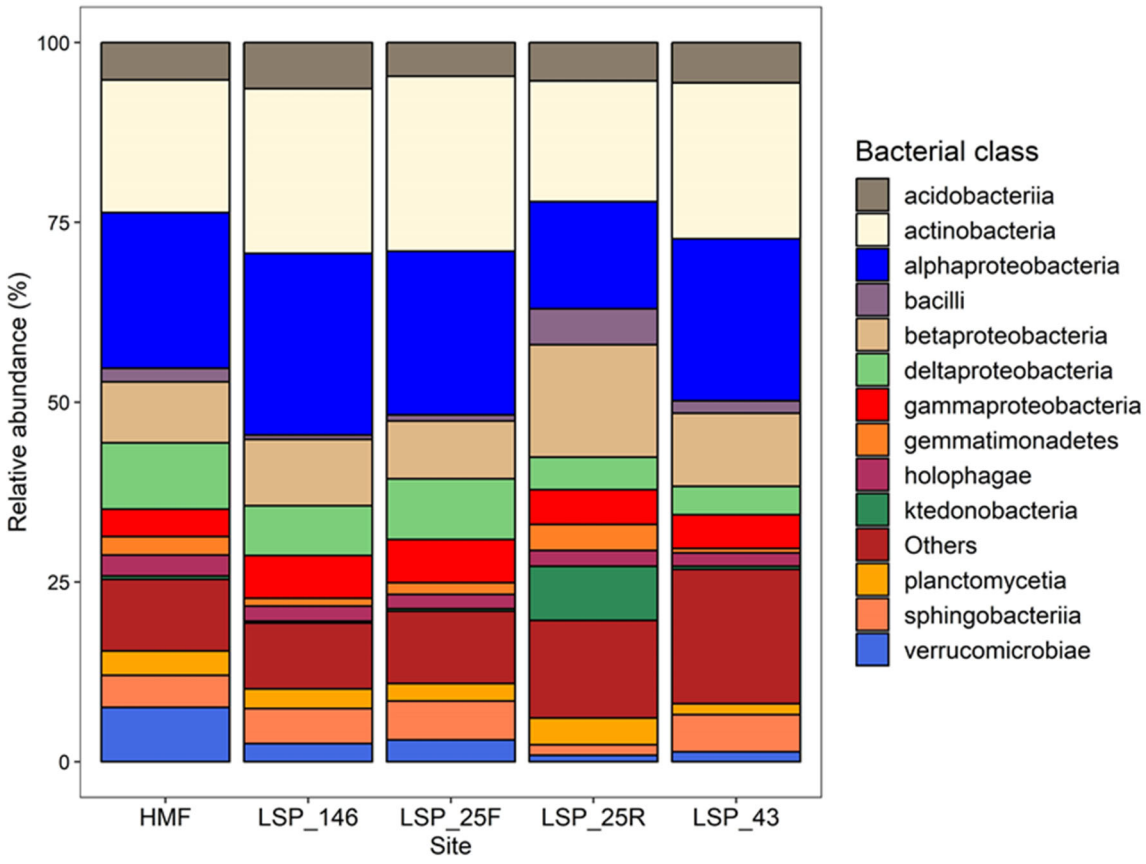

Figure 3-7: Relative abundance of the dominant classes of bacteria found across the sites.

After surveying the fungal community using ITS1 region of the fungal rRNA gene, we found that the fungal community composition appeared to be different at our reference site HMF compared to our experimental study sites at LSP (Figure 7A). We observed that Agaricomycetes was the most abundant taxa at LSP ranging from 57.3 % at 25R to 83.1 % at LSP 146 (Figure 7B). The relative abundance of Agaricomycetes at HMF was very low (only 6.4 %) (Figure 7B). While the relative abundance of Agaricomycetes was found to be low at HMF, sordariomycetes and mortierellomycotina were found to be at higher (26.4 % and 21.8 % respectively) at the same site (Figure 7B). Sordariomycetes abundance at LSP ranged from 4.9 % at LSP 43 to 19.3% at LSP 25R. Mortierellomycotina was found to be at lower abundance at LSP compared to HMF

and ranged from 0.83 % at LSP 43 to 2.7 % at LSP 146. Additionally, pezizomycetes abundance was also found to be high (13.3 %) at HMF compared to LSP sites where they were less than 5 %.

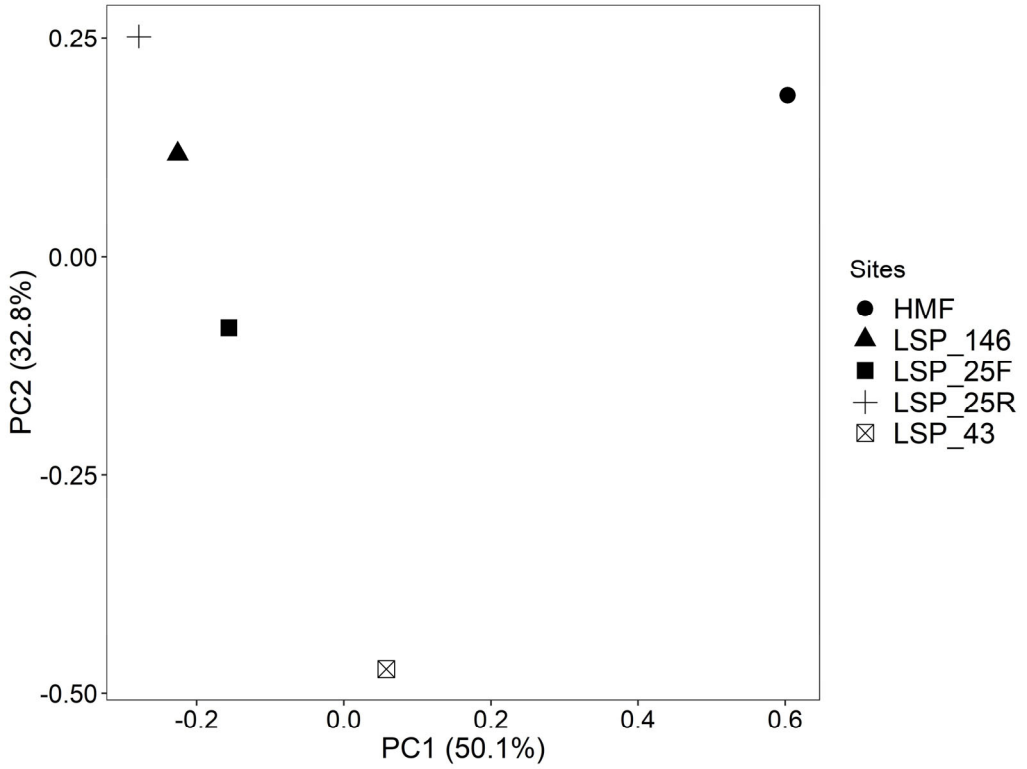

Figure 3-8: PCoA representing the differences in fungal community composition among the sites.

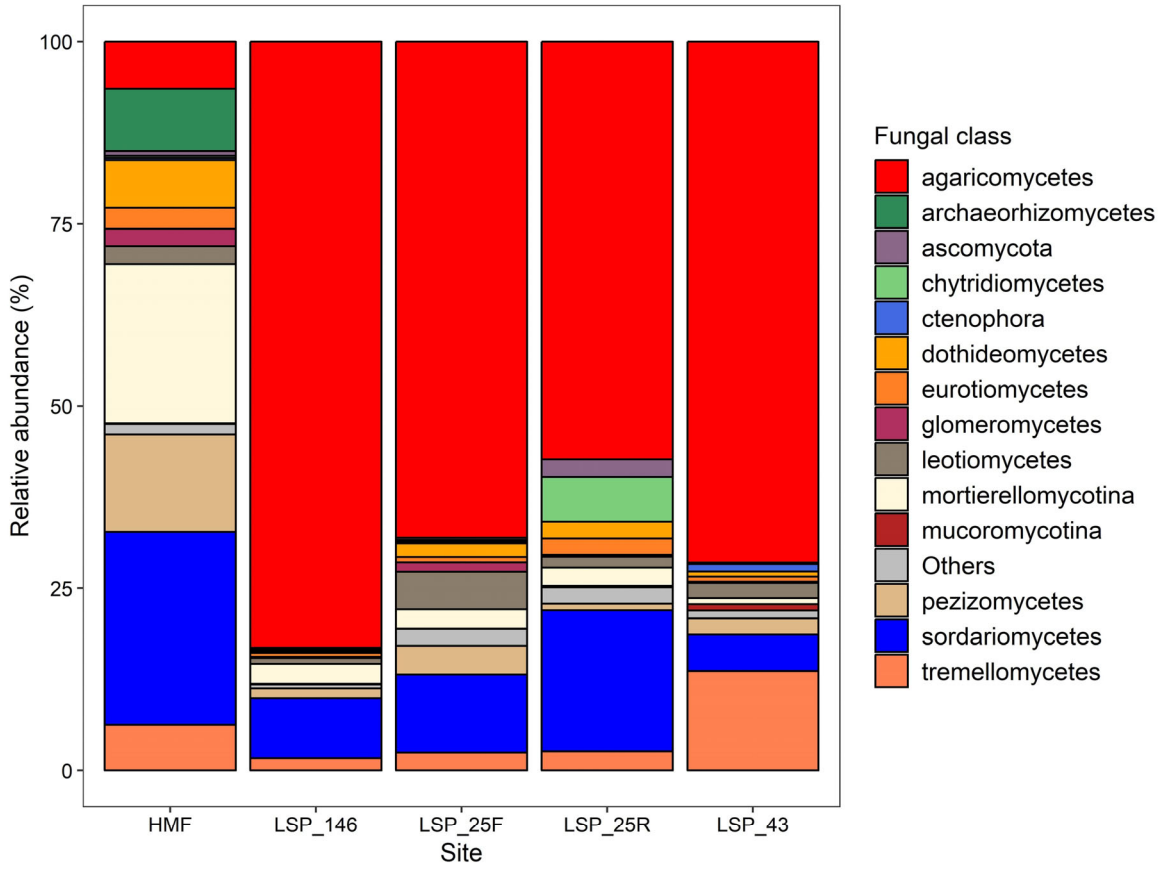

Figure 3-9: Relative abundance of the dominant classes of fungi found across the sites.

We also quantified the metal contaminants at the sites (Table 1). Metal contamination generally was high at 25R and 25 F. Cu, Zn, As and Pb were very high in 25R and 25F. Contamination at HMF and 43 were lower than the rest of the site. V concentration at LSP 146 was the highest. We correlated the metal contaminants with the microbial community to study how they responded to metals.

Table 3-1:Concentration of metals found at the study sites. All data are shown in ppm ± standard error of the mean (n=3)

	V	Cr	Cu	Zn	As	Pb
HMF	38.29±0.53	26.67±0.47	20.4±2.52	42.48±1.73	4.78±0.12	29.97±0.43
LSP 43	44.68±0.62	33.41±0.64	99.12±5.08	50.52±1.54	19.39±0.83	230.09±11.40
LSP 25F	114.23±13.39	119.57±2.93	2714.37±469.94	8450.88±1971.05	849.69±265.24	8824.21±1816.56
LSP 146	205.58±5.7	159.25±2.34	121.17±6.80	103.96±12.22	40.88±5.46	389.31±22.94
LSP 25R	105.92±16.79	216.04±32.07	7460.25±1659.09	20735.59±4690.23	1324.88±268.75	21964.46±4485.46

To study how the microbial communities (both fungal and bacterial) varied with soil heavy metal load, we carried out Pearson correlation between the two principal coordinates (first and second) and heavy metal concentrations. We found strong negative correlation between the first principal coordinate axis and Cu ($r = -0.89$, $p<0.05$), Zn ($r = -0.88$, $p<0.05$) and Pb ($r = -0.88$, $p<0.05$) (Figure 8A). Other heavy metals such as Cr, As and V displayed no such relationship with the bacterial first principal coordinate axis. We did not find any significant correlation between the bacterial second principal coordinate axis and heavy metals. Fungal principal coordinate axes were not correlated to any of the heavy metals.

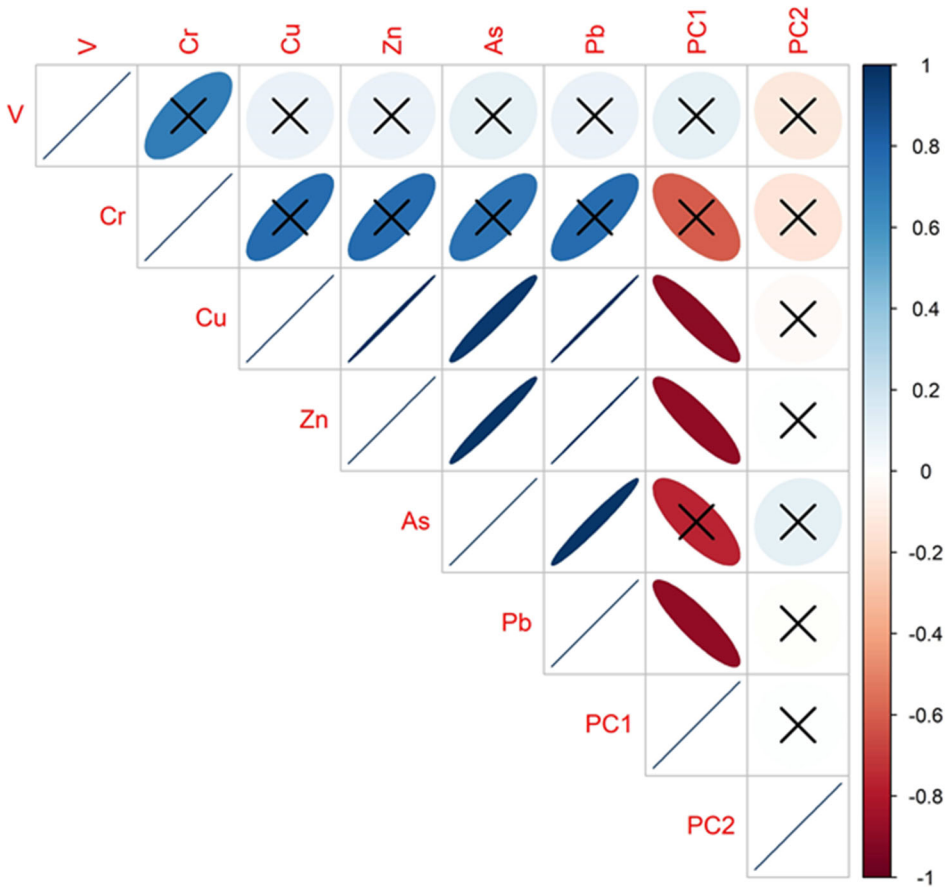

Figure 3-10: Correlation matrix of bacterial principal coordinate axes (PC 1 and PC 2) with Arsenic (As), Chromium (Cr), Copper (Cu), Lead (Pb), Zinc (Zn).

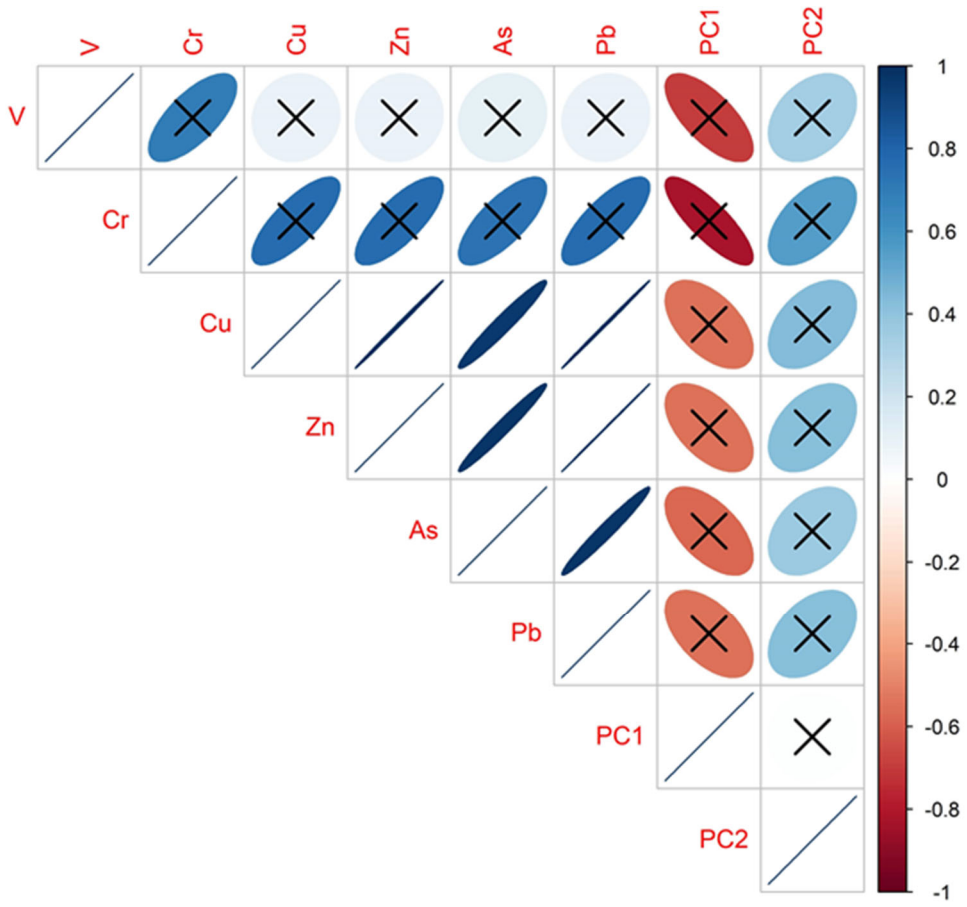

Figure 3-11: Correlation matrix of fungal principal coordinate axes (PC 1 and PC 2) with Arsenic (As), Chromium (Cr), Copper (Cu), Lead (Pb), Zinc (Zn).

3.5 DISCUSSION

Liberty State Park is an urban brownfield that displays different levels of contaminants across sites (Table 1). This heterogeneity provides a unique opportunity to study the soil function and microbial community of a contaminated soil ecosystem. We chose phosphatase activity as a proxy for soil function at LSP because phosphatases catalyze the hydrolysis of ester bonds to release phosphate into the soil ecosystem (Nannipieri et al. 2011). As such, it serves as a good measure of soil nutrient cycling, allowing us to investigate the functionality of a brownfield that boasts a surprisingly robust early successional hardwood forest and plant community. We found

that LSP 146 and 25F had the highest phosphatase activity of all sites, including the reference site, HMF. The high phosphatase activity suggests that soil at LSP 146 and 25F is particularly functional in phosphorus cycling and microbial activity. Our results concur with a previous study on LSP where the authors also found site 146 to have the highest phosphatase activity of multiple LSP sites studied (Hagmann et al. 2015). In contrast, LSP 25R, the site with no vegetation exhibited little phosphatase activity, suggesting minimal microbial nutrient cycling.

Our correlation analyses show a strong positive relationship between phosphatase activity and LOI, an estimation of soil organic carbon. LSP sites 146 and 25F exhibited a high LOI as well as a high phosphatase activity. Previous studies have reported that soil organic carbon positively influences phosphatase activity in uncontaminated soils (Bonmati et al. 1991, Boerner et al. 2005) and here we observe a similar phenomenon at contaminated LSP. Our results suggest that organic matter also increases soil enzymatic activity in contaminated sites such as LSP. Soil organic carbon improves not only microbial activity (Kuzyakov and Blagodatskaya 2015) but also increases microbial biomass; LSP 146 has significantly higher bacterial abundance compared to other sites within LSP (Figure 2). We also observed a strong positive correlation between LOI, bacterial density and phosphatase activity indicating a potentially crucial role played by soil organic carbon at LSP. Besides improving microbial activity and biomass; soil organic carbon is known to reduce the readily extractable fraction of heavy metals in soils (Yang et al. 2016), thereby minimizing the stress exerted by heavy metals and allowing ecosystems damaged by metal contamination to recover.

To understand how heavy metal contamination influences the microbial community, we sequenced the microbial ribosomal RNA gene for both bacteria and fungi, and studied the relationship between community composition and heavy metal concentrations. Our findings

indicate that the bacterial communities were sensitive to metal contaminants, particularly to Cu, Zn, and Pb. The concentrations of these three metals were especially high at LSP 25R (Table 1). We also observed a strong negative correlation between Cu, Zn, Pb and the composition of the bacterial community (Figure 8A). Dai et al. (2004) have previously reported that Cu and Zn affect microbial activity by influencing carbon and nitrogen cycling and this provides a plausible explanation for the lack of enzymatic activity observed at 25R. Cu, Pb, and Zn are also known to shape microbial communities (Wang et al. 2007, Li et al. 2015, Kou et al. 2018). It was interesting to see the lack of any relationship between the composition of the fungal communities and heavy metals suggesting no associations between metals and fungi. The results indicate that the bacterial communities may be more sensitive to metal load than fungi. Past results also suggest that fungi are more tolerant to heavy metals (Hiroki 1992). This tolerance can be attributed to the presence of transporter proteins, stress enzymes and different metallotionein (Hildebrandt et al. 2007). It was also interesting to notice that As and Cr, known for exhibiting high toxicity, did not have a relationship with either bacterial or fungal community composition. Fungi are known to be resistant to different heavy metals (Gadd 2007) because they perform redox transormations and release of biomolecules and sometimes accumulate metals in their filaments (Bruins et al. 2000, Gadd 2007, Zafar et al. 2007). Their ability to persist in metal contaminated soils and their active involvement in nutrient cycles could be harnessed to restore and reclaim contaminated sites.

3.6 CONCLUSION

Natural attenuation of disturbed sites by microbes is possible through bioaugmentation (Wubs et al. 2016). Disturbed ecosystems such as brownfields can be rehabilitated using microbes and plants. Singh et al. (2019) show that soil inoculation can improve soil phosphatase activity but

within the context of abiotic soil properties. Therefore, a greater understanding of microbial responses to soil properties and contaminants is required. Our study provides critical insights into the soil function and microbial ecology of a metal contaminated soil system. Here, we established a strong association between metals such as Cu, Zn, and Pb on microbial community composition and likely functioning. Our results suggest that bacterial abundance and soil organic matter play a crucial role in regulating soil phosphatase activity in an urban brownfield. Soil organic matter and microbial biomass facilitate nutrient cycling in the soil thereby promoting recovery of damaged ecosystems. While the results show that the bacterial community is sensitive to metal contaminants, it also indicates that the fungal community is more resistant to heavy metal contaminants at LSP. Our results have applications in management and planning of brownfield sites, which can can help mitigate risks posed by the contaminated sites. This information could be leveraged to delve deeper into microbial responses to different heavy metal contaminants, and whether the ability of microbes to survive and thrive in stressed environments can be harnessed to ameliorate the effects of heavy metals to reclaim and restore damaged ecosystems. With urbanization threatening biodiversity and ecosystem productivity, these brownfield sites can be re-established with vegetation with help from bioaugmentation and soil management practices. Such practices can be beneficial to the urban community.

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4 ABIOTIC FACTORS DETERMINE FUNCTIONAL OUTCOMES OF MICROBIAL INOCULATION OF SOILS FROM A METAL CONTAMINATED BROWNFIELD

4.1 INTRODUCTION

Interactions between the biotic (living) and abiotic (non-living) components of soil are complex and dynamic, especially in the presence of contamination (Krumins et al., 2015). Contaminants can impact soil properties by serving as a filter on microbial community composition and functioning. While it is known that both biotic and abiotic soil properties shape soil microbial community composition (Torsvik et al., 2002; Zak et al., 2003; Fierer and Jackson, 2006; Lauber et al., 2009), subsequently affecting soil function (Strickland et al., 2009), biotic factors, including the composition of bacterial and fungal communities, are often perceived to be more important in defining soil extracellular enzyme function compared to abiotic factors. For the purposes of this paper, 'abiotic' properties refer to all non-living properties of the soil including heavy metals, total C, N, and P content, and pH; the abiotic properties of the soils studied here have been and are under ongoing investigation (Hagmann et al., 2015). The 'biotic' properties in this paper refer to the soil fungal and bacterial communities. While soil microbial community composition is shaped by both organic and inorganic contaminants (Frostegård et al., 1993; Smit et al., 1997; Turpeinen et al., 2004; Guo et al., 2017), the contaminants themselves are simultaneously chemically transformed by the microbes too (Gadd, 2007; Falkowski et al., 2008). Because the microbial communities of soil also drive plant diversity and terrestrial productivity (Van der Heijden et al., 1998; Van Der Heijden et al., 2008, Schnitzer et al., 2011; Paul, 2014), understanding factors that impact soil function, particularly in contaminated soils, will guide our understanding of ecosystem functioning in post-industrial and contaminated urban

systems. Separating the effects of biotic from abiotic factors is necessary to get a deeper understanding of these complex and interdependent systems.

Targeted microbial inoculation is one approach to increase soil function and extracellular enzymatic activities (Khan et al., 2000; Rajkumar et al., 2012). Targeted inoculation has been used to support the colonization of microbes on plant seeds and roots to improve biomass productivity (Ahmad et al., 2018; Afzal et al., 2012; Panke-Buisse et al., 2015) and soil quality (Raj et al., 1981). It can also enhance remediation of soil contaminants (Prenafeta-Boldú et al., 2004; Afzal et al., 2012; Zhu et al., 2018), facilitate plant establishment (Mengual et al., 2014), enhance soil phosphorus uptake (Raj et al., 1981; Rodrıǵ uez and Fraga, 1999), improve nitrogen fixation (Bhattacharjee et al., 2008), and suppress plant pathogens (Mendes et al., 2011). In most targeted inoculation studies, one or few microbial species were used. Therefore, there is a need to study the effects of introducing whole soil microbial communities on soil function (Strickland et al., 2009; Wubs et al., 2016; Calderón et al., 2017). Moreover, whole microbial community inoculation studies have been typically done in uncontaminated field soils and the effects of introducing whole communities into contaminated soils remain poorly understood (Wubs et al., 2016; Calderón et al., 2017). Studies show that a microbial community can be inoculated to field soils (Wubs et al., 2016; Calderón et al., 2017) where the native microbial community has been experimentally disrupted by irradiation or via anthropogenic degradation, but these results are in part inconclusive. Both Wubs et al. (2016) and Calderón et al. (2017) found that microbial community composition of soil shifted after they were inoculated. Wubs et al. (2016) found that the macro and microbial community moved in the direction of the origin of inoculum. However, the compositional shifts did not necessarily result in improvement in soil function. While Wubs et al. (2016) did not use any soil function parameter (such as enzymatic

activity or soil productivity) to measure soil health, Calderón et al. (2017) found that re-inoculation did not significantly improve nitrogen cycling. Therefore, more studies are required to understand the effects of whole community inoculation as a means to restore or improve soil health. Via sterilization, we can isolate the effects of base soil (abiotic factors) and the introduced microbial communities (biotic factors).

We here present a case study in which we examined the outcomes of a microbial inoculation experiment in contaminated soils in terms of enzymatic activity by isolating the role of the abiotic context from the biotic inocula. Specifically, we sought to answer 1.) Can a microbial community from a high functioning contaminated soil be used to enhance enzymatic activity in a low functioning contaminated soil? And 2.) How do the abiotic properties of the soil base or biotic properties of the inoculum influence the resulting enzymatic activity? To address these questions, we established cross-inoculation experiments, in which we cross- and self-inoculated sterilized soils that were known to have high and low phosphatase activities, respectively, and originate from a metal contaminated urban brownfield in New Jersey, USA. By sterilizing soil samples and inoculating them with inocula from high- or low functioning soils, we investigated the relative importance of biotic and abiotic factors on function of metal contaminated soil after inoculation. We inoculated our experimental units (i.e. soil pots) to (re)introduce microbial communities, a source of enzymatic activity in soils (Paul, 2014), and measured extracellular soil phosphatase activity as well as analyzed microbial community compositions and diversity. The railroad yard was active from 1919 to1967 and was used to store commodities including coal (Gallagher et al., 2008). After the railroad went bankrupt, all operations and most anthropogenic activity stopped in the 1970's when the region was fenced off with limited human access. Behind

this fence a robust temperate deciduous forest has been naturally succeeding (Gallagher et al., 2011).

4.2 MATERIALS AND METHODS

4.2.1 SOIL COLLECTION FROM LSP

We collected soils from two well studied sites within the park, 43 and 146 (Figure. 1). Specifically, we collected soil samples from the top 10 cm at five different points four meters apart along three different mapped transects. We consolidated the soils from the five sampling points of each of the three transects into a single composite sample making 3 replicates (n=3). Back in the lab, we removed large debris and plant litter by coarse sieving followed by finer sieving through a 2mm sieve. We sterilized all the sieved soil by autoclaving to denature enzymes and eliminate microbes (Serrasolsas and Khanna, 1995), and all soils were stored at 4 °C until the start of the experiment.

Figure 4-1.Aerial view of liberty state park showing site 43 and 146.

4.2.2 EXPERIMENTAL DESIGN

We carried out a microbial inoculation study in which microbial communities from sites 43 and 146 (Figure. 1) were re-inoculated to sterilized pots of the same and alternate soil bases (Figure. 2). Our experimental design consisted of soil base (from site 43 and 146), inoculum (from 43, 146 and non-inoculated control (NI)), and time (8- time points over 7 months) as factors (Figure. 1.2). For example, experimental treatments for soil base from 146 was self-inoculated with soil suspension from 146, cross-inoculated with a suspension from 43 and left non-inoculated, and the same was done for soil base from 43. All six treatment combinations were replicated three times with eight discrete sampling time points (144 pots total).

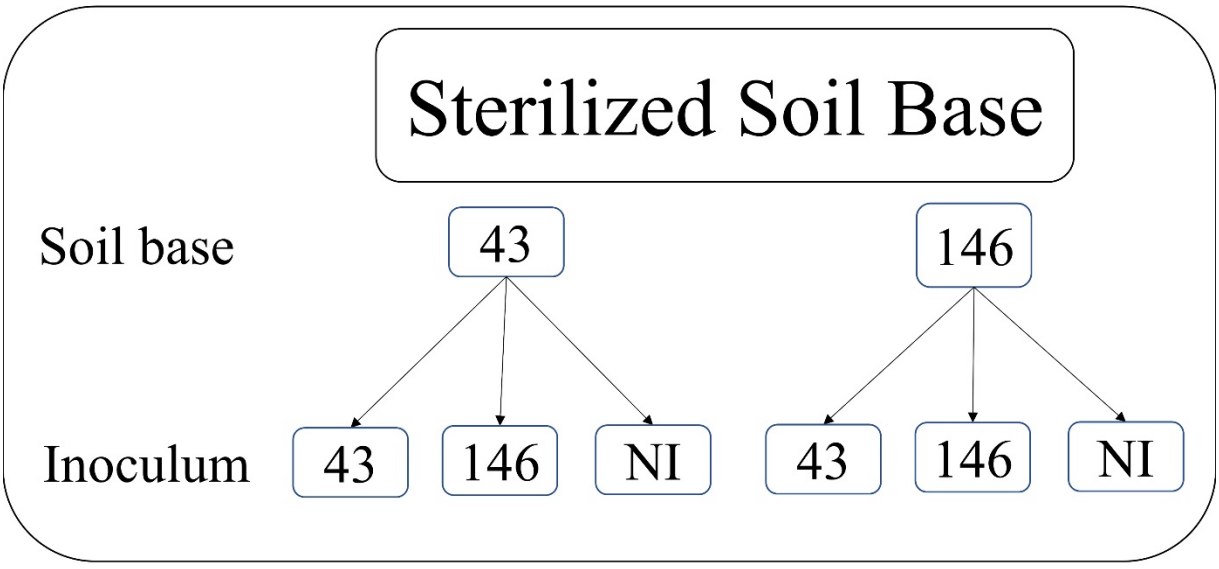

Figure 4-2: Experimental design for the experiments showing two different soil bases and different inoculation levels. NI indicates non-inoculated.

4.2.3 PHYSICO-CHEMICAL PROPERTIES OF THE SOIL SAMPLES

As previously described (Hagmann et al., 2015), soils from site 146 exhibit significantly higher extracellular soil phosphatase activity compared to soils from site 43. Besides soil phosphatase, L-leucineamino- peptidase and cellobiohydrolase activities were also significantly higher at site 146 compared to site 43. The 'abiotic' physico-chemical properties of the soils, including heavy metals, total C, N, and P content, and pH, have been previously reported and their relationship to extracellular enzyme activities has been investigated (Hagmann et al., 2015). Further, to investigate differences in abiotic factors between the two soils in this study, we used the physico-chemical parameter data from Hagmann et al. (2015) and carried out a MANOVA. We used pH, organic matter and moisture as the three dependent variables and the sites as the independent variable. The three dependent variables were chosen because these three variables are considered important for structuring microbial communities and subsequently soil function (Fierer, 2017). We found significant differences (Pillai = 0.99383, $F = 107.47$, $p < 0.01$) between the two soils based on these parameters. Further, we carried out another MANOVA on three metal contaminants (arsenic, lead and chromium) present at the two sites that are ranked higher in the ATSDR substance priority list (ATSDR, 2017). We again found that the two sites were significantly different (Pillai=0.99315, $F = 96.694$, $p < 0.05$) in terms of metal contaminant concentrations. Therefore, our analysis of these response variables suggest that the two soil bases appear to be different from one another. We also carried out a nonmetric multidimensional scaling analysis using pH, organic matter, moisture, arsenic, lead and chromium as response variables to visualize the differences across the two soils (Figure.1.3).

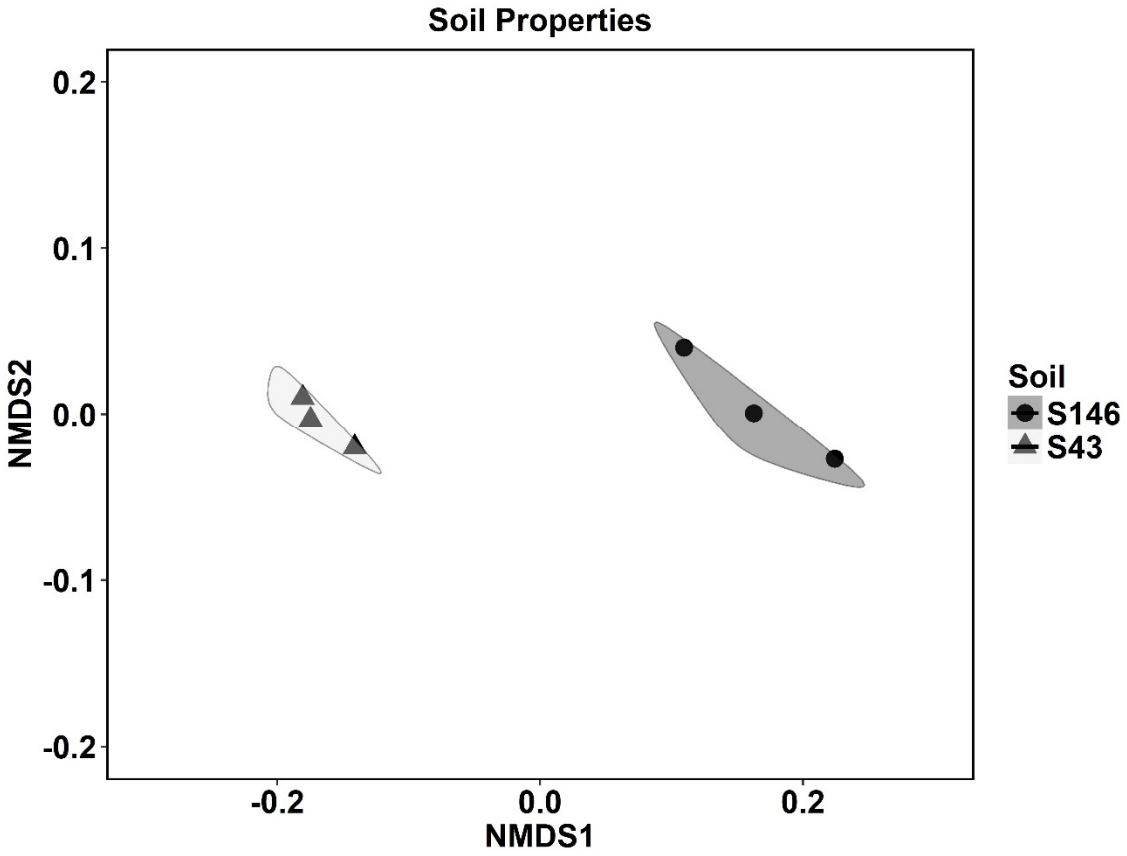

Figure 4-3: NMDS exhibiting differences in physico-chemical properties across the two soil bases.

4.2.4 EXPERIMENTAL INOCULATION AND MAINTENANCE

We filled each pot with 25 g of soil and either inoculated or left uninoculated following the experimental design (Figure.1.2). As per the experimental design, we inoculated pots with either 2 mL of a 10% weight/volume soil suspension for test pots or 2 mL sterile water for negative controls. The soil inocula was prepared by suspending 5 g of soil in 50 mL of sterile distilled water to achieve a final concentration of 10% weight/volume. The pots were then manually homogenized to ensure thorough mixing and kept in a climate-controlled chamber, maintaining a temperature of 24 °C during day and 19 °C at night and a photoperiod of 16/8 h day night cycle.

The relative humidity was 65%, and lighting intensity was 550 μmoles/m2/sec during daytime. We maintained moisture in the pots by adding 2 mL sterile water five days a week.

4.2.5 MEASUREMENT OF EXTRACELLULAR PHOSPHATASE ACTIVITY

We measured acid phosphatase activity in the contaminated soils. Phosphatase activity is a proxy for enzyme function in the soils because phosphatase activity is often viewed as a fundamental metabolic process that will capture the functioning of active microbial taxa (Nannipieri et al., 1979) and extracellular soil phosphatase activity has been found to correlate with microbial biomass and nitrogen mineralization and thus indicate soil functional potential (Clarholm, 1993). We employed a destructive soil sampling method for soil collection from pots after 15 days and monthly thereafter for a total of eight-time points, such that each sample was a discrete time factor. Phosphatase activity of the collected soils were then calculated fluorimetrically in a 96 well plate at 30 °C following a previously described method (Hagmann et al., 2015) with the modification that the slopes of the standard curves, which were found to not be significantly different between different experiments, were averaged and the best fit linear equation for the mean standard curve was used to convert emission intensities to rates of product formation.

4.2.6 MICROBIAL COMMUNITY ANALYSIS

We performed a 16s rRNA amplicon survey on soils destructively sampled from the sixth month of the sampling time points. We extracted DNA from 0.25 g of soil using the MoBio PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's instructions. Following extraction, DNA concentration was quantified on a nanodrop (Thermo Scientific Nanodrop 2000) and stored at −22 °C. The DNA samples were sent to MR DNA (Shallowater, TX, USA) for sequencing and bioinformatics analysis. Briefly, the V4 region of the 16s rRNA gene was amplified by polymerase chain reaction using universal bacterial primers

515f/806r. Fungal ribosomal rDNA was amplified using primer set ITS 1. The amplicons were subsequently sequenced on an Ion Torrent PGM platform. The bioinformatics analysis was done by MR DNA using proprietary codes. Briefly, the sequences were demultiplexed and depleted of barcodes and primers. Sequences shorter than 150 bp, sequences with ambiguous base calls, and those with homopolymer runs exceeding 6 bp were removed from downstream analysis. Further, the sequences were denoised, chimeras removed and Operational Taxonomic Units (OTUs) generated. The OTUs were defined by clustering at 3% divergence (97% similarity). Finally, the OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes, RDPII and NCBI (DeSantis et al., 2006).

4.3 DATA ANALYSIS

We analyzed the results via a 3-way ANOVA in which phosphatase activity was the response variable and soil inocula, soil base, and time were the explanatory variables. Within the experiment, each measurement of phosphatase activity was normalized to its respective sterilized, non-inoculated control, to remove background enzyme response. For example, phosphatase activity in sterilized soil base 146, self-inoculated with soil 146, was divided by phosphatase activity measured in sterilized, non-inoculated soil base 146. The ANOVA model included sampling time points as one of the significant explanatory variables but the trends across eight sampling time points for both experiments were idiosyncratic (Fig. SI 3). Therefore, we averaged all time points and presented only results of analysis with respect to soil base or inocula.

We used genus level taxonomic hierarchy to study the microbial community composition of the soils. We carried out a non-metric multidimension scaling (NMDS) analysis to observe the β-diversity in the soil. We also carried out PERMANOVA using the adnois function from the

vegan package (Oksanen et al., 2018) in R (R Core Team, 2018) to determine statistically significant differences between the microbial communities of source soils and inoculum treatments. We also calculated the Shannon-Weaver diversity for both bacterial and fungal genera and carried out a 2-way ANOVA with Shannon-Weaver index as the response variable and, soil base and inoculum as predictors. For all data transformation and statistical analysis, JMP®13 pro (Institute 2012) and R (R Core Team, 2018, Version 3.4.0) were used. Tidyr and Dplyr packages from tidyverse were used for transforming data (Wickham, 2016). JMP®13 pro (SAS Institute Inc., 2017) and ggplot2 package from tidyverse (Wickham, 2016) were used for visual representation of the data.

4.4 RESULTS AND DISCUSSION

We sterilized all soils used in the study employing the same method to remove the original biotic communities. Therefore, we can see that abiotic soil characteristics were more important in predicting phosphatase activity than the origin and composition of the inoculum that was added to the soil (Fig. 1.4). The experimental units with soil base 146 had significantly higher phosphatase activities than those with soil base 43 (indicated as S146 and S43 respectively in Fig. 4A, $F = 9.195$; $p < 0.01$). Conversely, the two soil inocula did not yield significantly different normalized phosphatase activities (indicated as I146 and I43 in Fig. 1.4, $F = 0.003$; $p=0.95$). In addition of measuring phosphatase activity, we also carried a microbial community survey using 16s rRNA marker gene. Sequencing resulted in identification of 351 bacterial and 176 fungal genera in the soil samples destructively sampled from the sixth month point after soil cross- and self-inoculation. NMDS followed by PERMANOVA analysis indicated a significant difference in both bacterial (pseudo $F = 5.85$, $p < 0.01$; Fig. 1.5) and fungal (pseudo $F = 14.31$, $p < 0.01$; Fig. 1.6) community compositions between the two soil inocula. The differences in

microbial community compositions for the two soil inocula did not lead to significantly different extracellular phosphatase activities for the different inocula (described above). We found no significant difference in either the bacterial (pseudo F= 1.04, p > 0.1; Fig. 1.5) or the fungal (pseudo F = 0.92, p > 0.1; Fig. 1.6) communities across the two soil bases.

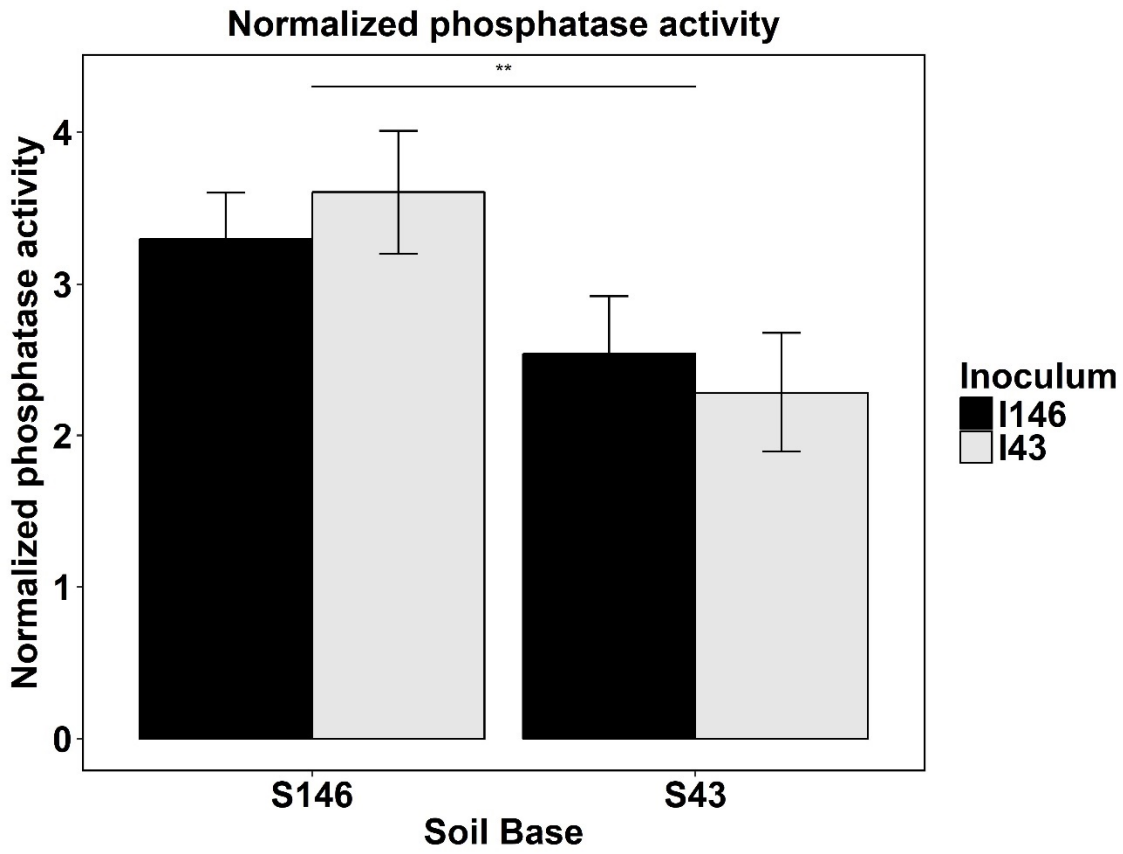

Figure 4-4: Normalized phosphatase activity in the sterilized soil.

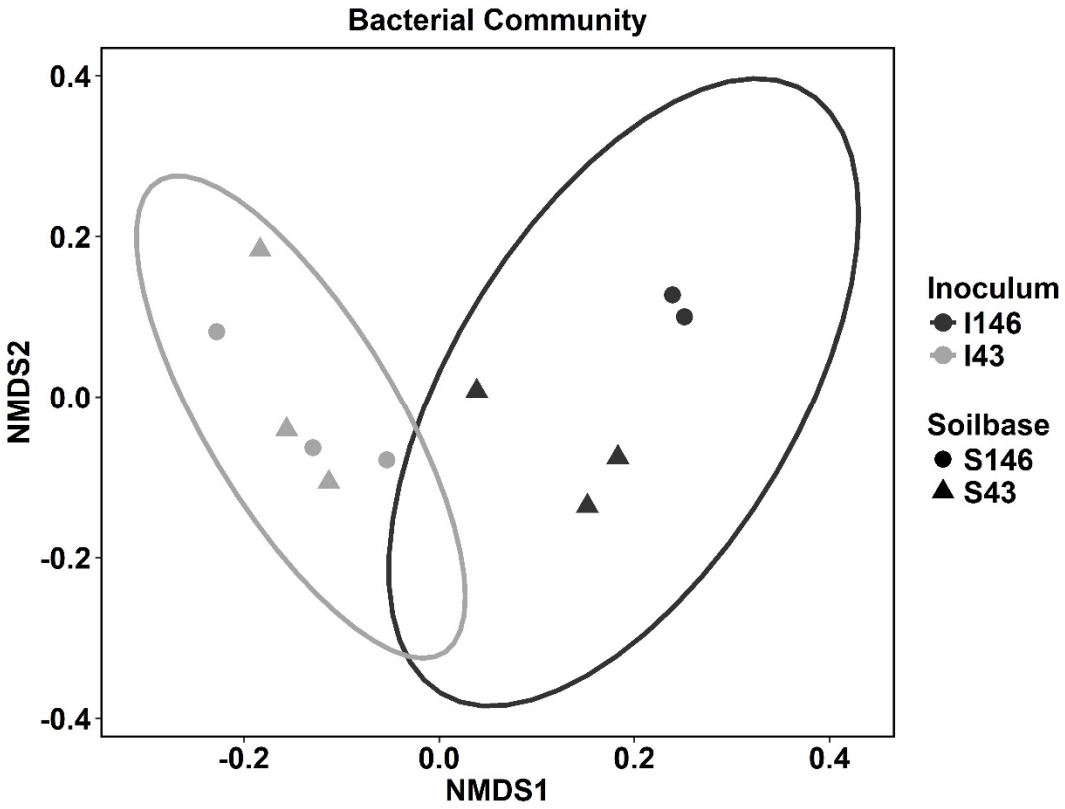

Figure 4-5: NMDS of bacterial genus across the soil treatments

The results we found here are likely specific to the soils and timescale of our experiment, as in many studies soil abiotic factors have been found to determine microbial community composition (Haack et al., 1995; Zhou et al., 2002; Fierer et al., 2003; Fierer and Jackson, 2006; Buyer et al., 2010; Brockett et al., 2012). The differences in extracellular phosphatase activities for the two soil bases described above (Fig.1.4) do not result from significantly different microbial communities. As the data show no direct link between microbial community composition and extracellular phosphatase activity, factors other than community composition must impact extracellular enzyme activity in these soils.

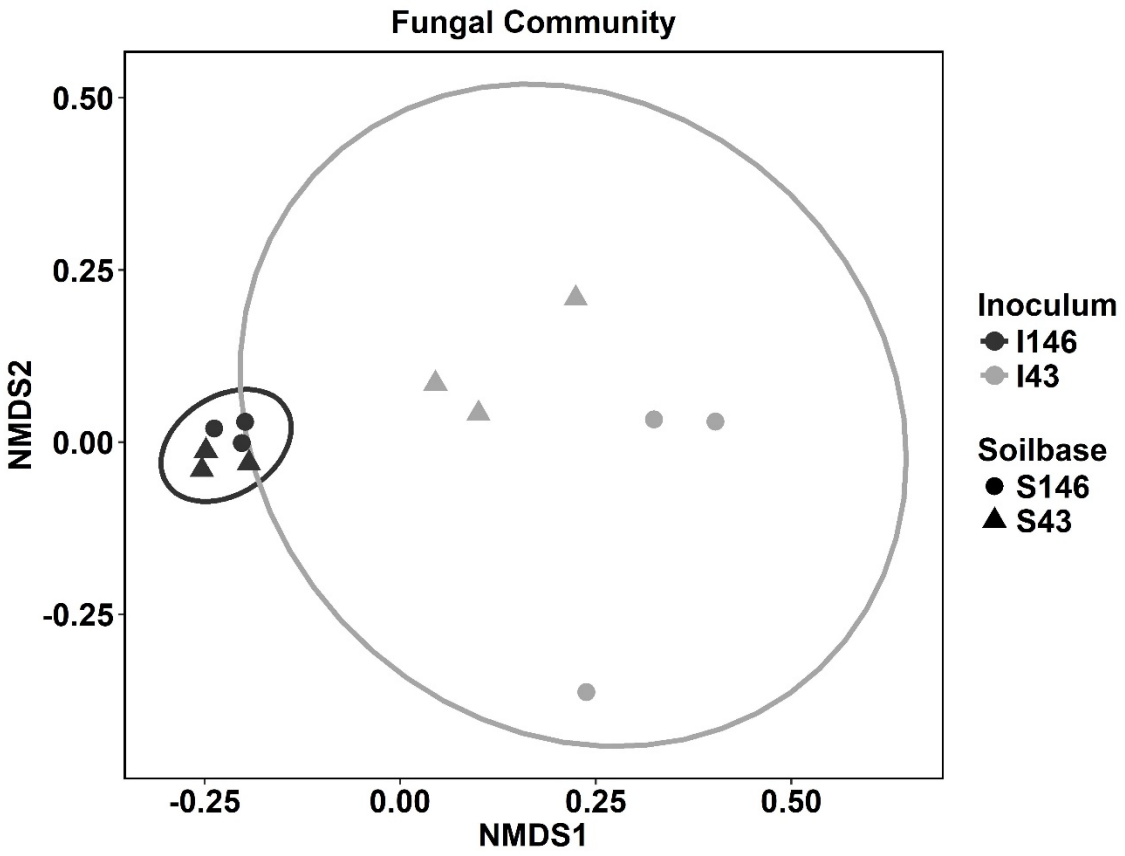

Figure 4-6: NMDS of fungal genus across the soil treatments

The two base soils in this study are chemically different (Fig.1.3) and factors including soil physico-chemical properties may influence the extracellular enzymatic activities of the inoculated soils (Narendrula-Kotha and Nkongolo, 2017). Pertinently, Schimel et al. (2007) have suggested that environmental stress can lead to large changes in physiological responses that may subsequently affect nutrient flow even when the microbial communities are not changing significantly.

The dominant classes of bacteria in the inoculated LSP soils, accounting for> 90% of the bacterial class composition, were gammaproteobacterial, alphaproteoba0cterial, actinobacteria, sphingobacteriia, betaproteobacteria, acidobacteriia, and planctomycetia (Fig. 1.7). For fungi, the

dominant taxa were tremellomycetes, sordariomycetes, mucoromycotina, and eurotiomycetes (Fig.1.8). The relative abundances of bacterial and fungal classes were found to be different for the soils inoculated with microbial communities. For example, on average, gammaproteobacteria were 65% higher in soil bases inoculated with 146 than with 43 (Fig.1.7) and soils inoculated by 43 exhibited more alphaproteobacteria and actinobacteria (26% and 24%, respectively, Fig. 1.7).

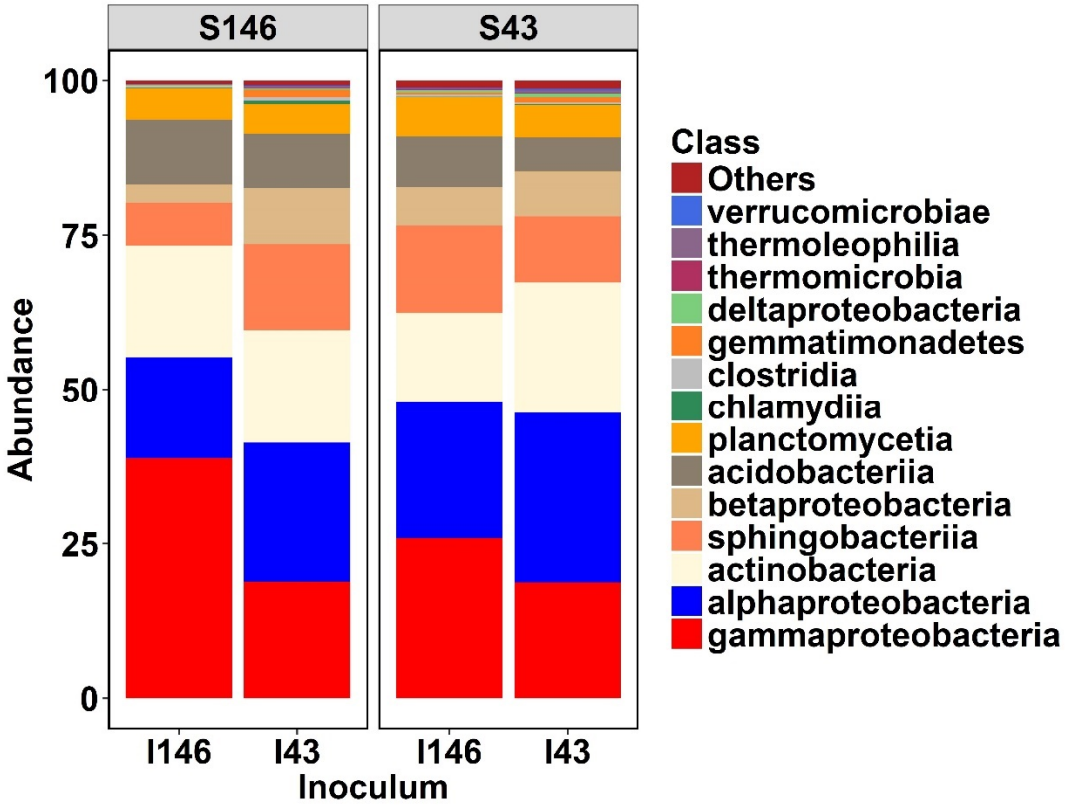

Figure 4-7: Relative abundance of bacterial classes across the soil and inocula treatments.

Sordariomycetes was found to be> 86% higher in soils inoculated with 146 than soil inoculated with 43 (Fig. 1.8). Tremellomycetes and mucoromycotina were found to be higher in soils inoculated with 43 than 146 (39% and 202%, Fig. 1.8).

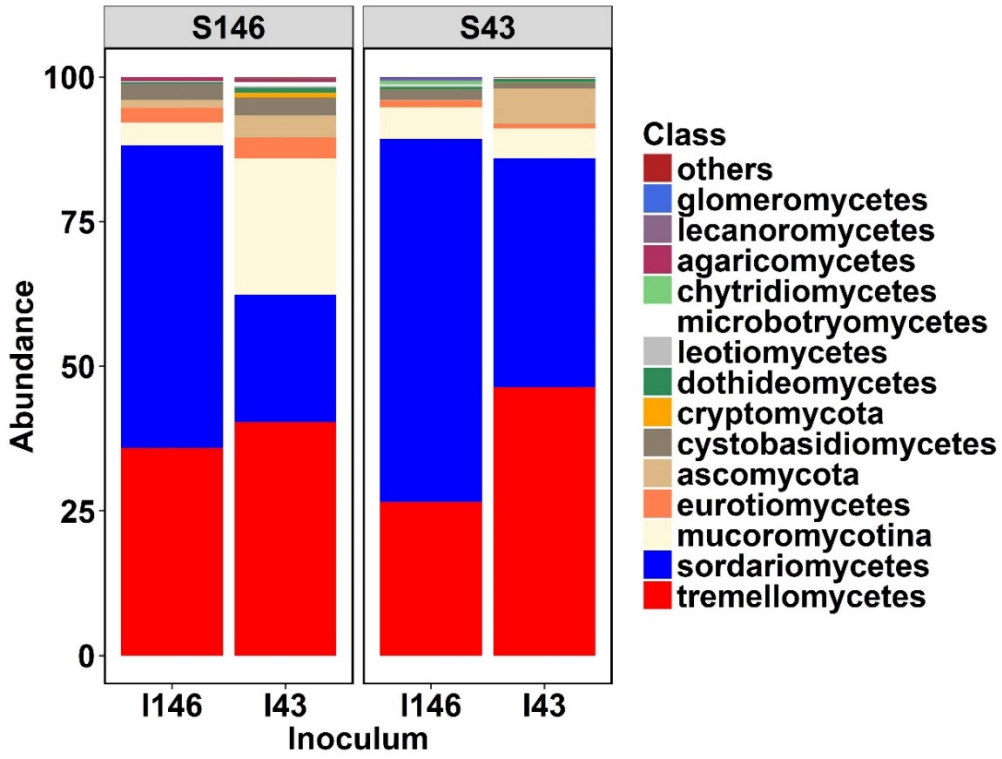

Figure 4-8: Relative abundance of bacterial classes across the soil and inocula treatments.

Microbes from the taxa Verrucomicrobia, considered metabolically active and abundant in uncontaminated soil (Janssen, 2006; Brewer et al., 2016; Buckley and Schmidt, 2001; Dunfield et al., 2007; Hou et al., 2008; Sangwan et al., 2005), were almost absent in LSP soils, accounting for less than 0.4% of the total microbial community composition. Recent studies suggest the taxa includes species of microorganisms that follow methanotropic metabolism (Dunfield et al., 2007; Hou et al., 2008). These differences in end-point microbial communities resulting from different inocula are expected, but it is surprising that the extracellular phosphatase activities do not vary as a result. The differences in abiotic conditions likely impact the functional outcomes of the microbial community. Moreover, a portion of the community may consist of ruderal species, which tend to thrive first in a disturbed situation like the one in our experiment. Grime's (1997) adaptation of stress tolerator-competitor-ruderal model in soil microbiology by Fierer (2017)

suggests that ruderal species provide little or no function to the soil, possibly further contributing to why differences in microbial community composition in our data do not coincide with differences in phosphatase activity.

We found that bacterial genus level diversity across soil base ($F = 9.30$; $p < 0.05$) and inoculum ($F = 23.12$; $p < 0.005$) from site 146 was significantly lower (Fig. 1.9).

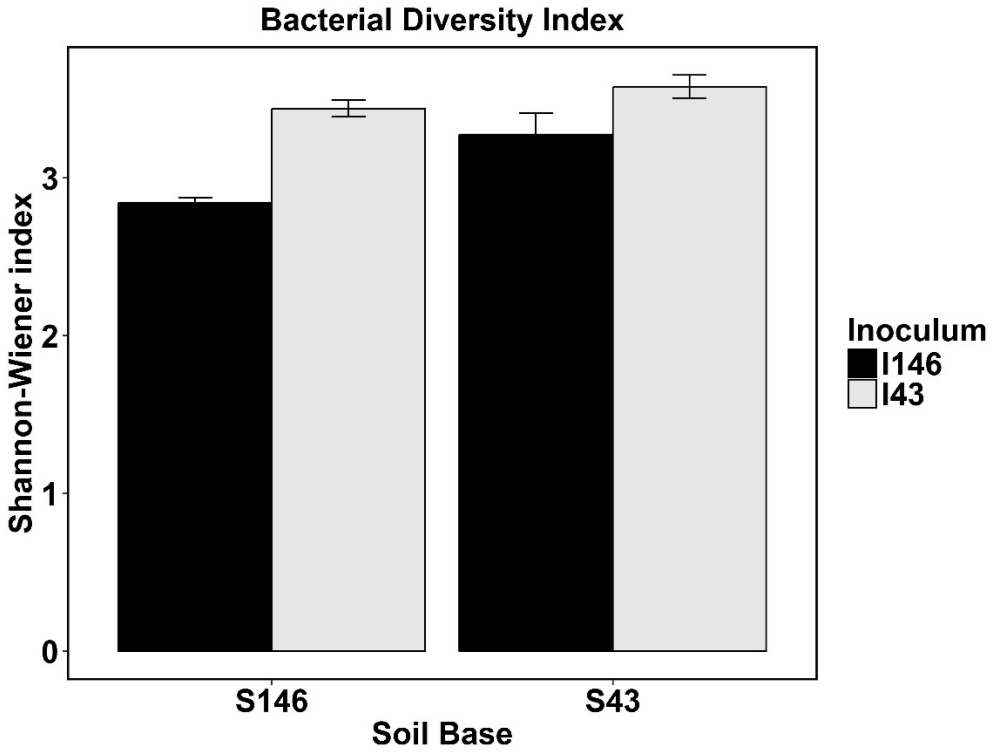

Figure 4-9: Shannon-Wiever diversity index of bacterial genus.

Further analysis of fungal diversity reveals that when experimental pots were inoculated with soil slurry from site 146, the genus diversity was significantly lower than when inoculated with slurry from 43 ($F = 16.695$; $p < 0.005$; Fig.1.10)

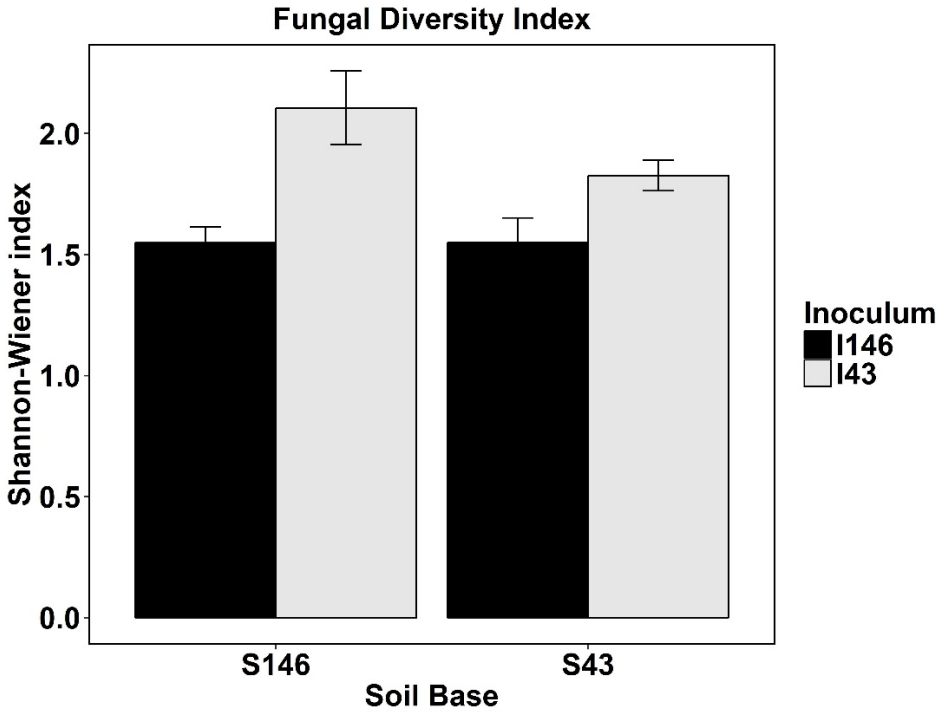

Figure 4-10: Shannon-Wiever diversity index of fungal genus.

Fungal diversity was not different between the two soil bases ($F=1.83$, $p > 0.05$) (Fig. 1.10). The results are similar to those of Calderón et al. (2017) who found that phylogenetic diversity reduced when a microbial community was re-introduced after disrupting the native microbial community composition. In the current study, soil samples that exhibited reduced diversity also displayed high extracellular soil phosphatase activity, which is believed to increase phosphate cycling in soil. The inverse relationship could be associated either with ruderal species (Fierer, 2017) or competition across species (Maynard et al., 2017). An increase in phosphate cycling leads to increased plant productivity (Speir and Cowling, 1991). Increasing primary productivity can be especially useful in contaminated sites where revegetation can stabilize and phytoremediate heavy metals and other organic pollutants (Ali et al., 2013; Salt et al., 1995; Schnoor et al., 1995; Dudka and Adriano, 1997; Raskin and Ensley, 2000; Pérez-Esteban et al., 2014).

4.5 CONCLUSION

In conclusion, in the metal-contaminated soils studied here, soil phosphatase activity increased with microbial whole community inoculation, but the increase was contingent upon the soil base and not the composition of the microbial community inoculated. Soil extracellular enzymes such as phosphatase are thought to predominantly originate from soil fungi and bacteria (Gadd, 2007). Here, the increase in phosphatase activity was not dependent on the source or identity of the microbial inocula introduced, but phosphatase supports a fundamental metabolic function that is not likely tied to specific microbes. In summary, the data indicate that the outcome of microbial inoculation in terms of phosphate cycling depends on soil abiotic properties but that the microbial community composition is driven by the inoculum introduced. This finding implies the absence of a link between microbial community composition and phosphatase activity on the time-scale of this experiment in our soils. This result has implications for understanding the ecology and remediation of contaminated soils. Each contaminated system is unique in physico-chemical properties and flora and fauna, and successful remediation approaches rely on an understanding of these differences. Whole community inoculation can be a rapid and inexpensive tool to improve soil function such as phosphate cycling, but the degree to which soil function is enhanced will depend on abiotic soil properties. As long as the microbial community introduced is resistant to the contamination in the base soil, the community composition of the microbial inoculum introduced may be less important for the resulting phosphatase activity than the abiotic properties of metal contaminated base soil.

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5 EFFECT OF MICROBIAL INOCULATION ON TRANSLOCATION OF CHROMIUM FOR SOIL TO SWITCHGRASS

5.1 INTRODUCTION

Heavy metal contamination of soil has been a major concern because of its toxicity towards human beings, animals and plants. Soil being a primary reservoir of heavy metal plays a significant role in heavy metal cycles (Liu et al., 2013). Heavy metals cannot be degraded so it continues to accumulate in soils which when exposed to humans is damaging to their health. Due to the hazardous nature of heavy metals, remediation or decontamination of soil becomes an absolute necessity. Ex situ methods have been employed for heavy metal clean-up which strip the heavy metals of soil. Established ex-situ methods such as thermal processes, physical separation, electrochemical methods, washing, stabilization/solidification and burial are very expensive and disrupt the normal microbial flora and the existing ecosystem (Rajkumar, Sandhya, Prasad, & Freitas, 2012).

Various amendments have also been utilized either to immobilize the heavy metal or to solubilize them and make them available to plants that can accumulate heavy metals. Synthetic chelators such as ethylenediaminetetraacetic acid (EDTA), ethylenetrinitrilopentaacetic acid (ETPA), have been used to enhance metal solubility during phytoextraction. There are certain limitations to the above method too. Soil amendments can be phtyotoxic and cause stress to the plants. These amendments can also be toxic to the soil's microbial flora and ultimately affect the health of the soil. In addition to that, the chelators rapidly solubilize the heavy metal which may lead to leaching of chelator-heavy metal complexes to the ground water and contaminate it (Punamiya et al., 2010)

An alternative could be low input long term and sustainable remediation approaches (ITRC, 2009). These sustainable approaches include the use of plants, fungi, and bacteria to increase soil function and mitigate risks from the contaminants (Cundy et al., 2016; Megharaj and Naidu, 2017). These approaches leverage the use of soil-plant-microbe interaction to aid movement of metal contaminants from the soil to harvestable parts of a plant.

In this study, we focus on harnessing soil-plant-microbe interaction to examine the potential of switchgrass to phytoextract chromium from soil. Chromium, a known carcinogen, is in the priority list of chemicals of ATSDR. It interferes with cell signaling and causes cell death (Wu et al. 2013). Chromium is also one of the major contaminants at LSP, our study site. We choose switchgrass as a test plant since it is found at LSP and is native to the United States It is also known to uptake Chromium. Moreover, switchgrass has dense and fibrous root allowing ample surface area for soil-plant-microbe interaction.

The main objective of the paper is to study the effect of microbial inoculations on switchgrass productivity and its potential to extract chromium in a chromium-spiked soil. Specifically, we investigated the translocation of chromium from soil to above and belowground biomass of switchgrass.

5.2 METHODS

5.2.1 STUDY SITE

We collected soil for our mesocosm experiment from Liberty State Park (LSP). LSP, an urban brownfield, is contaminated with a suite of heavy metals such as vanadium, chromium, arsenic, copper, lead, and zinc (Hagmann et al. 2015, Singh et al. 2019a). LSP originally was a low lying marshland, which was filled with construction debris from New York City to build a railway yard in the early 1900s (Gallagher et al. 2008). The railway yard operation began in the 1920s

and continued until the late 1960s. The railway yard was used for storage and transportation of several goods, which led to heavy metal contamination of the site (Gallagher et al. 2008). The site was left abandoned after the railway yard ceased its operations in the 1970s. While a section of the site was remediated by soil capping, approximately 100 hectares was fenced off and restricted for public access (Gallagher et al. 2011). The undisturbed and heavy metal contaminated 100 hectares of land showed signs reclamation and a dense early successional vegetation developed at LSP (Gallagher et al. 2011).

5.2.2 SOIL COLLECTION

We collected soil from a pre-determined site at LSP (LSP 146), which is not only contaminated with approximately 100 ppm chromium contamination but also exhibits high enzymatic activity (Hagmann et al. 2015, Singh et al. 2019a). We collected soil samples from the top 10 cm along a transect at five different pins that were four meters apart. We passed the bulk soil through 2 mm sieve to remove larger plant debris and rocks and mixed them make a composite sample. We stored the soil at 4 °C until the start of the experiment.

5.2.3 EXPERIMENTAL DESIGN AND SET UP

We sterilized the sieved soil by autoclaving to denature enzyme and disrupt the microbial community. We divided the autoclaved soil into four treatment groups, each weighing at 750 grams. We spiked three groups with potassium dichromate solution but left one group unspiked (we call it level one; table 1). We measured the final concentration of the four treatment group and found it to range from 102.34 ± 1.33 ppm to 214.68 ± 7.706 ppm. Table 1 represents the concentration of four groups of chromium spiked soil. After spiking the soil, we used 125 gm of soil from each treatment to set up 24 pots (six pots per treatment * four treatments = 24 pots). We then inoculated half of all the pots (three pots per treatment * four treatments = 12 pots) with

five ml inoculum prepared from unsterilized LSP 146. We prepared the inoculum by suspending 10 g of unsterilized soil in 100 mL of DI water (10% weight/volume). The other 12 pots were left uninoculated. Instead, we added 5 ml of sterile DI water to all the pots. After the pots were set up as per experimental design, we transplanted two seedlings of switchgrass in each of the 24 pots. For transplantation, we grew switchgrass from seeds provided by USDA (germplasm accession number - PI 422006) on an uncontaminated soil for four weeks. We then selected switchgrass seedlings that were 4 inches tall for transplantation. After choosing 48 switchgrass seedlings, we surface sterilized the selected seedlings with H2O2/ethanol mixture (Kereszt et al. 2007). We then transplanted two seedlings per pot (2 seedlings * 24 pots = 48 seedlings). After setting up the pots, we kept them in a climate-controlled chamber, maintaining a temperature of 24 °C during day and 19 °C at night and a photoperiod of 16/8 h day night cycle. The relative humidity was 65%, and lighting intensity was 550 μmoles/m2/sec during daytime. We maintained moisture in the pots by adding 10 mL sterile water three days a week. We allowed the switchgrass to grow for eight months. After eight months, we harvested the switchgrass plants and measured the above ground and below ground biomass. We also quantified the chromium concentration in the harvested soils and plant tissue. Further, we analyzed the microbial community composition of the rhizosphere using amplicon sequencing. Finally, we measured the phosphatase activity of the soil collected from the rhizosphere of the harvested plants.

Table 5-1: Chromium concentration in spiked soil

Chromium treatment group	**Chromium concentration (ppm)**
Level one	102.34 ± 1.33

Level two	130.26 ± 2.77
Level three	145.80 ± 0.71
Level four	214.68 ± 7.706

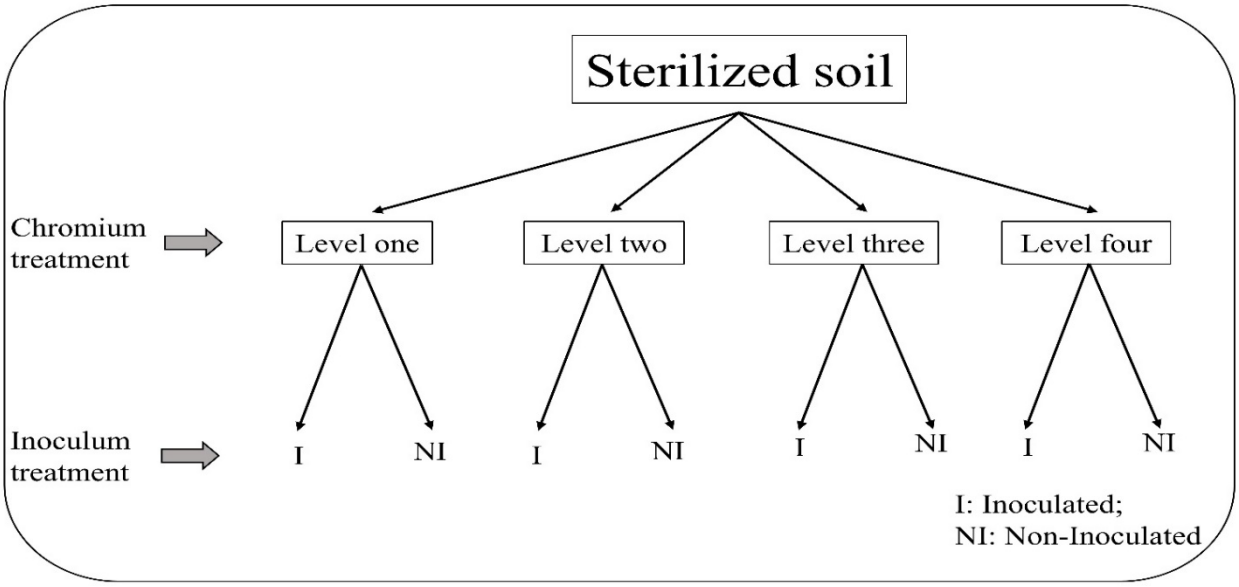

Figure 5-1: Experimental design showing four levels of chromium treatment and two levels of inoculum treatment. Each treatment combination was replicated three times (n=3).

5.2.4 DNA EXTRACTION AND SEQUENCE ANALYSIS

We studied the bacterial community of the switchgrass rhizosphere by analyzing the 16S rRNA gene amplicons. We extracted DNA from the 0.25 g of soil attached to the switchgrass root using the MoBio PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's instructions. We stored the extracted DNA at -22 ℃ before sending it to the Molecular Research DNA laboratory (MR DNA, Shallowater, TX) for DNA sequencing and bioinformatic analysis. The V4 region of the 16s rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers 515f/806r. Following the PCR step, the PCR

products were barcoded, and equimolar concentrations of amplicons from all samples were consolidated and sequenced on the Ion Torrent PGM system using the manufacturer's guidelines at MR DNA. The sequences were processed using MR DNA's proprietary pipeline. Briefly, the sequences were demultiplexed, and the barcodes and primer sequences were removed. Sequences with ambiguous base calls and with homopolymer run exceeding 6 bp and sequences<150 bp were also removed. Sequences were then denoised, and operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity) followed by removal of singleton sequences and chimeras. Final OTUs were taxonomically classified using BLASTN against a curated database derived from GreenGenes, RDPII, and NCBI (DeSantis et al. 2006).

5.2.5 PLANT BIOMASS MEASUREMENT

We measured the above and belowground plant biomass after destructively harvesting the experimental pots. We separated the belowground biomass from the aboveground biomass and washed it thoroughly with DI water to remove soil particles. We dried the above and belowground biomass in a hot air over at 70 ℃ for seven days. After drying the samples, we measured the aboveground and belowground biomass. After recording their weights, we used 0.5 g of plant tissues to quantify the chromium content in switchgrass using inductively coupled plasma mass spectrometry (ICP-MS).

5.2.6 CHROMIUM QUANTIFICATION IN SOIL AND PLANT TISSUES

We quantified the metal concentrations of the harvested soil samples using ICPMS employing the EPA 3050B method. Briefly, we digested 0.5 gm of soil with 10 mL 50% HNO_3 (15.7 M, ACS plus certified) at 95 ± 5 °C for 15 min and allowed it to cool. After cooling, we refluxed 5 mL of 15.7M HNO_3 at 95 ± 5 °C for 30 min and repeated the steps until white fumes replaced

the brown fumes. We maintained a constant heat of 95 ± 5 °C without boiling until the volume reduced to 5 mL. Subsequently, we cooled the samples and added 2 mL of deionized water and 3 mL of H2O2. We reheated the samples and added more H2O2 until the effervescence stopped. We then diluted the samples to a final volume of 50 mL and filtered using 0.4 μm filters to remove any soil particles. We further diluted the samples by a factor of 100 with 1% HNO3 and quantified the metals on ICP-MS. For measuring chromium in plant samples, we kept the 0.5 g of plant tissue in 10 ml of 50% HNO3 (15.7 M, ACS plus certified) for 12 hours at room temperature before using EPA 3050B method. After digesting the plant samples, we used the same dilution factor to dilute the samples and analyzed them in the ICP-MS.

5.2.7 MEASUREMENT OF EXTRACELLULAR PHOSPHATASE ACTIVITY

We measured acid phosphatase activity of the harvested soil samples. Phosphatase activity is sometimes used as a proxy for soil function because it is a fundamental metabolic process that captures the functioning of active microbial taxa (Nannipieri et al. 2011). We employed the method from Hagmann et al. (2015) for phosphatase activity measurement. Briefly, we suspended approximately 0.1 g of soil in 100 mL 0.1M 2-(N-morpholino ethanesulfonic acid (MES) buffer and sonicated at an output of 25W for 3 min. Subsequently, we added 160 μl of sample suspension along with 40 μl of the 350 μM fluorescent analog substrate (4-methylumbelliferyl-phosphate) to a 96 well plate. Concurrently, we generated a standard curve on the same 96 well plate using four different concentrations (0, 500 μM, 1000 μM, 1500 μM, and 2500 μM) of the product (4- methylumbelliferone). We then measured the fluorescence of the samples and the standard curve in a plate reader at 320 nm excitation and 450 nm emission at 30 °C with every measurement taken 7.5 minutes apart over three hour period. We calculated the

phosphatase activity using the change in product concentration and normalized it over unit dry weight of soil.

5.3 DATA ANALYSIS

We recorded several responses to investigate the effect of inoculum and chromium treatment on proxies for plant-soil-microbe interaction. Our responses were bacterial community composition, Shannon diversity index, phosphatase activity in the soil, aboveground and belowground biomass, chromium concentration in above and belowground biomass, and bioconcentration factor of chromium in above ground and belowground biomass. We ran a principal coordinate analysis on the relative abundance of the genus to visualize the bacterial community composition in an ordination scale. We used “vegan” and “ape” packages to run the principal coordinate analysis. To visualize the bacterial community composition, we used “ggplot2” from the “tidyverse” package in R studio. We also carried out permanova to analyze the differences in bacterial community structure between inoculated and non-inoculated groups. To investigate the effect of inoculum and chromium treatments on Shannon diversity index, phosphatase activity, aboveground and belowground biomass, chromium concentration in above and belowground biomass, and bioconcentration factor of chromium in above ground and belowground biomass, we used a two-way anova analysis. We used Shannon diversity index, phosphatase activity aboveground and belowground biomass, chromium concentration in above and belowground biomass, and bioconcentration factor of chromium in above ground and belowground biomass as our responses and inoculum and chromium treatments as the predictors. All data analysis is done in R studio.

5.4 RESULTS AND DISCUSSION

We measured phosphatase activity as a proxy for soil enzyme function and analyzed using two-way anova. Extracellular soil phosphatase activity was our response variable with chromium and inoculum treatment as the two independent variables. We found no significant difference in phosphatase activity for either inoculum treatment (Fig. 2, F = 1.1.25, p > 0.05) or chromium treatment (Fig. 3, F = 2.79, p > 0.05). We have previously shown that phosphatase activity is dependent on the soil base used regardless of the inoculum treatment (Singh et al. 2019a). Therefore, phosphatase activity in the same soil base would exhibit similar activity. As such, we do not see any significant differences in phosphatase activity between inoculum and chromium treatment.

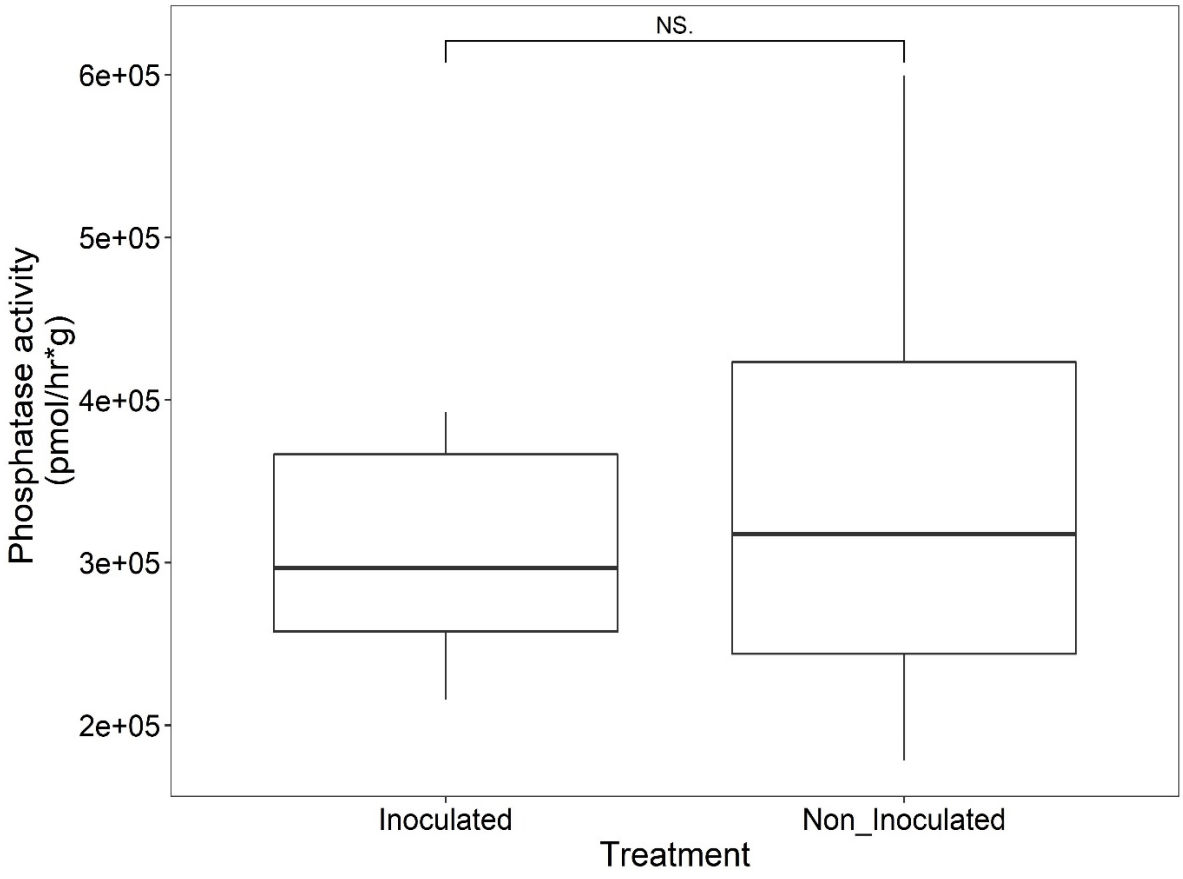

Figure 5-2: Phosphatase activity does not show any significant difference between inoculated and non-inoculated treatment. Box-whisker plot shows the median and the spread of data. (n=12)

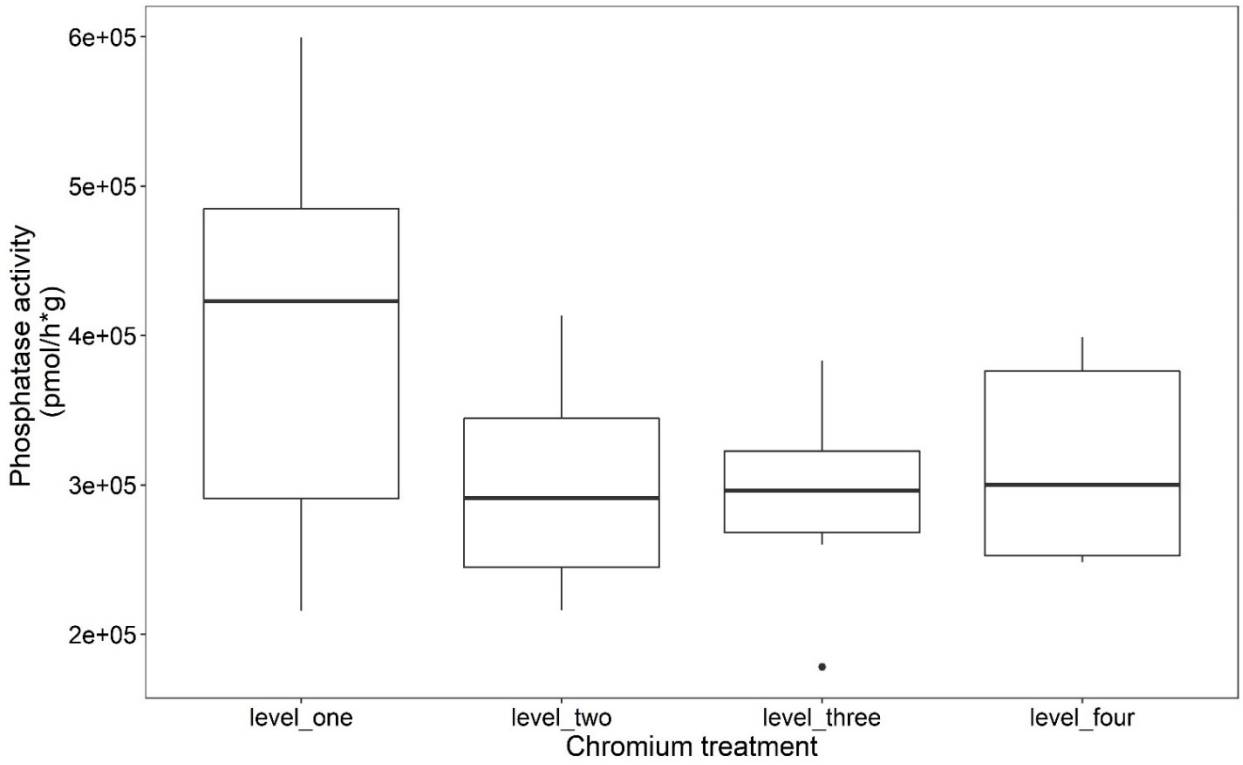

Figure 5-3: Phosphatase activity does no show any significant difference among different level of chromium. Box-whisker plot shows the median and the spread of data. (n=6)

To ensure that our bacterial inoculation was able to colonize, we analyzed the bacterial community composition of the soil samples. We used principal coordinate analysis to visualize the bacterial community (Fig .4). We found that while the first principal coordinate explained 53.63 % of the variation, the second principal coordinate explained 12.05 % of the variation.

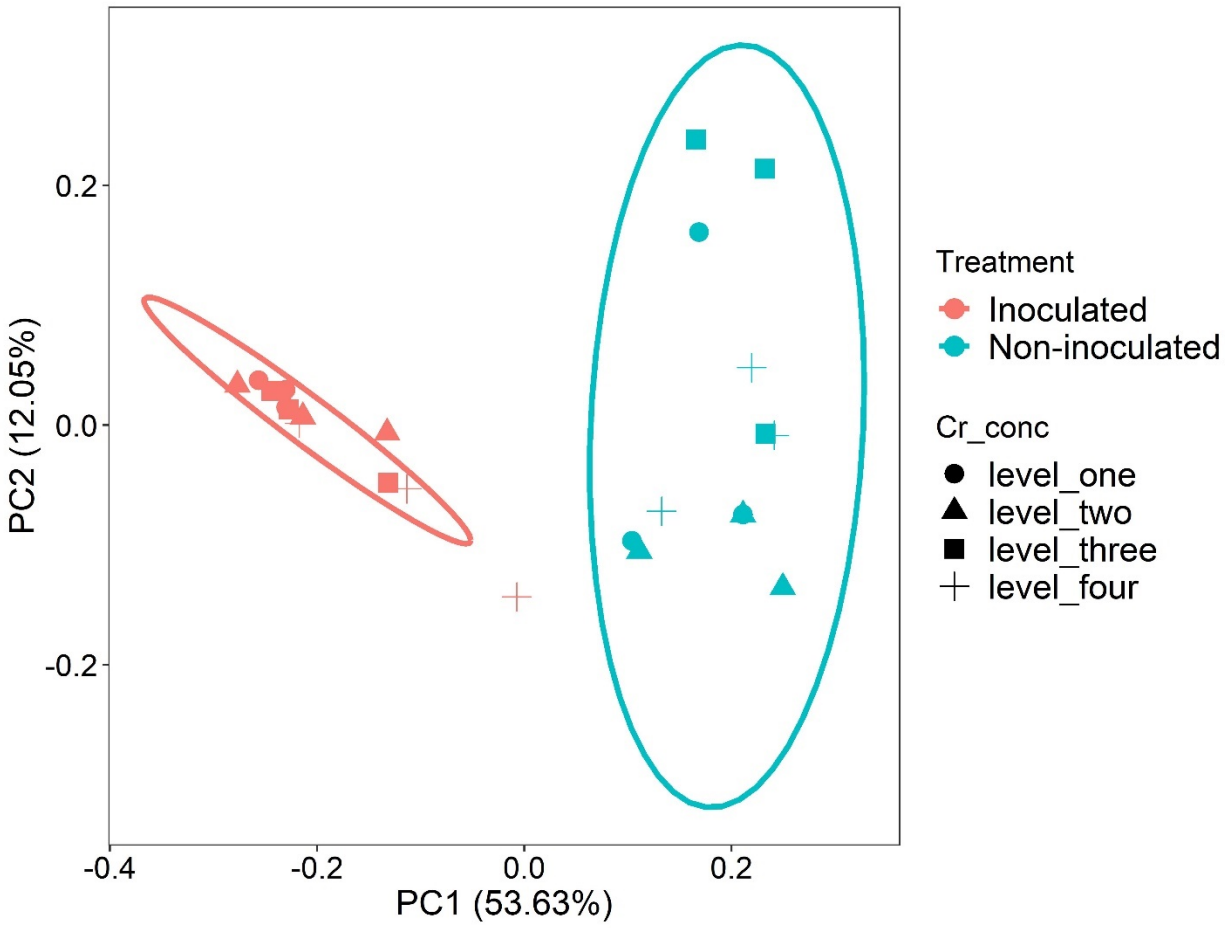

Figure 5-4: Principal coordinate analysis representing the differences the bacterial community between the inoculum treatments. PC1 explains 53.63% of the variation associated with the data, while PC2 explains 12.05% of the variation.

We subsequently used permanova to examine differences between the bacterial community. We compared bacterial community across inoculum and chromium treatment and found that the bacterial community was significantly different (pseudo $F = 0.09$, $p > 0.001$) across the inoculum. However, we did not see any significant difference (pseudo $F = 20.837$, $p < 0.05$). The significant difference between inoculum treatment indicates that the inoculation helped colonize native bacterial community in the inoculated experimental pots, while a random bacterial community was established in the non-inoculated experimental pots. Moreover, we did not

observe any significant difference in the bacterial community across chromium treatment suggesting that the chromium concentration did not impact the microbial community composition. In previous studies we have shown that bacterial community in soils at LSP did not significantly change with chromium contamination (Singh et al. 2019b). Moreover, other studies have hypothesized that continuous exposure to metal concentration may help microbial communities evolve to become resilient to contamination (Krumins et al. 2015). Therefore, it is possible that the bacterial community at LSP through decades of exposure to chromium have becomes resilient to chromium contamination. As such we do not see a significant impact of chromium treatment on the bacterial community.

We also calculated the Shannon diversity index to examine the diversity of bacterial community in our experiment (Fig 5). After performing two-way anova with Shannon diversity as the response variable, and chromium and inoculum treatment as independent variable, we found the model highly significant ($F = 15.79$, $p < 0.0001$). On further inspection, the model reveled inoculum treatment to be the significant independent factor with inoculated pots exhibiting significantly higher ($F = 108.5$, $p < 0.0001$) diversity than its non-inoculated counterparts. Here again, we did not see any significant effect ($F = 063$, $p > 0.05$) of chromium treatment on the Shannon diversity index. The higher Shannon diversity was expected in the inoculated pot as has been observed in past studies where microbial diversity increased with inoculation (Calderón et al. 2017, Gottshall et al. 2017, Molineux et al. 2017).

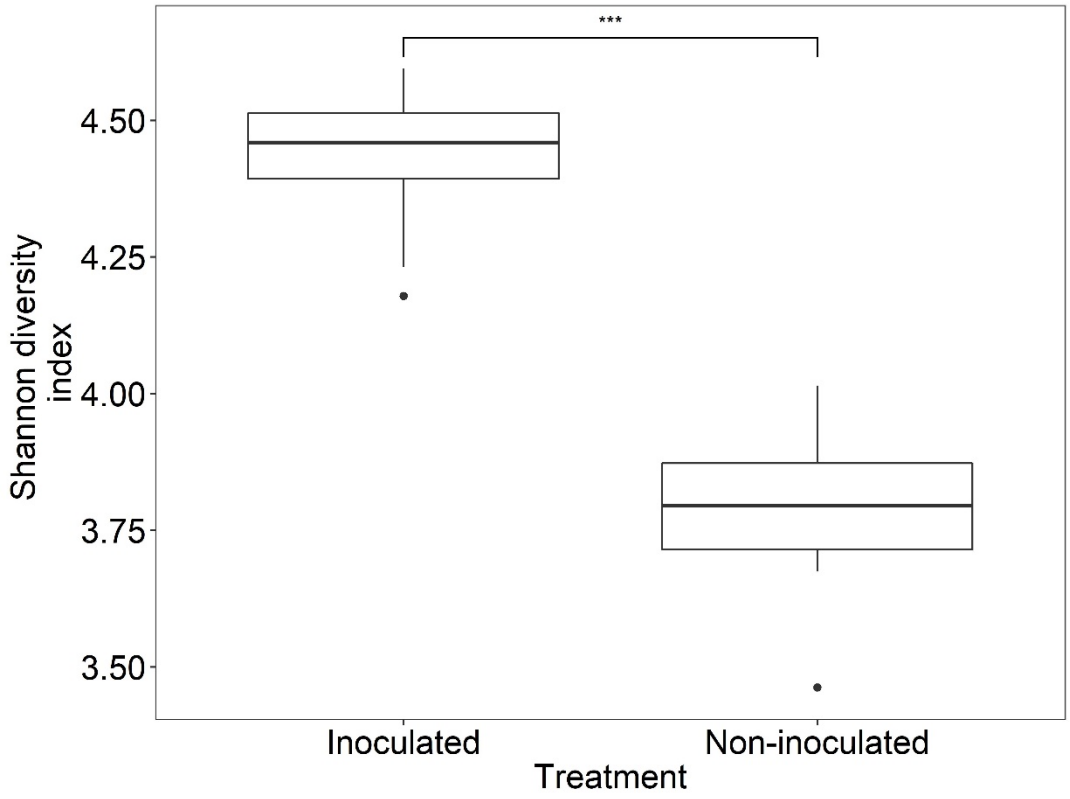

Figure 5-5: Shannon diversity is significantly higher in inoculated experimental pots compared to the non-inoculated pots. Box-whisker plot shows the median and the spread of data. *** represents p value less than 0.0001.

After harvesting the plants, we measure the shoot and root biomass of switchgrass. We used two-way anova to analyze the data with the root (or shoot) biomass as the dependent variable and the chromium and inoculum as dependent variables. We found our model to be significant ($F = 5.01$, $p < 0.005$). On further inspection, we found both inoculum ($F = 5.28$, $p < 0.05$) and chromium ($F = 7.69$, $P < 0.005$ treatment to be significant. Since we had four levels of chromium, we carried out a tukey test and found that level one of chromium treatment to be significantly lower than level three and level four of the treatment. The results indicate that experimental pot with

inoculuted soil had significantly high shoot biomass. We also found that treatment with higher level of chromium had higher biomass.

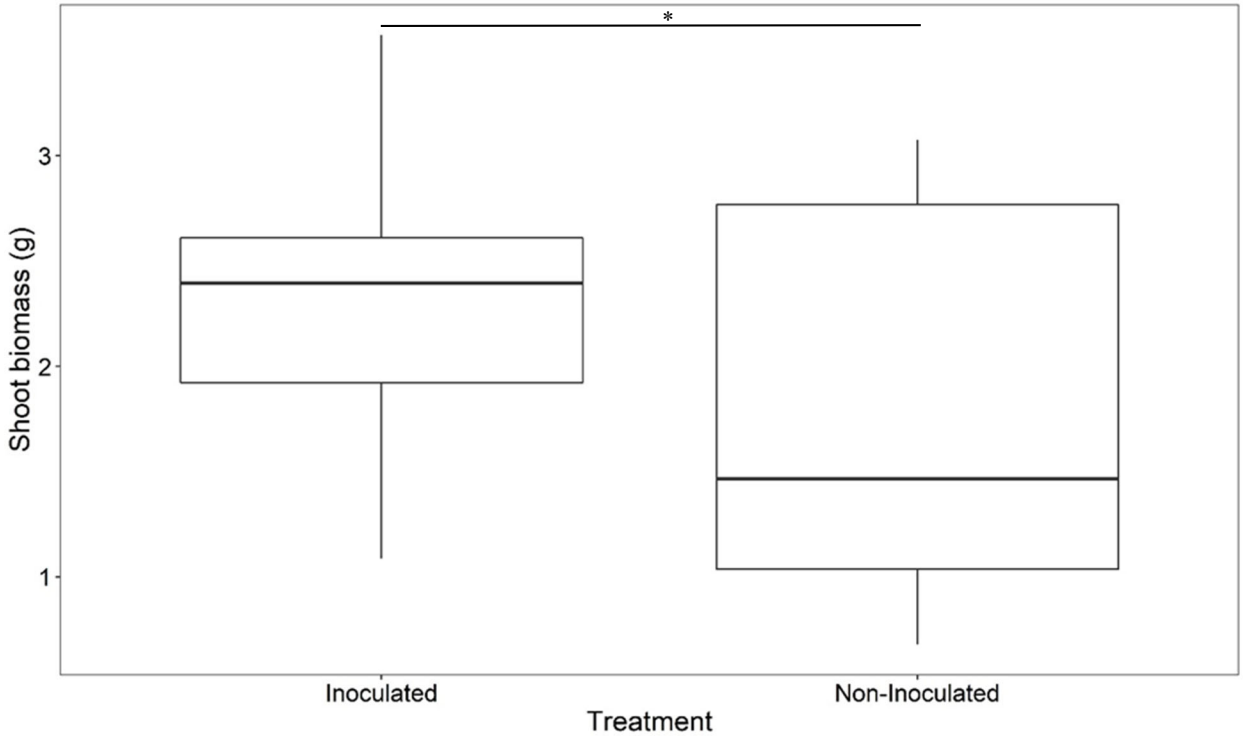

Figure 5-6: Inoculated experimental pot have significantly higher shoot biomass than their non-inoculated counterparts. Box-whisker plot shows the median and the spread of data. * indicate p value at 0.05

We did the same analysis on root biomass using the belowground biomass as the response variable and inoculum and chromium as the independent variable. We found the two-way anova model to be significant ($F = 9.86$, $p < 0.0001$). We also found inoculum ($F = 17.37$, $p < 0.001$) and chromium ($F = 13.81$, $p < 0.0001$) treatment to be highly significant. Root biomass in inoculated soil were significantly higher than the non-inoculated counterpart. We also ran a tukey test on the chromium treatment since we had four treatment levels and found the

experimental pots with the highest chromium concentration had significantly higher root biomass than level one and level two.

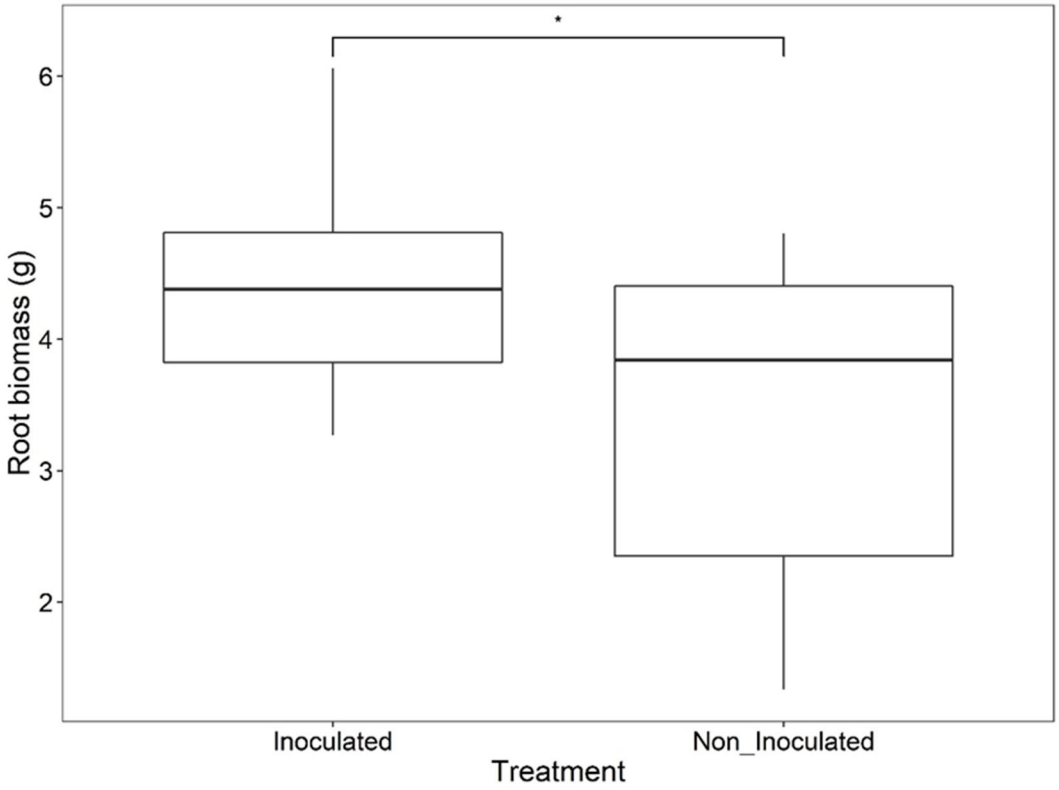

Figure 5-7: Inoculated experimental pot have significantly higher root biomass than their non-inoculated counterparts. Box-whisker plot shows the median and the spread of data. ** indicate p value at 0.005

We measured chromium concentration in above and belowground biomass. We use a two-way anova to compare chromium concentration in the biomass (response variable) across inoculum and chromium treatment (independent variables). We did not find any significant different in chromium concentration in root ($F = 3.73$, $p > 0.05$) and shoot ($F = 3.49$, $p > 0.05$) F across inoculum treatment. However, we found significant difference in chromium concentration in root ($F = 6.20$, $p < 0.01$) and shoot ($F = 6.26$, $p < 0.01$). Upon further examination, we found that

with chromium concentration in both root and shoot expectedly increased with chromium treatment. Our results are similar to Chen et al.(2012) research where chromium concentration in plants biomass increase with increase exposure to chromium.

We also measured the translocation of chromium from root and to shoot. This movement of metal from soil system to belowground and above ground biomass can be measured by translocation factor (TF)

$$TF_{shoot} = \frac{Chromium\ concentration\ in\ shoot}{Chromium\ concentration\ in\ root}$$

$$TF_{root} = \frac{Chromium\ concentration\ in\ root}{Chromium\ concentration\ in\ soil}$$

We used a two anova analysis to compare the shoot and root TF under different inoculum and chromium treatment. We did not find any significant difference in translocation of chromium from root to shoot ($F = 1.5$, $p > 0.5$). The result indicated that movement of chromium from the roots to shoots of switchgrass across inoculum chromium treatment are same. However, we found significant difference in movement of chromium from soils to the roots. We found that there was significant higher ($F = 6.12$, $p < 0.05$) chromium translocation from soil in uninoculated soils. We did not find any significant difference in chromium translocation across different chromium treatment. It was interesting to see that the soil that were uninoculated had higher translocation of chromium to root while translocation in inoculated had significantly lower translocation.

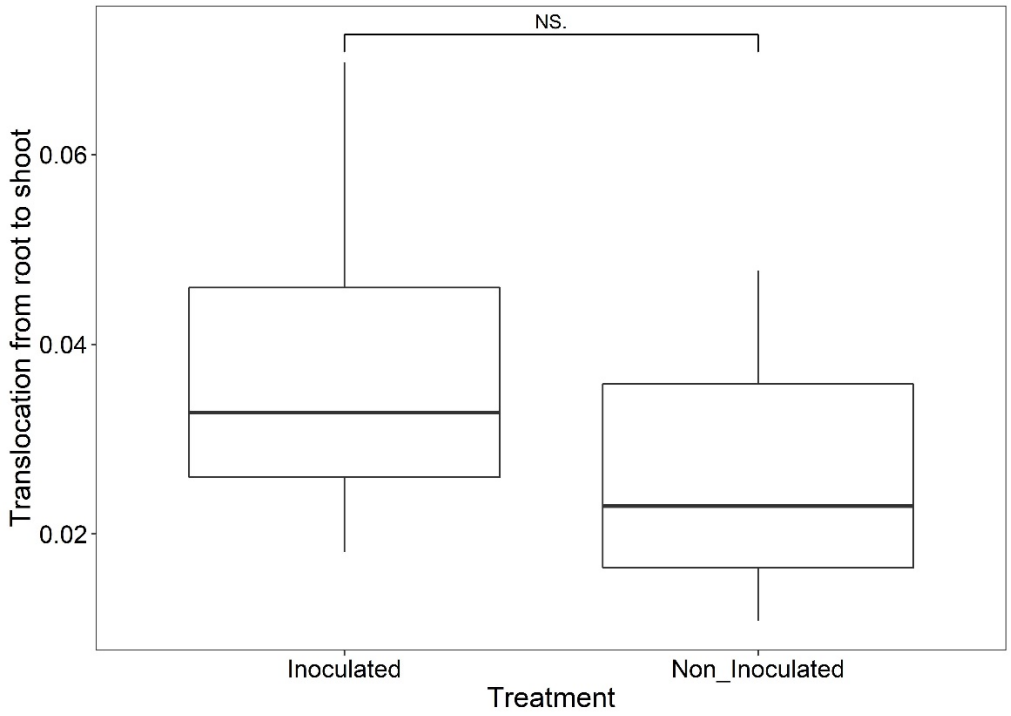

Figure 5-8: Translocation of chromium from root to shoot between inoculum treatments is not significantly different. Box-whisker plot shows the median and the spread of data.

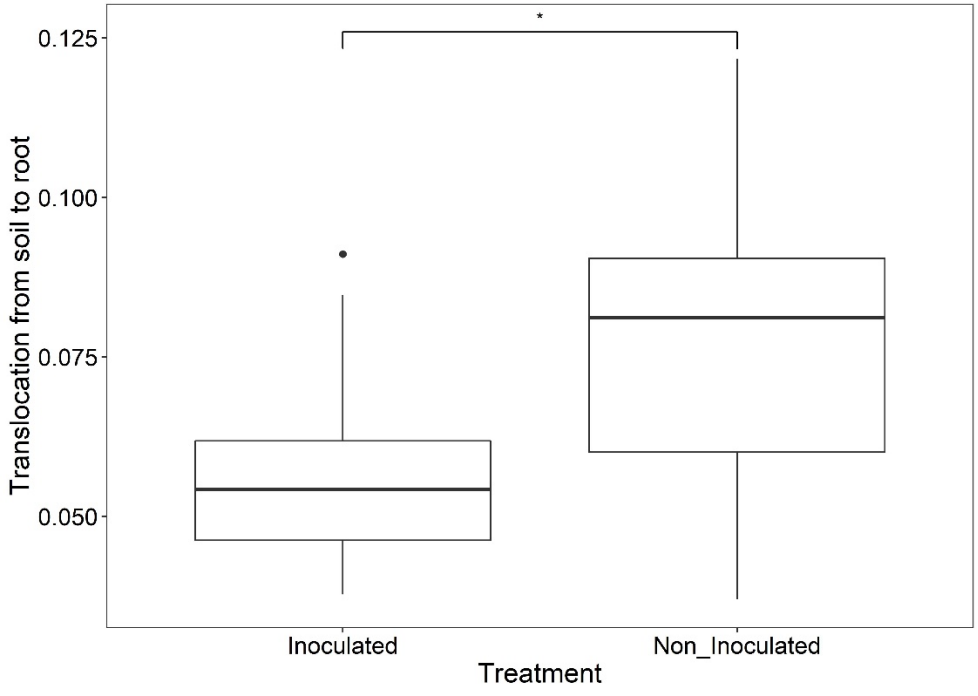

Figure 5-9: Translocation of chromium from soil to root between inoculum treatments is significantly different. Box-whisker plot shows the median and the spread of data. * indicate significance level at 0.05

Previous studies have shown that microbes impact chromium movement to plants (Juwarkar and Jambhulkar 2008, Braud et al. 2009, Chatterjee et al. 2009). Studies have shown that chromium movement to plants can be reduced (Cervantes et al. 2001), which is also seen in our results. This reduction can be attributed to binding of chromium with liposaccharides, cell wall or capsules of microorganisms present in soil (Snyder et al. 1978, Flemming et al. 1990, McLean et al. 1990). This reduction in mobility of chromium from soil to switchgrass root potentially impacted the biomass. Therefore, we saw a significantly higher aboveground and belowground biomass in inoculated treatment.

5.5 CONCLUSION

The results from the study sheds important light into the plant-microbe interaction. We found that bacterial community at LSP potentially alleviates metal stress, thereby increasing the biomass of the plant. We see significant increase in both aboveground and belowground biomass which could potentially improve overall productivity of in contaminated sites such as brownfields. Improving biomass is known not only to stabilize metal contaminants but also improves overall soil health. We also observed that inoculation treatment reduced translocation of chromium in roots. Bioaugmentation of sites with microbes from sites such as LSP (that have shown signs of recovery) can boost revegetation efforts, which is turn can improve green space in urban areas. Such gentle remediation efforts can improve biodiversity and overall health of soil.

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6 CONCLUSION

Natural attenuation of disturbed sites by microbes is possible through bioaugmentation (Wubs et al., 2016). Disturbed ecosystems such as brownfields can be rehabilitated using microbes and plants. With this dissertation, we have shown that soil inoculation can improve soil phosphatase activity but within the context of abiotic soil properties. Therefore, a greater understanding of soil, microbes, and contaminants is required. Our study provides critical insights into the soil function and microbial ecology of a metal contaminated soil system. We have also shown some heavy metals have a strong association with microbial community composition and likely functioning. Our results have applications in management and planning of brownfield sites, which can help mitigate risks posed by the contaminated sites. This information could be leveraged to delve deeper into microbial responses to different heavy metal contaminants, and whether the ability of microbes to survive and thrive in stressed environments can be harnessed to ameliorate the effects of heavy metals to reclaim and restore damaged ecosystems.

We also show that soil enzymatic activity (particularly soil phosphatase activity) increased with microbial whole community inoculation, but the increase was contingent upon the soil base and not the composition of the microbial community inoculated. We also show the increase in phosphatase activity, an indicator of soil health, was not dependent on the source or identity of the microbial inocula introduced, but phosphatase supports a fundamental metabolic function that is not likely tied to specific microbes. The outcome of microbial inoculation in terms of phosphate cycling depends on soil abiotic properties but that the microbial community composition is driven by the inoculum introduced. Results from the dissertation have implications for understanding the ecology and remediation of contaminated soils. Each contaminated system is unique in physico-chemical properties and flora and fauna, and

successful remediation approaches rely on an understanding of these differences. Whole community inoculation can be a rapid and inexpensive tool to improve soil function such as phosphate cycling, but the degree to which soil function is enhanced will depend on abiotic soil properties.

With the research, we have also provided critical insights into the mechanism of translocation of metal in switchgrass. We found that the switchgrass biomass increased when we used native bacterial community. We see significant increase in both aboveground and belowground biomass which could potentially improve not only the stability of contaminated soil but also bioremediation potential. We also observed that inoculation treatment reduced the concentration in roots

With urbanization threatening biodiversity and ecosystem productivity, contaminated sites such as brownfield can be re-established with vegetation with help from bioaugmentation and soil management practices. Such practices can be beneficial to the urban community and human health in general. Gentle remediation options can not only restore contaminated site but also improve the green space within urban concrete jungle. It improves the quality of soil as well as biodiversity with derelict sites.

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