UNIVERSITY OF CALIFORNIA
RIVERSIDE

Phyto-Mycoremediation of Metals-Contaminated Brownfields in Southern California

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Environmental Toxicology

by

Danielle Stevenson

December 2023

Dissertation Committee:
Dr. Samantha Ying, Chairperson
Dr. Jason Stajich
Dr. Ying-Hsuan Lin
Dr. Emma Aronson

The Dissertation of Danielle Stevenson is approved:

_____

_____

_____

_____

Committee Chairperson

University of California, Riverside

ABSTRACT OF THE DISSERTATION

Phyto-Mycoremediation of Metals-Contaminated Brownfields in Southern California

by

Danielle Stevenson

Doctor of Philosophy,
Graduate Program in Environmental Toxicology
University of California, Riverside, December 2023
Dr. Sam Ying, Chairperson

Soil contamination with metals and metalloids is a serious environmental and public health issue. Contaminated sites cover 22 million acres in the United States and 96,000 acres in California in California, exposing nearby communities to metals and other contaminants via entrained dust, surface water and groundwater. Conventional remediation involves excavation, capping, and disposal of contaminated soils, but is cost prohibitive and has environmental and social downsides. Biological remediation utilizes plants, fungi, and microbes to remediate soils and is more cost-effective than soil excavation plus has additional environmental and social co-benefits. However, bioremediation strategies using combinations of plants, especially native plants, and fungi, have not been tested in field conditions, particularly in semi-arid/arid regions.

In this dissertation, a series of studies were carried out to explore the role of arbuscular mycorrhizal fungal-plant systems in metals dynamics in bioremediation systems using a multi-scale, field and greenhouse-based approach including a field

survey of plant and microbial life on seven contaminated sites in Southern California, a controlled greenhouse pot experiment identifying controls on plant-AMF chromium (Cr) uptake under drought conditions, and a field-based phyto-mycoremediation study on three contaminated sites (brownfields) in Los Angeles.

In Chapter 2, we report the findings from a field survey of contaminated sites throughout Southern California, where several California native plant accumulators of metals such as lead (Pb), chromium (Cr), copper (Cu), arsenic (As) and nickel (Ni), were identified. Microbial taxa previously identified as contaminant remediators were seen at all study sites, as well as some yet untested taxa which may be candidates for use in bioremediation. Most of the variation in microbial community composition was explained by differences across sites; while water-extractable organic carbon, silt and sand content, and cationic exchange capacity were the most significant drivers of microbial community composition within brownfields. Our findings identified potential plant and fungal candidates for further exploration in bioremediation applications suited to Southern California's climate.

In Chapter 3, we provide results from a greenhouse study that investigated the interactions between AMF and Cr in *Medicago sativa* under two different soil moisture regimes, drought and optimal moisture, in unsterilized field soils collected from a contaminated brownfield. Chromium translocation to *Medicago sativa* shoots was reduced by AMF inoculation and significantly increased by soil moisture and soil organic carbon content. *Medicago* plants grown under drought stress showed reduced Cr accumulation in plant shoots. AM fungal inoculation reduced soil Cr concentration by

45.8% and the greatest Cr reductions were seen in droughted soils with higher soil carbon concentrations and AMF inoculation. Inversely, significant increases in Cr(VI) concentrations (123% on average) occurred when there was no AMF inoculation and at low AMF colonization percentages (<20%) as well as at higher SOC percentages. These findings suggest it may be beneficial to apply AMF for the phytoremediation of Cr and Cr(VI) contaminated soils, especially in semi-arid regions, where similar drought or redox-fluctuating conditions may exist.

Finally, in Chapter 4, we evaluated a novel biological soil metal remediation approach utilizing California native plants with different fungal treatments including arbuscular mycorrhizal fungi (AMF) inoculum and *Pleurotus ostreatus* inoculum in the form of spent mushroom blocks on three brownfields. Metals uptake by plant-fungal systems were examined with different levels of organic and inorganic contaminants at brownfield sites under irrigated and unirrigated water regimes. To our knowledge, this was the first field study of phyto-mycoremediation in Southern California, which was carried out in partnership with community groups, city, and regulatory agencies. Treatment with plants and fungi reduced soil concentrations of all metals of concern, and produced statistically significant reductions for Pb, Cu and As, when compared to control plots. Although the native plants tested were not as performant in metal accumulation and extraction into aboveground parts as the control plant, *Chrysnopogon zizanioides* (dryland remediator Vetiver), they had much greater survival and biomass production in both irrigated and unirrigated plots. *Heterotheca grandiflora* had the highest mean Pb uptake of the native plants tested, but was not significantly different than *Erigonium*

fasciculatum, suggesting both may be suitable native plant options for phytoremediation efforts in Southern California. Irrigation and inoculation with AMF significantly increased metal accumulation in all plants tested, although AMF-mediated effects on Pb, Cr, and As translocation from plant roots to shoots was plant species dependent. Unexpectedly, significant increases to plant germination, survival, biomass, and metal uptake were observed with *Pleurotus ostreatus* (Oyster mushroom) inoculation which has not been reported in past studies. Study results suggest that longer timelines are necessary for Pb reductions in highly contaminated brownfield soils using plant-fungi combinations. Regional pilot-scale studies of the best plant-fungal treatment combinations could be explored over longer timeframes for *in situ* brownfields bioremediation.

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Tables

Chapter 2

Chapter 3

Chapter 4

Appendices

Chapter 2

Chapter 3

Chapter 4

Chapter 1: Introduction to Arbuscular Mycorrhizal Fungi and Their Role in Soil Remediation

1. Background and Significance

1.1 Environmental Exposures to Metal(loid)s Through Soils

Soil contamination with metals and metalloids is a serious environmental and public health issue worldwide (Alloway, 2013; GWRTAC, 1997; Noll, 2003; Wuana, 2011; Zwolak et al., 2019). It is well-known that potentially hazardous metals exist in unsafe concentrations in many urban and peri-urban soils, including residential soils, sites of food production, as well as former industrial sites (brownfields and Superfund sites) (Filippelli & Laidlaw, 2010). Both 'Superfund' sites and 'brownfields' are contaminated sites where hazardous substances occur at higher levels than background concentrations. Superfund sites are regulated under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), while brownfields are considered sites where “reuse may be complicated by the presence or potential presence of a hazardous substance, pollutant, or contaminant” ((USEPA), 2021). These sites are typically contaminated through industrial operations such as mining, transportation (i.e., railyards), manufacturing, military operations and weapons production, agricultural chemicals application and hazardous waste disposal (formal or through improper handling) but can also become contaminated through accidental spills and natural disasters (i.e., hurricanes, floods) (USEPA, 2017). In the US there are 13,000 Superfund sites and 450,000 brownfields, covering ~22 million acres nationally (Brodsky, 2007; Kiaghadi et al., 2021; Simons, 1998). California is thought to host ~150,000 brownfields covering 96,000 acres

(Brodsky, 2022). Contaminated sites usually contain complex mixtures of organic and inorganic compounds, rather than a single metal or type of contaminant (Ajmone-Marsan & Biasioli, 2010; Wuana & Okieimen, 2011). Soil metal contamination arises from both natural sources, such as parent material, and anthropogenic sources, such as historical or present-day industrial emissions or wastes, transportation throughways, and other sources (Alloway, 2013; Wuana & Okieimen, 2011; Zwolak et al., 2019). Soils are the major sink for metals released into the environment from these activities, and unlike organic contaminants which can be metabolized to non-toxic forms through microbial action, most metals cannot be degraded and instead accumulate in soils (Kirpichtchikova et al., 2006; Noll, 2003). The continued increase of metal concentrations in soils poses a health hazard to humans and other living organisms, particularly through consumption of contaminated food and water, and ingestion of metal-containing dust (CDC, 2010; Filippelli & Laidlaw, 2010; Rai et al., 2019; Wuana & Okieimen, 2011). People living near contaminated sites can be exposed to hazardous contaminants through direct contact with the soil and indirect contact with soil through entrained dust and through air, surface water, and ground water (USEPA,2017). Many contaminated sites within urban and residential areas can remain unremediated for decades due to the high cost of conventional remediation.

While some trace metals such as zinc (Zn), manganese (Mn), chromium (Cr) are needed by humans, plants, and other organisms in small quantities, these metals are toxic above certain quantities; others, such as lead (Pb), arsenic (As), and cadmium (Cd) have no known biological function and are harmful even in small doses. The most common

metals found across brownfields, in order of abundance, are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (GWRTAC, 1997; USEPA, 1996). Urban soils often contain 200-1500 mg/kg Pb (Ajmone-Marsan & Biasioli, 2010; CDC, 2010; McClintock, 2012; Mielke et al., 1997; Paltseva, 2019) which is much higher than the California human health screening level of 80 mg/kg (Carlisle, 2009). The Agency for Toxic Substances and Disease Registry has classified As and Pb as their top two priority substances to address with Cd as number seven and Cr(VI) number seventeen based on their frequency of occurrence, toxicity and potential for human exposure (Registry, 2019).

1.2 Predominant forms of Pb, As, Cd and Cr in soils

Lead is a transition metal belonging to group IV and period 6 of the periodic table, and the cation Pb2+ is the most common form of Pb in soils. Lead soil mobility and bioavailability are largely controlled by phosphorous (P), iron (Fe), organic matter content and pH. Iron oxides including oxyhydroxides such as goethite and organic matter can form strong surface complexes create surface bonding sites for Pb cations, and the presence of phosphate can lead to formation of Pb phosphates, also decreasing the mobility of Pb in soil (Clark et al., 2006). Similarly, increased pH decreases Pb mobility and bioavailability since less H+ ions are available to compete with Pb cations for binding sites (Hettiarachchi & Pierzynski, 2004). Although Pb2+ is not redox-active, in oxidizing conditions the concentration in solution is controlled by ion exchange and specific adsorption reactions, as well as the solubility of carbonate, hydroxycarbonate and

phosphate minerals (Essington, 2003). In reducing conditions, Pb concentrations in solution are controlled by the solubilities of sulfide minerals (PbS), which are more stable than minerals found in oxidized systems, making PbS very low to immobile (Essington, 2003). The ingestion of Pb at low doses can cause toxicity to every organ system including the neurological, gastrointestinal, reproductive, cardiovascular, endocrine and renal systems, and lead to increased risk for multiple cancers (Baldwin & Marshall, 1999). Health authorities have determined that no level of Pb intake is safe for children, who are particularly vulnerable to Pb's neurotoxic effects and can experience associated reduced learning and behaviour outcomes ((ATSDR). 2020; (CDC). 2021; Xintaras, 1992).

Arsenic is a redox-active metalloid that can be present in several oxidation states (−3, 0, +3, +5) though the +3 and +5 are the most common oxidation states in soil. The mobility of arsenic in soils depends on the type and amount of adsorbing soil constituents, the pH value, and the redox potential. In well aerated soils, arsenate [As(V), $H_xAsO_4^{x-3}$] is the predominant inorganic form of arsenic that is generally less mobile due to its strong adsorption on common soil minerals (Fe and Mn oxides), while arsenite [As(III), $H_XAsO_3^{x-3}$] is the predominant form under suboxic to anoxic conditions and acidic conditions and is generally more mobile due to adsorption on a more selective number of soil minerals (Gupta & Chen, 1978; Masue et al., 2007). Long-term As exposure at high doses via oral ingestion can increase risk of a wide range of cancers, and lead to skin disorders, an increased risk for diabetes and high blood pressure (Registry, 2007).

Cadmium is a transition metal with no known essential biological function. Cadmium mostly occurs as Cd(II) in soils and exhibits chemistry similar to nutrient cations essential for plants and animals, Zn, P, Ca, Cu, Mn and Fe (Qin et al., 2020). Because of its structural similarity to these nutrients, competitive adsorption occurs at the root surface and Cd is readily taken up by roots of plants through transport systems involved in micronutrient uptake, mainly through members of the zinc regulated/iron-regulated transporter protein (ZIP) and translocated to shoots of plants (Qin et al., 2020). Many studies have demonstrated that Cd can enter the food chain easily through uptake into food crops, resultantly, Cd exposure through ingestion of contaminated crops is the second greatest route of exposure, after cigarette smoking (Satarug et al., 2003; Wagner, 1993). Cadmium(II) has a similar redox chemistry to Pb2+, where it is moderately mobile under oxidizing conditions, controlled by ion exchange, adsorption reactions and the solubility of phosphate, carbonate and hydroxycarbonate, but has low mobility or immobility under reducing conditions, controlled by sulfide mineral solubility in the form CdS. Cadmium is typically found in the labile, exchangeable phase (Ajmone-Marsan & Biasioli, 2010), when soils are acidic with low organic matter content. Cadmium is a known human carcinogen (prostate, kidney, bladder, and breast cancers) which also damages the renal system and bone health ((ATSDR), 2012a; Humans, 1993; Rai et al., 2019).

Chromium is a redox-active metal which is present predominantly as Cr(VI) under oxidizing conditions and insoluble Cr(III) hydroxides under reducing conditions. At most anthropogenically contaminated sites, Cr(VI) is the most common oxidation state

present in soils and is also the more toxic and more mobile (soluble) form compared to Cr(III). Chromium(VI) can be immobilized through abiotic reduction [e.g. by Fe(II)] (Wielinga et al., 2001) or microbial reduction (Wang, 2000); conversely, Cr(III) can be abiotically oxidized by Mn(IV) oxides (Kim et al., 2002) which are commonly found in soils and, in particular, under redox fluctuating conditions. Exposure to Cr(VI) via ingestion can cause kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer and other adverse health effects ((ATSDR), 2012b).

1.3 Metals Exposures Through Soils

Soils are a major metals exposure pathway for humans across many land-use types, but community gardens and urban farms (Cheng et al., 2015; Clark et al., 2006; Cooper et al., 2020; McClintock, 2012; Paltseva et al., 2018; Surls et al., 2016) as well as residential housing on or in proximity to contaminated sites (brownfields and Superfund sites) in densely populated urban areas can increase likelihood of exposure because residents come into direct contact with the soil, consume crops grown in metal-contaminated soil, and can come into indirect contact with soil through entrained dust (Filippelli & Laidlaw, 2010; Kiaghadi et al., 2021; Wuana & Okieimen, 2011; Zota et al., 2011). Chronic, low-dose exposure to metals from ingestion of soil dust from living on or near contaminated sites can lead to decreased immunological function, increased risk of certain cancers, kidney damage and endocrine disruption (Humans, 1993; Jaishankar et al., 2014; Järup, 2003; Järup & Åkesson, 2009). A recent study found that living within 1

mile of a Superfund site is correlated with decreased life expectancy by as much as 1.22 years in areas that are highly socioeconomically disadvantaged (Kiaghadi et al., 2021). Typical contaminants found on brownfields include petroleum hydrocarbons, heavy metals such as lead, hexavalent chromium and cadmium, industrial solvents (e.g. trichloroethylene [TCE], tetrachloroethene [PCE], and trichloroethane), asbestos, volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), polycyclic aromatic hydrocarbons (PAHs), and/or polychlorinated biphenyls (PCBs) (USEPA, 2010). Few biogeochemical and remediation studies have considered mixtures of metals, or mixtures of metals with other soil contaminants. This is an important area for research attention, outreach and mitigation because additive or synergistic health effects can be seen from exposure to multiple metals and metals and organic contaminants (Mielke et al., 2005; Rai et al., 2019).

1.4 Potential Impacts of Climate Change on Exposures via Soils

Because legacy metals (e.g., Pb, As and Cr) persist and bioaccumulate in the environment, long-term environmental processes related to global climate change could influence their fate and transport and change ecosystem and human exposures (Balbus et al., 2013; Landis et al., 2014). Several recent studies predict that risk of exposure to lead, arsenic and other pollutants from contaminated sites will increase under climate change, especially in urban areas (Augustsson et al., 2011; Office, 2019; Shukla et al., 2019). Climate changes that may alter metal mobility include higher temperatures, reduced soil moisture, increased frequency and severity of extreme weather and natural disasters (such

as flooding and wildfires), and increased global atmospheric CO2 concentrations (Shukla et al., 2019).

Reduced soil moisture is thought to be a significant factor influencing soil metal mobility under climate change (Augustsson et al., 2011; Berg & Sheffield, 2018; Garten et al., 2009). Augustsson (2011) used models to determine how risk of exposure to Pb from contaminated sites may be altered under climate change and found that groundwater infiltration, hydraulic conductivity, soil moisture and soil: water distribution were the variables with the strongest influence on risk. Climate models have predicted global changes -20-28% in soil moisture over the next 100 years within the Southwestern US (Berg & Sheffield, 2018; Cook et al., 2015; Lenihan et al., 2003; Warter et al., 2021). Decreasing soil moisture was predicted for most areas, even where precipitation is expected to increase, as a result of increased surface temperatures resulting in higher evaporation rates (Berg & Sheffield, 2018). Reduced soil moisture content and increased evapotranspiration may also reduce the formation of stable (and thus less bioavailable) complexes between metal(loid)s with phosphate, SOM and minerals, due to the lack of water required for these chemical reactions to occur (Garten et al., 2009). Another consequence of reduced soil moisture is the higher resuspension of soil dust particles, which creates additional exposure pathways for toxic metals within soils and increases their transport (Balbus et al., 2013; Paltseva & Neaman, 2020).

1.5 Relevance to Health Disparities and Policy

Racial/ethnic and income disparities have been documented with respect to exposure to hazardous metals in soils specifically, and with exposure to environmental contaminants in general. In California, more than 18 percent of Latinxs and more than 17 percent of African Americans live in one of the top 10 percent most pollution-burdened communities; in comparison, less than 3 percent of whites reside in the top 10 percent most impacted communities (California Environmental Protection Agency (CalEPA), 2018). Contaminated sites are known to be concentrated in socioeconomically disadvantaged areas, especially in communities of color and on or near tribal reserves (Anderton D, 1997; Johnstone et al., 2020; Di Fonzo et al, 2022). A recent scoping review found that ~34% of Superfund sites are categorized under 'Native American Interest' by the Environmental Protection Agency (EPA), while Native American people make up only 2.9% of the total population (Chong, 2022). 'Disadvantaged' and low-income communities comprise a disproportionate number of brownfield locations within California at 40% and 57% respectively (CPCFA, 2017). This leads to health disparities and disproportionate health burdens for neighboring communities because living near a contaminated site increases the likelihood of exposure to the contaminants present. This is evidenced by corresponding high blood Pb (and other contaminant) levels in children and residents of neighborhoods with higher soil contamination and contaminated sites (Aelion et al., 2013; Jones et al., 2009; McClintock, 2012; Mielke et al., 2017; Rothenberg et al., 1996; White et al., 2016). The reality that people of color and low-

income residents tend to experience disproportionate environmental burdens and related health issues, is known as environmental injustice (Bullard et al, 1994 and 2018).

Despite strong evidence of soil as an important exposure pathway for Pb and other metals, there are not any city-level policies that have been implemented to remediate metals, nor guidelines for soil remediation options (LeBrón et al., 2019). To develop policy that supports remediation of metals and reduce exposures to contaminants from brownfields, there is a need for studies into the effectiveness of alternative, cost-effective approaches. The governmental and regulatory agencies responsible for remediation of contaminated sites within the City of Los Angeles (the Bureau of Engineers, Brownfields Department, and Department of Toxic Substances Control), have requested more information on the feasibility, costs, timelines, and effectiveness of myco-phyto-remediation strategies, with a preference for locally implemented studies and plants and fungi adapted to this climate. These data have the potential to inform policy changes (City of LA Brownfields, personal communication). However, not a single local study (and few continental) could be identified where these strategies had been implemented in field conditions, pointing to a need to undertake such studies. Community-academic-governmental partnerships are well-positioned to close this gap between community needs, scientific evidence, and policies that are needed to promote health equity and environmental justice with regards to soil contamination.

2. Current and Potential Remediation Strategies to Reduce Exposure

The most common form of conventional soil remediation of urban brownfields involves physical removal and replacement or isolation via capping and covering of contaminated soil (Sharma et al., 2018; USEPA, 2021). Other physical methods such as heat treatment, vitrification or electro-remediation, or chemical methods of washing, nano-remediation or precipitation are also used, depending on the site (Sharma et al., 2018; USEPA, 2021). These conventional are generally cost prohibitive in most urban areas, farms, and over large brownfields and Superfund sites, and often leave residual contamination (Hunter et al., 2019; Mielke et al., 2006; Mulligan et al., 2001). Soil excavation and removal costs between $57,000-$4,000,000/acre (not including the cost of soil replacement) (Mielke et al., 2006; Paull, 2008; Raskin et al., 1997). The cost of remediation is a commonly cited reason for the persistence of metals in the environment (Elias & Gulson, 2003). A study completed by Russell et al. (1991) projected that cumulative clean-up costs for all contaminated sites (for the period 1990 through 2020), using current remediation practices, will cost approximately $750 billion, with plausible upper bound at $1trillion. This cost is inclusive of Superfund sites regulated by the US Environmental Protection Agency, but not of all brownfields and contaminated soils. Given the scale and spread of contaminated soils across the United States and in California and the cost of cleanup using conventional methods, remediation via excavation and landfilling are infeasible remedies for all sites and lower-cost remediation methods that treat soil in place are desirable.

Further, conventional remediation methods have environmental and social costs, and while they physically contain the contaminants, they do not eliminate them. Additionally, the moving and hauling of contaminated soil with conventional remediation is a hazard that creates additional routes of potential for exposure for neighboring communities (Khan et al., 2000; Sparks, 2003). Excavated soils are often transported to landfills which are often located in areas that are disproportionately socioeconomically disadvantaged which further externalizes the problem (Bullard, 1994, 2018; Eckerd & Keeler, 2012; Hoffman, 1985).

2.1 Remediation Alternatives: Biological Remediation

Alternative strategies to reduce risk from or remediate metal-contaminated soils include biotic methods that utilize native or introduced communities of fungi, bacteria, and plants to extract metals or stabilize them in soils. These biological approaches are promising alternatives to conventional remediation which can be done *in situ*, removing the need for soil excavation thus minimizing exposure pathways from the remediation and providing significant cost savings (Jabeen et al., 2009; Sparks, 2003; USEPA, 1996).

2.2 Phytoremediation

Phytoremediation utilizes plants and their associated rhizosphere microbial communities (fungi and bacteria) to accumulate metals within plants (phyto-extraction), and/or stabilize (phyto-stabilization) metals or degrade organic contaminants in the soil (Banks et al., 2003; Garbisu & Alkorta, 2001; Henry et al., 2013; Khan, 2006).

Phytostabilization stabilizes or immobilizes contaminants in the roots or root zone of plants, but does not absorb the contaminants into plant shoots, which reduces the bioavailability of pollutants in the environment (Raskin et al., 1997). Phytostabilization is a less-researched area of phytoremediation (Raskin et al., 1997). Phytoremediation of metal-contaminated sites requires a functional microbial community for plant community establishment, soil development, and biogeochemical cycling.

Phytoextraction employs certain plants that have been identified as metal hyperaccumulators: plants capable of storing metals in their tissue at concentrations that are much higher (100-500 times) than the surrounding environment (Garbisu & Alkorta, 2001; Gawronski, 2007). A wide variety of plant species have been identified as phytoremediators ranging from large trees to small grasses, the majority in the plant families Asteraceae, Poaceae, Brassicaceae, Solanaceae, Salicaceae, Chenopodiaceae, Careophylaceae and Fabaceae (Gawronski, 2007; Office & Support, 1999; van der Ent et al., 2015). Many hyper-accumulator plants remain yet to be discovered and identified (Khan et al., 2000). Species are often used together, but research to date has not systematically considered the potential benefits of planting diverse native communities (Office & Support, 1999). Use of native plants growing on contaminated sites may have advantages over conventional agricultural plant species, especially in Southern California, due to their dense, deep root systems adapted to the seasonal rainfall of the Mediterranean climate (Hellmers et al., 1955) and the likelihood that native plants have native mycorrhizal fungal associates in the soil, which affect the availability of root exudates and can thereby enhance microbial composition in the rhizosphere (Barea et al.,

1975; Rambelli, 1973). Further, due to their adaptation to local ecosystems and climate, native plant species will have a lower cost to maintain (Devinny et al., 2005).

Phytoremediation has become increasingly popular because of its cost effectiveness (50-80% cost savings compared to soil excavation and replacement) and its environmental benefits such as the creation of soil on site ((USEPA), 2001; Farraji et al., 2016; Jabeen et al., 2009). Some of the limitations of phytoremediation include remediation zone restricted to rooting depth, long time scales for thorough remediation (depending on the initial concentration), and disposal of the contaminated plant material ((USEPA), 2001; Farraji et al., 2016; Wuana & Okieimen, 2011). Phytoextractor plants rich in accumulated metals are typically processed by ashing (controlled incineration) or composting. Some extracted metals can also be reclaimed from the ash, generating recycling revenues (Corzo Remigio et al., 2020; van der Ent et al., 2015). Because disposal of plant material used in phytoextraction has an ecological footprint and cost, this problem should be addressed in future research to build a circular economy for remediation. In addition to reuse of precious metals in ash, the use of biofuel plants as extractors and subsequent transformation of plant matter into fuel, and use of extractor cereals as animal fodder or feed have been proposed; alternatively, phyto-stabilization is encouraged over extraction (Conesa et al., 2012; Garbisu & Alkorta, 2001; Grispen et al., 2006; Mulligan et al., 2001; Sharma et al., 2018; van der Ent et al., 2015; Watanabe, 2001). Additionally, reduced bioavailability of contaminants can limit the effectiveness of phytoextraction. Metals, such as Pb, often form complexes with organic matter or precipitate with carbon, phosphorous, and hydroxide which limit Pb's availability for

plant uptake (Blaylock et al., 1997; Hettiarachchi & Pierzynski, 2004). While surfactants, or chelating agents, can be added to increase the bioavailability of metals and enhance phytoextraction (Blaylock et al., 1997; do Nascimento et al., 2006), these simultaneously increase the potential for the metal to leach from the site thus increasing risk of exposure and spread of the contaminant (Wu et al., 2004), making it unlikely to gain regulatory approval in field settings.

Further research gaps need to be addressed to bring phytoremediation to a stage where local regulatory agencies can be confident that these methods are safe, more cost-effective and more effective than conventional methods (Watanabe, 2001). Firstly, most phytoremediation research has focused on a single contaminant, but most contaminated sites contain multiple contaminants; therefore, studies examining phytoremediation effectiveness on contaminant mixtures is more relevant for field application. Second, there is a paucity of phytoremediation field studies in hot and arid/semi-arid areas to derive protocols for myco-phytoremediation of mixed-contaminated soils from. Plants in the field experience stresses that are not present in greenhouse and laboratory conditions, such as nutrient deficiencies and harsh weather- in southern California drought and high temperatures are additional stressors. Further, few field studies tested native plants, fewer still field studies tested combined approaches utilizing fungi and microbial to enhance phytoremediation. A search of the USEPA's Contaminated Sites Clean-up Information database returned <20 phytoremediation field studies have been performed in the USA on soil Pb, As, Cr and Cd (CLU-IN, 2019); none of which used native plants indigenous to the site nor microbial-associated plants. Additionally, no studies have been performed in

Southern California or similar semi-arid Mediterranean climate zone. A search of the global phytoremediation database, Version 1.0 published by Kansas State University Department of Agronomy and the NRCS Plant Materials Center (Hettiarachchi et al., 2012) yielded a similar lack of field studies in Mediterranean climate (one study on cadmium remediation in Australia).

2.3 Arbuscular Mycorrhizal Fungi-Assisted Phytoremediation

Plant-associated fungi (and microbes) may enhance the phytoremediation of metals-contaminated soils. Arbuscular mycorrhizal fungi (AMF) are among the most common soil microorganisms occurring in almost all habitats and climates, from the deserts to the boreal forest, who live in association with plants through the roots (Smith & Read, 2010). AMF are one type of mycorrhizae belonging to the phylum Glomeromycota; the various types of mycorrhizal fungi and their structures are shown in Figure 1. AMF are the generalist mycorrhizae, who associate with 80% of all plants worldwide, including most agricultural crops, shrubs, grassland plants and some trees (Smith & Read, 2010). ~200 species of AMF have been identified, but AMF diversity may be much higher (Nilsson et al., 2019). They live in obligate, symbiotic relationships with plants through the roots, trading nutrients and water in exchange for photosynthetic products from the plant and significantly reducing plant stress (Giasson et al., 2008; Turnau & Haselwandter, 2002).

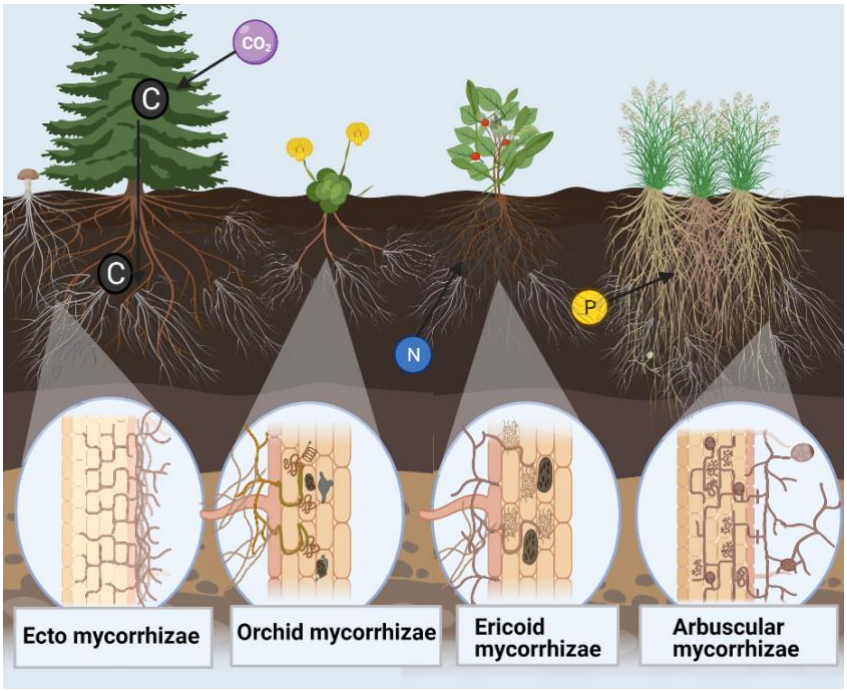

Figure 1. Different types of mycorrhizae and their structures.

AMF play key roles in soil biogeochemical cycles and are capable of many important metal and mineral transformations, particularly with regards to phosphorus (Gadd, 2007; Giasson et al., 2008; Smith & Read, 2010). Mycorrhizae contribute up to 80% of the phosphorus, 25% of the nitrogen, 10% of the potassium, 25% of the zinc and 60% of the copper absorbed by plants, and can receive 10–30% of the host plant's net carbon fixation (Bago et al., 2000; Smith & Smith, 2012). AMF increase the plant contact area with soil. They were shown to enhance root absorption area up to 47-fold (Smith & Read, 2010) and improve water absorption (Cui & Nobel, 1992; Sweatt & Davies Jr, 1984). Accordingly, mycorrhizal colonization improves plant establishment and survival particularly in adverse conditions such as in low fertility and arid soils (Allen et al., 1996; Allen et al., 1991). Contaminated sites are generally poor in nutrients and contain a

highly altered soil structure, thus mycorrhizal fungi can play an important role in plant establishment for phytoremediation purposes (Giasson et al., 2008; Turnau & Haselwandter, 2002). In addition, these mycorrhizae have been shown to protect plants from harmful effects of excess metals (Diaz et al., 1996; Khan, 2006; Khan et al., 2000; Killham & Firestone, 1983; Shi et al., 2019). For example, a meta-analysis of 299 studies found AMF inoculation significantly reduced arsenic uptake in crops, and at the same time, positively modify the nutrient supply and the biomass in host crops that are grown under arsenic stress (Neidhardt, 2021). Interestingly, this effect is not uniform; both inhibition and enhancement of metal uptake have been reported (Diaz et al., 1996; Khan, 2006; Tan et al., 2015; Yang et al., 2015).

Although a vast number of publications on the effect of AMF on the uptake of metals by plants is available (Chaudhry & Khan, 2002; Chen et al., 2003; Chen et al., 2007; Diaz et al., 1996; Gildon & Tinker, 1983; Gonzalez-Chavez et al., 2002; Joner & Leyval, 2001; Joner et al., 2000; Khan, 2006; Khan et al., 2000; Krishnamoorthy et al., 2015; Li et al., 2018; Tan et al., 2015; Turnau & Haselwandter, 2002; Wu* et al., 2010; Xavier & Boyetchko, 2002; Yang et al., 2015) and others, the majority of studies were done in a laboratory setting or in greenhouses, overlooking the conditions present on contaminated sites and other field settings. Further, since AMF have at times been shown to enhance phytoextraction, and at times enhance phyto-stabilization, the controls on this are not clear and remain unresolved and the mechanisms are still poorly understood (Audet & Charest, 2007; Ferrol et al., 2016; Gaur & Adholeya, 2004; Harms et al., 2011). These questions, if resolved, could be of great use in agriculture, where AM fungi may be

applied as crop-protectants to inhibit metal uptake into edible parts of crops, among other co-benefits, and in phytoremediation (coined “mycorrhiza-remediation” by Khan, 2006) and ecological restoration efforts, where they can help plants survive harsh conditions and facilitate greater uptake and removal of metals.

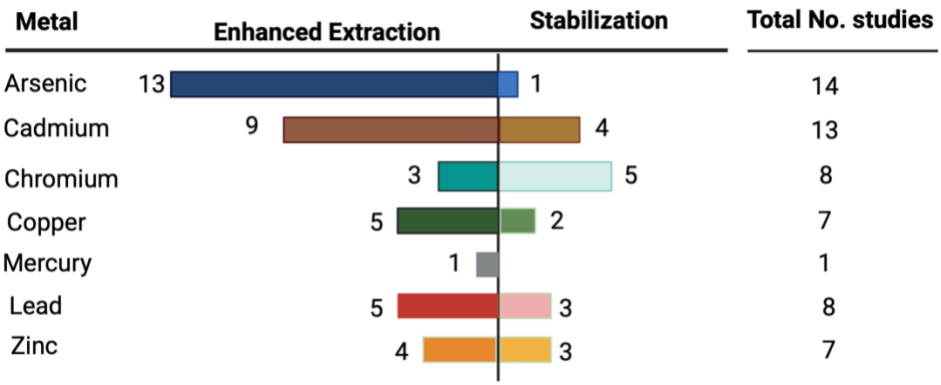

Figure 2. Number of pot studies showing inhibited vs. enhanced metal uptake when plant crops associate with arbuscular mycorrhizal fungi. 52 studies were reviewed through a Web of Science search using the keywords “arbuscular mycorrhizal fungi and trace metals” “arbuscular mycorrhizal fungi and metals and crops.” All studies were greenhouse pot culture studies using edible plant crops.

3. Mechanisms of AMF Interactions with Metal(loids)

The most important mechanisms (observed or hypothesized) through which AM fungi influence metal transfer in plants and the soil properties and environmental factors are summarized below. A full review of these was undertaken (Stevenson and Ying, unpublished). These include processes of mobilization and immobilization in the fungal extraradical hyphae, sorption to fungal cell walls and uptake into fungal cells. After being incorporated into the fungus, metals can be chemically transformed, stored in different parts of the cell, or translocated along fungal hyphae and into plant symbionts.

Immobilization of metal(loids) by AMF and resultant phyto-stabilization is thought to occur primarily through ion exchange, complexation, adsorption, and precipitation mechanisms with [1] fungal hyphal cell wall components and [2] extracellularly in the soil via organic acids, polysaccharides, and proteins extracellularly excreted by AMF.

[1] Since AMF extraradical mycelium has a large surface area with an overall negative charge, a variety of functional groups (carboxyl, sulfate, phosphate, hydroxyl, amino, sulfhydryl) for metal ions to bind to, and a high redox potential, AMF extraradical mycelium has a strong capacity to adsorb metal(loid)s from soil (Bahafid et al., 2017; Gonzalez-Chavez et al., 2002; Joner et al., 2000; Smith & Read, 2010; Turnau & Haselwandter, 2002). Once on the fungal surface, AMF may facilitate chemical oxidation, reduction, methylation and dealkylation reactions to reduce the metal toxicity by changing the metals' speciation and/or oxidation state. For reduction to occur, the metal must first bind to the positively-charged complexing groups on fungal surfaces and then be reduced by adjacent electron donors (carboxyl groups or dissolved organic carbon) (Wu et al., 2015). Synchrotron-based X-ray absorption near edge structure (XANES) and SEM image analysis revealed that Cr(VI) was reduced to Cr(III) and then combined with phosphate and histidine-like ligands on the fungal surface, immobilizing Cr in the AM fungal biomass (Wu et al., 2015). Li, S. (2018) found that arsenic immobilization and detoxification in AM fungi occurs largely through influencing As speciation and partitioning through As methylation; this occurs through up-regulation of the phosphorus transporter gene *MsPT4 and* metallothionein gene *MsMT2* expression.

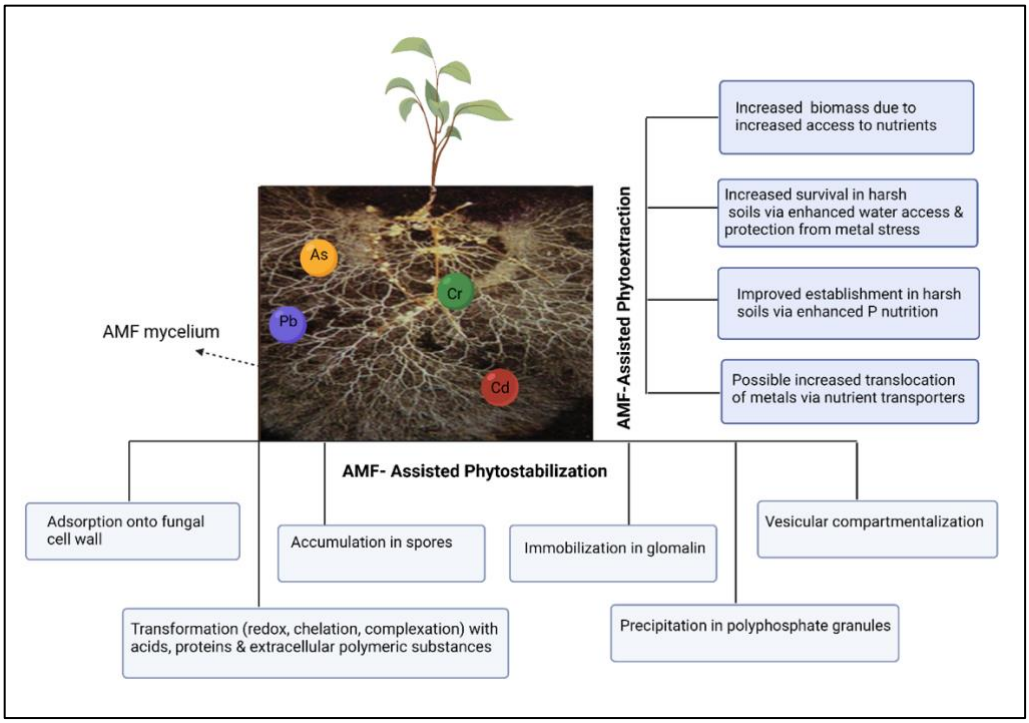

Figure 3. Hypothesized and observed mechanisms of AMF-mediated metal transfer and immobilization in plant partners.

[2] The most important AMF extracellular product for the immobilization of metal(loids) is glomalin. Glomalin is a glycoprotein family of heat shock proteins excreted extracellularly into the soil around AM fungal hyphae, a sticky substance that binds soil particles into soil aggregates (Gadkar & Rillig, 2006) and has been found to complex with metal ions in soils, immobilizing them before they reach the living fungal cell wall (Gonzalez-Chavez et al., 2004; Khan, 2006). Studies have found a correlation between the quantity of glomalin in soil and the quantity of immobilized metals for the metal(loids) As, Cu, Cd, Zn and Pb (Gonzalez-Chavez et al., 2004) and Cr and Hg (Wu et al., 2010). AM fungal strains with significant secretion of glomalin may be more suitable for metal immobilization efforts. How stable and persistent glomalin is in soils remains

contradictory in the literature and has only been tested in the tropics, where estimates range from two years (Lovelock et al., 2004) to 25 years (Rillig et al., 2001). Literature on glomalin content in polluted soils are extremely scarce and there is a need to gather data on this aspect.

Once within AM fungal cells, chelation of metal ions by ligands, compartmentation within vacuoles or vesicles, and coping with oxidative stress occur. Vesicles (found only in mycorrhizal fungi) are internal storage compartments that help regulate cytosolic concentrations of metals by making them unavailable to create toxic effects (Gadd, 1999; Turnau & Haselwandter, 2002; Wu et al., 2018). Metal chelators (metallothionines, poly P granules) are found in these vacuoles which further complex and immobilize the metals. It has been observed that AMF found on metal-polluted sites have more vesicles and other storage organelles than fungi on non-metal-polluted sites (Khan, 2006; Khan et al., 2000; Turnau & Haselwandter, 2002).

Enhancement of metal(loids) transfer from AMF to plant and resultant phytoextraction is thought to occur primarily [1] indirectly by enhancing plant survival in poor soils and increasing plant biomass and [2] directly by enhancing plant symbiont uptake of toxic metals for removal (Gadd, 2009) via nutrient transporters in the extraradical mycelium. [1] Under toxic metal(loid) conditions, increased biomass of mycorrhizal plants resulting from enhanced P nutrition and water absorption and protection leads to increased removal of metal(loid)s from the soil, with AM thus contributing to metal(loid) phytoextraction (Ferrol et al., 2016). [2] The direct pathway involves high-affinity metal transporters located in root hairs and epidermal cells and in

the extraradical mycelium (ERM). Metals enter the cytosol via transporters in the cell membrane which are used for the uptake of essential metals; sometimes nonessential metals are co-transported using the same transporters due to their low specificity (Joutey et al., 2015; Viti et al., 2014; Wu et al., 2018; Wu et al., 2015). Fungal sulfur (S), calcium (Ca), potassium (K), copper (via COPT transporters), manganese (Mn), zinc (Zn) and phosphate (P04) transporters are the main proteins involved in metal uptake by these cells; Cr is transported via the sulfate transporter, As via the phosphate transporter, Cd via the zinc regulated/iron-regulated transporter protein (ZIP) (Gadd, 2009; González-Guerrero et al., 2016). Metals are translocated along the hyphae to intracellular structures (intraradical mycelium (IRM)) and then transferred to the root at the symbiotic interface, the arbuscules.

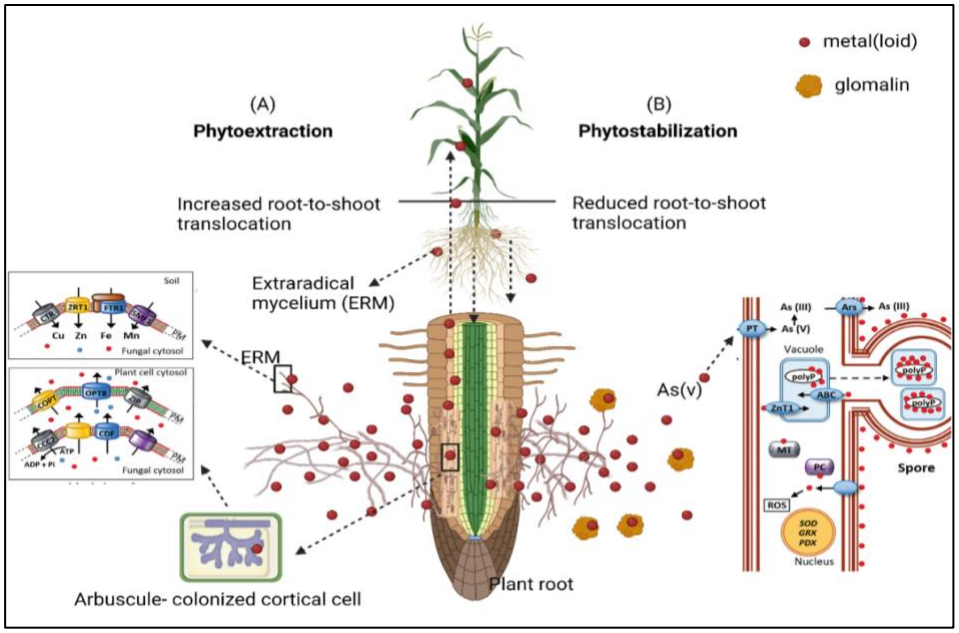

Figure 4. Schematic representation of AM contribution to plant metal(loid) uptake and immobilization (After Ferrol, 2016). The putative fungal and plant metal transporters involved in the mycorrhizal pathway are CTR, fungal Cu transporter; CCC2, Cu-ATPase; CDF, cation diffusion facilitator; COPT, plant Cu transporter; FTR1, Fe permesase; OPT, oligopeptide

transporter; SMF1, Mn transporter; ZRT1, Zn transporter; ZIP, Zn–Fe permease (some ZIP members transport Mn) (Ferrol, 2016). ABC, ABC transporter; Ars, arsenite pump; GRX, glutaredoxin; MT, metallothionein; PC, phytochelatin; PDX, pyridoxine synthase; PT, P transporter; ROS, reactive oxygen species; SOD, superoxide dismutase; ZnT1, Zn transporter.

(A) Enhanced uptake and shoot transfer. Under low-metal conditions, metals are taken up directly through the plant pathway and the mycorrhizal pathway via nutrient transporters, with AM thus contributing to enhanced metal accumulation. Indirectly, AMF increase plant partner biomass and survival via enhanced P nutrition and water absorption, thus enhancing phytoremediation.

(B) Soil metal(loid) immobilization. Metal(loid)s are immobilized in the arbuscular mycorrhizal fungal structures, with AM thus contributing to phyto-stabilization. Mechanisms of metal(loid) tolerance in the fungus are illustrated in the lower right-hand corner of the diagram. These mechanisms include metal(loid) binding to the cell wall, chelation by glomalin, decreased uptake and/or increased efflux to the exterior, chelation of metal ions in the cytosol, metal(loid) compartmentalization in the vacuoles, and activation of antioxidant defenses. In the vacuoles, metal(loid)s are stabilized with polyp and some of these vacuoles are translocated to the spores.

3.1 Controls on AMF Metals Inhibition vs. Enhanced Uptake in Plant Partners

[1] AMF species and strain differences in metal tolerance. AM fungal species differ in their sensitivity to metal(loid)s. “Metal tolerance" is the ability of an organism to survive metal toxicity by means of intrinsic properties and/or environmental modification of toxicity (Gadd, 1994). Many AM fungal species are tolerant to metals; this has been

observed under field conditions with the high prevalence of these fungi on metal-contaminated sites: AMF can reputedly be isolated from any soils polluted by toxic metals (Amir et al., 2014; Gadd, 2007; Hassan et al., 2011; Hildebrandt et al., 2007; Pawlowska et al., 1997). Sometimes, AMF species can develop resistance to metals, forming metal-resistant strains of AMF. Strains represent adaptations that occur through physiological and/or morphological changes including genetic adaptation, typically via upregulation genes relating to metal detoxification (Gadd, 1994). “Metal resistance" is the ability of an organism to survive metal toxicity by means of a mechanism produced in direct response to the metal species concerned (Gadd, 1994). These metal-resistant strains are also isolated from metal-contaminated habitats. Mycorrhizal fungal species isolated from metal contaminated sites confer that tolerance or resistance to their plant partner, whereas AMF strains without previous exposure to metals may not. For example, Diaz G. et al. (1996) observed lower Pb concentration in the shoots of plants inoculated with an AM isolate from contaminated soil (*G. mosseae*) than in the shoots of plants inoculated with an isolate from noncontaminated soil (*G. macrocarpum*). These metal-tolerant and resistant species of AMF may have applications in phytoremediation and as crop-protectants (Audet & Charest, 2007; Gaur & Adholeya, 2004; Gonzalez-Chavez et al., 2004; Joner & Leyval, 2001; Khan, 2006; Khan et al., 2000; Tan et al., 2015; Xavier & Boyetchko, 2002; Yang et al., 2015).

[2] Soil biotic, abiotic, and environmental factors affect AMF-metal-plant dynamics. Soil and environmental factors observed to affect AM fungal-metal-plant dynamics include: pH, CEC, soil temperature and moisture, soil texture, the

concentration and availability of metal(s), the presence of other pollutants, nutrients and minerals (especially phosphorus), and the presence of and association with other soil microorganisms (Gadd, 1994; Giasson et al., 2008).

Metal sorption in AMF is frequently shown to be strongly pH dependent (Dhankhar & Hooda, 2011; Fomina et al., 2005; Joner & Leyval, 2001; Turnau & Haselwandter, 2002). Soil pH affects the solution chemistry of metals, the activity of the functional groups (and surface charge) of the fungal biomass and the competition of metallic ions (Abbas et al., 2014). Generally, adsorption is reduced and thus metal uptake increases significantly when pH of the metal solutions decreases from pH 6.0 to 2.5 (is more acidic); and greater adsorption and reduced uptake occurs at pH 6-9 (Abbas et al., 2014; Comte et al., 2008). A study by (Viti et al., 2014) observed that low pH values contributed to Cr(VI) detoxification by providing the protons needed for reduction reaction and increasing the protonation level of the adsorbent surface, which is thus more positively charged and more attractive for negative Cr. However, the pH optimal for adsorption/immobilization vs transport and accumulation varies by fungal species, and AMF fungi are also known to modify the pH of their environment (usually lowering pH) through the excretion of organic acids, creating appropriate physicochemical conditions for precipitation of metals to take place (Fomina et al., 2005; Gadd, 1999).

Soil metal concentration has been shown to affect AMF-metal-plant dynamics: metal accumulation in their plant symbionts is reported to be lower under elevated soil metal concentrations and higher under lower metal conditions (Toler et al., 2005). In a literature survey, (Audet & Charest, 2007) observed an increased metal uptake in AMF-

associated plants at low metal concentrations and reduced metal uptake in AMF-associated plants at high soil metal levels, resulting in enhanced plant tolerance through metal stress avoidance. The exact metal concentrations at which uptake vs inhibition are observed and how those levels correspond with typical agricultural or urban agricultural and brownfield soil metal levels is undescribed. It is well documented that AMF coexist with other soil microorganisms which might have positive or negative effects on metal uptake (Giasson et al., 2008). AMF improves plant growth, health, and productivity in metal contaminated soils by altering the activity and abundance of rhizosphere microbiota. Most studies aimed at improving phytoremediation have shown synergistic effects of dual inoculation of AM fungi with other microbes, such as P-solubilizing microbes, N-fixing bacteria, and other fungi; with combined effects greater than each on their own. Increased plant growth and metal tolerance and/or uptake has been seen when various AMF species (including *G. mosseae* and *G. deserticola*) were combined with: a plant-growth-promoting (PGP) bacterium (*Brevibacillus brevis*), both isolated from Cd-contaminated soil (Vivas et al., 2005); *Trichoderma koningii* (Arriagada et al., 2007); earthworms (Ma et al., 2006); *Aspergillus niger* (Azcon et al., 2009; Medina et al., 2010). Yet again, while plant growth is generally enhanced by AMF in combination with other microbes, diverse effects are seen regarding whether these combinations enhance or inhibit metal-uptake in plants.

While some of the mechanisms of AMF-metal(loid) immobilization are understood, there are still many questions that remain to be resolved before AMF can be applied to enhance phytoremediation of metal-contaminated soils. The mechanisms

underlying enhancement of metal(loid) uptake in plants via AMF need to be determined so that AMF-assisted phytoremediation strategies can be explored. To get there, an understanding of the soil abiotic, biotic, and environmental factors that influence metal uptake vs immobilization in AMF-plants, and evidence from testing AMF application in the field are necessary gaps to address. Further isolation of indigenous AMF from metal-contaminated soils and a greater understanding of the mechanisms of tolerance and resistance involved can advance potential for these AMF to be biotechnological tools for remediation and crop protection.

3.2 Potential Effects of Climate Change on AMF-Plant Dynamics in Remediation Systems

Climate change is altering the interactions among plants and soil microorganisms in ways that will alter the structure and function of ecosystems and may increase exposures to soil metal contaminants (Balbus et al., 2013; Landis et al., 2014). Higher temperatures have generally been shown to increase metal uptake and distribution in plants due to increased evapotranspiration translocating metals from lower horizons upward to the rhizosphere, thus becoming more readily available for plant uptake (Paltseva & Neaman, 2020). Several studies of phytoextraction plants have shown increased uptake and accumulation of As, Cd, Cr, Ni, Pb, and Zn with increased temperature (Antoniadis & Alloway, 2001; Baghour et al., 2001; Hooda & Alloway, 1993). This could mean that metal(loid) translocation into food crops may also increase

under climate change. The effects on metal mobility and uptake into plants through drought (decreased soil moisture) is unresolved.

Arbuscular mycorrhizal fungi may be able to buffer against this, should the controls on AMF-assisted immobilization vs uptake in plants become better understood. Yet little information is available on the effects of mycorrhizal inoculation on metal uptake by plants under climate stress conditions via changes to soil moisture and temperature. More research is required to understand the impact of climate change on soil metal mobility in soil-mycorrhizal-plant systems and the direct and/or indirect effects of soil moisture on plant–AMF interactions and their consequences in metal-contaminated soils.

4. References

(ATSDR), A. f. T. S. a. D. R. (2012a). Toxicological profile on Cadmium. . https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=48&tid=15

(ATSDR), A. f. T. S. a. D. R. (2012b). Toxicological profile on Chromium. https://www.atsdr.cdc.gov/ToxProfiles/tp7-c3.pdf

(ATSDR)., A. f. T. S. a. D. R. (2020). Toxicological profile for Lead. https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=96&tid=22

(CDC)., C. f. D. C. (2021). *Childhood Lead Prevention Program* National Center for Environmental Health, Division of Environmental Health Science and Practice. https://www.cdc.gov/nceh/lead/prevention/default.htm

(CLU-IN), C. S. C.-U. I. (2019). *Phytotechnology Project Profiles*

(USEPA), E. P. A. (2001). Brownfields Technology Primer: Selecting and Using Phytoremediation.

(USEPA), U. S. E. P. A. (2021). *Overview of EPA's Brownfields Program*. US EPA https://www.epa.gov/brownfields/overview-epas-brownfields-program#:~:text=What%20is%20a%20Brownfield%3F%20A%20brownfield%20is%20a,presence%20of%20a%20hazardous%20substance%2C%20pollutant%2C%20or%20contaminant.

Abbas, S. H., Ismail, I. M., Mostafa, T. M., & Sulaymon, A. H. (2014). Biosorption of heavy metals: a review. *J Chem Sci Technol*, *3*(4), 74-102.

Aelion, C. M., Davis, H. T., Lawson, A. B., Cai, B., & McDermott, S. (2013). Associations between soil lead concentrations and populations by race/ethnicity and income-to-poverty ratio in urban and rural areas. *Environmental geochemistry and health*, *35*(1), 1-12.

Ajmone-Marsan, F., & Biasioli, M. (2010). Trace Elements in Soils of Urban Areas. *Water, Air, & Soil Pollution*, *213*(1), 121-143. https://doi.org/10.1007/s11270-010-0372-6

Allen, M., Figueroa, C., Weinbaum, B., Barlow, S., & Allen, E. (1996). Differential production of oxalate by mycorrhizal fungi in arid ecosystems. *Biology and Fertility of Soils*, *22*(4), 287-292.

Allen, M. F., Allen, M. F., & Allen, M. F. (1991). *The ecology of mycorrhizae*. Cambridge University Press.

Alloway, B. J. (2013). Sources of heavy metals and metalloids in soils. In *Heavy metals in soils* (pp. 11-50). Springer.

Amir, H., Jourand, P., Cavaloc, Y., & Ducousso, M. (2014). Role of Mycorrhizal Fungi in the Alleviation of Heavy Metal Toxicity in Plants. In Z. M. Solaiman, L. K. Abbott, & A. Varma (Eds.), *Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration* (pp. 241-258). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-45370-4_15

Antoniadis, V., & Alloway, B. (2001). Availability of Cd, Ni and Zn to ryegrass in sewage sludge-treated soils at different temperatures. *Water, Air, and Soil Pollution*, *132*(3), 201-214.
Audet, P., & Charest, C. (2007). Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives. *Environmental pollution*, *147*(3), 609-614.

Augustsson, A., Filipsson, M., Öberg, T., & Bergbäck, B. (2011). Climate change — An uncertainty factor in risk analysis of contaminated land. *Science of the total environment*, *409*(22), 4693-4700. https://doi.org/https://doi.org/10.1016/j.scitotenv.2011.07.051

Azcon, R., del Carmen Perálvarez, M., Biro, B., Roldán, A., & Ruíz-Lozano, J. M. (2009). Antioxidant activities and metal acquisition in mycorrhizal plants growing in a heavy-metal multicontaminated soil amended with treated lignocellulosic agrowaste. *Applied Soil Ecology*, *41*(2), 168-177.

Baghour, M., Moreno, D., Hernandez, J., Castilla, N., & Romero, L. (2001). Influence of root temperature on phytoaccumulation of As, Ag, Cr, and Sb in potato plants (Solanum tuberosum L. var. Spunta). *Journal of Environmental Science and Health, Part A*, *36*(7), 1389-1401.

Bago, B., Pfeffer, P. E., & Shachar-Hill, Y. (2000). Carbon metabolism and transport in arbuscular mycorrhizas. *Plant physiology*, *124*(3), 949-958.

Bahafid, W., Joutey, N. T., Asri, M., Sayel, H., Tirry, N., & El Ghachtouli, N. (2017). Yeast biomass: an alternative for bioremediation of heavy metals. *Yeast-Industrial Applications*, *559*.

Balbus, J. M., Boxall, A. B., Fenske, R. A., McKone, T. E., & Zeise, L. (2013). Implications of global climate change for the assessment and management of human health risks of chemicals in the natural environment. *Environmental toxicology and chemistry*, *32*(1), 62-78.

Baldwin, D. R., & Marshall, W. J. (1999). Heavy metal poisoning and its laboratory investigation. *Annals of clinical biochemistry*, *36*(3), 267-300.

Banks, M. K., Schwab, P., Liu, B., Kulakow, P. A., Smith, J. S., & Kim, R. (2003). The Effect of Plants on the Degradation and Toxicity of Petroleum Contaminants in Soil: A Field Assessment. In D. T. Tsao (Ed.), *Phytoremediation* (pp. 75-96). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-45991-x_3

Barea, J., Azcon, R., & Hayman, D. (1975). Possible synergistic interactions between Endogone and phosphate-solubilizing bacteria in low-phosphate soils vera. Endomycorrhizas; Proceedings of a Symposium.

Berg, A., & Sheffield, J. (2018). Climate Change and Drought: the Soil Moisture Perspective. *Current Climate Change Reports*, *4*(2), 180-191. https://doi.org/10.1007/s40641-018-0095-0

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., . . . Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science & Technology*, *31*(3), 860-865.

Brodsky, C. (2007). How Many Brownfields Does California Have? https://www.cclr.org/sites/default/files/how-many-brownfields-in-ca.pdf

Bullard, R. D. (1994). Unequal protection: Environmental justice and communities of color.

Bullard, R. D. (2018). *Dumping in Dixie: Race, class, and environmental quality*. Routledge. California Environmental Protection Agency (CalEPA), O., CEJA. . (2018). *CalEnviroScreen3.0: A Critical Tool for Achieving Environmental Justice in California*

Carlisle, J. (2009). Revised California human health screening levels for lead. *California Environmental*.

CDC, C. f. D. C. (2010). Case Studies in Environmental Medicine (CSEM): Lead Toxicity. In: Agency for Toxic Substances and Disease Registry

Chaudhry, T., & Khan, A. (2002). Role of symbiotic micro-organisms in sustainable plant growth on heavy metal contaminated industrial sites. *Biotechnology of microbes and sustainable utilization*, 270-279.

Chen, B., Li, X., Tao, H., Christie, P., & Wong, M. H. (2003). The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. *Chemosphere*, *50*(6), 839-846.

Chen, B., Zhu, Y.-G., Duan, J., Xiao, X., & Smith, S. (2007). Effects of the arbuscular mycorrhizal fungus Glomus mosseae on growth and metal uptake by four plant species in copper mine tailings. *Environmental pollution*, *147*(2), 374-380.

Clark, H. F., Brabander, D. J., & Erdil, R. M. (2006). Sources, Sinks, and Exposure Pathways of Lead in Urban Garden Soil. *Journal of Environmental Quality*, *35*(6), 2066-2074. https://doi.org/https://doi.org/10.2134/jeq2005.0464

Clarke, L. W., Jenerette, G. D., & Bain, D. J. (2015). Urban legacies and soil management affect the concentration and speciation of trace metals in Los Angeles community garden soils. *Environmental pollution*, *197*, 1-12.

Comte, S., Guibaud, G., & Baudu, M. (2008). Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. *Journal of hazardous materials*, *151*(1), 185-193.

Conesa, H. M., Evangelou, M. W. H., Robinson, B. H., & Schulin, R. (2012). A Critical View of Current State of Phytotechnologies to Remediate Soils: Still a Promising Tool? *The Scientific World Journal*, *2012*, 173829. https://doi.org/10.1100/2012/173829

Cook, B. I., Ault, T. R., & Smerdon, J. E. (2015). Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances*, *1*(1), e1400082.

Cooper, A. M., Felix, D., Alcantara, F., Zaslavsky, I., Work, A., Watson, P. L., . . . Scavo, A. J. (2020). Monitoring and mitigation of toxic heavy metals and arsenic accumulation in food crops: A case study of an urban community garden. *Plant direct*, *4*(1), e00198.

Corzo Remigio, A., Chaney, R. L., Baker, A. J. M., Edraki, M., Erskine, P. D., Echevarria, G., & van der Ent, A. (2020). Phytoextraction of high value elements and contaminants from mining and mineral wastes: opportunities and limitations. *Plant and soil*, *449*(1), 11-37. https://doi.org/10.1007/s11104-020-04487-3

Cui, M., & Nobel, P. S. (1992). Nutrient status, water uptake and gas exchange for three desert succulents infected with mycorrhizal fungi. *New Phytologist*, *122*(4), 643-649.

Devinny, J., Longcore, T., Bina, A., Kitts, C., & Osborne, K. H. (2005). Phytoremediation with native plants. *University of Southern California, Los Angeles*.

Dhankhar, R., & Hooda, A. (2011). Fungal biosorption–an alternative to meet the challenges of heavy metal pollution in aqueous solutions. *Environmental technology*, *32*(5), 467-491.

Diaz, G., Azcón-Aguilar, C., & Honrubia, M. (1996). Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of Lygeum spartum and Anthyllis cytisoides. *Plant and soil*, *180*(2), 241-249.

do Nascimento, C. W. A., Amarasiriwardena, D., & Xing, B. (2006). Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. *Environmental pollution*, *140*(1), 114-123. https://doi.org/https://doi.org/10.1016/j.envpol.2005.06.017

Eckerd, A., & Keeler, A. G. (2012). Going green together? Brownfield remediation and environmental justice. *Policy Sciences*, *45*(4), 293-314.

Elias, R. W., & Gulson, B. (2003). Overview of lead remediation effectiveness. *Science of the total environment*, *303*(1-2), 1-13.

Essington, M. E. (2003). *Soil and Water Chemistry: An Integrative Approach* CRC Press.

Farraji, H., Zaman, N. Q., Tajuddin, R., & Faraji, H. (2016). Advantages and disadvantages of phytoremediation: A concise review. *Int J Env Tech Sci*, *2*, 69-75.

Ferrol, N., Tamayo, E., & Vargas, P. (2016). The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. *Journal of experimental botany*, erw403.

Filippelli, G. M., & Laidlaw, M. A. (2010). The elephant in the playground: confronting lead-contaminated soils as an important source of lead burdens to urban populations. *Perspectives in biology and medicine*, *53*(1), 31-45.

Fomina, M., Alexander, I. J., Colpaert, J., & Gadd, G. (2005). Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biology and Biochemistry*, *37*(5), 851-866.

Gadd, G. M. (1994). Interactions of fungi with toxic metals. *The Genus Aspergillus*, 361-374.

Gadd, G. M. (1999). Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. *Advances in microbial physiology*, *41*, 47-92.

Gadd, G. M. (2007). Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological research*, *111*(1), 3-49.

Gadd, G. M. (2009). Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *84*(1), 13-28.

Gadkar, V., & Rillig, M. C. (2006). The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. *FEMS microbiology letters*, *263*(1), 93-101.

Garbisu, C., & Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology*, *77*(3), 229-236. https://doi.org/https://doi.org/10.1016/S0960-8524(00)00108-5

Garten, C. T., Classen, A. T., & Norby, R. J. (2009). Soil moisture surpasses elevated CO2 and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment. *Plant and soil*, *319*(1), 85-94. https://doi.org/10.1007/s11104-008-9851-6

Gaur, A., & Adholeya, A. (2004). Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Current Science*, 528-534.

Gawronski, S. W., Gawronska, A. Helena (2007). *Plant Taxonomy for Phytoremediation* (N. Marmiroli, B. Samotokin, & M. Marmiroli, Eds.). Springer Netherlands.

Giasson, P., Karam, A., & Jaouich, A. (2008). Arbuscular mycorrhizae and alleviation of soil stresses on plant growth. *Mycorrhizae: Sustainable agriculture and forestry*, 99-134.

Gildon, A., & Tinker, P. (1983). Interactions of Vesicular arbuscular mycorrhizal infections and heavy metals in plants: II. The effects of infection on uptake of copper. . *New Phytologist*, *95*(2), 263-268.

Gonzalez-Chavez, C., D'haen, J., Vangronsveld, J., & Dodd, J. (2002). Copper sorption and accumulation by the extraradical mycelium of different Glomus spp.(arbuscular mycorrhizal fungi) isolated from the same polluted soil. *Plant and soil*, *240*(2), 287-297.

Gonzalez-Chavez, M., Carrillo-Gonzalez, R., Wright, S., & Nichols, K. (2004). The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environmental pollution*, *130*(3), 317-323.

González-Guerrero, M., Escudero, V., Saéz, Á., & Tejada-Jiménez, M. (2016). Transition Metal Transport in Plants and Associated Endosymbionts: Arbuscular Mycorrhizal Fungi and Rhizobia [Review]. *Frontiers in Plant Science*, *7*(1088). https://doi.org/10.3389/fpls.2016.01088

Gorospe, J. (2012). *Growing greens and soiled soil: Trends in heavy metal contamination in vegetable gardens of San Francisco*. San Jose State University.

Grispen, V. M. J., Nelissen, H. J. M., & Verkleij, J. A. C. (2006). Phytoextraction with Brassica napus L.: A tool for sustainable management of heavy metal contaminated soils. *Environmental pollution*, *144*(1), 77-83. https://doi.org/https://doi.org/10.1016/j.envpol.2006.01.007

Gupta, S. K., & Chen, K. Y. (1978). Arsenic removal by adsorption. *Journal (Water Pollution Control Federation)*, 493-506.

GWRTAC. (1997). Remediation of metals-contaminated soils and groundwater. *Tech. Rep. TE-97-01, GWRTAC*.

Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology*, *9*(3), 177-192. https://doi.org/10.1038/nrmicro2519

Hassan, S. E. D., Boon, E., ST-ARNAUD, M., & Hijri, M. (2011). Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. *Molecular Ecology*, *20*(16), 3469-3483.

Hellmers, H., Horton, J., Juhren, G., & O'keefe, J. (1955). Root systems of some chaparral plants in southern California. *Ecology*, *36*(4), 667-678.

Henry, H. F., Burken, J. G., Maier, R. M., Newman, L. A., Rock, S., Schnoor, J. L., & Suk, W. A. (2013). Phytotechnologies--preventing exposures, improving public health. *International Journal of Phytoremediation*, *15*(9), 889-899. https://doi.org/10.1080/15226514.2012.760521

Hettiarachchi, G. M., & Pierzynski, G. M. (2004). Soil lead bioavailability and in situ remediation of lead-contaminated soils: A review. *Environmental progress*, *23*(1), 78-93.

Hildebrandt, U., Regvar, M., & Bothe, H. (2007). Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry*, *68*(1), 139-146.

Hoffman, A. (1985). A Community under Siege: A Chronicle of Chicanos East of the Los Angeles River, 1945–1975. By Rodolfo F. Acuña.(Los Angeles: Chicano Studies Research Center, University of California, Los Angeles, 1984. xvi+ 523 pp. Maps and notes. Paper, $21.95.). In: Organization of American Historians.

Hooda, P., & Alloway, B. (1993). Effects of time and temperature on the bioavailability of Cd and Pb from sludge-amended soils. *Journal of Soil Science*, *44*(1), 97-110.

Humans, I. W. G. o. t. E. o. C. R. t. (1993). Cadmium and cadmium compounds. In *Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry*. International Agency for Research on Cancer.

Hunter, C. M., Williamson, D. H., Gribble, M. O., Bradshaw, H., Pearson, M., Saikawa, E., . . . Kegler, M. (2019). Perspectives on heavy metal soil testing among community gardeners in the

United States: a mixed methods approach. *International journal of environmental research and public health*, *16*(13), 2350.

Jabeen, R., Ahmad, A., & Iqbal, M. (2009). Phytoremediation of heavy metals: physiological and molecular mechanisms. *The Botanical Review*, *75*(4), 339-364.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60-72.

Joner, E., & Leyval, C. (2001). Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. *Biology and Fertility of Soils*, *33*(5), 351-357.

Joner, E. J., Briones, R., & Leyval, C. (2000). Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and soil*, *226*(2), 227-234.

Jones, R. L., Homa, D. M., Meyer, P. A., Brody, D. J., Caldwell, K. L., Pirkle, J. L., & Brown, M. J. (2009). Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988–2004. *Pediatrics*, *123*(3), e376-e385.

Joutey, N. T., Sayel, H., Bahafid, W., & El Ghachtouli, N. (2015). Mechanisms of hexavalent chromium resistance and removal by microorganisms. *Reviews of Environmental Contamination and Toxicology Volume 233*, 45-69.

Järup, L. (2003). Hazards of heavy metal contamination. *British medical bulletin*, *68*(1), 167-182.
Järup, L., & Åkesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicology and applied pharmacology*, *238*(3), 201-208.

Khan, A. G. (2006). Mycorrhizoremediation—an enhanced form of phytoremediation. *Journal of Zhejiang University Science B*, *7*(7), 503-514.

Khan, A. G., Kuek, C., Chaudhry, T., Khoo, C. S., & Hayes, W. J. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, *41*(1-2), 197-207.

Khan, S., Cao, Q., Zheng, Y., Huang, Y., & Zhu, Y. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental pollution*, *152*(3), 686-692.

Kiaghadi, A., Rifai, H. S., & Dawson, C. N. (2021). The presence of Superfund sites as a determinant of life expectancy in the United States. *Nature Communications*, *12*(1), 1-12.

Killham, K. a., & Firestone, M. (1983). Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. *Plant and soil*, *72*(1), 39-48.

Kim, J. G., Dixon, J. B., Chusuei, C. C., & Deng, Y. (2002). Oxidation of chromium (III) to (VI) by manganese oxides. *Soil Science Society of America Journal*, *66*(1), 306-315.

Kirpichtchikova, T. A., Manceau, A., Spadini, L., Panfili, F., Marcus, M. A., & Jacquet, T. (2006). Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence, EXAFS spectroscopy, chemical extraction, and thermodynamic modeling. *Geochimica et Cosmochimica Acta*, *70*(9), 2163-2190.

Krishnamoorthy, R., Kim, C.-G., Subramanian, P., Kim, K.-Y., Selvakumar, G., & Sa, T.-M. (2015). Arbuscular mycorrhizal fungi community structure, abundance and species richness changes in soil by different levels of heavy metal and metalloid concentration. *PloS one*, *10*(6), e0128784.

Landis, W. G., Rohr, J. R., Moe, S. J., Balbus, J. M., Clements, W., Fritz, A., . . . Stahl, R. G. (2014). Global climate change and contaminants, a call to arms not yet heard? In: Wiley Online Library.

LeBrón, A. M., Torres, I. R., Valencia, E., Dominguez, M. L., Garcia-Sanchez, D. G., Logue, M. D., & Wu, J. (2019). The state of public health lead policies: Implications for urban health inequities and recommendations for health equity. *International journal of environmental research and public health*, *16*(6), 1064.

Lenihan, J. M., Drapek, R., Bachelet, D., & Neilson, R. P. (2003). Climate change effects on vegetation distribution, carbon, and fire in California. *Ecological applications*, *13*(6), 1667-1681.

Li, J., Sun, Y., Jiang, X., Chen, B., & Zhang, X. (2018). Arbuscular mycorrhizal fungi alleviate arsenic toxicity to Medicago sativa by influencing arsenic speciation and partitioning. *Ecotoxicology and environmental safety*, *157*, 235-243.

Lovelock, C. E., Wright, S. F., Clark, D. A., & Ruess, R. W. (2004). Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *Journal of ecology*, 278-287.

Ma, Y., Dickinson, N., & Wong, M. H. (2006). Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous trees on Pb/Zn mine tailings. *Soil Biology and Biochemistry*, *38*(6), 1403-1412.

Masue, Y., Loeppert, R. H., & Kramer, T. A. (2007). Arsenate and arsenite adsorption and desorption behavior on coprecipitated aluminum: iron hydroxides. *Environmental Science & Technology*, *41*(3), 837-842.

McClintock, N. (2012). Assessing soil lead contamination at multiple scales in Oakland, California: Implications for urban agriculture and environmental justice. *Applied Geography*, *35*(1-2), 460-473.

Medina, A., Vassilev, N., & Azcon, R. (2010). The interactive effect of an AM fungus and an organic amendment with regard to improving inoculum potential and the growth and nutrition of Trifolium repens in Cd-contaminated soils. *Applied Soil Ecology*, *44*(2), 181-189.

Mielke, H. W., Berry, K. J., Mielke, P. W., Powell, E. T., & Gonzales, C. R. (2005). Multiple metal accumulation as a factor in learning achievement within various New Orleans elementary school communities. *Environmental Research*, *97*(1), 67-75.

Mielke, H. W., Dugas, D., Mielke Jr, P. W., Smith, K. S., & Gonzales, C. (1997). Associations between soil lead and childhood blood lead in urban New Orleans and rural Lafourche Parish of Louisiana. *Environmental Health Perspectives*, *105*(9), 950-954.

Mielke, H. W., Gonzales, C. R., & Powell, E. T. (2017). Soil lead and children's blood lead disparities in pre-and post-Hurricane Katrina New Orleans (USA). *International journal of environmental research and public health*, *14*(4), 407.

Mielke, H. W., Powell, E. T., Gonzales, C. R., Mielke, P. W., Ottesen, R. T., & Langedal, M. (2006). New Orleans soil lead (Pb) cleanup using Mississippi River alluvium: Need, feasibility, and cost. *Environmental Science & Technology*, *40*(8), 2784-2789.

Mulligan, C., Yong, R., & Gibbs, B. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering geology*, *60*(1-4), 193-207.

Neidhardt, H. (2021). Arbuscular mycorrhizal fungi alleviate negative effects of arsenic-induced stress on crop plants: A meta-analysis. *PLANTS, PEOPLE, PLANET*, *3*(5), 523-535. https://doi.org/https://doi.org/10.1002/ppp3.10122

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., & Tedersoo, L. (2019). Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology*, *17*(2), 95-109.

Noll, M. R. (2003). Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. *Journal of Environmental Quality*, *32*(1), 374.

Ochiai, E.-I. (1987). Toxicity of Heavy Metals. *General Principles of Biochemistry of the Elements*, 361-395.

Office, U. S. E. P. A. T. I., & Support, E. M. (1999). *Phytoremediation resource guide*. US Environmental Protection Agency, Office of Solid Waste and Emergency ….

Office, U. S. G. A. (2019). *Superfund: EPA Should Take Additional Actions to Manage Risks from Climate Change* (Report to Congressional Requestors Issue. https://www.gao.gov/assets/gao-20-73.pdf

Paltseva, A., Cheng, Z., Deeb, M., Groffman, P. M., Shaw, R. K., & Maddaloni, M. (2018). Accumulation of arsenic and lead in garden-grown vegetables: Factors and mitigation strategies. *Science of the total environment*, *640*, 273-283.

Paltseva, A. A. (2019). *Lead and Arsenic Contamination in Urban Soils in New York City* City University of New York].

Paltseva, A. A., & Neaman, A. (2020). An Emerging Frontier: Metal (loid) Soil Pollution Threat under Global Climate Change. *Environmental toxicology and chemistry*.

Paull, E. (2008). The environmental and economic impacts of brownfields development.

Pawlowska, T. E., Błaszkowski, J., & Rühling, Å. (1997). The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. *Mycorrhiza*, *6*(6), 499-505.

Qin, S., Liu, H., Nie, Z., Rengel, Z., Gao, W., Li, C., & Zhao, P. (2020). Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: A review. *Pedosphere*, *30*(2), 168-180. https://doi.org/https://doi.org/10.1016/S1002-0160(20)60002-9

Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K.-H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment international*, *125*, 365-385.

Rambelli, A. (1973). The rhizosphere of mycorrhizae. *Ectomycorrhizae. Their ecology and physiology*, 299-349.

Raskin, I., Smith, R. D., & Salt, D. E. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. *Current opinion in biotechnology*, *8*(2), 221-226.

Registry, A. f. T. S. a. D. (2007). Toxicological profile on Arsenic https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=22&tid=3

Registry, A. f. T. S. a. D. (2019). *Toxic Substances Priority List*

Rillig, M. C., Wright, S. F., Nichols, K. A., Schmidt, W. F., & Torn, M. S. (2001). Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant and soil*, *233*(2), 167-177.

Rothenberg, S. J., Williams Jr, F. A., Delrahim, S., Khan, F., Kraft, M., Lu, M., . . . Wooten, D. J. (1996). Blood lead levels in children in south central Los Angeles. *Archives of Environmental Health: An International Journal*, *51*(5), 383-388.

Satarug, S., Baker, J. R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P. E., Williams, D. J., & Moore, M. R. (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicology letters*, *137*(1-2), 65-83.

Sharma, S., Tiwari, S., Hasan, A., Saxena, V., & Pandey, L. M. (2018). Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. *3 Biotech*, *8*(4), 216. https://doi.org/10.1007/s13205-018-1237-8

Shi, W., Zhang, Y., Chen, S., Polle, A., Rennenberg, H., & Luo, Z. B. (2019). Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. *Plant, cell & environment*, *42*(4), 1087-1103.

Shukla, P., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H., Roberts, D., . . . Van Diemen, R. (2019). IPCC, 2019: Climate Change and Land: an IPCC special report on climate

change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.

Simons, R. A. (1998). How Many Urban Brownfields are Out There?: An Economic Base Contraction Analysis of 31 U.S. Cities. *Public Works Management & Policy*, *2*(3), 267-273. https://doi.org/10.1177/1087724x9800200310

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Academic press.

Smith, S. E., & Smith, F. A. (2012). Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia*, *104*(1), 1-13.

Sparks, L. D. (2003). *Environmental Soil Chemistry* Elsevier Science.

Surls, R., Borel, V., & Biscaro, A. (2016). Soils in Urban Agriculture: Testing, Remediation, and Best Management Practices.

Sweatt, M. R., & Davies Jr, F. T. (1984). Mycorrhizae, water relations, growth, and nutrient uptake of geranium grown under moderately high phosphorus regimes. *Journal of the American Society for Horticultural Science*, *109*(2), 210-213.

Tan, S.-Y., Jiang, Q.-Y., Zhuo, F., Liu, H., Wang, Y.-T., Li, S.-S., . . . Jing, Y.-X. (2015). Effect of inoculation with Glomus versiforme on cadmium accumulation, antioxidant activities and phytochelatins of Solanum photeinocarpum. *PloS one*, *10*(7), e0132347.

Toler, H., Morton, J., & Cumming, J. (2005). Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. *Water, Air, and Soil Pollution*, *164*(1), 155-172.

Turnau, K., & Haselwandter, K. (2002). Arbuscular mycorrhizal fungi, an essential component of soil microflora in ecosystem restoration. In *Mycorrhizal technology in agriculture* (pp. 137-149). Springer.

USEPA. (1996). Report: recent developments for in situ treatment of metals contaminated soils. In: US Environmental Protection Agency, Office of Solid Waste and Emergency Response.

USEPA. (2021). Remediation Technologies for Cleaning Up Contaminated Sites. https://www.epa.gov/remedytech/remediation-technologies-cleaning-contaminated-sites

van der Ent, A., Baker, A. J., Reeves, R. D., Chaney, R. L., Anderson, C. W., Meech, J. A., . . . Morel, J. L. (2015). Agromining: farming for metals in the future? In: ACS Publications.

Viti, C., Marchi, E., Decorosi, F., & Giovannetti, L. (2014). Molecular mechanisms of Cr (VI) resistance in bacteria and fungi. *FEMS microbiology reviews*, *38*(4), 633-659.

Vivas, A., Barea, J., & Azcón, R. (2005). Interactive effect of Brevibacillus brevis and Glomus mosseae, both isolated from Cd contaminated soil, on plant growth, physiological mycorrhizal fungal characteristics and soil enzymatic activities in Cd polluted soil. *Environmental pollution*, *134*(2), 257-266.

Wagner, G. J. (1993). Accumulation of cadmium in crop plants and its consequences to human health. *Advances in agronomy*, *51*, 173-212.

Wang, Y. T. (2000). Microbial reduction of chromate. *Environmental Microbe-Metal Interactions*, 225-235.

Warter, M. M., Singer, M. B., Cuthbert, M. O., Roberts, D., Caylor, K. K., Sabathier, R., & Stella, J. (2021). Drought onset and propagation into soil moisture and grassland vegetation responses during the 2012–2019 major drought in Southern California. *Hydrology and Earth System Sciences*, *25*(6), 3713-3729.

Watanabe, M. E. (2001). Can bioremediation bounce back? *Nature Biotechnology*, *19*(12), 1111-1115.

White, B. M., Bonilha, H. S., & Ellis, C. (2016). Racial/ethnic differences in childhood blood lead levels among children< 72 months of age in the United States: a systematic review of the literature. *Journal of racial and ethnic health disparities*, *3*(1), 145-153.

Wielinga, B., Mizuba, M. M., Hansel, C. M., & Fendorf, S. (2001). Iron promoted reduction of chromate by dissimilatory iron-reducing bacteria. *Environmental Science & Technology*, *35*(3), 522-527.

Wu, L., Luo, Y., Xing, X., & Christie, P. (2004). EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agriculture, Ecosystems & Environment*, *102*(3), 307-318.

Wu, S., Hu, Y., Zhang, X., Sun, Y., Wu, Z., Li, T., . . . Zheng, L. (2018). Chromium detoxification in arbuscular mycorrhizal symbiosis mediated by sulfur uptake and metabolism. *Environmental and Experimental Botany*, *147*, 43-52.

Wu, S., Zhang, X., Sun, Y., Wu, Z., Li, T., Hu, Y., . . . Zhang, Z. (2015). Transformation and immobilization of chromium by arbuscular mycorrhizal fungi as revealed by SEM–EDS, TEM–EDS, and XAFS. *Environmental Science & Technology*, *49*(24), 14036-14047.

Wu, S. C., Wong, C. C., Shu, W. S., Khan, A. G., & Wong, M. H. (2010). Mycorrhizo-Remediation of Lead/Zinc Mine Tailings Using Vetiver: A Field Study. *International Journal of Phytoremediation*, *13*(1), 61-74. https://doi.org/10.1080/15226511003671353

Wuana, R. A., & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology*, *2011*, 402647. https://doi.org/10.5402/2011/402647

Xavier, L., & Boyetchko, S. (2002). Arbuscular mycorrhizal fungi as biostimulants and bioprotectants of crops. In *Applied mycology and biotechnology* (Vol. 2, pp. 311-340). Elsevier.

Xintaras, C. (1992). *Impact of lead-contaminated soil on public health*. Agency for Toxic Substances and Disease Registy, US Department of Public Health.

Yang, Y., Han, X., Liang, Y., Ghosh, A., Chen, J., & Tang, M. (2015). The combined effects of arbuscular mycorrhizal fungi (AMF) and lead (Pb) stress on Pb accumulation, plant growth parameters, photosynthesis, and antioxidant enzymes in Robinia pseudoacacia L. *PloS one*, *10*(12), e0145726.

Zota, A. R., Schaider, L. A., Ettinger, A. S., Wright, R. O., Shine, J. P., & Spengler, J. D. (2011). Metal sources and exposures in the homes of young children living near a mining-impacted Superfund site. *Journal of Exposure Science & Environmental Epidemiology*, *21*(5), 495-505. https://doi.org/10.1038/jes.2011.21

Zwolak, A., Sarzyńska, M., Szpyrka, E., & Stawarczyk, K. (2019). Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: a Review. *Water, Air, & Soil Pollution*, *230*(7), 164. https://doi.org/10.1007/s11270-019-4221-y

Chapter 2: Microbial and Plant Diversity on Contaminated Sites in Southern California

1. Abstract

Certain microbes are capable of degrading organic contaminants and enhancing the phytoremediation of contaminated soils. Understanding the diversity and abundance of microbes, as well as plants, on contaminated sites helps identify suitable species for successful application in remediation and restoration. In the first study of the biology of contaminated sites in Southern California, seven contaminated brownfields and Superfund sites were surveyed for plant, bacterial and fungal volunteers with the goal of identifying locally adapted species tolerant of the mixed organic and metal contaminated soils and the arid and semi-arid climatic conditions found regionally. Several California native shrubs previously unidentified as plant hyper-accumulators of copper (Cu), including *Baccharis salcifolia* and *sarothroides*, *Eriogonum fasciculatum* and *Brickellia californica,* as well as one unstudied non-native Cu hyperaccumulator, *Helminthotheca echiodes*. Other California native accumulators of metals such as lead (Pb), chromium (Cr), and nickel (Ni), were identified. Fungal and bacterial communities were dominated by the Ascomycota and Proteobacteria phyla, and the Dothideomycetes, Sordariomycetes and Spizellomycetes fungal and Gammoproteobacterial and Planstomycetes bacterial classes. Several microbial taxa previously identified as contaminant remediators were seen at all study sites, as well as some yet untested taxa which may be candidates for use in bioremediation. Ordinations, including RDA and PCA, illustrated that most of the variation in microbial community composition was explained by differences across sites.

Water-extractable organic carbon, silt and sand content, and cation exchange capacity were the most significant drivers of microbial community composition within sites. Our findings identified potential plant and fungal candidates for further exploration in bioremediation applications suited to Southern California and the most significant soil and environmental drivers influencing their communities.

2. Introduction

Bioremediation is the process of employing living organisms e.g., plants, fungi, bacteria, to biodegrade or detoxify pollutants in the environment. Fungi play major roles in ecosystem processes and biogeochemical cycles relevant to the fate and transport of organic and inorganic contaminants in soils, however, fungal diversity of contaminated sites remains poorly characterized and fungal potential in remediation remains "untapped" (Czaplicki et al., 2016; Harms et al., 2011). Studying the biological diversity of contaminated sites can yield insights into novel fungal, plant and bacterial species that could have applications in biotechnology, bioremediation, ecological restoration, and the development of sustainable solutions for pollution control (Bourdel et al., 2016; He & Yang, 2007; Pilon-Smits, 2005; Rohrbacher & St-Arnaud, 2016).

Contaminated sites can harbor a diversity of fungal species due to the selective pressures imposed by the presence of pollutants (Mejra et al, 2023). These selective pressures favor the growth of taxa that are tolerant or resistant to pollutants and may be involved in biodegradation or bioaccumulation (Gadd et al, 1991). This may include fungi that use complex organic pollutants as a carbon source, enzymatically degrading

them and, in doing so, potentially reduce their toxicity and aide in soil creation (Harms et al., 2011), as well as plant-associated fungi (e.g., mycorrhizal fungi and/or endophytic fungi) which may confer metal and other contaminant tolerance to their plant partner and may be applied to enhance plant based remediation (i.e., phytoremediation) of metals (Amir et al., 2014; Leyval et al., 1995; Tullio et al., 2003).

Plants that putatively tolerate the presence of metals (metallophytes) can be classified in three general categories: (1) excluders, which limit metal concentrations in shoots; (2) accumulators, which translocate metals into above-ground tissues; and (3) indicators, which demonstrate a linear relationship between metal concentrations in plant tissues and soil (Baker et al., 2000). The Global Hyperaccumulator Database operationally defines hyperaccumulators as that plants which contain in their dry weight foliar tissue > 100 μg/g cadmium, thallium or selenium, > 300 μg/g of cobalt, copper or chromium, > 1000 μg/g of nickel, arsenic, lead, > 3000 μg/g of zinc, or > 10 000 μg/g of manganese, when growing in their natural habitat (Reeves et al, 2017; Baker & Brooks, 1989; Reeves, 2003; van der Ent *et al*., 2013). Phytoextraction is a type of phytoremediation that employs 'hyperaccumulator plants' which can accumulate metals in their tissue at concentrations much greater than the surrounding environment (Garbisu & Alkorta, 2001; Gawronski, 2007). A variety of phytoremediation plants ranging from large trees to small grasses have been identified, yet few hyperaccumulator plants tolerant of semi-arid environments have been identified (Khan et al., 2000; Office & Support, 1999; van der Ent et al., 2015). California native plant metal hyperaccumulators are not as extensively studied or well-documented as hyperaccumulators in other parts of the

world, particularly native plants from Southern California and other semi-arid to arid regions within California. The only California native metal accumulating plants identified to date are: *Sequoiadendron giganteum* (Giant sequoia; Pb, Cu and Zn) (Gateva et al., 2004), *Streptanthus polygaloides* (purple jewelflower; Ni and Zn) (Boyd and Davis, 2001; Meindl et al., 2014; Sanchez-Mata et al., 2014), *Streptanthus farnsworthianus* (Ni) (Reeves et al., 1981; Reeves, 1983), *Pinus radiata* (Monterey pine; Zn and Cu) (McLaren, 2007; Palma, 2003; Rodriguez et al., 2012), *Lupinus species* (Lupine; Cu) (Ximénez-Embún, 2001 and 2002; Dary et al., 2010; Page et al., 2006).

Use of native plants for phytoremediation may have advantages over agricultural plant species, especially in arid regions, due to their dense, deep root systems adapted to the seasonal rainfall of the Mediterranean climate found in southern California and other regions (Hellmers et al., 1955). Although no extensive survey of plant and microbial diversity of contaminated sites in Southern California, or California more broadly, has been undertaken thus far, using California native plants for phytoremediation, along with co-occurring soil fungi and bacteria, could be beneficial for addressing the statewide soil contamination. Microbial diversity in soil is influenced by a variety of soil properties that create suitable habitats for different fungal and bacterial species. While the relationships can be complex and context-dependent, several soil properties are known to be key drivers of microbial diversity, including pH, high organic matter, moisture, texture, nutrient availability, cation exchange capacity (CEC), temperature, oxygen levels, land use and level of disturbance, plant diversity, interactions with other microbes, climatic region and environmental factors and soil depth (Aguilar-Trigueros et al., 2015; Yang, et.

al., 2017). Currently, the soil and environmental properties that influence fungal diversity on southern California contaminated sites are not known (Aguilar-Trigueros et al., 2015; Yang, et. al., 2017).

In this study, we assessed microbial and plant diversity on seven contaminated brownfields and Superfund sites in Southern California (Figure 1). We explored the following questions: 1) Which fungi and bacteria inhabit contaminated soils in Southern California? 2) Are rhizosphere-associated microbial communities different from those in the bulk soil? 3) Which soil physicochemical factors influence microbial diversity on the contaminated sites surveyed? 4) Which plant volunteers on contaminated sites in Southern California hyperaccumulate metals of concern?

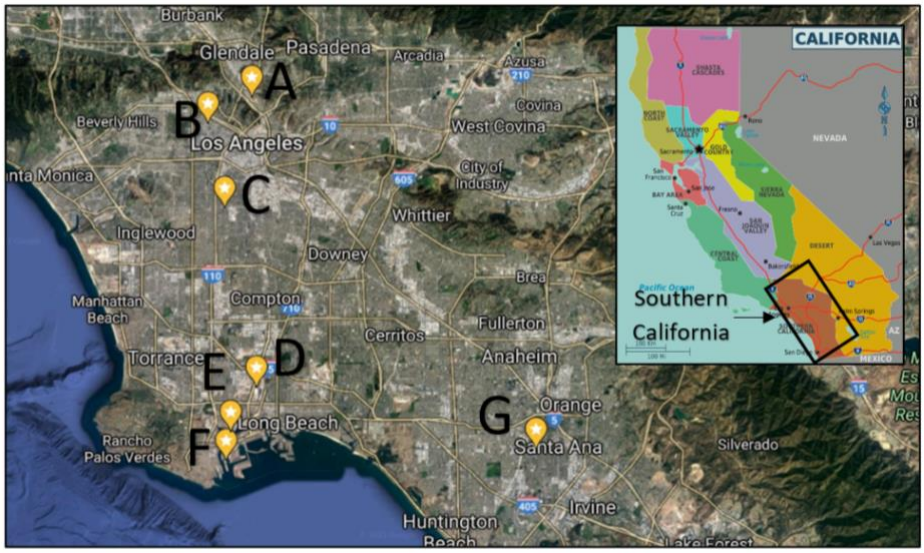

Figure 1. Map of sites sampled in southern California. Taylor Yard (A), LA Ecovillage (B); Slauson and Wall (C); Berths 70-71 (D); GATX Annex (E); San Pedro Boat Works (F); Daisy and Walnut (G).

3. Materials and methods

3.1 Study system

A majority of Southern California's climate is Mediterranean and classified as warm and arid (Köppen-Geiger climate classification *Csa*). The average annual temperature in Los Angeles County is 18.7ºC (high of 23.7ºC and low of 13.7ºC) and average precipitation is 36.2 cm/year (NOAA, 2020). The average annual temperature in Orange County is 17.2ºC (high of 23.1ºC and low of 13.4ºC) and average precipitation is 37.5 cm/year (NOAA, 2020). Sites experienced different amounts of rainfall in the year 2021. Site A had 43.6 inches of rainfall, Site B had 28.25 inches of rainfall, Site C had 26.48 inches of rainfall, sites D and F had 24.02 inches of rainfall, Site E had 20.18 inches and Site G had 33.55 inches of rainfall (DPW, 2023). Sites had varying altitudes: site A is 120 m above sea level, site B is 78 m above sea level, site C is 26 m and sites D, E and F are between 1 - 8 m above sea level, site G is 39 m above sea level (California, 2023). The soil order in Los Angeles County is entisol, sub-order and great group is Xeropsamments (dry, sandy), family is sandy, mixed, thermic typic Xerofluvents and coarse-loamy, mixed, super active, thermic Fluventic Haploxerolls and series is Metz, Urban (anthropogenic) and Pico (USDA, 2021). The soils at these sites are generally hydrophobic, compact, and nutrient-depleted, thus, much of these properties are barren apart from patches of weeds and plant volunteers. The soils are mostly capped with asphalt or concrete, with patches of soil where cracks have formed, or plants have broken through. All sites contain soil concentrations of metal(loid)s exceeding regulatory limits including Pb, As, Cu and other inorganic contaminants; most sites have organic co-

contamination such as solvents (TCE, PCE), gasoline and diesel-range organics and polycyclic aromatic hydrocarbons exceeding regulatory limits. Screening limits (SL) for each contaminant are available in Table 1 (DTSC, 2020b; EPA, 2020). A detailed description of each site's past and present land use and contaminant levels is provided.

Study Sites

Taylor Yard ("G2 parcel"; Site A) (34.0990 -118.2375) is owned by the City of LA and regulated by the Department of Toxic Substances Control (DTSC). The location served as a former rail yard with Pb concentrations between 100 - 25,000 ppm, diesel concentrations between 98 - 36,000 mg/kg, and 12 – 16 mg/kg As in soils 0 – 152 cm depth. The City of LA will re-develop the lot for use as a public park by 2028 (City of LA, Bureau of Engineers, 2021). As of 2023, Much of Taylor Yard is under concrete, but patches of soil are exposed along patchy and dispersed vegetation on the site.

LA Ecovillage ("Songs"; Site B) (34.072651507669214, -118.29065692967363) is a community-owned land trust. The lot was formerly an automotive repair shop and soils contain Pb concentrations between 85 - 300 mg/kg, As concentrations ~20 mg/kg, Cd concentrations between 10 - 15 mg/kg, Cu concentrations between 6,600 mg/kg, diesel concentrations ~ 400 mg/kg, and benzo(a)pyrene concentrations ~7.7 mg/kg, benzo(a)anthracene concentrations ~15 mg/kg, benzoflouranthracene concentrations ~14 mg/kg and dibenz(a)anthracene concentrations ~0.36 mg/kg in soils (0- 61cm) (EPA, 2017). Soils are currently capped by asphalt. The land trust plans to redevelop this site as

a community hub, tiny-home village, and demonstration urban agriculture site by 2025 (USTU Board, personal communication).

Table 1. Screening limits for contaminants found on sample sites (US EPA and DTSC).

Contaminant	Residential screening limit (mg/kg)	Industrial screening limit (mg/kg)
Lead (Pb)	80	320
Arsenic (As)	1.9	24
Cadmium (Cd)	1.7	980
Antimony (Sb)	30	470
Copper (Cu)	3000	47,000
Chromium (VI)	0.3	17
Diesel	98	400
B(a)P	0.038	11
TCE	0.016	480
PCE	0.015	460

Slauson and Wall (Site C) (33.98762527296143, -118.27117757601722) is owned by the City of LA and regulated by DTSC. It is a former site of the U.S. Electrical Manufacturing Company and has also been occupied for manufacturing, printing, metal plating, metal polishing, wood and furniture finishing, and vehicle maintenance and repair. Soils on the site contain Cr(VI) concentrations between 790 - 1,100 mg/kg, Pb concentrations between 520 - 1,200 mg/kg, trichloroethylene (TCE) concentrations ~19.7 mg/kg and perchloroethylene (PCE) concentrations ~55.67 mg/kg in soils (0 –152cm) (City of LA, personal communication). Soils are currently capped under concrete.

Berths 70-71 ("B70-71"; Site D) (33.73596602491816, -118.2816410080327) was formerly a US Navy submarine base and chemical waste disposal and storage facility operated by various oil companies (179). It is owned by the Port of LA and regulated by the California Water Board. Soils contain As concentrations between 5.35-15.3 mg/kg,

Pb concentrations ~124 mg/kg, TCE concentrations ~130 mg/kg and PCE concentrations up to 1,100 mg/kg (Board, 2003).

GATX (Site E) (33.72596602491813, -118.1816410080327) is a former military base and coast guard site that burnt in a fire (180). It is owned by the Port of LA and regulated by the DTSC. The site currently contains soil concentrations of metals and solvents that exceed regulatory limits (Port of LA site manager, personal communication).

San Pedro Boat Works ("B44"; Site F) (33.71596602491812, -118.1816210080325) previously operated as a commercial boat yard (DTSC, 2021b). It is owned by the Port of LA and regulated by the Department of Toxic Substances Control. The site contains elevated concentrations of soil metal(loid)s Sb, As, Cd, Cu, Pb, Hg, TI, Ni and Zn as well as PCBs (Aroclor-1248 and Aroclor-1254), PCE, TCE, diesel and benzo(a)pyrene above regulatory limits.

Walnut and Daisy (Site G) (33.74489438426813, -117.89509197358704) is a vacant site owned by the City of Santa Ana and leased to CRECE Urban Farming Coop and THRIVE Santa Ana, a community land trust. The site was identified by Orange County Environmental Justice and UC Irvine's School of Public Health as having Pb concentrations above 100 mg/kg (Masri et al., 2020). It is being redeveloped into a community farm (THRIVE, personal communication).

Figure 2. Aerial photographs of sites from Google Earth (Google, 2023): From top left: Site A, Taylor Yard (Los Angeles); Site B, LA Ecovillage (Los Angeles); Site C, Slauson and Wall (Los Angeles); Site G, Daisy and Walnut (Santa Ana); Site F, San Pedro Boat Works (Long Beach); Site E, GATX Annex (Long Beach); and Site D, Berths 70-71 (Long Beach).

3.2 Soil and plant sampling

At each site, using sterile technique, five soil cores (0 - 10 cm) were taken from interspace soils (i.e., soils between plant islands) in randomized sample locations using a steel telescoping auger. Samples were deposited into sealed bags (Whirl Pak), homogenized within the sealed bag, and stored on dry ice immediately. An additional five soil cores (0 – 10 cm) were taken from the rhizosphere (root zone) of plants that have

volunteered on each site from random locations. Corresponding above-ground plant parts were harvested with clippers into zipper storage bags and stored at 4^{o}C until analysis. Photos of plants were taken for identification purposes and GPS coordinates of all sampling locations were determined using a handheld GPS (Garmin). Before each sampling, auger, buckets, and sampling tools were decontaminated with deionized water and 70% ethanol. Samples were stored at 4^{o}C during transport then stored at -80^{o}C until DNA extraction.

3.3 Plant identification and chemical analyses

Plants growing on each site were photographed using a smartphone and identified using *Picture This* application (Glority, LLC) and verified by iNaturalist as needed. Aboveground parts of plants were oven dried at 60^{o}C for 3 days, then ground in a blender and microwave digested following EPA method 3052. Briefly, 0.45 g of dried ground plant and 5 ml HNO_3, 3 ml H_2O_2 and 1 ml HCl was added to the microwave vessel. Digestate (~7.5 ml) was then poured into 50 ml polypropylene tubes and diluted to 50 ml with deionized water. 6 ml of digestate was then acidified by 2% with trace metal grade HNO_3 and analyzed for concentrations of total dissolved metals in the digested samples using Agilent 7700 inductively coupled plasma mass spectrometry (ICP-MS) (U.S. EPA. 2007).

3.4 Soil physical and chemical characterization

Soil moisture and temperature at each sampling depth (10 cm and 152 cm) were measured using TM5 soil temperature and moisture probes (Campbell Scientific) and recorded with a EM50 data logger (Meter Corp). Soil texture was determined using the LS-13-320 Grain Size Analyzer (Beckman-Coulter) after hydrogen peroxide digestion of organic carbon and deflocculation with sodium hexametaphosphate (SHMP). pH was measured in air-dried soil slurries (2:1 DDI water: soil vol./vol.) using a field soil pH meter (Hannah instruments, Smithfield, RI). CEC was determined using the $BaCl_2$ compulsive exchange method (Gillman and Sumpter, 1986). Soils were air-dried, ground, and sieved (2 mm) prior to measuring total soil elemental concentrations using X-ray fluorescence (XRF) spectrometry (SPECTRO XEPOS HE). Sequential extractions following the method outlined in (Tessier et al., 1979) was used to quantify metals (Pb, As, Cd, Cr, Zn, Cu) associated with three fractions: water extractable, exchangeable, and weak acid extractable. Dissolved metals in extracts were analyzed using ICP-MS (Agilent 7700).

Organic carbon and nitrogen content and stable isotopes were measured, and C/N calculated using an elemental analyzer (Costech ECS 4010-Delta V Plus IRMS, Valencia, CA). Soil samples were prepared by drying in an oven at 105^{o}C, ground and sieved to 100 mesh, then 40 mg weighed into foil tins. Inorganic carbon content (carbonate) was determined using an ELTRA CS500 Carbon/Sulfur Analyzer (Newtown, PA). Water-extractable organic carbon (WEOC) was extracted in DI water by shaking soil samples for three hours followed by centrifugation and quantified using the

Shimadzu TOC-VCPH Total Organic Carbon Analyzer (as per Homyak et al., 2018). Soil phosphate (PO_4) concentrations were measured in air-dried soils using the Olsen sodium bicarbonate extraction method (Olsen & Sommers, 1982; Olsen, 1954), where samples were shaken with 0.5 M $NaHCO_3$ (pH 8.5), filtered and filtrates stored at -20^oC until analysis. Nitrate (NO_3^-) and ammonium (NH_4^+) were extracted from fresh soils (stored at 4^oC) by shaking with 2.0 M KCl solution, filtering the extract, and filtrates were stored at -20^oC until analysis (188). PO_4, NO_3^- and NH_4^+ concentrations were measured using a discrete analyzer (Alpkem Flow Solution IV Automated wet chemistry system).

3.5 Analysis of Microbial Biomass

To measure the mass of the viable microbial community, a modified chloroform fumigation extraction method (Horwath & Paul, 1994) was used (Fierer, 2003) with the chloroform-exposure and extraction step combined, coupled with a reduced amount of chloroform exposure time. Briefly, organic C from lysed microbial cells in soil samples was directly extracted in a potassium sulfate salt (KCl) solution after chloroform-fumigation and TOC determined using a TOC-V Analyzer (Shimadzu). Microbial biomass (μg C/g) was calculated as the difference between extracted organic C from fumigated and non-fumigated soil samples without correcting for extraction efficiency and therefore may represent a 'flush' of C, rather than total microbial biomass.

3.6 Soil arbuscular mycorrhizal fungal spore counts and diversity

Spores were extracted from soils using a sucrose density centrifugation method (Allen et al., 1979; Ianson & Allen, 1986) and counted under a dissecting microscope at

30 - 50x (Brundrett et al., 1996). Subsequently, phenotypic characteristics were observed under the microscope for taxonomic classification, according to the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM, 2023).

3.7 Soil microbial community composition

The PowerSoil DNeasy extraction kit (Qiagen Inc., Germantown, MA) was used to extract DNA from ten (0.4 g/sample) samples from each site; to account for soil heterogeneity, these ten samples consisted of five biological replicates each within both rhizosphere and interspace zones. The manufacturer's extraction protocol was followed, with the following modifications: a heated lysis step was added (25 minutes at 65^{o}C) before homogenization (Rubin et al., 2014) and cellular lysis with the MP Bio Fastprep (Qiagen Inc., Germantown, MA) bead beater (5 cycles at 6.5 m/sec for 1 min). Samples were purified from residual contaminants with the PEG-based magnetic bead protocol using a 1:2 sample: magnetic bead ratio (Rohland & Reich, 2012). DNA concentrations were determined by fluorescence using the DS-11 nanodrop (DeNovix, Wilmington, DE, USA) and each sample was normalized to equimolar concentrations at ~10 ng/µL. After extraction, DNA was stored at -20^{o}C until further analysis. The fungal community was assessed by targeting the ITS2 subregion using the universal fungal primers 5.8SFun and ITS4Fun (Taylor et al., 2016) and the bacterial community with the 16S rRNA region using the Earth Microbiome Project bacterial primers 515F/806R for the variable V4 region (Caporaso et al., 2011), forward: ATTAGAWACCCBNGTAGTCC and reverse: TTACCGCGGCKGCTGRCAC.

Fungal library construction was conducted in a two-step polymerase-chain-reaction (PCR) procedure for amplification and indexing (Berry et al., 2011). Bacterial library construction was adapted from Illumina MiSeq platform protocol for 16S metagenomic libraries (Beganskas et al., 2018). PCR reactions were carried out on an BioRad Thermocycler (Hercules, California). First-round amplifications for fungi were carried out with DIP-seq adapted primers with universal tails synthesized 5′ to the locus specific sequences (Alvarado et al., 2017). Besides template DNA, reactions contained 12.5 µL Phusion HotStart II DNA polymerase (Thermo Fisher Scientific, Waltham, MA), 5 µL each 1µM forward and reverse primer (Eurofins Genomics, Louisville, KY), 1.5 µL nanopure H20 and 0.1 µL BSA. Thermal cycler conditions were as follows: 95^{o}C for 2 min, 95^{o}C for 30 s, 60^{o}C for 1 min, 68^{o}C for 1 min, return to step two 40 times, and finally an indefinite hold at 10^{o}C, as per Phillips et al. (2020), until proceeding with downstream processing. The results of the reaction products were checked on a 1% agarose gel; successful amplifications were pooled by sample, and products were purified using a second PEG-bead cleanup and eluted in 20 µL Tris-Cl (pH 8.0). The two step PCR reaction then required an index PCR, carried out using primers with sequences matching the universal tails at the 3′ end, and matching Illumina MiSeq flowcell sequences at the 5′ end. Conditions for tailing reactions were identical to the first-round reaction except that only 100 nM of each indexing primer was used, only one was reaction conducted per sample, and only 15 total cycles were performed. Indexed PCR products were visualized on an agarose gel; PCR products were purified with the PEG-based magnetic bead cleanup, quantified via Nanodrop, and concentrations normalized

using SequalPrep Normalization Plates (Applied Biosystems, Waltham, MA) and pooled. The resulting pool was further concentrated with the PEG-bead protocol, quantified by qPCR and average fragment sizes estimated using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) prior to sequencing. Sequencing was carried out on a MiSeq Desktop Sequencer (Illumina Inc, San Diego, CA) running in paired end 2 × 300 mode by the UC Riverside Institute for Integrative Genome Biology Core (IIGB, Riverside, CA).

3.7.1 Bioinformatics

Sequences were demultiplexed by UCR's IIGB; sequence quality was evaluated using FastQC (Andrews, 2010) and initial quality filtering performed using QIIME (Caporaso et al., 2010) to remove sequences with average quality with phred scores < Q20. Reads were quality-controlled, sorted, and assigned into amplicon sequence variants (ASVs) using the Divisive Amplicon Denoising Algorithm (DADA2) module embedded in Anacapa (Callahan, 2016; Curd, 2017) and following the workflow outlined in 10.5281/zenodo.5794554 (Freund, 2023). Since ASVs are assigned based on 99% sequence identity, they are true representative of biological sequences and thus directly comparable across workflows (Prodan et al. 2020; Callahan, McMurdie, and Holmes 2017). The eestats2 program (Edgar & Flyvbjerg, 2015) was used to determine the ideal trimming length of the sequences. Locus-specific adapter, primer sequences, and contamination by PhiX (included in MiSeq runs by adding PhiX directly onto flowcells to promote read diversity as control sequences on the Illumina platform) were removed

using CutAdapt (Martin, 2011). Paired-end reads were merged with ea-utils (Aronesty, 2011). Merged-sequence quality was checked using FastQC (Andrews, 2010) and trimmed as needed, with expected error of 5 per forward read and 10 per reverse read permitted. 16S sequences were truncated and retained only if they retained 95% of initial sequence length (p =0.95) (Vargas-Albores et al., 2017). A 10% quality score threshold was used for truncating reads, and the minimum read length allowed was 50 bp and 0 unknown bases. Demultiplexed reads were screened for chimeras with usearch_ref mode against the UNITE-based fungal chimera dataset for ITS (Nilsson et al., 2015) and the NCBI-based bacterial chimera dataset for 16S (Balvočiūtė & Huson, 2017), one chimera was removed. QIIME was used to remove singletons, dereplicate the sequences, and remove de novo chimeras. ITS2 forward and reverse reads could not merge so the DADA2 pipeline proceeded with only filtered forward reads, the reverse complement of forward reads searched against the UNITE database to assign ASV identifiers. Taxonomic assignment tables were used with a bootstrap confidence cutoff of 60. The R decontam package v. 3.4.2 (Davis, 2018) was used to remove contaminant ASVs for each metabarcode marker (FITS) and sequencing run, based on their prevalence in extraction blank control samples. ASVs with decontamination scores below the specified threshold (0.1) or not assigned a taxonomic path were removed from the dataset unless they had the same taxonomic classification as an uncontaminated ASV. 6439 reads were returned for ITS2 rRNA data with an average of 96 per sample. 2,653,325 reads were returned for 16S rRNA data with an average of 39,019 per sample.

3.7.2 Statistical analyses

All statistical analyses were performed in R version 4.3.1 (R version 4.3.1; R Core Team 2023). The dplyr package was used to generate summary statistics. To evaluate whether any of the soil physico-chemical properties (pH, CEC, N, $N0_3$, NH_4, PO_4, P, moisture, temperature, OC, WEOC, and C/N) were significantly different between interspace and rhizosphere samples or sites we used one-way ANOVA (R packages “ggplot2", "ggpubr" and "tidyverse"). If the ANOVA test was significant, we performed multiple pairwise-comparisons between the means of groups using Tukey HSD (Tukey Honest Significant Differences) to see which group was different. Assumptions for one-way ANOVA were checked using 1) the normal probability plot of residuals and the Shapiro-Wilk test on the ANOVA residuals to check the assumption that the residuals are normally distributed, and 2) the residuals versus fits plot and/or Levene’s test, which is less sensitive to departures from normal distribution (in R package “car”) to check the homogeneity of variances.

To evaluate whether the mean microbial biomass of the interspace is significantly different from that of the rhizosphere, we used the unpaired two-samples t-test in R using the package “ggpubr”. To check independent t-test assumptions 1) whether the groups of samples being compared are normally distributed, we used the Shapiro-Wilk test and 2) whether the variances of the two groups are equal, we checked using F-test.

3.7.3 Comparison of rhizosphere and interspace microbial diversity

Differences between rhizosphere and interspace in overall general fungal (ITS2) community composition was compared across sites by performing permutational multivariate ANOVA (PERMANOVA) for each locus using the 'adonis' function in the 'vegan' package in R (999 permutations (Oksanen, 2011)). The R package "Vegan" and the diversity and specnumber functions were used to calculate Shannon Diversity and species richness (Oksanen et al. 2020). The Shapiro-Wilks test determined a p-value > 0.05, so species richness values are not normally distributed for this dataset and the Wilcoxon test was then used to compare the means of our sites and sample locations in a pairwise fashion. ASV count data was normalized, rather than rarefied, through centered log-ratio transformation prior to downstream analyses (Gloor et al., 2017; Weiss et al., 2017). Normalization of read data was chosen because of the demonstrated improvement in statistical power that normalization provides over rarefaction (McMurdie & Holmes, 2014; Weiss et al., 2017).

The R package "Vegan" was used to generate Bray-Curtis's indices. Beta-diversity was visualized using non-metric multidimensional scaling (NMDS) of the Bray-Curtis distances, using distance matrices generated from CLS-normalized data before filtering for functional group assignment. The NMDS was visualized in R using the ggplot2 package (Wickham, 2009) and the 'stat_ellipse' function with 95% confidence intervals. The fit of the data was assessed via the stress values associated with the NMDS, with stress values of less than 0.2 deemed acceptable.

3.7.4 Principal coordinates analysis (PCoA)

PCoA was carried out in R to determine how much of the variation in fungal diversity and richness could be explained by different sites, sample locations (rhizosphere or interspace) or soil properties (pH, CEC, WEOC, metal concentrations, etc.) within and between sites. This was assessed using the package "Vegan" in R, and ANOVA and Tukey's Honest Significant Difference Test were used to statistically compare the group dispersions and test the significance (p values </=0.05) of correspondence between soil properties and fungal species across sites.

4. Results and Discussion

4.1 Soil metal concentrations

All sites sampled had metal concentrations in surface soils (0-10 cm) above residential EPA screening limits for at least one metal, while most sites had metals concentrations lower than EPA defined industrial screening limits. Site D had the highest average concentrations of metals, with ranges for As between 52.7 – 1006µg/g, Pb between 371 – 4150µg/g, Cu between 239.2 – 28,640µg/g and Hg between 12.9 – 385.2µg/g. Site F had previously been reported to have elevated soil metal concentrations (Port of LA, 2019), but our testing revealed that metal concentrations were below screening levels except As (2.3 – 6.8µg/g) and Pb (38.1 – 109.1µg/g). Chromium and Zn soil concentrations at all sites were below the residential screening limits of 120,000µg/g and 230,000µg/g respectively (Appendix 4). Copper and Cd soil concentrations are not plotted except for Site D (Figure 7) since all other sites contained concentrations below

the residential screening limits of 3000µg/g and 7.1µg/g respectively, except for two outliers for Cu from Site C (11.1µg/g) and Site G (22.6µg/g) (Appendix 4).

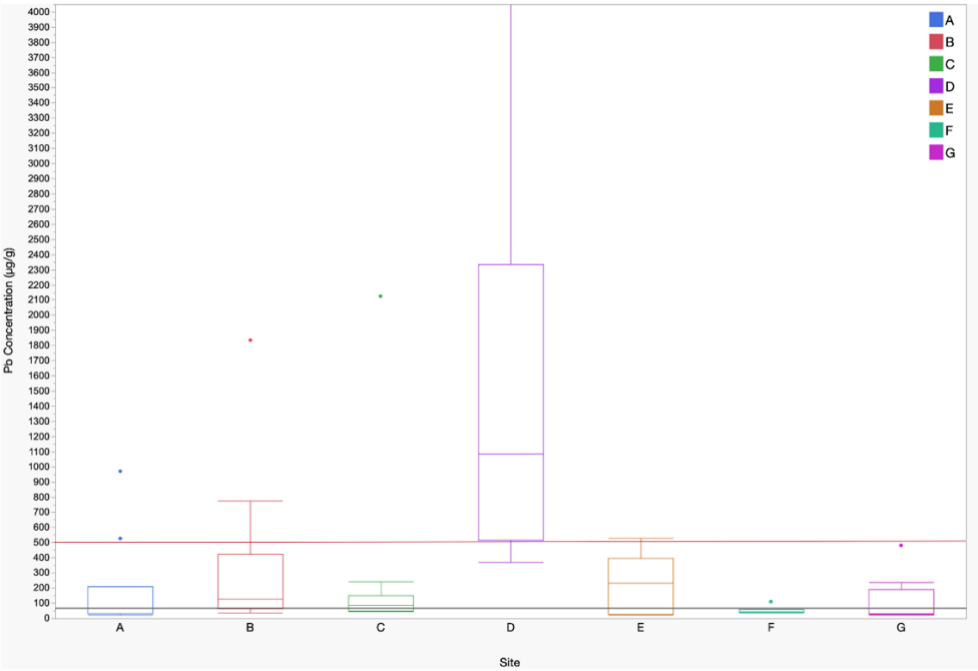

Figure 3. Soil Pb concentrations by site (n = 10 per site). The red line is the industrial screening limit for soil Pb (500µg/g) and the black line shows the residential/unrestricted screening limit for soil Pb (80µg/g) (DTSC, 2023). All samples at Site D contain Pb above the industrial screening limit, whereas Site F soil samples are all below the residential screening limit for Pb.

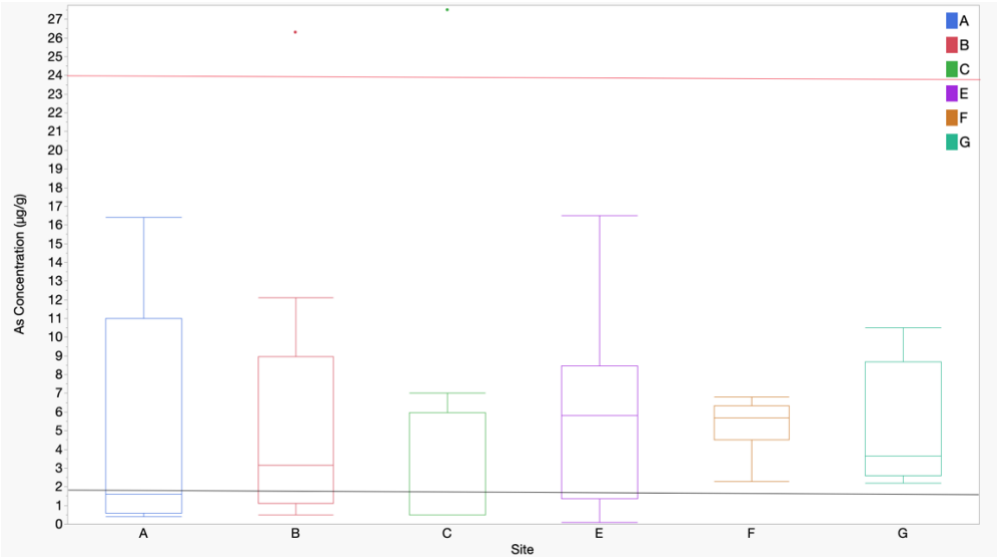

Figure 4. Soil As concentrations by site, excluding Site D provided in Figure 7 due to high concentration range relative to other sites (n = 10 samples per site). The red line shows the industrial screening limit for soil As (24µg/g) and the black line shows the residential/ unrestricted screening limit for soil As (1.9µg/g) (DTSC, 2023). All samples at Site F and G contain As above the residential screening limits, but no sites shown contained soil As concentrations above the industrial screening limit for As.

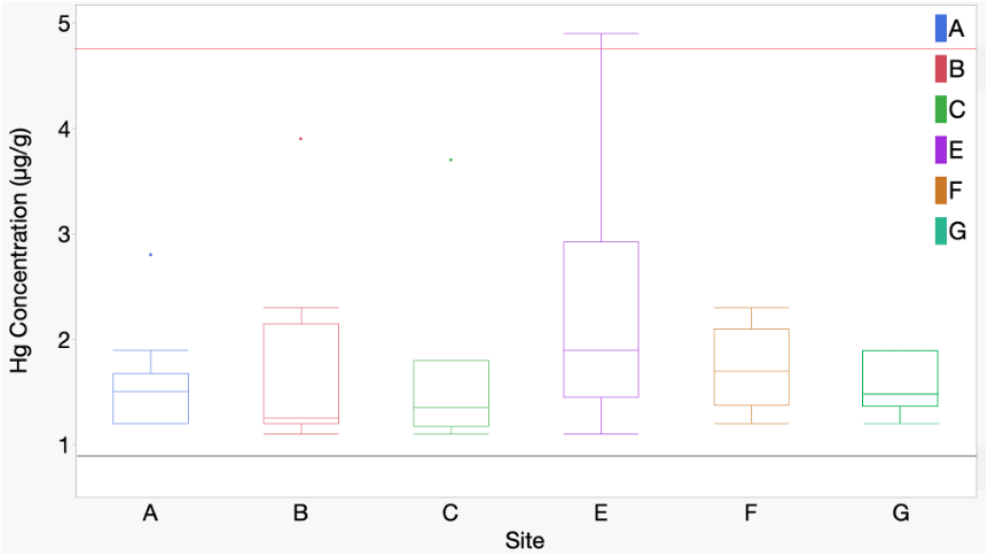

Figure 5. Soil Hg concentrations by site, excluding Site D provided in Figure 7 due to high concentration range relative to other sites (n= 10 samples per site). The red line shows the industrial screening limit for soil Hg (4.6µg/g) and the black line shows the residential/unrestricted screening limit for soil Hg (1.1µg/g) (DTSC, 2023). All samples at all sites shown contain Hg above the residential screening limits, but only site E contained at least

one sample with Hg concentrations above the industrial screening limit for Hg. Site G contained one only sample above the residential screening limit for Hg.

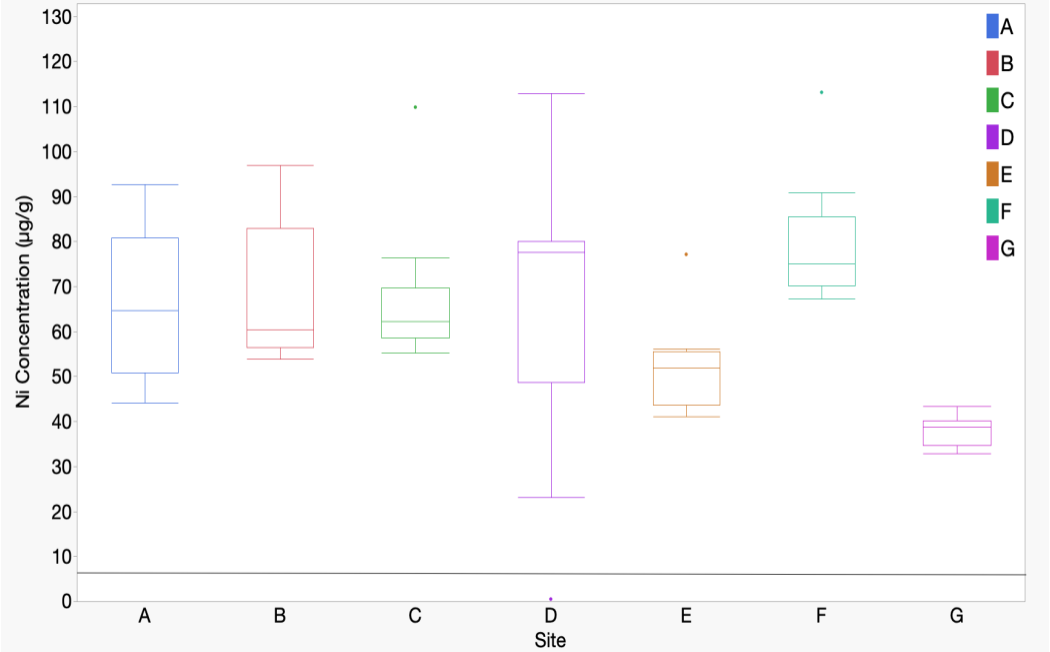

Figure 6. Soil Ni concentrations by site, excluding Site D provided in Figure 7 due to high concentration range relative to other sites (n= 10 samples per site). The black line shows the residential/unrestricted screening limit for soil Ni (7.1µg/g) and the industrial limit is not shown because no samples contained Ni exceeding industrial limits (820µg/g) (DTSC, 2023). All samples at all sites shown contain Ni above the residential screening limits for Ni.

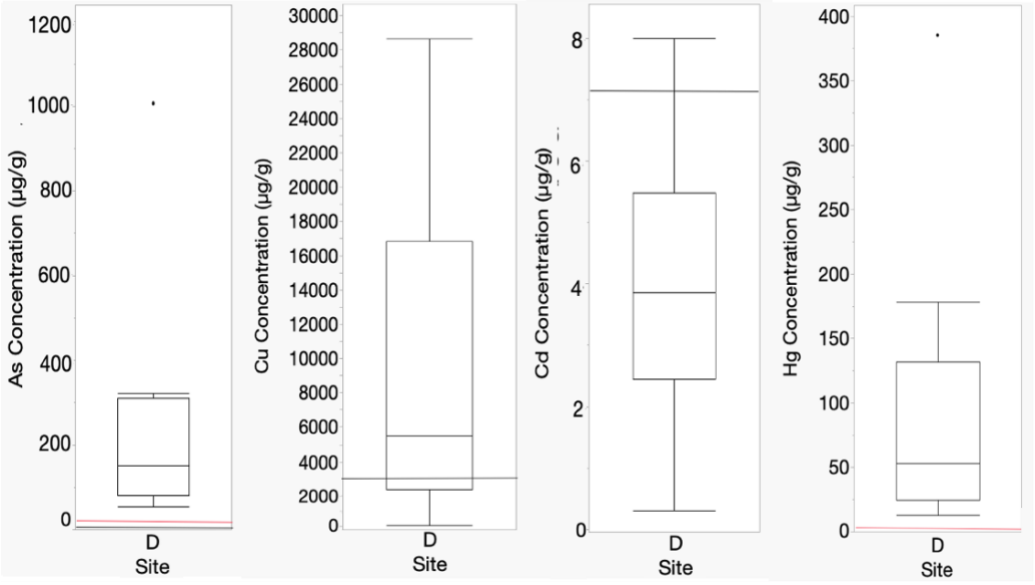

Figure 7. Soil As, Hg, Cu and Cd concentrations for Site D. The black line shows the residential/unrestricted screening limit for soil As (1.9µg/g), Cu (3000µg/g) and Cd (7.1µg/g) and the industrial limit is shown in red for As (24µg/g) and Hg (4.6µg/g) (DTSC, 2023). No samples at Site D contained Cu or Cd above the industrial screening limits, whereas all samples at Site D contained concentrations of As and Hg above the industrial screening limits.

4.2 Soil sequential metal extractions

Water extractable, exchangeable, and weak acid extractable fractions representing the most to least plant-available are shown (Figures 8-10) (Tessier et al., 1979). Weak acid extractions measure the metals adsorbed to poorly crystalline oxides and complexed with organic matter, carbonates, and secondary minerals (Harmsen et al., 1977). There was high variability between sites in metal availability to plants based on extraction data. As and Pb had some availability to plants, but Cu was not very available to plants according to these extraction data.

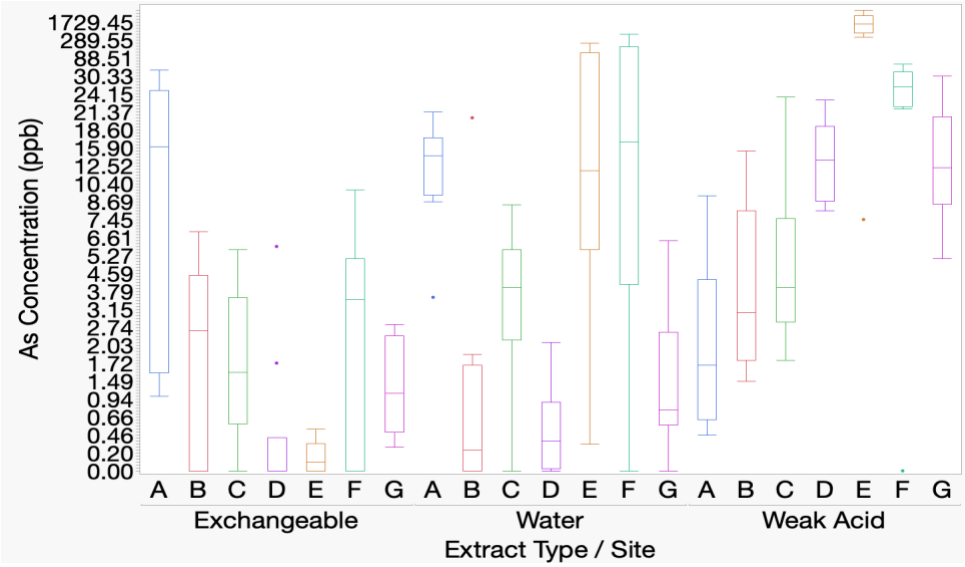

Figure 8. Water-extractable, exchangeable and weak acid extractable As concentrations by site (n = 10 samples per site).

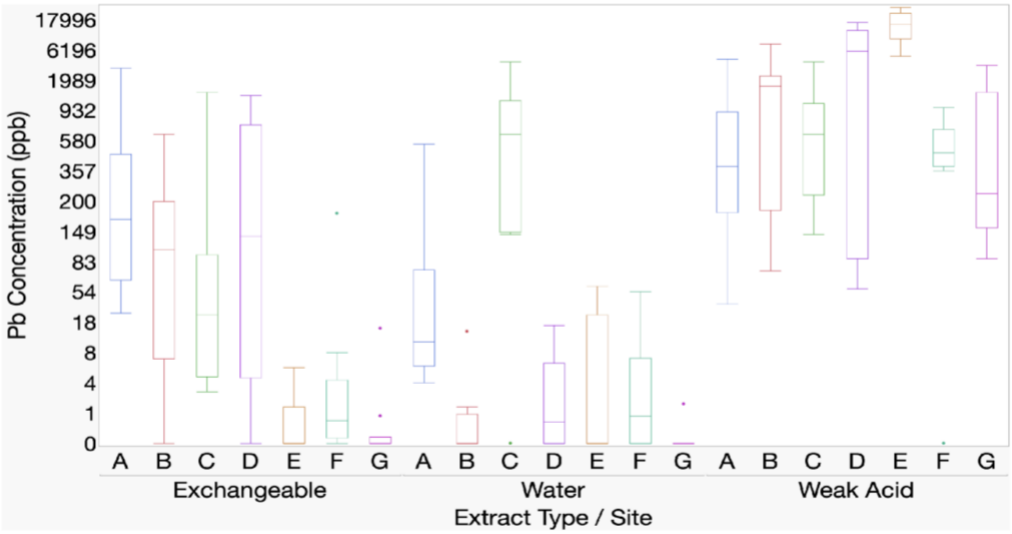

Figure 9. Water-extractable, exchangeable, and weak acid extractable Pb concentrations by site (n = 10 samples per site).

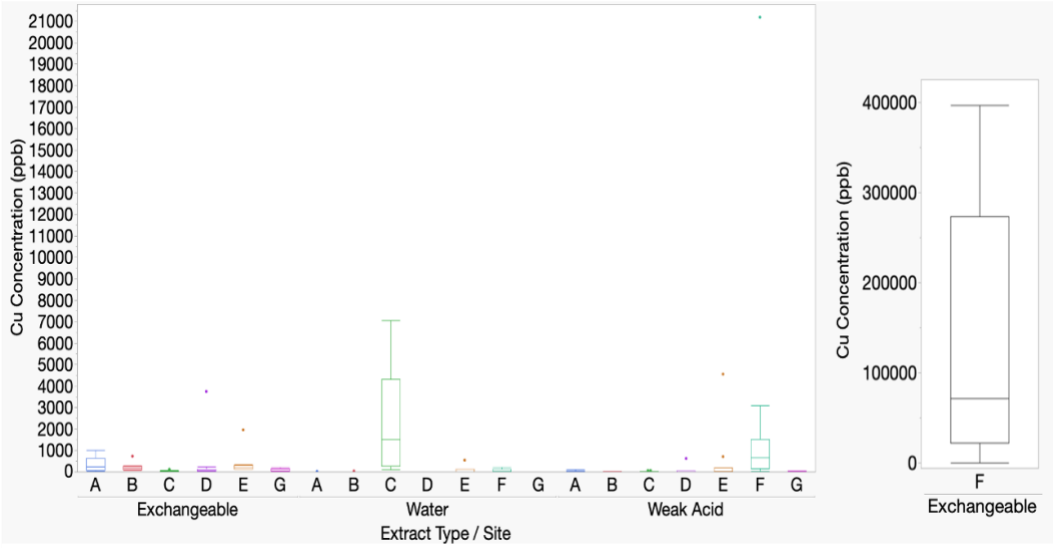

Figure 10. Water-extractable, exchangeable, and weak acid extractable Cu concentrations by site. Site F is shown on its own graph for Exchangeable Cu concentrations because it went as high as 400,000 ppb which was off the charts for the overall graph (n = 10 samples per site).

4.3 Plant Identification and Metal Uptake

Table 2. Plant Identification, Mycorrhizal Status and Native Status at Survey Sites.

Plant Common Name	Latin Name	Mycorrhizal status	California Native?	Plant Family	Sites Observed
Bristly oxtongue	*Helminthotheca echioides*	Arbuscular	No	*Asteraceae*	A, C and D
California brickellbush	*Brickellia californica*	Arbuscular	Yes	*Asteraceae*	A
California bush sunflower	*Encelia californica*	Arbuscular	Yes	*Asteraceae*	A
California buckwheat	*Erigonum fasciculatum*	Arbuscular	Yes	*Polygonaceae*	A
California sagebrush	*Artemisia californica*	Arbuscular	Yes	*Asteraceae*	A
Chinaberry tree	*Melia azedarach*	Arbuscular	No (invasive)	*Meliaceae*	A
Chinese elm	*Ulmus parvifolia*	Arbuscular	No (can be invasive)	*Ulmaceae*	A
Cliff aster	*Malacothrix saxatilis*	Arbuscular	Yes	*Asteraceae*	A
Desert broom	*Baccharis sarothroides*	Arbuscular	Yes	*Asteraceae*	A
Fig	*Ficus carica*	Arbuscular	No (can be invasive)	*Moraceae*	A
Fountain grass	*Cenchrus setaceus*	Arbuscular	No (invasive)	*Poaceae*	A
Mexican fan palm	*Washingtonia robusta*	Arbuscular	Yes	*Arecaceae*	A, B, C
Mule fat	*Baccharis salicifolia*	Arbuscular	Yes	*Asteraceae*	A and D
Pampas grass	*Cortaderia selloana*	Arbuscular	No (invasive)	*Poaceae*	A
Shortpod mustard	*Hirschfeldia incana*	Non	No	*Brassicaeae*	A, B and D
Telegraphweed	*Heterotheca grandiflora*	Arbuscular	Yes	*Asteraceae*	A and B
Tree of heaven	*Ailanthus altissima*	Arbuscular	No (invasive)	*Simaroubaceae*	A
Tree tobacco	*Nicotiana glauca*	Arbuscular	No	*Solanaceae*	A
Purple fountain grass	*Pennisetum setaceum*	Arbuscular	No (invasive)	*Poaceae*	A, C
Coyote brush	*Baccharis pilularis*	Arbuscular	Yes	*Asteraceae*	A

Mallow	*Malva neglecta*	Arbuscular	No	*Malvaceae*	B and C
Common sow thistle	*Sonchus oleraceus*	Low rates of arbuscular infectivity	No	*Asteraceae*	C
Hairy fleabane	*Erigeron bonariensis*	Arbuscular	No	*Asteraceae*	G
Prickly lettuce	*Lactuca serriola*	Arbuscular	No	*Asteraceae*	B, C and G
Nasturtium	*Tropaeolum majus*	Arbuscular	No	*Tropaeolaceae*	B
Black Nightshade	*Solanum nigrum*	Arbuscular	No	*Solanaceae*	C
Yellow sweet clover	*Melilotus officinalis*	Arbuscular	No	*Fabaceae*	C
Wall barley	*Hordeum murinum*	Arbuscular	No	*Poaceae*	A, B, C and D
Panic grass / Switchgrass	*Panicum virgatum*	Arbuscular	No but native to North America	*Poaceae*	C
Lambs' quarters	*Chenopodium giganteum*	Non	No	*Amaranthaceae*	B and C
Chickweed	*Stellaria media*	Arbuscular	No	*Caryophyllaceae*	B and C

Vegetation at all seven contaminated sites is made up of a mixture of native, naturalized, and invasive plant species (Table 2), all of which volunteered on these sites and none of which was planted. Among the volunteer plants tested, *Eriogonoum fasticulatus* and *Heterotecha grandiflora* were the California native plants that accumulated the largest quantity of Pb, and all four species of *Baccharis* found on these sites accumulated Pb, although none of the plant volunteers accumulated enough lead to be considered hyper-accumulators. Of the non-native (and invasive) plants found growing on these sites, *Melilotus officinalis* (sweet yellow clover) far surpassed other plants, even known remediator species "Switchgrass" (*Panicum virgatum*), by a factor of four.

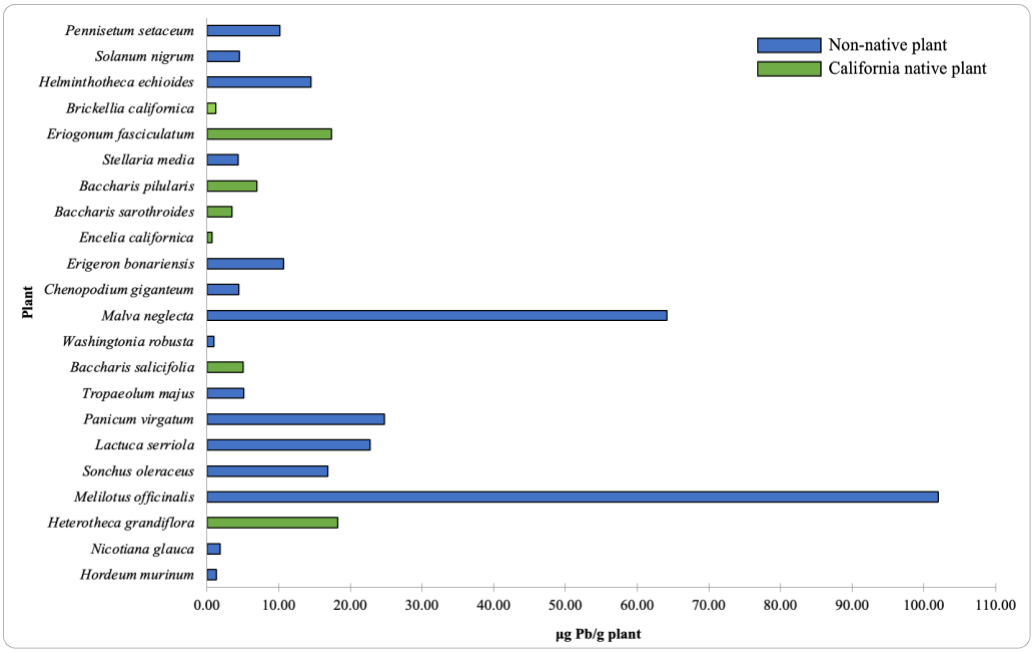

Figure 11. Mean lead uptake (µg Pb / g plant) averaged across replicates across sites. Blue bars represent non-native plants while green bars represent California native plants. Not all plants occurred at all sites.

Several previously unidentified Cu hyperaccumulators were identified in this study, including three California native plants: *Brickellia californica*, *Erigonium fasciculatum* and *Baccharis sarothroides*. Non-native plant volunteers that hyperaccumulated Cu included known metal accumulators *Pennisetum setaceum*, *Malva neglecta*, *Stellaria media* and *Nicotiana glauca* (Lu et al., 2017; Salinitro et al., 2020; Naz et al., 2022; Lin et al., 2018; Swaefy et al., 2020; Barazani et al., 2004; Shingu et al., 2005; Barba-Bioso et a., 2023) as well as previously unstudied *Helminthotheca echiodes*.

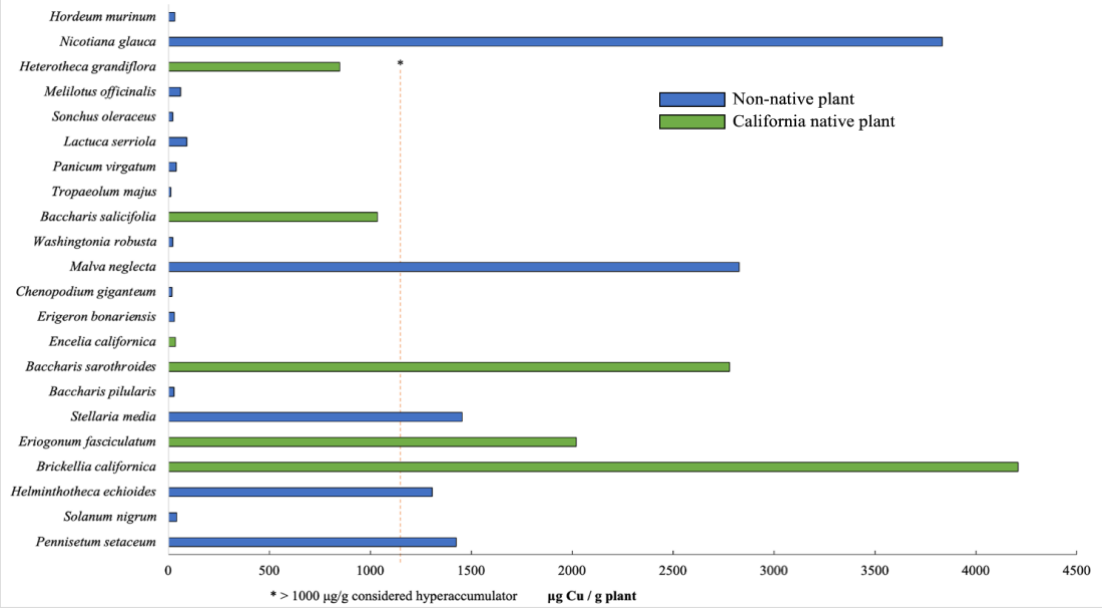

Figure 12. Mean copper uptake (µg Cu / g plant) averaged across replicates across sites. Blue bars represent non-native plants while green bars represent California native plants. Not all plants occurred at all sites.

Although no new As or Cr hyperaccumulators were identified in this study, four non-native 'weed' species (*Helminthotheca echioides*, *Lactuca serriola*, *Panicum virgatum* and *Hordeum murinum*) and one California native plant "Telegraphweed" (*Heterotheca grandiflora*), accumulated Cr. Appendix 3 has a table with mean volunteer plant metal uptake into foliar parts by metal.

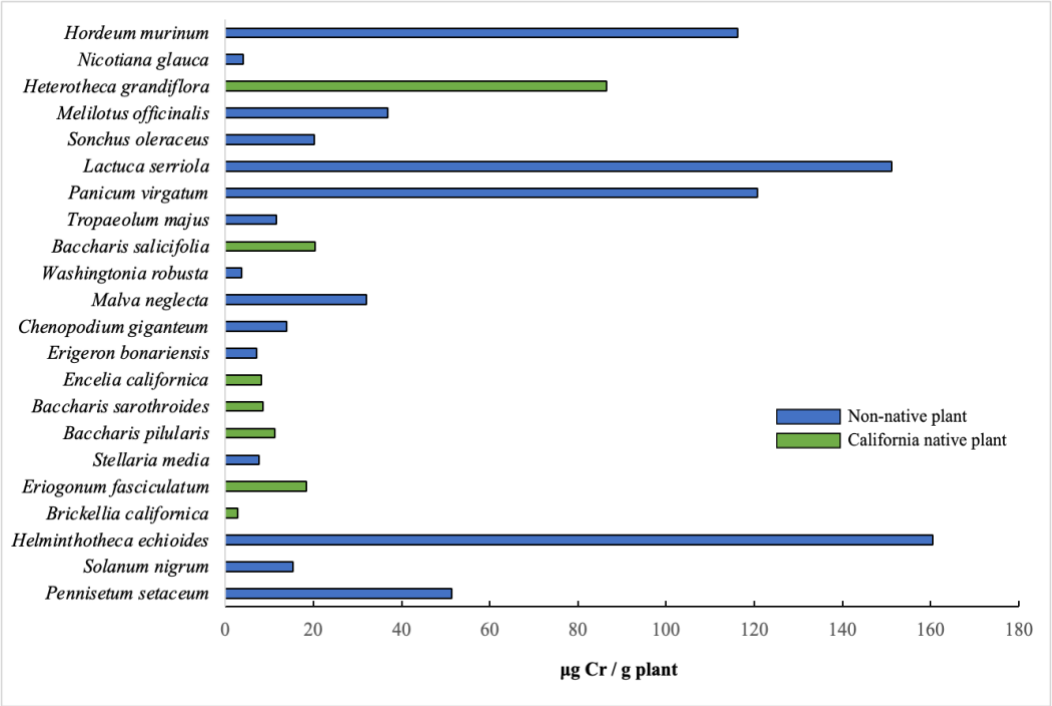

Figure 13. Mean chromium uptake (µg Cr / g plant) averaged across replicates across sites. Blue bars represent non-native plants while green bars represent California native plants. Not all plants occurred at all sites.

Overall, the plant volunteer metal-extractors on contaminated sites in order by concentration of total metals content were: *Helminthotheca echioides, Malva neglecta,* and *Heterotheca grandiflora,* with *Hordeum murinum* and *Melilotus officinalis* with particularly high uptake of Cr and Pb, respectively (Figure 14).

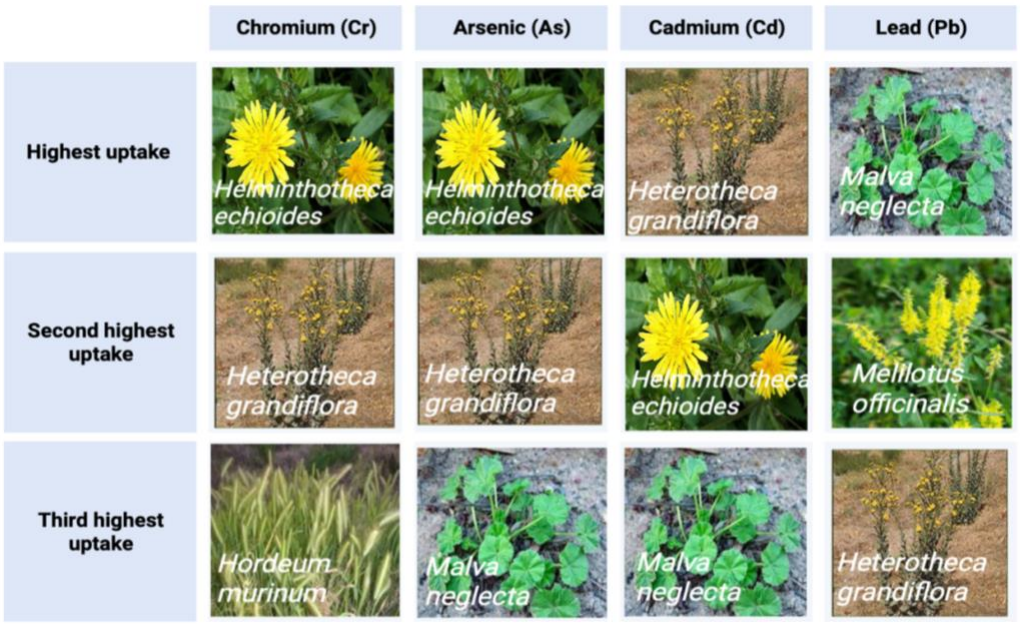

Figure 14. Ranking of plant volunteers on contaminated sites in Southern California for highest metal uptake by metal and uptake concentration.

Overall, the California native plant volunteer metal-extractors on contaminated sites in order by concentration of total metals content were: *Heterotecha grandiflora*, *Erigonium fasticulatus* and *Baccaris salsifolia* (Figure 15), with *H. grandiflora* containing the highest concentration of all metals of concern (Pb, Cr, as and Cd).

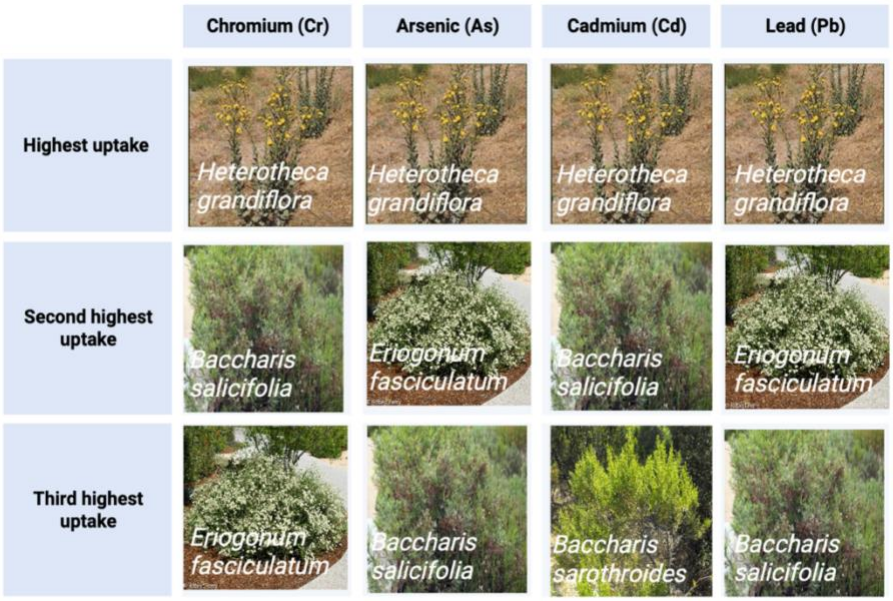

Figure 15. Ranking of California native plant volunteers on contaminated sites in Southern California for highest metal uptake by metal and uptake concentration.

4.4 Soil Microbial biomass

Soil microbial biomass is the part of the organic matter of the soil that constitutes living organisms smaller than 10 μm^3 (Horvath & Paul,1994). There was a significant difference in microbial biomass between sites ($p = <0.0001$), with a range of 0.92 – 476.46 µg/g. Site D, the site with the highest soil metal concentrations and most exceedances of industrial screening limits, contained the highest microbial biomass. However, there was not a clear pattern with regards to overall contamination level and microbial biomass at these sites. There was no significant difference in mean microbial biomass in the rhizosphere soils as compared to in interspace soils at these contaminated sites ($p = 0.3102$).

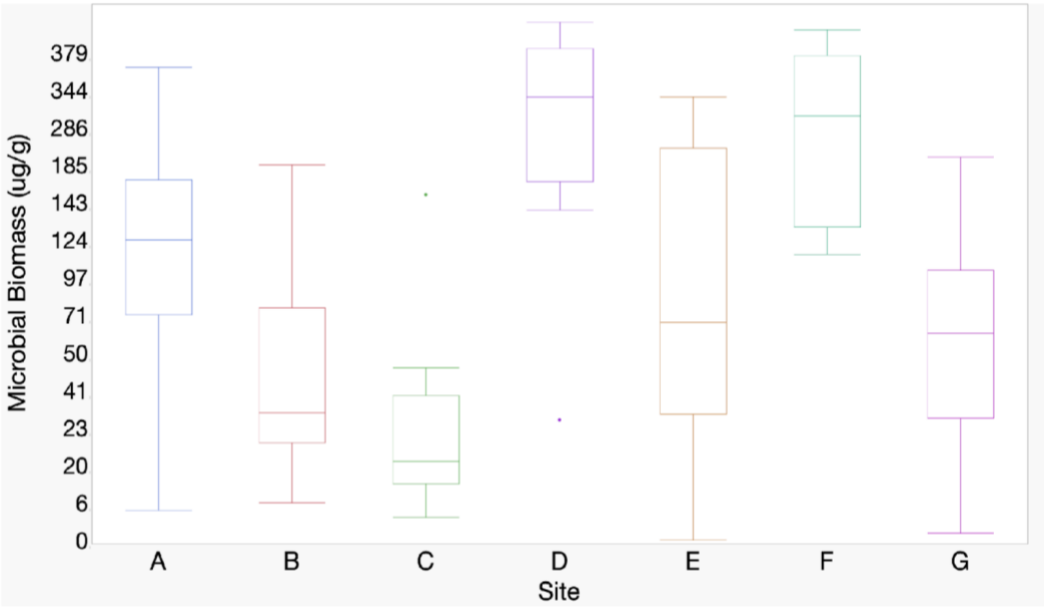

Figure 16. Microbial biomass by site.

4.5 Arbuscular Mycorrhizal Spore Counts and Diversity

Nine morphotypes from the Glomeromycota were detected in spore extractions, corresponding to six genera. There was a predominance of species from the *Glomus* and *Acaulospora* genera, which have been found to adapt to contaminated soil conditions. *Rhizophagus*, *Gigaspora, Entrophospora* and *Scutellospora* were also observed. Mean AMF spore counts from all sites surveyed was 144 per gram of soil, and mean AMF diversity was seven morphotypes. Site F, the uncontaminated site, had the highest spore counts, likely due to the lack of disturbance and pollution pressure.

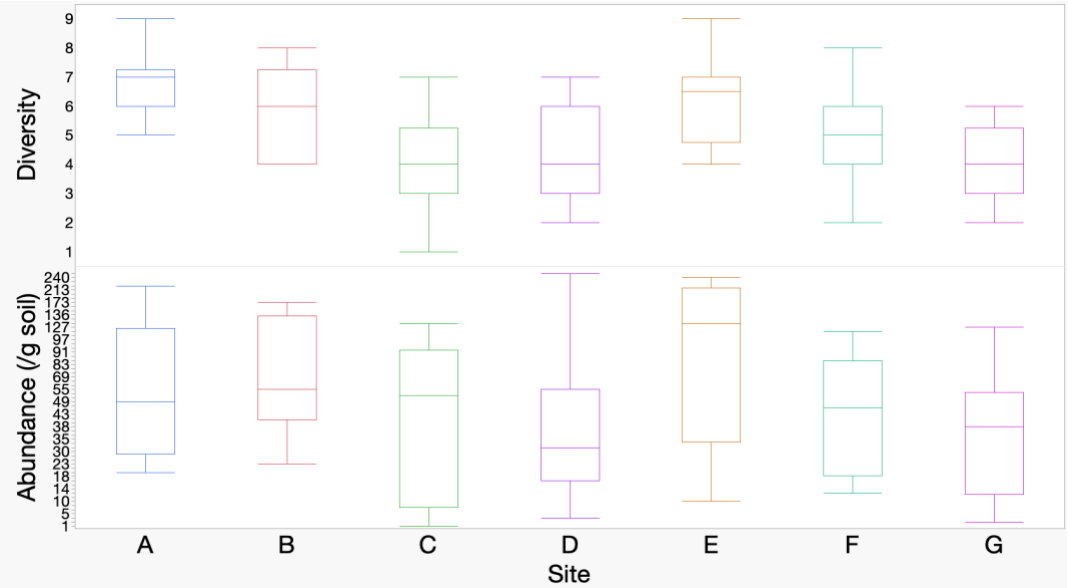

Figure 17. Spore abundance (per g of soil) (averaged from 10 replicates per site including five rhizosphere and five interspace samples) and diversity (by number of morphotypes) at the seven contaminated sites in Southern California tested.

4.6 Soil Microbial Community Analysis

Fungi from the Ascomycota were the most dominant phylum across all sites surveyed (68%), specifically the Dothideomycetes and Sordariomycetes classes were most abundant. Fungi from the Chytridiomycota were detected in six of the seven study site soils (17%) (the Spizellomycetes class was most abundant), and Basidiomycota in five of the seven study site soils (13.4%) (the Wallemiomycetes class most abundant) (Appendix 5). While no fungi from the Glomeromycota were detected in ITS2 sequence data, spores from arbuscular mycorrhizal fungi were detected in all soils surveyed (Figure 17). Proteobacteria were the dominant bacterial phylum across all sites surveyed (34.2%), specifically the Gammoproteobacterial class was found in highest abundance. The Chloroflexi (12.1%) and Planctomycetota (7.086%) phyla were detected in relatively

higher abundances and were dominated by the Chloroflexia and Planctomycetes bacterial classes, respectively (Appendix 6).

4.6.1 Microbial Community Composition by Genera

The most abundant fungal genera (in order of abundance) were *Cladosporium* (8.2%)*, Alternaria* (7.5%), *Botryotrichum* (6.3%), *Fusarium* (5%), *Spizellomyces* (4.5%) *and Cystobasidium* (4%).

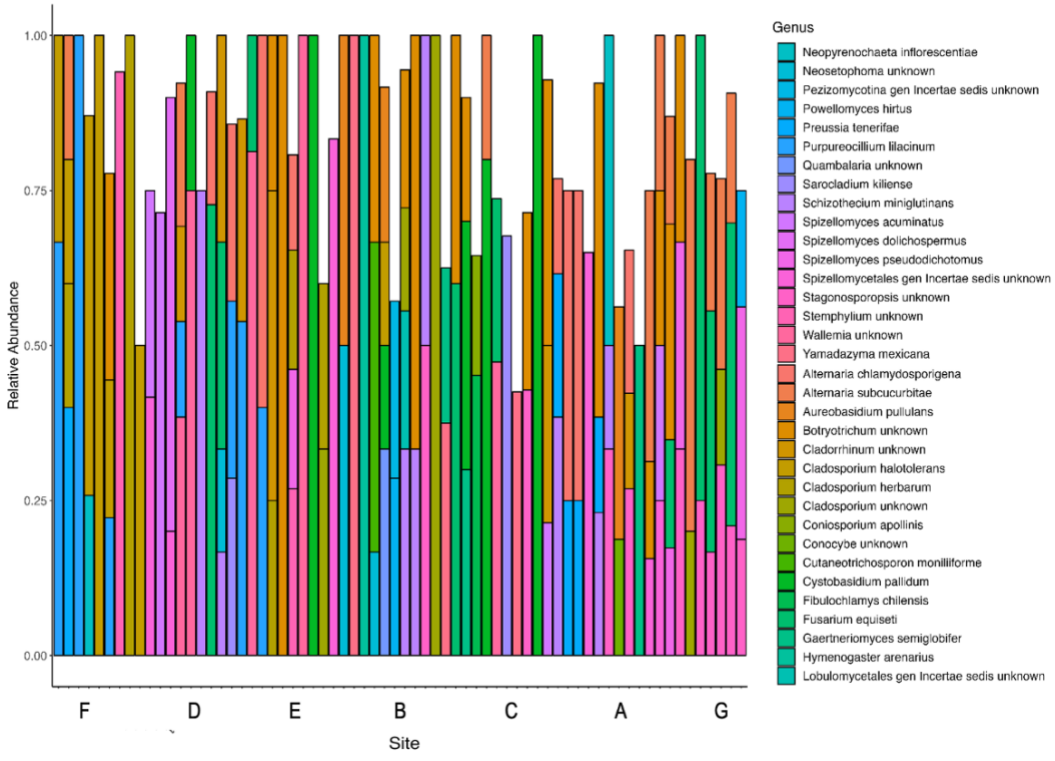

Figure 18. Relative abundance of fungal genera with legend.

Bacterial genera present in the highest relative abundance were Massilia (0.3293 %), mnd1 (0.19205 %), Bryobacter (0.1409 %), Noviherbaspirillum (0.1367 %), Halomonas (0.125 %) and Cthoniobacter (0.1045 %). Unknown genera were present in 2.936 % of samples.

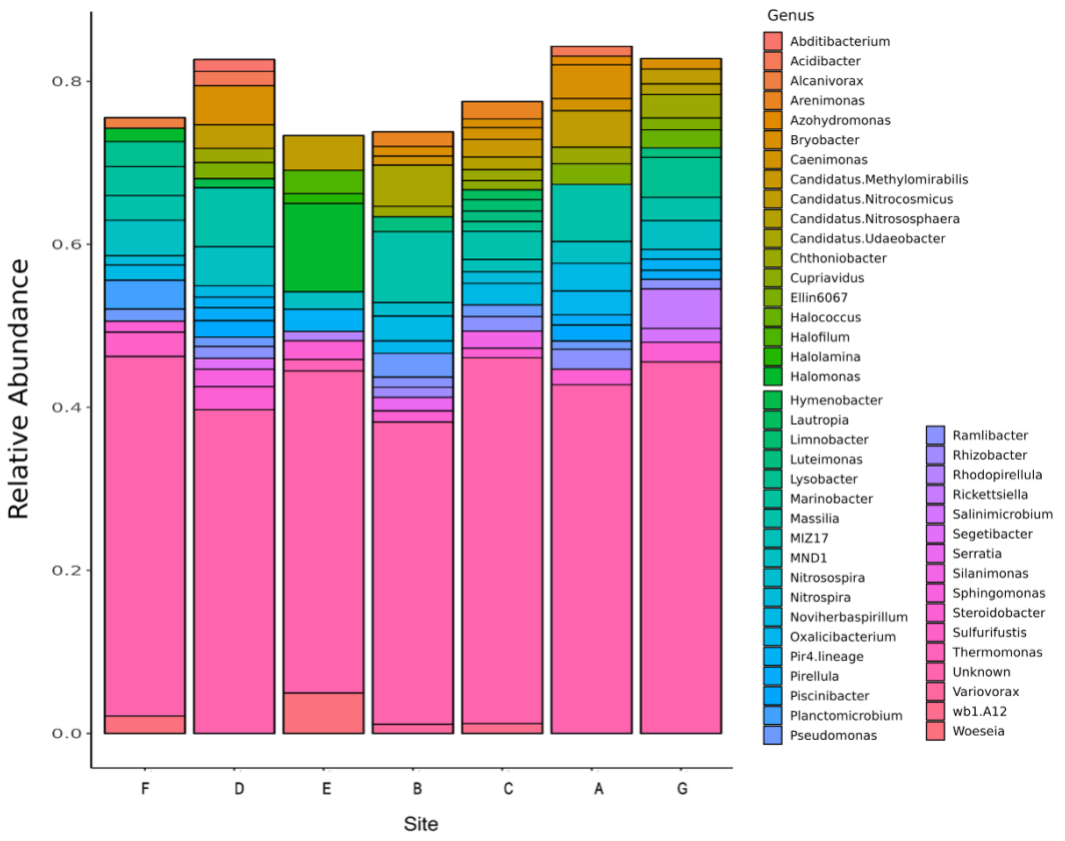

Figure 19. Relative abundance of bacterial genera with legend.

4.6.2 Microbial Richness and Diversity by Site

Fungal diversity was significantly different between sites (p = 0.0009), but not fungal richness (p = 0.6277). The most contaminated site, Site D, had the lowest fungal

richness. Site G had the highest fungal Shannon diversity while containing the lowest levels of (known) metal concentrations among the contaminated sites, whereas Site F had the lowest fungal Shannon diversity although it was entirely uncontaminated. Site A soils contained the second highest Shannon diversity despite being one of the sites with the highest metal (and organic contaminant) concentrations contaminated sites and largely covered in concrete, similar to Site C, which are both highly contaminated and covered by 5-feet of concrete for > 60 years.

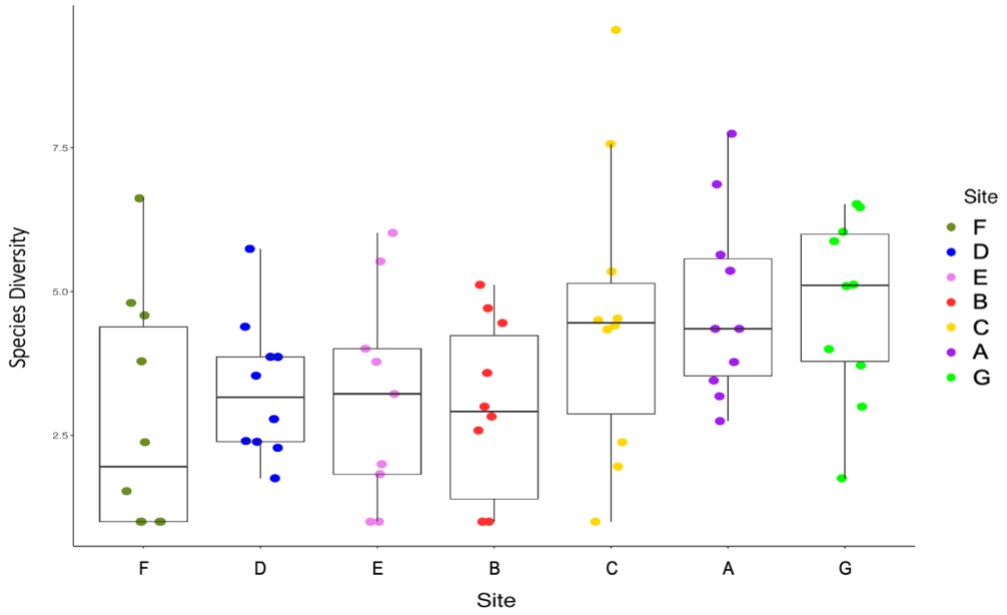

Figure 20. Fungal species Shannon diversity by site.

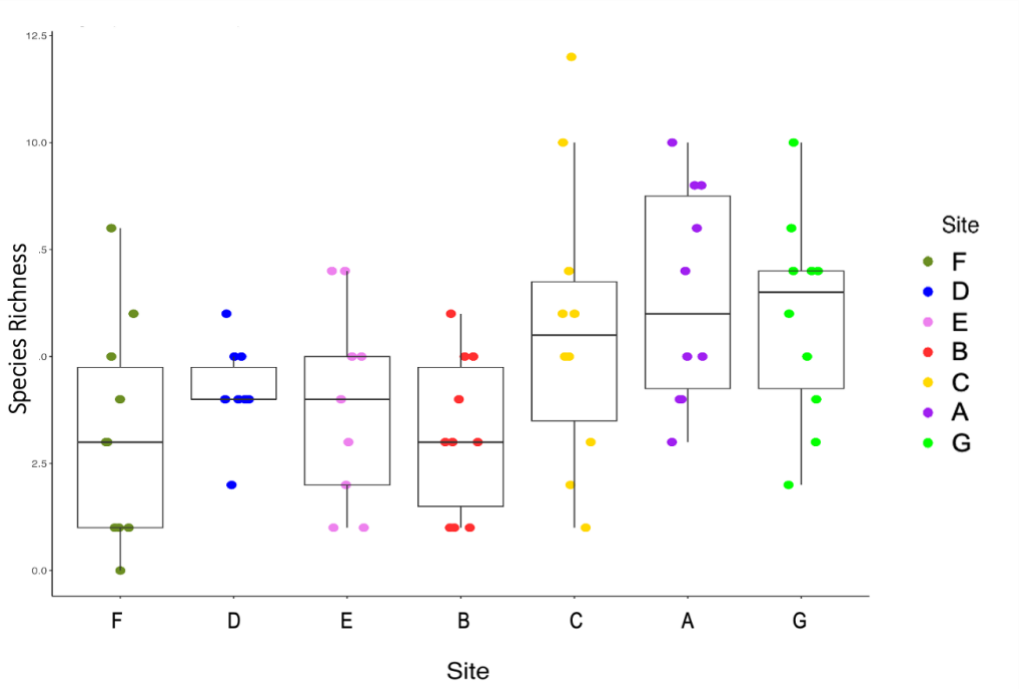

Figure 21. Fungal species ASV richness by site.

Bacterial Shannon diversity and richness were significantly different between sites ($p_{diversity}$ = 0.011196; $p_{richness}$ = 0.002953). The most contaminated site, Site D, had the highest bacterial diversity and richness. Generally, bacterial diversity and richness were highest at the most contaminated sites, except for Site F which had comparable bacterial richness to some of the contaminated sites despite being uncontaminated.

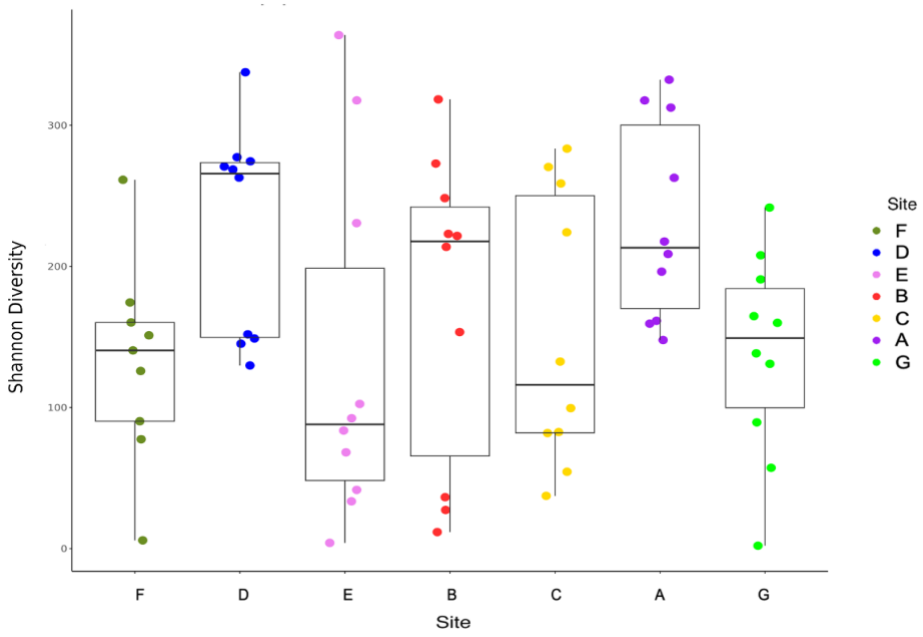

Figure 22. Bacterial species Shannon diversity by site.

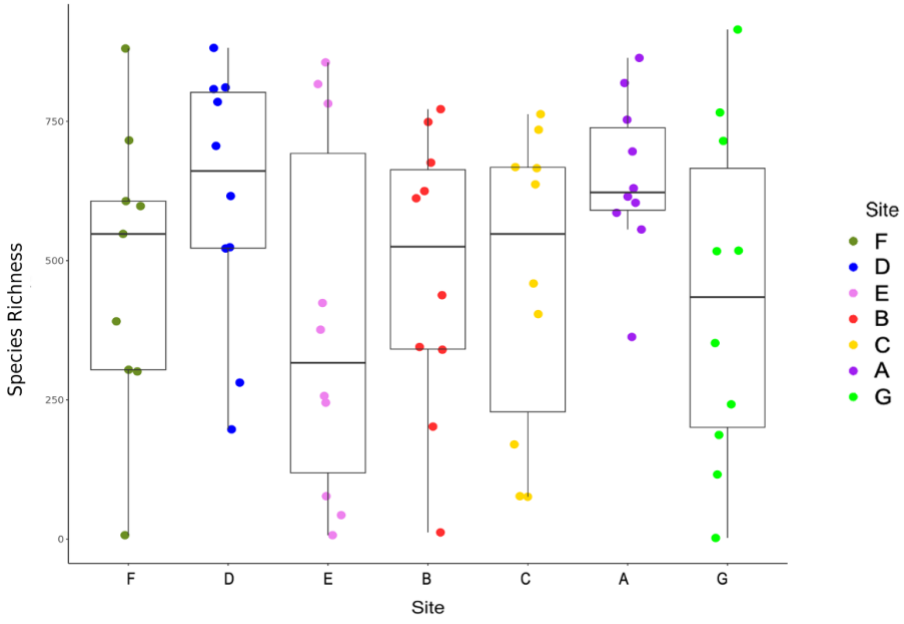

Figure 23. Bacterial species ASV richness by site.

When principal components ordination analysis (PCoA) was used to look at environmental variables and fungal diversity by site (sites A through G) and sample location (rhizosphere vs interspace), *site* explained 42.76% of the variation in fungal diversity, whereas *sample location* explained only 20.93% of the variation. Site G was a noticeable outlier that showed very dissimilar community composition as compared to other sites as indicated by the PCoA (Figure 24). The only obvious difference between Site G and the other sites in terms of soil properties was that Site G soils contained no known organic contaminants, while all other sites had organic contaminant concentrations above industrial screening limits.

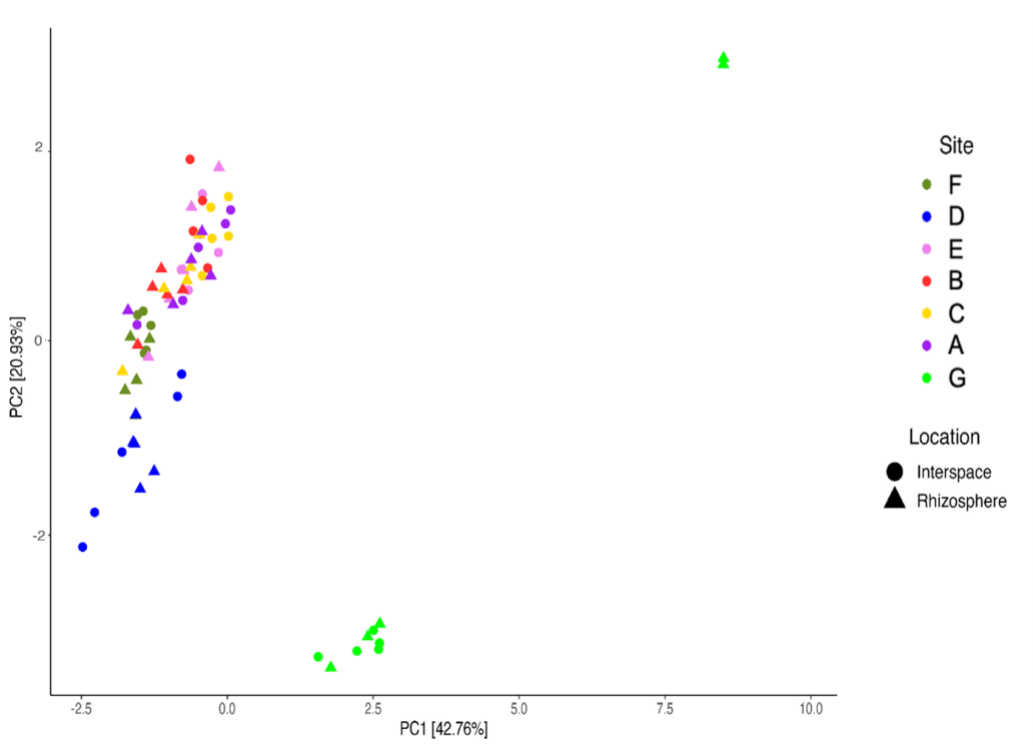

Figure 24. PCoA of fungal diversity using log transformed environmental data by Site and location.

When the PCoA was run without Site G, it is apparent that while there is high variation between the sites, Site D, the most contaminated site, tended to be the most dissimilar to the other sites. Site F, the uncontaminated site, was also clustered together and dissimilar to the other sites.

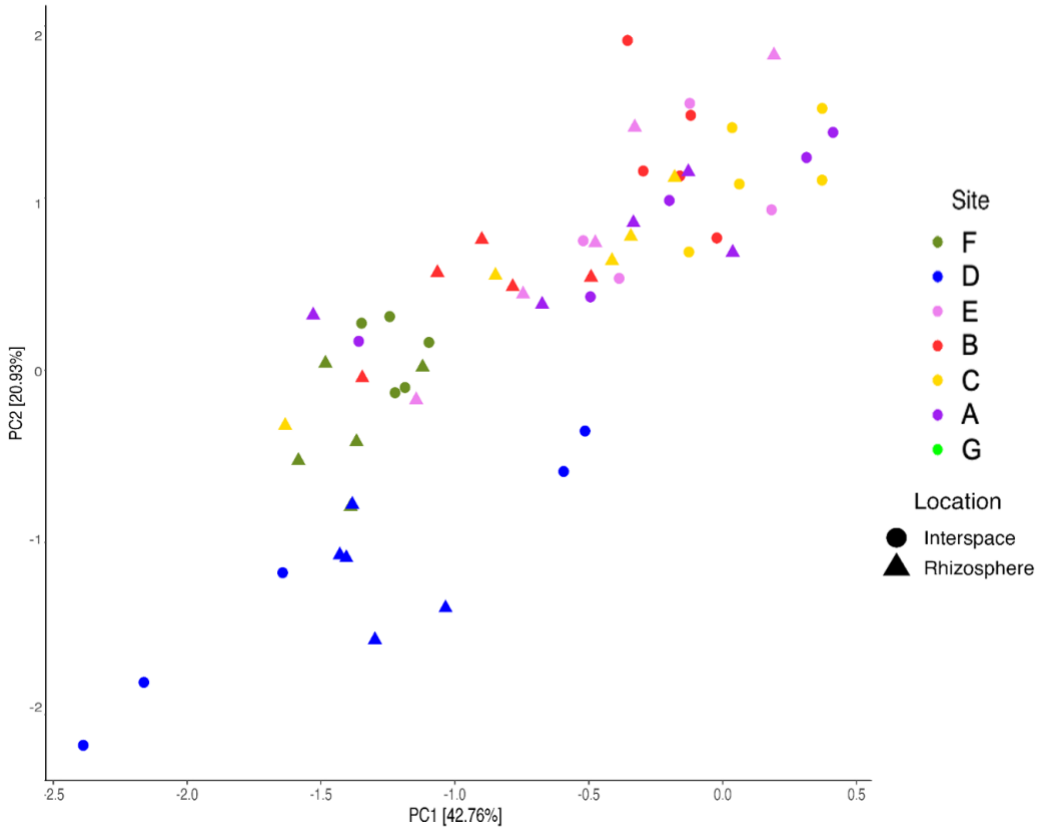

Figure 25. PCoA for fungal ASV's using log transformed environmental data by Site and location, excluding Site G.

Bacterial diversity was also best explained by site (42.7%), and less so by sample location (20.93%). Site D, the most contaminated site, was most dissimilar to other sites in terms of bacterial Shannon diversity, consistent with findings for fungal diversity (Figure 26).

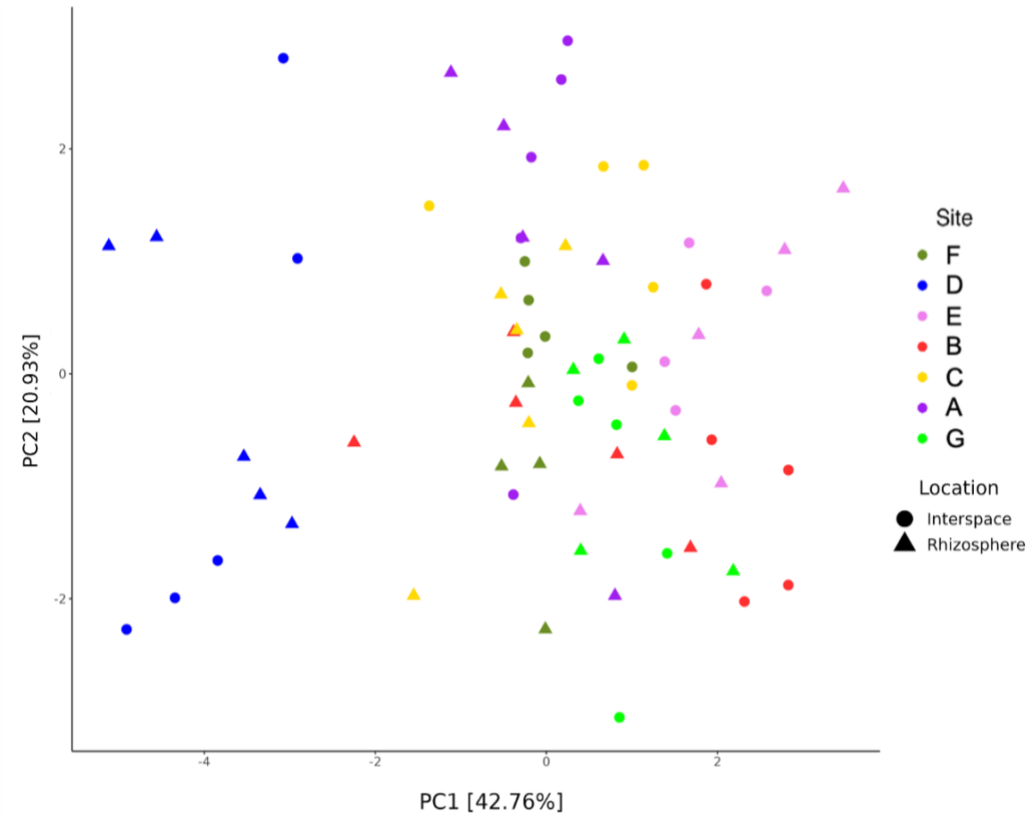

Figure 26. PCoA for bacterial ASV's using log transformed environmental data by Site and location.

4.6.3 Microbial Diversity and Richness by Sample Location

There was higher fungal and bacterial ASV richness and Shannon alpha diversity within the rhizosphere soils than the interspace soils (Figures 27-30), however, this difference was not statistically significant ($p = 0.159$) for fungi but was for bacteria ($p = 0.0112$).

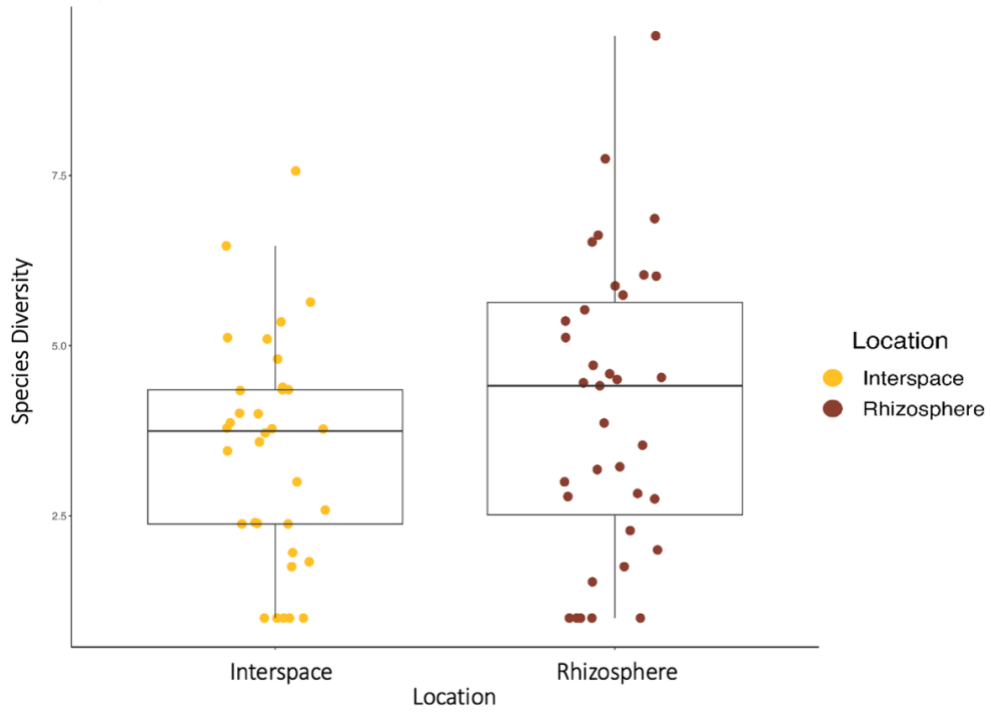

Figure 27. Fungal species diversity by sample location.

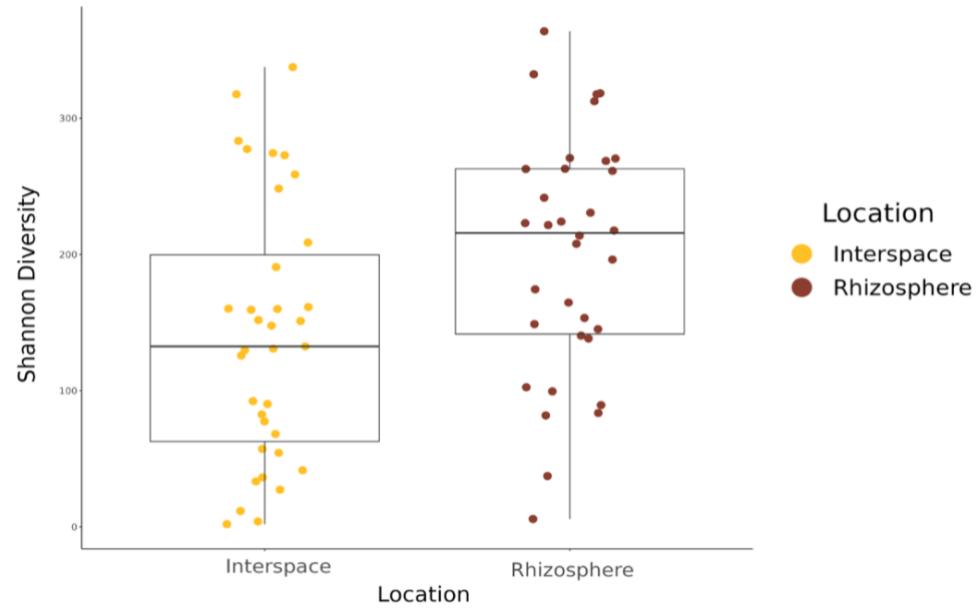

Figure 28. Bacterial species diversity by sample location.

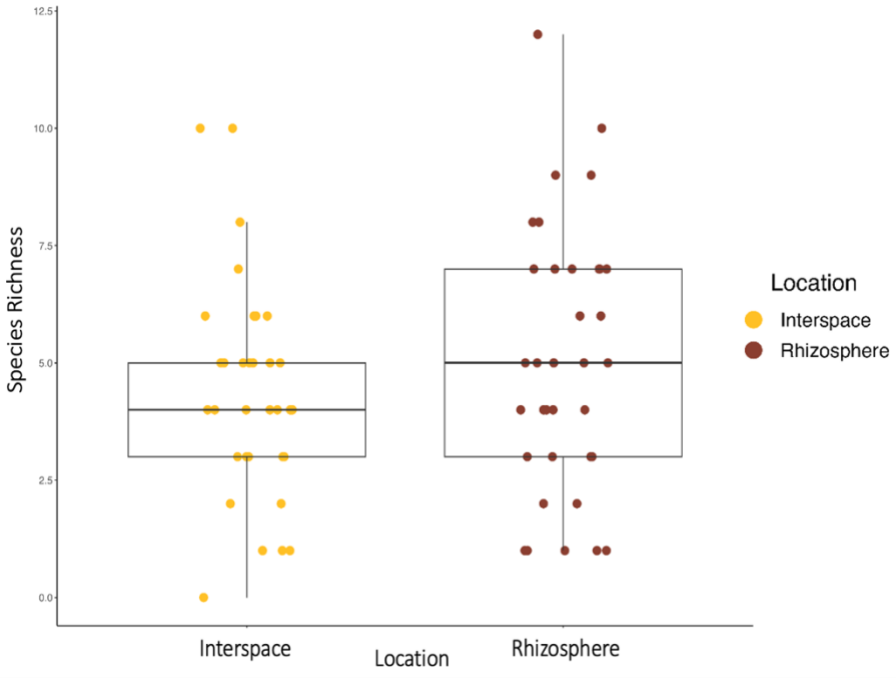

Figure 29. Fungal species richness by sample location.

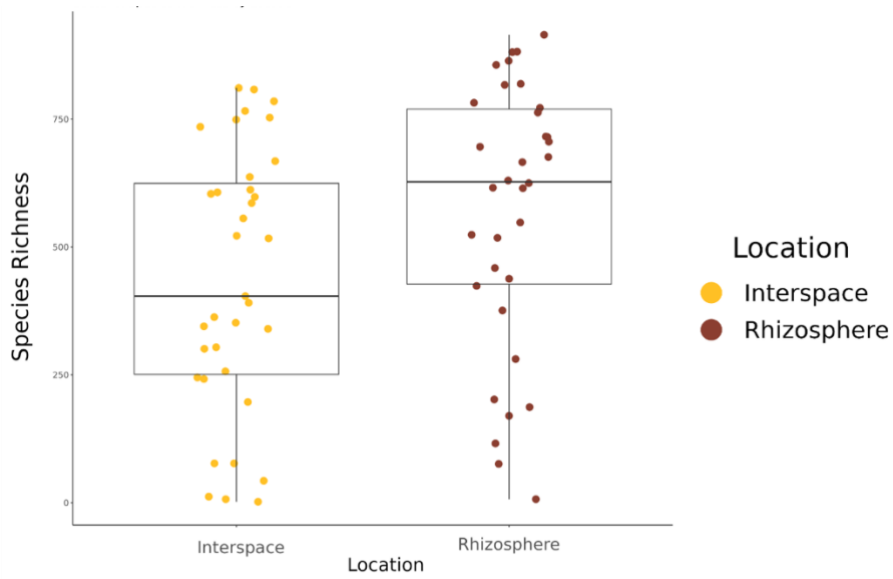

Figure 30. Bacterial species richness by sample location.

4.6.4 Relationships between Microbial Composition and Soil Variables

Bray-Curtis Distances were used to complete redundancy analysis to identify which soil parameters influenced fungal and bacterial composition on the contaminated sites surveyed in Southern California. The first two axes of the RDA accounted for only 5.26% of the variability in the relative abundance of fungi and bacteria. The variability in relative abundance of fungi was best explained by four of the measured soil parameters (WEOC, $p = 0.002$; percent silt, $p = 0.002$; percent sand, $p = 0.013$; CEC, $p = 0.022$; PERMANOVA). Water-extractable organic carbon (WEOC), the fraction of organic carbon compounds in soil that can be dissolved in water, was the most significant driver of fungal composition ($p = 0.002$). Soil texture, particularly % silt ($p = 0.002$) and % sand ($p = 0.013$) were significant drivers of fungal composition. Cation exchange capacity (CEC) was also a significant explanatory variable of variation in fungal composition in these contaminated soils ($p = 0.022$). Soil metal concentrations and availability were not significant predictors of fungal composition in this study as predicted, although cadmium concentration was close to significance.

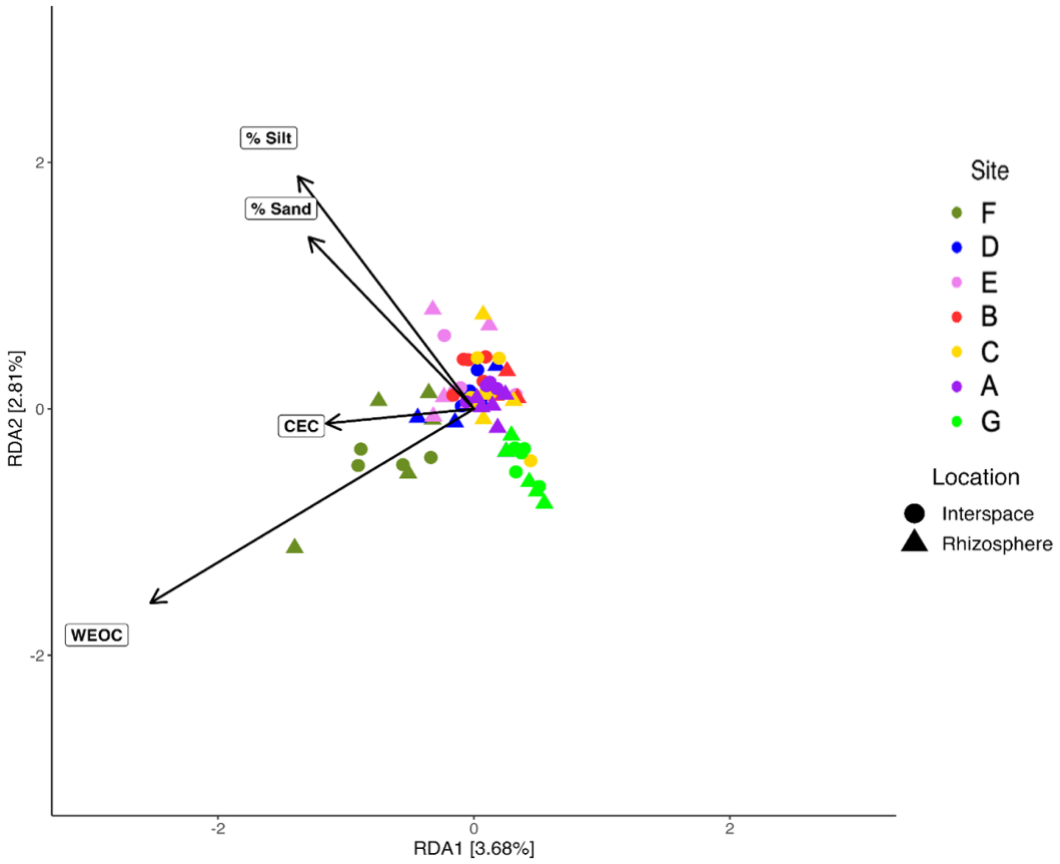

Figure 31. RDA of soil environmental variables effects on fungal composition using Bray-Curtis distances.

The variability in relative abundance of bacteria was best explained by four of the measured soil parameters (percent sand, $p = 0.013$; percent silt, $p = 0.015$; CEC, $p = 0.021$; WEOC, $p = 0.034$; PERMANOVA). These are the same soil parameters that best explained variation in fungal diversity, although in a different ranking. Soil texture (percent sand and silt) were the most significant drivers of variation in bacterial diversity on the contaminated sites surveyed. Cation exchange capacity and water-extractable organic carbon (WEOC) were also significant drivers of variation in bacterial diversity in study sites.

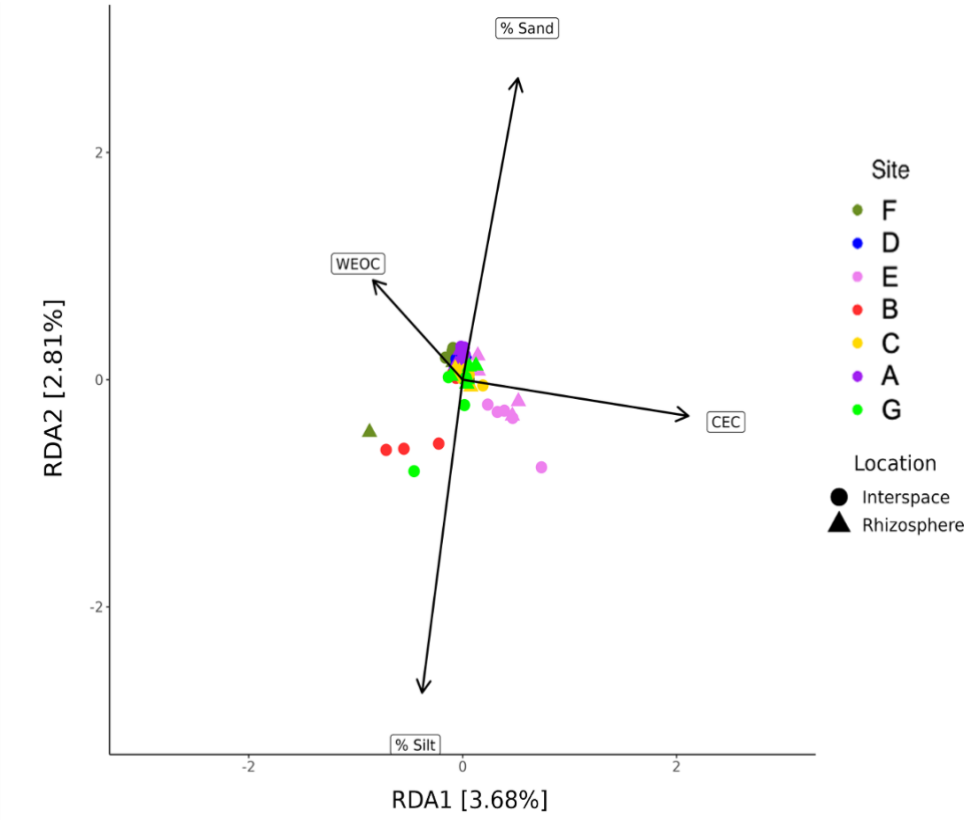

Figure 32. RDA of soil environmental variables effects on bacterial composition using Bray-Curtis distances.

5. Limitations

Plants were not washed prior to processing and analysis in this study, this leaves the potential for contamination of foliar samples with soil or aerial particulates. This may be a cause of erroneous hyperaccumulator designation in historic data, particularly for copper, cobalt, lead, and chromium. However, contamination of plant samples is of particular concern for plants sampled close to smelters or active mine sites which have a high probability of being contaminated by airborne metal-rich particles, which is not applicable in this study (Faucon et al., 2007).

Some authors suggest that in addition to the uptake thresholds listed for identifying hyperaccumulator plants, two additional screening criteria should be met and considered in evaluation of phytoextraction candidates: bioconcentration factor (BCF or shoot/soil concentration) >1, and translocation factor (TF or shoot/root concentration) > 1 (Egendorf, 2020; van der Ent *et al.*, 2013). While BCF was calculated for plants tested, TF was not due to resource constraints resulting in only plant foliar parts being analyzed for metal concentrations.

6. Discussion

6.1 Accumulators and hyperaccumulators of metals

Fifteen plant families were represented at the 7 contaminated sites, with the *Asteraceae* and *Poaceae,* two plant families known to host metal hyperaccumulator species, appearing in the highest abundance. No new Pb, As or Cr hyperaccumulator plants were discovered; however, those metal(loid)s were not available for plant uptake according to our soil metal extraction results. Generally, most metals had the highest concentrations in the weak-acid-extractable fraction, which is considered only slightly available to plants.

Lead was found in the highest concentrations in the weak acid extractions. The biogeochemistry of Pb in soil renders it largely immobile. Lead adsorbs strongly to organic matter and Fe and Mn oxides in soils and is not readily extractable by plants (Blaylock, 2000; R Blaustein, 2017; Egendorf, 2020; McBride et al., 1994). Further, plants have physiological mechanisms that prevent Pb from moving into aboveground

tissues (Egendorf, 2020). As a result, only nine Pb hyperaccumulator plants have been identified globally. The only California native plant that has shown capacity to accumulate Pb to date is *Sequoiadendron giganteum* (Giant sequoia) (Gateva et al., 2004), which is not a feasible species for use in phytoremediation.

Several soil properties contribute to Pb mobility (and thus potential for plant uptake): high cation exchange capacity (CEC), clay minerals, carbonates, Fe and Mn oxides, phosphates, soil organic matter (SOM), and neutral pH contribute to low Pb mobility whereas low content of these soil properties and either alkaline or acidic soils and sandy and silty soils contribute to increased Pb mobility and plant uptake (McBride et al., 1994; Kabata-Pendias et al., 2007). Plant-specific factors such as root surface area, root exudates, and mycorrhizal associations also affect Pb translocation into plants, though high soil Pb concentration is likely the most important contributor to observed high plant uptake (Salt et al., 1995). This contrasts with findings of the current study, as Site D had very high soil Pb concentrations (400 – 4400 µg/g) but plant volunteers at site D did not exhibit particularly high Pb accumulation.

The uptake of Pb by *Melilotus officinalis* (sweet yellow clover) far surpassed values reported for other plants, including taking up four times higher concentrations than known remediator species "Switchgrass" (*Panicum virgatum*). Both *Melilotus officinalis* and *Panicum virgatum* were only found on Site C, which had high lead concentrations along with the highest Pb mobility as shown in the water-extractable fraction. *Melilotus officinalis* has been identified in other studies (Fernandez et al., 2012; Ghazarayan et al.,

2021 and others) as a phytoextractor of Pb, Cu, Zn and Hg. *Malva neglecta* (the common mallow) exhibited the second highest Pb uptake of all plants sampled. *Malva neglecta* has been identified as a Zn and Cu hyperaccumulator (Malayeri et al. 2013; Nematian and Kazemeini 2013) but has not been reported as a Pb accumulator.

Copper is considered moderately difficult to extract from soils (Kennen, 2017). In the present study, three California native plants that meet hyperaccumulator criteria for Cu were discovered including *Brickellia californica*, *Erigonum fasciculatum,* and *Baccharis sarothroides,* as well as previously unstudied *Helminthotheca echiodes*. *Helminthotheca echiodes* is an edible weed from Europe that is common amongst cereal crops and consumed as a wild green. No reports of metal accumulation have previously been reported for this species.

Most phytoremediation research has focused on remediation of metal cations, including Zn, Ni, Cd and Pb, which are transported into plants via plant cation transporter systems and subsequently partitioned into cell walls or vacuoles, then bound, or complexed inside of plants via metal-binding proteins and phytochelatins (McGrath and Zhao, 2003; Hooda et al., 2007; Verbuggen et al., 2009; Memon and Schroder, 2009). All plants are essentially Cu, Zn, Ni and Co accumulators due to the ability to uptake these metals through divalent cation transporters (e.g., ZIP, COPT and NiCoT transporters); however, a select number of plants have the capacity to hyperaccumulate these metals (Ferrol et al., 2016; Gadd, 2009; González-Guerrero et al., 2016). In contrast, Cd and Pb are not required by plants and are therefore toxic to plants (Lui et al., 2018). NRAMP (Natural Resistance-Associated Macrophage Protein) transporters can uptake divalent

cations Cd and Pb due to the transporter's low specificity (Joutey et al., 2015; Viti et al., 2014; Wu et al., 2018; Wu et al., 2015).

Although no new As or Cr hyperaccumulators were identified in the present study, *Helminthotheca echiodes* had high Cr uptake, along with *Lactuca serriola,* a common weed that has been previously identified as capable of accumulating several metals including Cd, Pb, Cu, Ni and Zn (Porębska et al., 1999; Ważny et al., 2018; Abou-Shanab et al., 2017). *Hordeum murinum*, an invasive grass to California, also had high Cr uptake, and has been identified as a metal hyperaccumulator (Boularbah et al., 2006; Pirjol et al., 2017; Durães et al., 2015; Popoviciui et al., 2018). Arsenic and Cr interact differently with regards to plant uptake since their mobility and toxicity are highly species-dependent, with the Cr(VI) and As(V) oxyanions being the most toxic and mobile (Hayat *et al*., 2012). Since these oxyanions have no nutritional role in plants, their root uptake is facilitated by the transporter systems of anionic nutrients such as sulphate anions; arsenate can replace phosphate in the P transport into plants due to its chemical similarity (Ferrol et al., 2016; Hayat *et al*., 2012). The oxidizing nature of these species generates significant oxidative stress in plant tissues. The poor translocation of Cr has been identified as a possible barrier to effective phytoremediation of this metal, and the accumulation of Cr in granular deposits in plant roots may contribute to this poor translocation (Bishehkolaei *et al*., 2011).

6.2 Differences in Microbial Biomass and Diversity Between Sites and Sample Locations

Lead and other potentially toxic metals can impact microbial biomass, activity, and diversity (Kandeler et al., 2000; Chen et al. 2014; Hong et al., 2015; Fajardo et al., 2019). However, some studies show that persistent organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) can increase both microbial biomass and diversity because they can be used as a C source for microbes (Kabelitz et al., 2004; Teng et al., 2010). There was not a clear pattern with regards to overall contamination level and microbial biomass at these sites, although the most contaminated site (Site D) had the highest microbial biomass. Microbial biomass may be more linked to C/N ratios at each site than contaminant levels. The average C/N ratio at Site D was 27.2 (±47.75), which was higher than most of the other sites and more optimal for microbial growth, suggesting an equilibrium state between mineralization and immobilization of N (Sparks, 2003). The lack of significant difference between microbial biomass in rhizosphere and interspace soils was unexpected, as the microbial density in the rhizosphere is commonly between 5 and 20 times greater (and can be 100 times greater) than that in the surrounding soil (Gray & Parkinson, 1968; Katznelson, 1965). One explanation may be that the fumigation extraction method used in this study can underestimate total microbial biomass at low soil moisture contents because of the influence of moisture on the quantity of extractable C following chloroform fumigation (Harris & Steer, 2003). Given the low moisture content of all study site soils (0.001 - 0.2976 m^3/m^3 when measured during the summer months of 2021), it is likely that microbial biomass was

underestimated, though we would then expect underestimation at all sites. Alternatively, low soil moisture at study sites can also limit microbial growth and decomposition of organic carbon. In addition, the soils found in brownfields and Superfund sites are often nutrient-depleted, highly saline, and highly compacted, affecting plant and microbial communities and potentially altering or limiting microbial biomass (Mejia et al., 2023).

It is unsurprising that there were statistically significant differences between fungal diversity by site, but not by sample location (rhizosphere vs interspace). There were statistically significant differences in the soil variables that most explained variation in fungal diversity (silt and clay content), water-extractable organic carbon (WEOC), cation exchange capacity (CEC) between sites, but no statistically significant differences between sample locations for these soil variables. Generally, microbial diversity is shown to increase in rhizosphere soils (Edwards et al., 2015; Turner et al., 2013) as seen in the present study with bacterial diversity being significantly higher in rhizosphere soils than diversity within interspace soils. This is likely due to the differences in environmental conditions and nutrient bioavailability in the two ecological niches, and the presence of organic exudates provided by plant roots that promote microbial growth within rhizosphere soils. Plant roots have been shown to have selective effect on both rhizosphere and endophytic microorganisms (Bais et al., 2006; Hartmann et al., 2009; Yu et al., 2014).

In the present study, there was a trend towards higher bacterial and fungal diversity (and richness for bacterial communities but not fungal) in the more highly contaminated sites. This positive correlation between contamination level and microbial

diversity is opposed to findings reported by other studies (Okrasińska et al., 2022; Mejra e al, 2023). Lower microbial diversity is reported at more contaminated sites because those with lower tolerance cannot survive (Mejra et al., 2023; Cachada et al., 2018; Okrasińska et al., 2022). Soil contamination exerts selective pressures on soil microbial communities by eliminating sensitive taxa (Thion et al., 2012; Bourceret et al., 2016; Okrasińska et al., 2022). However, contaminant distribution and soil properties is heterogeneous across soils. Spatial variability can create a patchwork of microhabitats, each favoring specific microbial species or metabolisms. Contaminant type and concentration can strongly influence microbial diversity. Therefore, the composition of contaminants on these sites may be more important than the total contamination level. Okrasińska et al. (2022) found that total gasoline and BTEX concentrations were factors that correlated with soil fungal ASVs composition on contaminated sites (in Europe) rather than metallic contaminants. Further, some fungi and bacteria can tolerate or even thrive in the presence of specific contaminants, such as petroleum hydrocarbons they can utilize as a food source; others adapt over time and develop tolerance or resistance mechanisms. This can lead to a shift in microbial community composition towards those species that can thrive in contaminated soils and specific site conditions. Fungi with specialized metabolic capabilities to degrade or transform these contaminants may dominate in such environments (Okrasińska et al., 2022). Ecological succession can also occur at contaminated sites, where the microbial community changes over time as conditions become more or less suitable for different species. Microbial succession at contaminated sites over time and with remediation is an area of interest for further study.

The study sites examined in the present study have been vacant (and contaminated) for decades, allowing time for succession as well as adaptation to occur.

6.3 Soil and Environmental Drivers of Microbial Composition of Contaminated Sites in Southern California

Water extractable organic carbon (WEOC) was one of the most significant explanatory variables of both fungal and bacterial diversity in our study of contaminated sites in Southern California. WEOC significantly varied between sites ($p = < 0.0001$) but did not vary significantly between the rhizosphere (mean = 101.2 mg/L) and the interspace (mean = 94.5 mg/L) soils. Since WEOC is water-extractable, its availability is closely linked to soil moisture levels. Soil moisture affects microbial activity, including fungal growth and metabolism, with adequate moisture levels enhancing carbon cycling and promoting microbial, especially fungal, activity and less microbial activity occurring in drier soils (Homyak et al., 2018). However, WEOC can increase as soils dry and physical access to C is constrained for microbes resulting in microbial die-off (Homyak et al., 2018). Soils at study sites were generally hot (31 - 40 °C**)** and dry (0.001 - 0.2976 m^3/m^3) when measured during the summer months of 2021, which may have increased WEOC (and had a corresponding effect on soil fungal communities). Similar conditions might be expected on the regional scale. WEOC may have been the most significant environmental explanation of variation in fungal composition because water extractable organic carbon is the most labile and available form of carbon for saprobic fungi and other microbes, potentially promoting fungal growth and stimulating biomass production

and metabolic activity (Homyak et al., 2018). Saprobic microbes found on contaminated sites may be capable of metabolizing hydrocarbons and other organic contaminants, but other studies have found that soluble organic matter (e.g., WEOC) may also increase the solubility, and therefore facilitate the translocation, of poorly water-soluble pollutants such as polyaromatic hydrocarbons (Chlou et al., 1987, Herbert et al., 1993, Maxin and Kögel-Knabner, 1995, Janzen et al., 1996). This in turn could lead to increased decomposition of organic matter and possibly organic contaminants as well as increased nutrient cycling. The diversity of organic compounds and carbon substrates within WEOC can influence the diversity of microbial species. During decomposition processes, the changing availability of WEOC can influence the succession of microbial communities. Initially, fast-growing fungi and bacteria that are adapted to utilizing simple carbon sources might dominate. As these carbon sources are depleted, the composition of fungal communities can shift to species capable of breaking down more complex organic compounds.

Soil texture (specifically % sand and silt) were significant drivers of both bacterial and fungal composition on contaminated sites surveyed. This is consistent with other studies, as soil texture can filter for microbes who can tolerate the conditions found in sandy or silty soils, such as certain moisture contents and nutrient availability (Jansa et al., 2008; Morley & Gadd, 1995). Site D (88 %) and A (79 %) were the sandiest soils, although all sites were dominated by sand, these sites had some of the highest microbial diversity. Silt content was variable (9.15 – 49.88 %) across study sites- Site B soils contained the highest concentrations of silt and Sites C and E soils also contained higher

percentages of silt. Cation exchange capacity (CEC) was also a significant driver of microbial diversity on contaminated sites, possibly due to the relationship between CEC and metal tolerance observed in microbes (Joner et al., 2000; Audet & Charest, 2007; Ballen & Graham, 2002; Crowley & Alvey, 2002; Gadd, 1994; Jansa et al., 2008; Leyval et al., 1991; Marschner et al., 1998). CEC had a range of 2.64 – 24.57 meq/100 g with an average of 8.37 meq/100 g across study sites. There was a significant difference between sites ($p = <0.0001$) but not between the rhizosphere and interspace soils with regards to CEC ($p = 0.8661$). Positive correlations between CEC and other factors including pH, soil organic C, organic matter and soil clay have been reported (Moore et al., 1998; Wang et al., 2005). In this study, a low level of organic C and organic matter in metal contaminated soils may have resulted in low CEC values which in turn affected microbial community diversity.

Contrary to our hypothesis, metal type, concentration or availability did not significantly explain bacterial or fungal diversity on the contaminated sites surveyed. Okrasińska (2022) found that organic contaminant concentrations, rather than individual metal concentrations, correlated with fungal ASVs composition. However, other studies (Mejia et al., 2023; Lemmel et al., 2021) have found a correlation between various metal concentrations and fungal community composition for lead, cobalt, and zinc. In the present study, we found that Cd approached statistical significance as an explanatory variable for fungal diversity. Cadmium was found to have profound inhibitory effect on the decomposition potential of some of the same fungal genera as those found in our study (Kumar et al., 2015), which could be expected to reduce their abundance and possibly

affect diversity over time. Another study found Zn and Cd content were strongly correlated with fungal community composition but did not observe effects on fungal diversity (De Beeck et al., 2014). It is plausible that the microbes found on these sites are present because they are adapted to the metal (and other) contamination and site conditions thus other soil and environmental properties related to nutrient, carbon, and water access were more important.

6.4 Relevance of Microbial Taxa on Contaminated Sites in Southern California to Remediation

Ascomycota have been found to be the dominant fungal phylum on other contaminated sites (Czaplicki et al., 2020; Mejia et al., 2023; Okrasińska et al., 2022) and even generally in soils globally (Egidi et al., 2019). Many organic pollutant-degrading fungi are found within the Ascomycota (Harms et al., 2011). They may predominate on contaminated sites because they disperse easily via wind and are generalist decomposers with stress-tolerance qualities (e.g., produces melanin) that make them competitive in the harsh conditions prevalent on contaminated sites (Egidi et al., 2019). Melanin are phenolic pigments which act like a "fungal armor", forming a film outside of fungal cell walls which protects the cells from toxic metal entry (Eisenman & Casadevall, 2012; Mehra & Winge, 1991). Melanized fungi (e.g., *Cladosporium* spp., *Alternaria sp.* and *Aureobasidium spp.*), are often isolated from industrially contaminated soils containing high concentrations of metals (Eisenman & Casadevall, 2012; Mehra & Winge, 1991).

A recent analysis of a brownfield next to Site A found that fungal communities were dominated by a single phylum, Ascomycota (94%), specifically the Dothideomycetes (37.7%), Sordariomycetes (34.9%), and Eurotiomycetes (21.8%) classes and bacterial communities by the Proteobacteria (28%) (Mejia et al., 2023). Dothideomycetes were among the most dominant fungal classes on petroleum-hydrocarbon-polluted sites (Iffis et al., 2017), and some species belonging to Dothideomycetes can tolerate or break down a range of petroleum hydrocarbon compounds (Harms et al., 2011; Junghanns et al., 2008; Stefani et al., 2015). Fungi belonging to the Sordariomycetes have also been shown to metabolize PAHs (Marchand et al., 2017) are investigated for biotechnological applications, such as enzyme production and bioremediation (Yakop, F., 2019). Some fungi within the class Spizellomycetes are found in contaminated sites (Jerônimo et al. 2015), however, their tolerance to contamination and potential for bioremediation remains unknown (Georg and Gomes 2007; Jia et al. 2018).

The most abundant genus was *Cladosporium* (8.2%), common, melanized molds which may be saprotrophic, endophytic, or plant and animal pathogens and live in air as well as terrestrial and aquatic environments (Deshmukh & Rai, 2005). *Cladosporium* is one of the most diverse genera of fungi, and three species were present on the sites surveyed: *Cladosporium halotolerans* (3.4%), *Cladosporium herbarum* (2.4%) and one unknown *Cladosporium* species (2.4%). *Cladosporium halotolerans* is a marine fungus that is halophilic, meaning it can tolerate hypersaline environments. It has been studied for metal-oxidization and removal from water (mercury and manganese) (Bovio, E., et

al., 2017; Singh, VK., et al., 2021; Mota, E. A., 2020; Wang, M., et al., 2022), high density polyethylene (HDPE) biodegradation (Di Napoli, M., et al, 2023) and polyester polyurethane (Zhang et al., 2022). *Cladosporium herbarum* has been found to degrade Azo-dyes (Abubacker, MN, et al., 2017), petroleum hydrocarbons (Bakri et al., 2022) and fungicides (Papazlantani, CV, et al., 2022).

The second-most abundant genus was *Alternaria* (7.5%). This genus is commonly found in contaminated soils and are known to tolerate metal-contamination (Kubatova et al., 2002; Massaccesi et al., 2002). The two most abundant fungal genera at our study sites, *Alternaria* and *Cladosporium sp.*, detected both in soil and root datasets, have been shown to degrade crude oil and a variety of its derivative products such as phenanthrene, benzo[a]pyrene, fluoranthene, and anthracene (Giraud et al., 2001; Mohsenzadeh et al., 2012; Potin et al., 2004). Sequencing of an adjacent parcel to Site D (Taylor Yard) found *Alternaria* were enriched in contaminated soils compared to uncontaminated soils and represent taxa of interest for future bioremediation research (Mejia, 2023). *Alternaria chlamydosporigena* (3.5%) is a dark septate endophyte which has been shown to degrade polycyclic aromatic hydrocarbons in soil (Mohammadi, SS, et al., 2020), sorb metals, and enhance phytoremediation of metal-contaminated and saline soils (Hou, L., 2020; Mohammadi, SS, et al., 2017). *Alternaria subcucurbitae* (*4%*) is a plant pathogen that is lesser understood.

Botryotrichum (species unknown) showed up in third highest abundance (6.3%). This is a genus of fungi in the class Sordariomycetes and the order Xylariales containing fungi associated with plant diseases and others with decomposing organic matter.

Botryotrichum has scarcely been studied for bioremediation, it has shown tolerance and even resistance to metals such as Pb and Cd as well as conditions prevalent on contaminated sites, such as extreme soil pH, concrete-like soils, and low concentrations of nutrients (Liu et al., 2022; Kumar et al, 2012 and 2015; Van Wylick et al, 2023) and potential for biosorption of metals such as Zn and Ni (Wu et al., 2022; Shilpi et al, 2012).

Fusarium (5%) was also present in high relative abundance. Several *Fusarium* species have been studied for their efficient enzymatic degradation of organic contaminants including PAHs (Haritash & Kaushik, 2009; Jacques et al., 2008; Marco-Urrea et al., 2015), tolulene (Akbarian, H. et al, 2022), DDT (Mitra et al., 2001), textile wastewater (Darwesh, OM, 2023), dioxins (Delsarte, et al., 2021) and other contaminants such as metals (Sayed et al, 2020; Amatussalam et al., 2011; Ekinpelu et al., 2014). *Fusarium equiseti* is a soil-dwelling plant pathogen that has not yet been tested for bioremediation capacity.

Two fungi from the *Spizellomyces* (4.5%), *Spizellomyces acuminatus* (2%) and one unknown *Spizellomyces* (2.5%) were detected, although this genus have not been studied for bioremediation potential yet. The yeast *Cystobasidium pallidum* (4%) was found in relatively high abundance; it has been isolated from seawater and terrestrial environments (Menezes et al., 2019). *Wallemia sp.* (3.6% unknown) are considered extremophiles with high potential for bioremediation of saline and otherwise harsh soils and environments containing petroleum and other contaminants (Chamekh et al.., 2019; Patankar, S., et al., 2021; Bhapkar et al., 2022; Tiquia-Arashiro et al., 2019; Ali et al., 2019; Huang et al., 2019). *Stagonosporopsis unknown* (3.4%) are potential plant

pathogens (blight) that have been found to degrade low-density polyethylene film (Khruengsai et al, 2021). *Schizothecium miniglutinans* (3.1%) was observed but has no record of being found in contaminated soils or used in bioremediation. *Purpureocillium lilacinum* (3%) have been isolated from diverse environments including estuarine systems and sewage sludge, have a wide pH and temperature tolerance and can metabolize diverse substrates, acting as decomposers, rhizosphere fungi and/or entomopathic fungi (as in nematode eggs) (Inglis PW, 2006). *Aureobasidium pullulans* (1.5%) is a filamentous, melanized yeast that can be plant-associated (in roots and/or as an endophyte). It is known for its ability to produce a polysaccharide-rich substance called pullulan, been shown to sorb metals (Pb, Cd, Cu) and degrade organic pollutants such as petroleum hydrocarbons, pesticides, textile dyes and polycyclic aromatic hydrocarbons (PAH's) in both soil and water (Blasi et al., 2016; Cordero et al., 2017).

The phylum containing arbuscular mycorrhizal fungi (AMF), the Glomeromycota, were conspicuously absent in the ITS2 sequence data. However, members of Glomeromycota are poorly amplified with commonly used ITS primers, therefore diversity and abundance within this phylum were likely underestimated (Merges et al., 2020; Schadt & Rosling, 2015). AMF are commonly found in metal-contaminated soils (Amir et al., 2014; Gadd, 2007; Hassan et al., 2011; Hildebrandt et al., 2007; Pawlowska et al., 1997), and are known to associate with most plant species identified at our study sites. AMF presence in our study soils was confirmed as spores were extracted from all study site soils. Mycorrhizal spore counts provided a snapshot of the presence and abundance of these fungi in an area at a given time, and information about the potential

for AMF colonization of plant roots and their potential contribution to plant growth. AMF spore counts were low in study site soils (mean ~ 144 per gram of soil). Spore densities can range from hundreds to thousands or even tens of thousands of spores per gram of soil in fertile and undisturbed soil ecosystems (Anderson, 1988; Krishnamoorthy, R., 2015). AMF diversity, abundance, and richness can be altered by toxic metals in contaminated soils, and high levels of contaminants may inhibit AMF growth, sporulation, and survival, leading to a decrease in spore density as well as diversity (Hassan et al., 2011; Krishnamoorthy et al., 2015; Pawlowska et al., 1997). Some AMF species are more sensitive to contamination than others and there tends to be shifts in the species composition of AMF in contaminated soils, with more resilient species dominating the community. These resilient AMF populations could exhibit higher spore densities due to their ability to tolerate or even thrive in the presence of contaminants. Various authors have reported isolating spores of arbuscular mycorrhizal fungal taxa including several Glomus species (e.g., *G. mosseae*, *G. fasciculatum*, *G. intraradices*, *G. aggregatum*, and *G. constrictum*) along with *Scutellospora dipurpurescens*, *Gigaspora sp*., and *Entrophospora sp*. in metal-polluted soils (Griffioen 1994; Pawlowska et al. 1996, 2000; Regvar et al. 2001; Krishnamoorthy, R., 2015).

Within the bacterial domain, the phylum Proteobacteria is the largest and most diverse lineage and are often the most abundant bacterial phyla on contaminated sites (Hemmat-Jou, M.H., 2018; Sandaa et al, 1999; Zhang et al, 2012). Bacteria from the Proteobacteria have been tested in soils remediation strategies for metals, radioactive metals, hydrocarbons, and polycyclic aromatic hydrocarbons (Singleton et al., 2006;

Labbe et al., 2004; Bachate, 2012; Smith et a., 2015). The phylum in the second-highest abundance among study sites was the Chloroplexi, also commonly isolated from contaminated sites, including metal and organic contaminated soils (Shen et al, 2018; Peng, 2015; Zhu et al., 2023; Li et al, 2017; Shani et al, 2016).

The most dominant bacterial class on contaminated sites in the present study was Gammaproteobacteria, which not only has been found in contaminated soils but has been tested (successfully) in bioremediation of hydrocarbon contaminated soils (Smith et al, 2015; Dashti 2015; Militti et al, 2010; Ali et al, 2016). The second-most abundant bacterial class, the Planctomycetes, has also been tested in successful bioremediation applications, ranging from aerobic to anaerobic aquatic to terrestrial systems (aquifers, wastewater treatment and soil) for arsenic, hydrocarbon, polychlorinated biphenols (PCB's) and ammonium contamination (Carvalho, TFB 2017; Biswas et al, 2023; Petric et al, 2011; Nakajima et al, 2008; Militon et al, 2010).

The bacterial genera in the highest abundance also contain known remediator species, including *Massilia*, found to degrade phenanthrene, BTEX, and sorb metals (Wang et al, 2016; Gu et al, 2016; Son et al, 2021; Zondo et al, 2012). MND1 was the second-most abundant bacterial genera which has been applied to enhance phytoremediation (Wang et al, 2022; Shen et al, 2018) and degrade PAH's (Li et al, 2023). *Bryobacter*, the third-most abundant bacterial genera, is commonly found in contaminated soils and is a bioremediation candidate deserving of further research (Yang et al., 2022). Preliminary studies found it may enhance phytoremediation, play a role in

organochloride degradation in bioretention ponds, sorb Tl and Cd and reduce vanadium in groundwater (Wang et al., 2022; Rasool et al, 2020; Hao et., al 2018; Li et al, 2023).

7. Summary

Biological diversity on contaminated sites reflects the ability of plants, fungi, and bacteria to adapt, tolerate, and interact with pollutants. These diverse communities play potentially important roles in remediating contaminated environments, influencing ecosystem dynamics, and providing valuable insights for both scientific research and practical applications. This study was the first survey of contaminated sites in Southern California to assess plant, fungal and bacterial diversity, identify plant (including native plant) accumulators of metals of concern and investigate the factors that explain variation in microbial community composition. Several previously unidentified California native plant hyper-accumulators of copper, including *Baccharis salcifolia* and *sarothroides*, *Eriogonum fasciculatum* and *Brickellia californica,* as well as one unstudied non-native Cu hyperaccumulator, *Helminthotheca echiodes*, were identified. Other California native accumulators of metals such as Pb, Cr, and Ni, were also identified. These plants are potential candidates for metals phytoremediation in California that could be further tested in remediation studies in California because they are adapted to regional conditions. Fungal communities on the contaminated sites were dominated by a single phylum, Ascomycota, specifically by the Dothideomycetes and Sordariomycetes classes. Bacterial communities were dominated by the Proteobacteria and Chloroplexi phyla and the Gammoproteobacteria and Planctomycetes classes. Several fungal and bacteria species

known to possess remediative capacities were seen in study samples, as well as some yet untested potential bioremediation candidate species including extremophiles capable of thriving in dry, hot, and saline environments. In our study, variability between sites explained most of the variation seen in fungal and bacterial community composition. Water-extractable organic carbon, percentage of silt and sand, and cation exchange capacity were the most significant soil properties driving differences in fungal as well as bacterial community composition, although contributed to a small amount of variation compared to site differences and sample location (rhizosphere soils vs interspace soils).

8. References

Aguilar-Trigueros, C. A., Hempel, S., Powell, J. R., Anderson, I. C., Antonovics, J., Bergmann, J., et al. (2015). Branching out: towards a trait-based understanding of fungal ecology. *Fungal Biol. Rev.* 29, 34–41. doi: 10.1016/j.fbr.2015.03.001

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online. *17*.

Allen, M. F., Allen, M. F., & Allen, M. F. (1991). *The ecology of mycorrhizae*. Cambridge University Press.

Aronesty, E. (2011). ea-utils: Command-line tools for processing biological sequencing data. In: Durham, NC.

Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements. *Biorecovery* 1: 81–126.

Balvočiūtė, M., & Huson, D. H. (2017). SILVA, RDP, Greengenes, NCBI and OTT — how do these taxonomies compare? *BMC Genomics*, *18*(2), 114. https://doi.org/10.1186/s12864-017-3501-4

Balacco, J. R., Vaidya, B. P., Hagmann, D. F., Goodey, N. M., & Krumins, J. A. (2022). Mycorrhizal infection can ameliorate abiotic factors in urban soils. *Microbial ecology*, 1-8.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., . . . de Sousa, F. (2013). Improved software detection and extraction of ITS1 and ITS 2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in ecology and evolution*, *4*(10), 914-919.

Berry, D., Ben Mahfoudh, K., Wagner, M., & Loy, A. (2011). Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Applied and Environmental Microbiology*, *77*(21), 7846-7849.

Blackwell, M. (2011). The Fungi: 1, 2, 3… 5.1 million species? *American journal of botany*, *98*(3), 426-438.

Blasi, B., Poyntner, C., Rudavsky, T., Prenafeta-Boldú, F. X., De Hoog, S., Tafer, H., & Sterflinger, K. (2016). Pathogenic yet environmentally friendly? Black fungal candidates for bioremediation of pollutants. *Geomicrobiology journal*, *33*(3-4), 308-317.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., . . . Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science & Technology*, *31*(3), 860-865.

Bourdel, G., Roy-Bolduc, A., St-Arnaud, M., & Hijri, M. (2016). Concentration of petroleum-hydrocarbon contamination shapes fungal endophytic community structure in plant roots. *Frontiers in microbiology*, *7*, 685.

Błaszkowski, J., Chwat, G., Kozłowska, A., Ryszka, P., & Kovács, G. (2015). Two new genera, Dominikia and Kamienskia, and D. disticha sp. nov. in Glomeromycota. *Nova Hedwigia*, *100*. https://doi.org/10.1127/nova_hedwigia/2014/0216

California Topographic Map. Accessed at: https://en-gb.topographic-map.com/map-1z6z4/California/?center=33.91886%2C-118.17719&zoom=11&popup=34.03616%2C-118.2534

Callahan, B., McMurdie, P., Rosen, M., Han, A., & Johnson, A. (2016). JA, Holmes SP. 2016. *DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods*, *13*(7), 581-583.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., . . . Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the national academy of sciences*, *108*(Supplement 1), 4516-4522.

Colombo, J. C., Cabello, M., & Arambarri, A. M. (1996). Biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure cultures of imperfect and lignolitic fungi. *Environmental pollution*, *94*(3), 355-362.

Cordero, R. J., Vij, R., & Casadevall, A. (2017). Microbial melanins for radioprotection and bioremediation. *Microbial biotechnology*, *10*(5), 1186-1190.

Curd EE, Gold Z, Kandlikar GS et al (2019) Anacapa Toolkit: an environmental DNA toolkit for processing multilocus metabarcode datasets. Methods Ecol Evol 10:1469–1475. https://doi.org/10.1111/2041-210X.13214

Czaplicki, L. M., Cooper, E., Ferguson, P. L., Stapleton, H. M., Vilgalys, R., & Gunsch, C. K. (2016). A new perspective on sustainable soil remediation—case study suggests novel fungal genera could facilitate in situ biodegradation of hazardous contaminants. *Remediation Journal*, *26*(2), 59-72.

Czaplicki, L. M., Redfern, L. K., Cooper, E. M., Ferguson, P. L., Vilgalys, R., & Gunsch, C. K. (2020). Investigating the mycobiome of the Holcomb Creosote Superfund Site. *Chemosphere*, *252*, 126208.

Davis, M. R., Zhao, F. J., & McGrath, S. P. (2004). Pollution-induced community tolerance of soil microbes in response to a zinc gradient. *Environmental Toxicology and Chemistry: An International Journal*, *23*(11), 2665-2672.

Davis NM, Proctor DM, Holmes SP et al (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:226. https://doi.org/10.1186/s40168-018-0605-2

del Val, C., Barea, J., & Azcón-Aguilar, C. (1999). Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. *Applied Soil Ecology*, *11*(2-3), 261-269.

Deshmukh, S., & Rai, M. (2005). *Biodiversity of fungi*. Enfield: Science Publishers, Inc.

Devinny, J., Longcore, T., Bina, A., Kitts, C., & Osborne, K. H. (2005). Phytoremediation with native plants. *University of Southern California, Los Angeles*.

Dong, Z.-Y., Huang, W.-H., Xing, D.-F., & Zhang, H.-F. (2013). Remediation of soil co-contaminated with petroleum and heavy metals by the integration of electrokinetics and biostimulation. *Journal of hazardous materials*, *260*, 399-408.

DTSC. (2021a). *GATX Annex Terminal -San Pedro* https://www.envirostor.dtsc.ca.gov/public/profile_report.asp?global_id=19420029

DTSC. (2021b). *San Pedro Boat Works* https://www.envirostor.dtsc.ca.gov/public/profile_report.asp?global_id=70000023

Dumbrell, A. J., Ashton, P. D., Aziz, N., Feng, G., Nelson, M., Dytham, C., . . . Helgason, T. (2011). Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist*, *190*(3), 794-804. https://doi.org/https://doi.org/10.1111/j.1469-8137.2010.03636.x

Edgar, R. C. (2017). Updating the 97% identity threshold for 16S ribosomal RNA OTUs. *bioRxiv*, 192211. https://doi.org/10.1101/192211

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics*, *31*(21), 3476-3482. https://doi.org/10.1093/bioinformatics/btv401

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., . . . Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature Communications*, *10*(1), 2369. https://doi.org/10.1038/s41467-019-10373-z

Eisenman, H. C., & Casadevall, A. (2012). Synthesis and assembly of fungal melanin. *Applied microbiology and biotechnology*, *93*(3), 931-940.

Engineering, C. o. L. A. D. o. P. W. a. B. o. (2021). *Taylor Yard G2 River Park Project Draft Final Implementation Feasibility Report* https://eng2.lacity.org/projects/tayloryard/TaylorYardG2RiverParkProject_IFR_February2021_FullReport.pdf

Essington, M. E. (2003). *Soil and Water Chemistry: An Integrative Approach* CRC Press.

Ferrol, N., Tamayo, E., & Vargas, P. (2016). The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. *Journal of experimental botany*, erw403.

Fierer, N. G. (2003). *Stress ecology and the dynamics of microbial communities and processes in soil*. University of California, Santa Barbara.

Fomina, M., Burford, E. P., & Gadd, G. M. (2005). Toxic metals and fungal communities. In *The Fungal Community* (pp. 753-778). CRC Press.

Freund, L. Dada2 pipeline at http://hlfreund.github.io/

Gadd, G. M., & Gadd, G. M. (2001). *Fungi in bioremediation*. Cambridge University Press.

Garbisu, C., & Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology*, *77*(3), 229-236. https://doi.org/https://doi.org/10.1016/S0960-8524(00)00108-5

Gawronski, S. W., Gawronska, A. Helena (2007). *Plant Taxonomy for Phytoremediation* (N. Marmiroli, B. Samotokin, & M. Marmiroli, Eds.). Springer Netherlands.

Giraud, F., Guiraud, P., Kadri, M., Blake, G., & Steiman, R. (2001). Biodegradation of anthracene and fluoranthene by fungi isolated from an experimental constructed wetland for wastewater treatment. *Water research*, *35*(17), 4126-4136.

Glassman, S. I., & Martiny, J. B. H. (2018). Broad-scale alpha- and beta- diversity patterns are robust to use of exact sequence variants versus operational taxonomic units. *bioRxiv*, 283283. https://doi.org/10.1101/283283

Gray, T., & Parkinson, D. (1968). *The ecology of soil bacteria* (Vol. 681). University of Toronto Press.

Haritash, A., & Kaushik, C. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *Journal of hazardous materials*, *169*(1-3), 1-15.

Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology*, *9*(3), 177-192. https://doi.org/10.1038/nrmicro2519

Harris, J., & Steer, J. (2003). Modern methods for estimating soil microbial biomass and diversity: an integrated approach. In *The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions* (pp. 29-48). Springer.

Hassan, S. E. D., Boon, E., ST-ARNAUD, M., & Hijri, M. (2011). Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. *Molecular Ecology*, *20*(16), 3469-3483.

Hellmers, H., Horton, J., Juhren, G., & O'keefe, J. (1955). Root systems of some chaparral plants in southern California. *Ecology*, *36*(4), 667-678.

Hettiarachchi, G. M., & Pierzynski, G. M. (2004). Soil lead bioavailability and in situ remediation of lead-contaminated soils: A review. *Environmental progress*, *23*(1), 78-93.

Hildebrandt, U., Regvar, M., & Bothe, H. (2007). Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry*, *68*(1), 139-146.

Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.-P., . . . Plassard, C. (2011). Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? *Plant and soil*, *348*(1), 29-61.

Homyak, P. M., Blankinship, J. C., Slessarev, E. W., Schaeffer, S. M., Manzoni, S., & Schimel, J. P. (2018). Effects of altered dry season length and plant inputs on soluble soil carbon. *Ecology*, *99*(10), 2348-2362.

Horwath, W. R., & Paul, E. A. (1994). Microbial Biomass. In *Methods of Soil Analysis* (pp. 753-773). https://doi.org/https://doi.org/10.2136/sssabookser5.2.c36

Hutchinson, S., Schwab, A., & Banks, M. (2003). Biodegradation of petroleum hydrocarbons in the rhizosphere. *Phytoremediation: transformation and control of contaminants*, 355-386.

Hu L, Robert CAM, Cadot S et al (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat Commun 9:2738. https://doi.org/10.1038/s41467-018-05122-7

Hua Z, Ouellette M, Makkay AM et al (2021) Nutrient supplementation experiments with saltern microbial communities implicate utilization of DNA as a source of phosphorus. ISME J 15:2853–2864. https://doi.org/10.1038/s41396-021-00960-8

Ianson, D. C., & Allen, M. F. (1986). The effects of soil texture on extraction of vesicular-arbuscular mycorrhizal fungal spores from arid sites. *Mycologia*, *78*(2), 164-168.

Iffis, B., St-Arnaud, M., & Hijri, M. (2017). Petroleum Contamination and Plant Identity Influence Soil and Root Microbial Communities While AMF Spores Retrieved from the Same Plants Possess Markedly Different Communities [Original Research]. *Frontiers in Plant Science*, *8*(1381). https://doi.org/10.3389/fpls.2017.01381

Jabeen, R., Ahmad, A., & Iqbal, M. (2009). Phytoremediation of heavy metals: physiological and molecular mechanisms. *The Botanical Review*, *75*(4), 339-364.

Jacques, R. J., Okeke, B. C., Bento, F. M., Teixeira, A. S., Peralba, M. C., & Camargo, F. A. (2008). Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. *Bioresource Technology*, *99*(7), 2637-2643.

Jin L, Son Y, Yoon TK et al (2013) High concentrations of single-walled carbon nanotubes lower soil enzyme activity and microbial biomass. Ecotoxicol Environ Saf 88:9–15. https://doi.org/10.1016/j.ecoenv.2012.10.031

Jenkinson, D. S., & Ladd, J. (1981). Microbial biomass in soil: measurement and turnover. *Soil biochemistry*, *5*(1), 415-471.

Joner, E. J., Briones, R., & Leyval, C. (2000). Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and soil*, *226*(2), 227-234.

Junghanns, C., Krauss, G., & Schlosser, D. (2008). Potential of aquatic fungi derived from diverse freshwater environments to decolourise synthetic azo and anthraquinone dyes. *Bioresource Technology*, *99*(5), 1225-1235.

Juwarkar, A. A., Singh, S. K., & Mudhoo, A. (2010). A comprehensive overview of elements in bioremediation. *Reviews in Environmental Science and bio/technology*, *9*(3), 215-288.

Katznelson, H. (1965). Nature and importance of the rhizosphere. *Ecology of soil-borne plant pathogens*, 187-207.

Kersters K, De Vos P, Gillis M, Swings J, Vandamme P, Stackebrandt E . (2006). Introduction to the Proteobacteria. In: Dwarkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds). The Prokaryotes, 3rd edn, vol. 5. Springer: New York, pp 3–37.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic acids research*, *41*(1), e1-e1.

Krishnamoorthy, R., Kim, C.-G., Subramanian, P., Kim, K.-Y., Selvakumar, G., & Sa, T.-M. (2015). Arbuscular Mycorrhizal Fungi Community Structure, Abundance and Species Richness Changes in Soil by Different Levels of Heavy Metal and Metalloid Concentration. *PloS one*, *10*(6), e0128784. https://doi.org/10.1371/journal.pone.0128784

Kubatova, A., Prasil, K., & Vanova, M. (2002). Diversity of soil microscopic fungi on abandoned industrial deposits. *Cryptogamie Mycologie (France)*.

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F., Bahram, M., . . . Callaghan, T. M. (2013). Towards a unified paradigm for sequence-based identification of fungi. In: Wiley Online Library.

Lee, J., Lee, S., & Young, J. P. W. (2008). Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS microbiology ecology*, *65*(2), 339-349.

Marchand, C., St-Arnaud, M., Hogland, W., Bell, T. H., & Hijri, M. (2017). Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. *International Biodeterioration & Biodegradation*, *116*, 48-57.

Marchiol, L., Fellet, G., Boscutti, F., Montella, C., Mozzi, R., & Guarino, C. (2013). Gentle remediation at the former "Pertusola Sud" zinc smelter: evaluation of native species for phytoremediation purposes. *Ecological Engineering*, *53*, 343-353.

Marco-Urrea, E., García-Romera, I., & Aranda, E. (2015). Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. *New biotechnology*, *32*(6), 620-628.

Marschner, P., Jentschke, G., & Godbold, D. L. (1998). Cation exchange capacity and lead sorption in ectomycorrhizal fungi. *Plant and soil*, *205*(1), 93-98.

Martin, F. M., Perotto, S., & Bonfante, P. (2000). Mycorrhizal fungi: a fungal community at the interface between soil and roots. In *The Rhizosphere* (pp. 279-312). CRC Press.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12.

Massaccesi, G., Romero, M. C., Cazau, M. C., & Bucsinszky, A. M. (2002). Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina). *World Journal of Microbiology and Biotechnology*, *18*(9), 817-820.

Masue, Y., Loeppert, R. H., & Kramer, T. A. (2007). Arsenate and arsenite adsorption and desorption behavior on coprecipitated aluminum: iron hydroxides. *Environmental Science & Technology*, *41*(3), 837-842.

Maynard, D., Kalra, Y., & Crumbaugh, J. (1993). Nitrate and exchangeable ammonium nitrogen. *Soil sampling and methods of analysis*, *1*.

McMurdie, P. J., & Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLOS Computational Biology*, *10*(4), e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Mejia, M.P., Rojas, C.A., Curd, E. *et al.* Soil Microbial Community Composition and Tolerance to Contaminants in an Urban Brownfield Site. *Microb Ecol* 85, 998–1012 (2023). https://doi.org/10.1007/s00248-022-02061-1

Mohsenzadeh, F., Rad, A. C., & Akbari, M. (2012). Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum-polluted soils. *Iranian journal of environmental health science & engineering*, *9*(1), 1-8.

Morley, G. F., & Gadd, G. M. (1995). Sorption of toxic metals by fungi and clay minerals. *Mycological research*, *99*(12), 1429-1438.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., . . . Kennedy, P. G. (2016). FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241-248.

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., & Tedersoo, L. (2019). Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology*, *17*(2), 95-109.

Nilsson, R. H., Tedersoo, L., Ryberg, M., Kristiansson, E., Hartmann, M., Unterseher, M., . . . De Sousa, F. (2015). A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. *Microbes and environments*, ME14121.

Noll, M. R. (2003). Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. *Journal of Environmental Quality*, *32*(1), 374.

O'Brien, H. E., Parrent, J. L., Jackson, J. A., Moncalvo, J.-M., & Vilgalys, R. (2005). Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology*, *71*(9), 5544-5550.

O'Reilly, S. E., & Hochella, M. F. (2003). Lead sorption efficiencies of natural and synthetic Mn and Fe-oxides. *Geochimica et Cosmochimica Acta*, *67*(23), 4471-4487. https://doi.org/https://doi.org/10.1016/S0016-7037(03)00413-7

Oksanen, J. (2011). Vegan : community ecology package. R package version 1.17-9. http://cran.r-project.org/package=vegan.

Olsen, S., & Sommers, L. (1982). Phosphorus in AL Page,(Ed). Methods of Soil Analysis. Part2. Chemical and Microbiological Properties. *Agronomy Mongraphs*, *9*(2).

Olsen, S. R. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. US Department of Agriculture.

Pawlowska, T. E., Błaszkowski, J., & Rühling, Å. (1997). The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. *Mycorrhiza*, *6*(6), 499-505.

Pinto, L. J., & Moore, M. M. (2000). Release of polycyclic aromatic hydrocarbons from contaminated soils by surfactant and remediation of this effluent by Penicillium spp. *Environmental Toxicology and Chemistry: An International Journal*, *19*(7), 1741-1748.

Potin, O., Rafin, C., & Veignie, E. (2004). Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil. *International Biodeterioration & Biodegradation*, *54*(1), 45-52.

Project Management Team Bureau of Engineering (2020) Taylor Yard G2 Parcel 3rd Quarter Status Report. City of Los Angeles, Los Angeles, CA. Accessed at:https://eng2.lacity.org/sites/g/files/wph726/f/Final%20Taylor%20Yard%20G2%20Status%20Report_January-March%202020.pdf

Reeves, R.D., Baker, A.J.M., Jaffré, T., Erskine, P.D., Echevarria, G. and van der Ent, A. (2018), A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytol, 218: 407-411. https://doi.org/10.1111/nph.14907

Roth AL, Vander Haar EL (2006) Media standing of urban parkland movements: the case of Los Angeles' Taylor Yard, 1985–2001. City Community 5:129–151. https://doi.org/10.1111/j.1540-6040.2006.00166.x

Yang, Y., Dou, Y., Huang, Y., & An, S. (2017). Links between soil fungal diversity and plant and soil properties on the Loess Plateau. *Frontiers in Microbiology*, *8*, 2198.

Rubin, B. E., Sanders, J. G., Hampton-Marcell, J., Owens, S. M., Gilbert, J. A., & Moreau, C. S. (2014). DNA extraction protocols cause differences in 16S rRNA amplicon sequencing efficiency but not in community profile composition or structure. *Microbiologyopen*, *3*(6), 910-921.

Schadt, C. W., & Rosling, A. (2015). Comment on "Global diversity and geography of soil fungi". *science*, *348*(6242), 1438-1438.

Stefani, F. O., Bell, T. H., Marchand, C., de la Providencia, I. E., El Yassimi, A., St-Arnaud, M., & Hijri, M. (2015). Culture-dependent and-independent methods capture different microbial community fractions in hydrocarbon-contaminated soils. *PloS one*, *10*(6), e0128272.

Taylor, D. L., Hollingsworth, T. N., McFarland, J. W., Lennon, N. J., Nusbaum, C., & Ruess, R. W. (2014). A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. *Ecological Monographs*, *84*(1), 3-20.

Taylor, D. L., Walters, W. A., Lennon, N. J., Bochicchio, J., Krohn, A., Caporaso, J. G., & Pennanen, T. (2016). Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. *Applied and Environmental Microbiology*, *82*(24), 7217-7226.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., . . . Suija, A. (2014). Global diversity and geography of soil fungi. *science*, *346*(6213).

Tessier, A., Campbell, P. G., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical chemistry*, *51*(7), 844-851.

USEPA, Regional Screening Level (RSL) Summary Table (TR=1E-06, HQ=1) (2020). https://semspub.epa.gov/work/HQ/400431.pdf

van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant and Soil* 362: 319–334.

Vargas-Albores, F., Ortiz-Suárez, L. E., Villalpando-Canchola, E., & Martínez-Porchas, M. (2017). Size-variable zone in V3 region of 16S rRNA. *RNA Biology*, *14*(11), 1514-1521. https://doi.org/10.1080/15476286.2017.1317912

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A., & Pigna, M. (2010). Mobility and bioavailability of heavy metals and metalloids in soil environments. *Journal of soil science and plant nutrition*, *10*(3), 268-292.

Appendix 1. Soil physical properties

Soils at study sites were generally hot (31 - 40 °C) and dry (0.001 - 0.2976 m^3/m^3), when measured during the summer months of 2021. While mean soil moisture was higher in the interspace soils (0.1016 m^3/m^3) than rhizosphere soils (0.0322 m^3/m^3) (averaged across 10 replicates per site and across the seven sites), and mean temperature was higher in the rhizosphere soils (38.19 °C) than interspace soils (36.45 °C), the difference was not statistically significant (p(temp)= 0.1183; p(moisture)= 0.3795); and while there were differences in average soil moisture and temperature between sites, the difference was not statistically significant (p(temp)= 0.0982; p(moisture)= 0.4135). Soils across sites were generally sandy loams (39 – 89% sand content). Clay content was variable (1.47 – 10.9 %) with Site B having the highest clay content. Silt content was variable (9.15 – 49.88 %) with Site B soils containing the highest concentrations of silt and C and E also containing higher percentages of silt. Site D (88 %) and A (79 %) were the sandiest soils, although all sites were dominated by sand.

Table S1. Mean Soil Physical Properties by Site and Location.

Rhizosphere and interspace means were calculated by averaging 5 replicates per site per location, whereas "All samples" are means of 10 replicates per site. Standard deviation values are beside each mean.

Site	Location	Mean Soil Temp (°C)	Mean Soil Moisture (m^3/m^3)	% Clay	% Silt	% Sand	Texture Class
Taylor Yard (A)	Interspace	37.54±9.56	0.5656±1.19	4.11±2.285	14.25±6.90	81.76±8.61	loamy sand
	Rhizosphere	38.60±7.43	0.0296±0.02	5.54±5.983	18.59±15.09	76.45±19.60	loamy sand
	All samples	38.07±8.09	0.2976±0.844	4.83±4.335	16.42±11.30	79.10±14.55	sandy
LA Ecovillage (B)	Interspace	31.41±4.31	0.0784±0.029	10.90±3.24	49.88±10.93	39.21±13.78	loam
	Rhizosphere	36.24±2.83	0.0204±0.011	4.63±2.93	29.73±14.04	65.62±16.91	loam
	All samples	33.82±4.27	0.0494±0.037	7.77±4.40	39.80±15.92	52.41±20.13	loam
Slauson (C)	Interspace	36.50±2.89	0.0091±0.007	2.85±1.34	25.62±9.29	71.51±10.62	sandy loam
	Rhizosphere	38.24±1.26	0.0005±0.0005	3.16±1.18	24.49±1.95	72.33±3.01	sandy loam
	All samples	37.37±2.29	0.0048±0.006	3.00±1.21	25.06±6.36	71.92±7.37	sandy loam
B70-71 (D)	Interspace	35.60±1.48	0.0298±0.006	1.47±0.76	11.38±6.53	87.14±7.24	loamy sand
	Rhizosphere	38.34±3.61	0.089±0.136	1.62±1.03	9.148±7.82	89.21±8.58	sandy
	All samples	36.97±2.97	0.0594±0.096	1.54±0.859	10.26±6.89	88.17±7.57	loamy sand
GATX (E)	Interspace	38.42±5.07	0.0055±0.007	7.12±2.01	38.69±4.24	54.15±6.05	sandy loam
	Rhizosphere	40.12±4.44	0.0058±0.002	5.81±1.13	33.79±10.04	60.38±10.95	sandy loam
	All samples	39.60±4.97	0.0058±0.005	6.70±1.59	38.45±3.48	57.07±4.78	sandy loam
B44 (F)	Interspace	37.95±1.02	0.001±0.002	4.65±1.91	23.49±5.22	71.84±96.104	sandy loam
	Rhizosphere	34.80±2.07	0.060±0.051	5.53±2.15	29.64±7.90	64.81±10.01	sandy loam
	All samples	36.37±2.26	0.031±0.046	5.09±1.97	26.56±7.10	68.33±8.65	sandy loam
Walnut & Daisy (G)	Interspace	40.04±4.82	0.00306±0.002	5.57±2.86	35.3±12.05	59.13±14.90	sandy loam
	Rhizosphere	38.62±1.75	0.0192±0.015	5.5±1.49	36.7±3.08	58.44±4.34	sandy loam
	All samples	39.33±3.50	0.011±0.013	5.85±2.68	36.9±10.75	57.26±13.35	sandy loam

Appendix 2. Soil chemical properties

Mean pH across all sites was circumneutral (7.32) with a range of 6.3 – 8.75 but there was no significant difference between rhizosphere and interspace soil pH ($p = 0.9151$), nor between the soil pH of different sites ($p = 0.1499$). Site F (6.85) and A (6.53) had slightly more acidic soils, whereas Site C had more basic soils (8.42). Soil organic carbon (SOC) content on all sites was low with a range of 0.48- 6.6 % and an average of 2.0%. Organic carbon enters the soil through the decomposition of plant and animal residues, root exudates, living and dead microorganisms, and soil biota. The SOC content may be low at these sites as SOC is generally low in sandy, low clay soils due to the lack of protection of OM by clay minerals and low soil moisture retention (Gregorich & Janzen, 2020; Van Veen et al., 1989). Rhizosphere soils across all sites had average SOC content (2.675µg/g) higher than interspace soils (1.763µg/g), though their difference was not significant ($p = 0.1244$); the difference between sites in SOC, however, was significant ($p = 0.0327$). There were undetectable amounts of inorganic carbon (carbonate) at all sites except for Site F (0.57µg/g) and D (0.88µg/g), the Port of LA / Long Beach sites near the ocean. Though SOC was generally low across sites, much of the carbon pool was water extractable (average 104.16 mg/L). Water-extractable organic carbon (WEOC) refers to the fraction of organic carbon compounds in soil that can be dissolved in water. It is a crucial component of soil organic matter and plays a significant role in various soil processes and interactions, including its influence on soil microbial diversity and abundance. Water extractable carbon is the carbon pool most available to microbes (Homyak et al., 2018). Water extractable organic carbon (WEOC) significantly varied

between sites ($p = < 0.0001$) but did not vary significantly between the rhizosphere (mean = 101.236 mg/L) and the interspace (mean = 94.494 mg/L) soils. Soil moisture controls microbial activity and soil carbon cycling, with less microbial activity occurring in drier soils (Homyak et al., 2018). However, WEOC can increase as soils dry and physical access to C is constrained for microbes resulting in microbial die-off (Homyak et al., 2018). The average C/N ratio at all sites was 23 (range of 11.2 – 43.81), suggesting an equilibrium state between mineralization and immobilization of N. Typical C/N ratio of soils is 10-12:1 (Sparks, 2003). CEC had a range of 2.64 – 24.57 meq/100 g with an average of 8.37 meq/100 g, which makes sense given the mean pH around 7 and soil texture of sandy loam across sites. There was a significant difference between sites ($p = <0.0001$) but not between the rhizosphere and interspace soils with regards to CEC ($p = 0.8661$).

The average extractable phosphate concentration in soils was 6.745µg/g (range 0.9 – 20.96µg/g). Site C, D and G had relatively low phosphate concentrations (1.36, 1.29 and 1.78µg/g respectively), whereas Site A, B, E and F had higher phosphate concentrations (1.36, 1.29 and 1.78µg/g respectively. Mean phosphate concentrations were not significantly different between the rhizosphere (7.43µg/g) and the interspace (6.01µg/g) soils ($p = 0.8551$). Soil phosphorus fractions can be higher in the rhizosphere soils than interspace soils due the release of OH^- ligands by plant roots into the surrounding soil to enhance phosphorus acquisition through ligand exchange (Hinsinger et al., 2011). But because soils at the study sites are generally sandy with low SOC content, phosphate adsorption is likely weaker and can leach more easily (Sparks, 2003). Mean ammonium (NH_4) concentrations were low at 1.85µg/g and mean nitrate (NO_3) was 35.82µg/g, with a

significant difference between sites for NH_4 ($p = <0.0001$) but not NO_3 ($p = 0.3419$) and high variability between the sites in their concentrations. There was no significant difference between interspace and rhizosphere soils for NH_4 nor NO_3 ($p = 0.9068$ and 0.875 respectively). Nitrate is held weakly by poorly developed soils and therefore can leach readily in arid and semi-arid regions (Sparks, 2003). Soils had a wide range of salinity (as measured by electrical conductivity) between 0.4319 – 1089 dS/M, although tended to be highly saline.

Table S2. Soil Chemical Properties.
Rhizosphere and interspace means were calculated by averaging 5 replicates per site per location, whereas "All samples" are means of 10 replicates per site.

Site	Location	WEOC (mg/L)	C/N	SOC (%)	NO_3 (mg/L)	pH	CEC (cmolc/kg soil)	PO_4 (mg/L)
Taylor Yard (A)	Interspace	15.68±7.02	20.04±8.41	2.97±4.13	0.67±0.91	6.30±3.67	7.38±4.86	10.56±8.25
	Rhizosphere	26.2±15.93	21.52±6.84	1.61±1.55	1.08±0.72	6.75±4.44	8.99±7.13	14.90±5.58
	All samples	20.91±12.87	20.78±7.27	1.71±2.99	0.87±0.81	6.53±3.94	8.19±6.19	12.73±6.31
LA Ecovillage (B)	Interspace	15.74±6.60	22.50±3.56	0.48±3.34	92.47±0.47	7.65±1.10	9.71±9.39	6.34±7.38
	Rhizosphere	21.61±13.78	19.52±8.62	0.99±1.47	10.68±0.24	7.33±3.34	11.83±2.20	3.86±2.34
	All samples	18.71±10.65	21.01±6.42	6.60±2.56	51.58±0.39	7.49±3.73	10.77±6.56	5.10±5.11
Slauson (C)	Interspace	19.50±12.33	23.82±4.58	1.65±0.58	2.13±0.159	8.75±2.82	6.31±0.88	1.24±6.77
	Rhizosphere	19.41±2.34	18.46±5.18	0.75±2.84	144.11±0.08	8.09±3.08	9.33±0.97	2.32±5.98
	All samples	19.49±11.63	21.14±5.04	1.63±1.96	73.12±0.36	8.42±2.79	7.82±1.04	1.78±6.12
B70-71 (D)	Interspace	69.39±0.50	39.6±53.11	2.80±23.80	2.95±2.87	6.75±3.93	4.03±2.46	1.28±0.58
	Rhizosphere	82.3±1.94	14.8±46.97	2.80±12.07	1.99±2.17	7.41±3.80	2.64±0.77	1.44±1.61
	All samples	75.84±1.34	27.2±47.75	2.53±22.07	2.47±2.45	7.08±3.66	3.33±1.87	1.36±1.14
GATX (E)	Interspace	23.78±13.67	17.80±2.28	2.38±0.186	18.77±0.90	7.29±9.75	24.57±2.57	6.05±195.4
	Rhizosphere	18.67±339.2	20.80±8.89	0.66±0.177	88.8±0.755	7.59±11.65	21.87±15.2	20.96±6.40
	All samples	26.99±11.51	20.33±5.74	0.99±0.201	55.38±0.79	7.53±9.89	24.54±13.6	13.99±136
B44 (F)	Interspace	488.36±180.4	11.20±0.83	0.63±0.126	11.04±0.52	6.87±0.52	17.33±6.40	14.89±10.3
	Rhizosphere	507.82±368.7	11.40±2.60	2.29±0.134	7.99±0.322	6.83±0.32	11.26±8.63	7.61±6.73
	All samples	498.09±273.8	11.30±1.82	0.95±0.127	9.51±0.412	6.85±0.41	14.29±7.85	11.25±9.09
Walnut and Daisy (G)	Interspace	18.95±5.12	43.81±24.7	2.50±0.44	109.58±0.4	7.28±4.81	7.86±2.59	1.67±1.86
	Rhizosphere	17.42±6.15	37.97±14.6	2.87±0.44	8.00±0.27	7.53±5.25	10.34±0.55	0.90±0.68
	All samples	18.19±5.4	40.89±19.4	4.09±0.60	58.79±0.38	7.40±4.93	9.10±1.81	1.29±1.34

Appendix 3. Plant Foliar Part Metal Concentrations

Table S3. Mean* Plant Volunteer Metal Uptake (Foliar Parts) with Standard Deviation.
*Averaged across biological and technical replicates across sites.

Plant Species	µg Cr / g plant	µg Ni / g plant	µg Cu / g plant	µg As / g plant	µg Cd / g plant	µg Pb / g plant
California Native Plants						
Brickellia californica	2.83±0.26	1.26±0.35	4209.63±5921.43	0.24±0.23	0.64±0.52	1.20±1.35
Eriogonum fasciculatum	18.40±15.05	8.10±8.94	2020.02±3973.52	1.89±3.57	0.00±2.64	17.39±31.94
Baccharis pilularis	11.21±1.92	4.47±1.92	27.25±3.39	0.00±0.06	1.48±0.09	6.96±10.55
Baccharis sarothroides	8.46±749	3.41±3.11	2778.87±4778.86	0.17±0.08	0.00±1.85	3.44±2.02
Encelia californica	8.22±6.39	3.45±2.85	35.01±6.13	0.00±0.07	0.66±0.58	0.72±0.16
Baccharis salicifolia	20.42±6.61	8.18±2.53	1036.02±2024.79	0.28±0.16	0.76±0.98	5.03±4.19
Heterotheca grandiflora	86.42±124.06	34.68±50.13	349.30±1396.77	0.90±1.24	3.04±2.82	18.21±0.19
Non-Native Plants						
Pennisetum setaceum	51.34±58.46	15.74±20.22	1425.45±30.56	0.45±0.59	1.13±3.78	10.14±9.56
Solanum nigrum	15.30±6.02	6.35±7.25	41.26±41.30	0.00±0.23	0.00±5.17	4.52±4.75
Helminthotheca echioides	160.56±135.38	61.96±50.56	1307.18±1805.41	1.36±1.48	2.50±1.41	14.49±12.29
Stellaria media	7.63±5.33	3.40±1.33	1454.37±1667.19	0.00±0.06	4.22±4.03	4.36±4.05
Erigeron bonariensis	7.07±2.06	3.81±0.25	28.87±16.83	0.58±0.40	4.88±4.09	10.69±8.47
Chenopodium giganteum	13.83±16.09	6.52±5.18	17.43±5.78	0.00±0.24	0.00±0.34	4.45±4.13
Malva neglecta	32.05±23.26	4.69±4.32	2828.54±575.16	0.99±2.07	2.62±1.80	64.18±51.09
Washingtonia robusta	3.66±1.23	1.75±0.05	22.25±18.75	0.07±0.06	0.85±0.60	0.99±0.59
Tropaeolum majus	11.58±1.37	4.92±3.17	11.88±9.09	0.00±0.00	4.11±5.47	5.12±2.98
Panicum virgatum	120.78±189.03	39.60±55.96	38.32±31.26	0.79±1.64	0.60±0.42	24.73±49.83
Lactuca serriola	151.08±191.39	44.90±58.71	91.21±111.38	0.00±2.01	0.00±1.00	22.77±33.85
Sonchus oleraceus	20.17±21.06	7.07±5.86	21.59±7.22	0.05±0.03	1.54±0.76	16.88±5.15
Melilotus officinalis	36.81±67.98	15.06±27.92	59.60±87.71	0.00±1.64	1.30±1.76	102.01±199.75
Nicotiana glauca	4.10±0.19	4.25±3.83	3834.02±5383.2	1.96±3.53	2.01±1.48	1.80±0.19
Hordeum murinum	116.26±126.67	35.73±39.91	31.52±39.39	0.83±1.84	1.33±2.54	1.33±29.34

Appendix 4. Soil Metal Concentrations

Table S4. Soil Metal Concentrations by Site (µg/g) *Individual sample concentrations are shown (n = 10 per site)							
Metal(loid)	**A**	**B**	**C**	**D**	**E**	**F**	**G**
As	0.5	0.5	0.5	305	5.6	5.5	9.2
	0.6	1.3	0.5	291.8	7.9	6.8	2.2
	1	1.8	0.5	1006	5.8	6.1	3.7
	7.5	0.5	0.5	81.3	5.9	6.5	3.5
	13.1	1.9	0.5	52.7	0.1	5	10.5
	0.7	4.4	7	162.4	0.4	3	3.4
	2.2	7.9	0.5	140.8	1.7	5.9	8.51
	0.4	7.2	5.6	74.9	16.5	2.3	3.6
	10.3	26.3	27.5	322.3	10.2	6.3	2.3
	16.4	12.1	3.1	83.1	5.8	5	7.1
Cd	0.1	0.7	0.5	5	0.4	0.3	2.6
	0.1	1	0.1	6.6	0.3	1	2.4
	0.2	1.1	0.3	8	0.5	0.6	2.2
	0.8	1.1	1	1.4	0.2	0.8	2.2
	3.5	0.2	0.9	0.3	0.4	0.8	22.6
	0.9	1.2	2.5	2.8	0.1	0.6	3.7
	0.7	1.7	1.1	4.1	0.3	1.6	9
	0.6	1.2	0.7	3.6	2.3	4	3
	0.5	5.4	11.1	3.2	0.5	2.5	2
	1.4	2.2	0.7	5.1	0.4	1.6	6.7
Cr	66.2	45.3	312.8	464.6	41.9	53.3	49.3
	73.3	45	437	312.8	46.8	67.5	46.7
	98.4	57.7	92.2	437	45.3	61.6	39.9
	84	48.5	61.4	92.2	19.7	69.9	38.1
	135.7	45.5	134.9	61.4	226.9	75	58.5
	68.3	56.7	191.6	134.9	23.7	59.5	42.3
	66.7	63.6	148.9	191.6	47.1	74.4	87.81
	98.7	50	158	148.9	123.7	85.3	39.6
	79.8	225.6	160.5	158	41.1	95.9	39.9
	186.9	55.9	66.2	160.5	45.2	57.7	58.5
Cu	11.2	34	32.5	18070	47.9	40.2	26.4
	10.9	66	25.1	16440	21	51.1	24.7

	56	63.6	18.7	28640	23.7	45.5	17.9
	53.7	103.5	46.3	1171	133.9	67.3	18.1
	86.4	75.7	25.4	239.2	14.3	43.9	66.2
	9.7	109.7	140.4	4952	39.9	49.1	19.6
	11.9	96.3	37.9	6652	40.9	108	25.75
	29.7	88.8	49.7	2733	594.5	73.3	13.5
	57.1	352.4	539.9	6031	20.9	130.8	24.8
	118	156.2	83.2	4083	18.9	55.6	107.2
Hg	1	1.8	1	178.3	2.1	1.2	0.7
	1.4	0.7	0.6	116.3	1.7	1.2	0.7
	0.7	0.8	1.3	385.2	1.1	1.6	0.7
	0.7	0.7	0.7	29.3	3.4	1.5	0.7
	1.1	0.6	0.7	12.9	0.6	1.6	0.7
	1	0.8	1.3	51.6	0.8	1.2	0.7
	0.7	0.7	0.7	54.3	1.2	1.8	0.7
	1.1	0.7	1.2	27.6	4.4	0.8	0.7
	0.7	3.4	3.2	116.2	1.6	0.9	0.7
	2.3	1.6	0.6	16.5	1	0.7	0.7
Ni	50.2	57.6	61.3	0.5	55.4	67.3	38
	51	54.3	63.3	23.1	52.9	76.9	33.8
	69.8	53.9	55.3	57.2	50.2	73.4	39.6
	69.3	63.4	66.2	79.2	41.1	76.7	39.8
	92.7	57.6	57.8	79	56.1	70.9	40.3
	44.2	96.9	76.4	66.7	41.5	71	35.4
	51.9	63.3	60.3	78.2	44.3	83.7	55.03
	60.2	57.2	58.9	112.9	77.1	90.9	32.8
	80.6	87.6	109.8	82.5	51.1	113.1	37.3
	81.3	81.4	67.5	77.1	52.6	67.7	43.3
Pb	23	37.6	71.3	1752	390.5	47.3	61.2
	33.3	65.3	45.5	4077	527.2	40.5	32
	89.2	63.2	44.1	4150	196.1	39.1	30.9
	97	95.8	82.3	487.4	39.8	41.9	23.3
	525.8	65.8	54.3	371	21.7	39.5	236.5
	32.4	159.3	242.4	885.1	24.5	38.1	34.5
	50.2	306.7	94.9	1287	26	59.1	65.38
	50.9	283	111.5	526.1	268.2	59.7	31.3
	104.4	1834	2124	1676	409.9	109.1	27.6

	969.4	777.1	122.8	537.5	289.3	47.2	479.8
Zn	32.1	67.7	189.2	4055	157.5	114.7	105.8
	47.6	124.2	165.9	4613	85.3	129.4	91.4
	172.9	77.6	101.3	8146	98.5	121.5	76.5
	142	125.2	216	645.3	56	146.5	73.2
	398.1	77.3	113.5	515.5	31.1	161.4	193.2
	44.9	382.9	2078	1593	34.2	129.6	99.4
	60.3	505	496	2226	48.4	259.4	175.06
	143.4	351.3	679.9	1150	807.9	305.8	74
	184.4	5639	7643	2030	80.8	241.2	83.1
	709	1246	494.5	3399	68.7	163.1	235

Appendix 5. Microbial Community Composition by Phylum

Fungi from the Ascomycota were the most dominant phylum across all sites surveyed, with 68% of samples containing Ascomycota (Figure 17). Site F, the site with lowest metal concentrations and least screening level exceedances, contained only fungi from the Ascomycota phylum, whereas Site C exhibited the highest fungal diversity at the phylum level. Aside from Ascomycota, Chytridiomycota (17%) was detected and occurred in six of the seven study site soils and Basidiomycota was detected in five of the seven study site soils (13.4%). Unidentified fungi (1.6%) were also detected, but were only found at Site C.

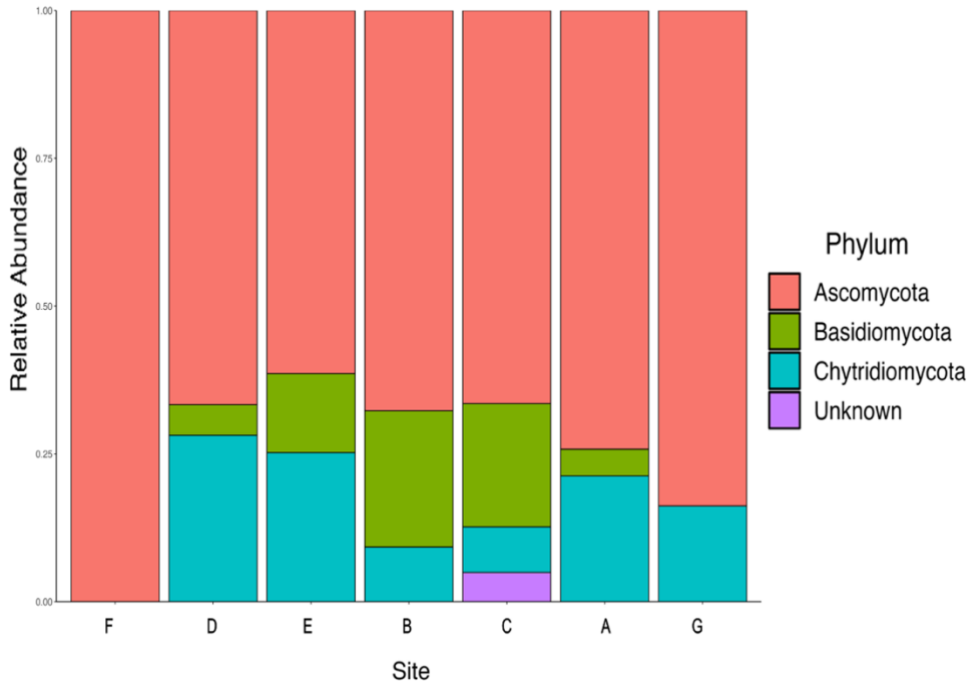

Figure S1. Relative abundance of fungal phyla on seven contaminated sites in Southern California.

Proteobacteria were the most dominant bacterial phylum across all sites surveyed; 34.2 % of samples contained Proteobacteria (Figure 18). Chloroflexi was the second most abundant bacterial phylum found within 12.144 % of samples. Planctomycetota was the third most abundant bacterial phylym with 7.086 % of samples. Generally, the sites with the highest concentrations of metals– A, B, C and D, contained more methanogenic and sulfate-reducing bacterial phylum as compared to the less contaminated sites. The bacterial phyla Crenarchaeota (2.973 %), Verrucomicrobiota (1.916%), Acidocateriota (1.895 %), Halobacteria (1.549%), Bacteroidota (1.148%) and RCP 2.54 (1.04%) were found in lesser abundance but > than 1%.

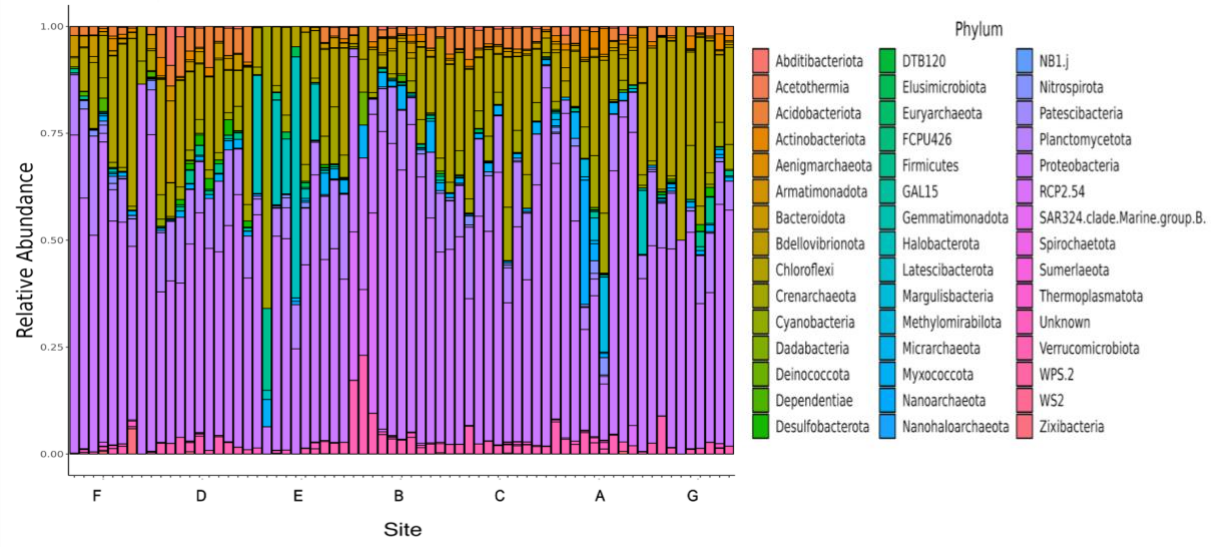

Figure S2. Relative abundance of bacterial phyla on seven contaminated sites in Southern California.

Appendix 6. Microbial Community Composition by Class

The most ubiquitous fungal classes found in our dataset were members of the Dothideomycetes (41%) and Sordariomycetes (21%) from the Ascomycota, and Spizellomycetes (15%) from the Chytridiomycota. To a much lesser extent, Wallemiomycetes (4.3%), Agaricomycetes (3%), Saccharomycetes (2.5%), Cystobasidiomycetes (2%), Eurotiomycetes (1%) and Exobasidiomycetes (1%) were present. The classes Lobulomycetes, Pezizomycotina and Tremellomycetes were present at abundances < 1%.

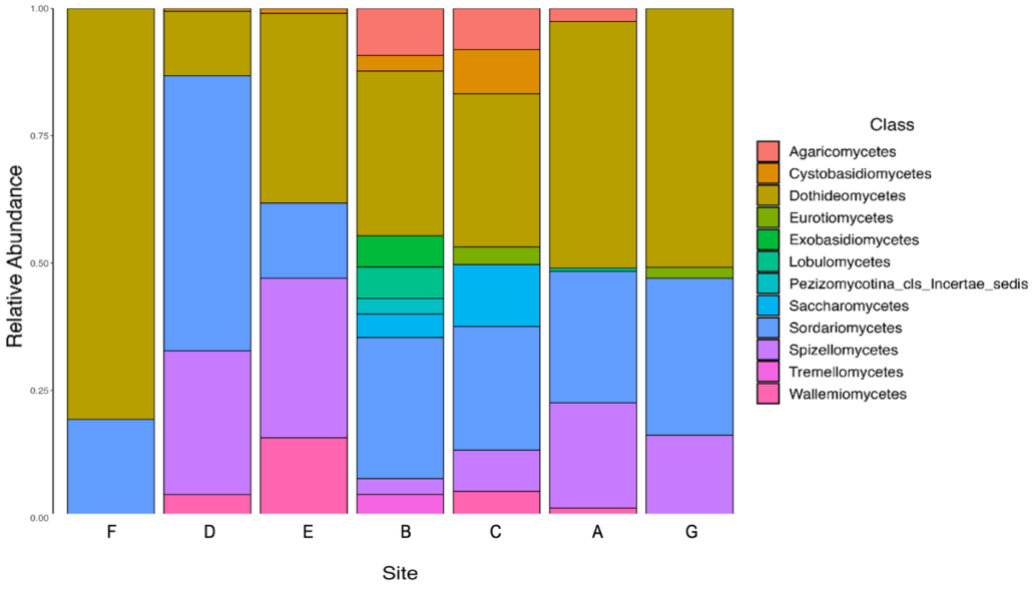

Figure S3. Relative abundance of fungal classes on seven contaminated sites in Southern California. Only including taxa with relative abundance > 1%.

The classes of bacteria in our dataset found in the highest abundance were the Gammaproteobacteria (32.77 %), Planctomycetes (5.064 %) and Gitt.GS.136 (3.781 %). The bacterial classes found in lesser abundance include: Nitrososphaeria (2.97 %), Chloroflexia (2.435 %), KD4.96 (2.422 %), Phycisphaera (1.84%), Verrucomicrobiae

(1.842 %), Acidobacteria (1.708 %), Halobacteria (1.549%), Alphaprotobacteria (1.316 %), Anaerolineae (1.208 %), JG30.KF.CM6 (1.146 %) and Bacteroidia (1.072 %). Unknown bacterial classes comprised 1.842 % of samples.

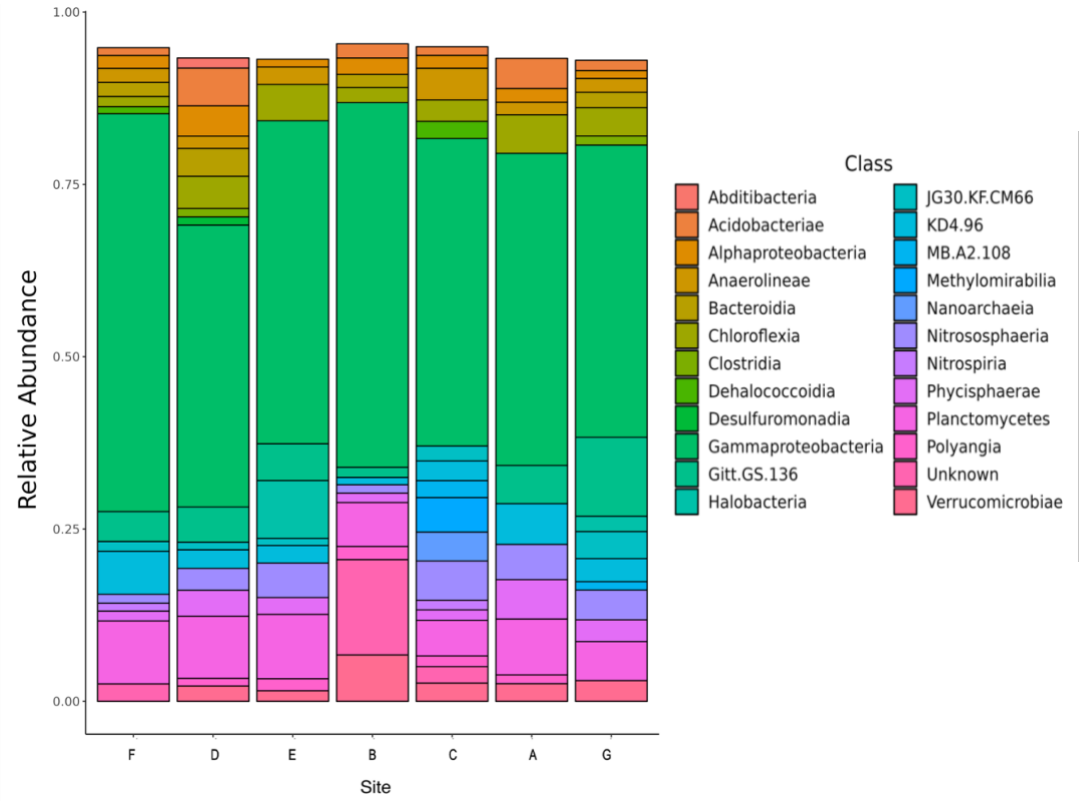

Figure S4. Relative abundance of bacterial classes on seven contaminated sites in Southern California. Only including taxa with relative abundance > 1%.

Chapter 3. Unraveling Controls on *Medicago sativa* - Arbuscular Mycorrhizal Fungi Chromium Interactions in Phyto-Mycoremediation Contexts

1. Abstract

Arbuscular mycorrhizal fungi (AMF) have been applied in past studies to enhance phytoremediation of chromium (Cr) by increasing plant survival in contaminated soils. Though AMF have been shown to mediate their plant partner's interactions with Cr, the conditions under which uptake is inhibited versus enhanced in AMF-plant partners is unclear; some studies have found that AMF can increase Cr phytoextraction, while others have found that AMF increases phyto-stabilization. The present work investigated the interactions between AMF and Cr in *Medicago sativa* under two different soil moisture regimes, drought and optimal moisture, in unsterilized field soils collected from a contaminated brownfield. Chromium translocation to *Medicago sativa* shoots was reduced by AMF inoculation and significantly increased by soil moisture and soil organic carbon content. *Medicago* plants grown under drought stress showed reduced Cr accumulation in plant shoots. AM fungal inoculation reduced soil Cr concentration by 45.8% and the greatest Cr reductions were seen in droughted soils with higher soil carbon concentrations and AMF inoculation. Inversely, significant increases in Cr(VI) concentrations (123% on average) occurred when there was no AMF inoculation and at low AMF colonization percentages (<20%) as well as at higher SOC percentages. These findings suggest it may be beneficial to apply AMF for the phytoremediation of Cr contaminated soils. These results have relevance in phytoremediation of Cr-contaminated

soils in semi-arid regions, where similar drought or redox-fluctuating conditions may exist.

2. Introduction

Chromium is a redox-active metal present in soils predominantly in one of two oxidation status: Cr(III) and Cr(VI). Chromium(III) is generally stable with a low mobility in soils, while Cr(VI) is highly mobile (Losi et al., 1994). Chromium(VI) is the most common oxidation state present in anthropogenically contaminated soils (Bartlett et al., 1988). Chromium(VI) is one of the Agency for Toxic Substances and Disease's (ATSDR) priority contaminants (2022) because of its toxicity and carcinogenicity as well as widespread presence in the environment (Losi et al., 1994). It has been reported to occur in concentrations ranging from few mg kg^{-1} to few thousands mg kg^{-1} in natural soils (Zayed and Terry, 2003) and up to 350 mg kg^{-1} in agricultural soils (Ertani et al., 2017). Chromium(VI) can be immobilized through abiotic reduction (e.g. by Fe(II)) (Wielinga et al., 2001) or microbial reduction (Wang, 2000); conversely, Cr(III) can be abiotically oxidized by Mn(IV) oxides (Kim et al., 2002) which are common in soils and, in particular, under redox fluctuating conditions. Possible remediation strategies for Cr and Cr(VI)-contaminated soils thus include abiotic or microbial reduction, alternatively, phytoremediation, utilizing plants to remove Cr from soils, and myco-assisted phytoremediation, utilizing fungi to enhance phytoremediation (Wani et al, 2022).

Arbuscular mycorrhizal fungi (AMF) are a type of generalist mycorrhizae that live in association with plants through the roots, trading nutrients and water in exchange

for photosynthetic products from the plant and significantly reducing plant stress (Giasson et al., 2008; Turnau & Haselwandter, 2002). AMF associate with ~80% of all plants worldwide, including most agricultural crops, shrubs, grassland plants and some trees and as such are among the most common soil microorganisms occurring in almost all habitats and climates, from the deserts to the boreal forest (Smith & Read, 2010). Although AMF have been shown to mediate their plant partner's interactions with metals, Cr has been less studied, and the conditions under which Cr uptake is inhibited versus enhanced in AMF-plant partners are unclear. Some studies have found that AMF can increase Cr phytoextraction in sunflowers (Davies et al, 2001), alfalfa (Li et al., 2023) and grass (Kullu et al. 2020), while others found that AMF increase phyto-stabilization in alfalfa (Wu et al, 2016), maize (Lourdes et al, 2021), sorghum (Kumar et al, 2021), tomato (Akhtar et al, 2020) and plantago (Estuan et al., 2010). Studies of AMF-plant-Cr interactions have mostly investigated these interactions in agricultural crops rather than phytoremediation contexts since Cr's known phytotoxicity threatens agriculture. However, soil Cr and Cr(VI) contamination is an issue affecting soils beyond just agricultural settings, such as contaminated sites (brownfields and Superfund sites). To successfully utilize plant-based soil remediation (aka "phytoremediation"), plants must be able to survive and grow in Cr-contaminated soils despite Cr phytotoxicity.

Species of Cr(III) and Cr(VI) have been reported to be absorbed by and accumulated within plants, although Cr(VI) species are thought to be more available to plants (Singh et al., 2013). Cr is not required in plant biological processes and can be phytotoxic at high concentrations (Hayat et al., 2012; Oliveira, 2012; Singh et al., 2013;

Shanker et al., 2005). Chromium-mediated phytotoxicity is seen initially as inhibition in seed germination, followed by reduced rates of growth leading to reduced yield (Hayat et al., 2012; Oliveira, 2012; Singh et al., 2013; Shanker et al., 2005). The mechanisms are thought to involve the suppression of enzyme activity and pigment synthesis reducing photosynthetic capacity (Ma et al., 2016; Rodriguez et al., 2012; Farid et al., 2017), reduced uptake and accumulation of essential nutrients by plants (Sundaramoorthy et al., 2010) and the generation of reactive oxygen species (ROS) disrupting redox homeostasis and inflicting oxidative damage on lipids, proteins, enzymes and DNA (Shanker et al., 2005; Panda, 2007). Cr is transported and accumulated in plants via carrier ions, such as iron, and is not directly absorbed by plants (Gajalakshmi et al., 2012, Singh et al., 2013). Soil-plant Cr transfer depends on Cr concentration and speciation and soil physico-chemical properties (soil pH, texture, organic matter, etc.) and is controlled by numerous factors relating to plant physiology (plant type, rate and type of root excretions, root surface area and transpiration) (Banks et al., 2006, Zeng et al., 2011b, Santos and Rodriguez, 2012). Once taken up, Cr(VI) can be reduced to Cr(III) and tends to remain sequestered in plant roots (Zayed et al., 1998). In the majority of plant species, Cr is poorly translocated towards aerial parts and is mainly retained in the root tissues (Jaison and Muthukumar, 2016).

Wu et al. (2014) demonstrated that AM symbiosis markedly alleviated plant Cr toxicity through immobilizing Cr within fungal structures (e.g. extra- and intraradical hyphae, spores) in roots. Besides direct Cr immobilization, AM symbiosis may also enhance plant Cr tolerance through improving plant mineral nutrition and facilitating the

production of key metabolites, such as sulfur (S)-metabolites and compounds, that may contribute to detoxification of Cr(VI) in plants (Wu et al., 2016). The influence of AMF on plant uptake of Cr may be affected by biotic edaphic factors such as the species and strain of AMF and its history of exposure to metals (Davis et al., 2004; Gadd, 1994; Glassman & Casper, 2012). Abiotic edaphic factors that may influence Cr uptake by AMF associated plants include the Cr chemical speciation and oxidation state, concentration, and solubility (Wu et al., 2016), pH (Viti et al, 2014) and phosphorous content (and availability) (Li et al., 1991). Soil metal concentration has been shown to affect AMF-metal-plant dynamics: metal accumulation in plant symbionts is reported to be lower under elevated soil metal concentrations and higher under lower metal conditions (Toler et al., 2005; Audet & Charest, 2007), resulting in enhanced plant tolerance through metal stress avoidance. However, the soil parameters (and Cr concentrations) under which AMF inhibit versus enhance Cr uptake into their plant symbiont have not been elucidated. Furthermore, worsening drought conditions caused by climate change and its influence on soil Cr mobility in AMF-plant systems has not been studied (Zhang et al., 2021).

In this study, we examined the effect of decreased soil moisture and adsorption on AMF-assisted metals uptake by plants. We hypothesized that Cr concentrations are important in AMF-mediated Cr translocation to plant partners grown in contaminated soils, and that translocation of redox-active Cr will increase under oxic conditions (drought simulation, with just enough pore water for transport). The addition of carbon-rich amendments (decomposer fungal mulch in the form of *Pleurotus ostreatus* mycelium

grown on sawdust) will create favorable conditions for reducing Cr(VI) to Cr(III) and for reducing Cr uptake into plants via adsorption processes.

3. Experimental Design

Experiments were conducted over 9 weeks in a completely randomized block design factorial scheme with 6 replicates of each treatment combination (total of 144 pots). *Medicago sativa* was grown with commercial AMF inoculum or without AMF (non-inoculated plants) at three Cr levels: 0 - 100 ug/g (uncontaminated), 100 - 2000 ug/g (low contamination) and > 2000 ug/g (high contamination), based on representative levels (USGS, 1984) and maintained at two soil moisture levels (optimal moisture and droughted).

Table 1. Experimental Design and Treatment Combinations

Plant type (1 type)	**Soil Total Cr Contamination Level (3 levels)**	**Irrigation Level (2 levels)**	**AMF Inoculation (2 treatments)**	***P. ostreatus* Carbon Amendment (2 treatments)**
Medicago sativa (Alfalfa)	Uncontaminated (<100 μg/g Cr)	35% WHC (0.476 L /day)	+ Commercial AMF inoculum	+ *P.ostreatus* sawdust spawn
	Low contamination (100 - 2000 μg/g Cr)	75% WHC (1.02 L /day)	- Commercial AMF inoculum	- *P.ostreatus* sawdust spawn
	High contamination (>2000 μg/g Cr)			

4. Methods

4.1 Soil collection

Field soil from a contaminated site was used because of the functional complexity of field soils and thus transferability of findings to remediation scenarios on contaminated

sites with similar contaminant mixtures and soil characteristics. Chromium-contaminated soils were collected from a brownfield called Slauson and Wall located in Los Angeles (33.98762527296143, -118.27117757601722) at 26 m above sea level (California, 2023). The site is classified as a warm and arid climate (Köppen-Geiger climate classification *Csa*) with an average annual temperature of 18.7ºC (high of 23.7ºC and low of 13.7ºC) and precipitation of 26.48 cm/year (NOAA, 2020). The soils are anthropogenic and capped under concrete, but arise from entisol Xeropsamments (Service, 2021). The site was reported by Ninyo and Moore Geotechnical and Environmental Sciences Consultants (2010) to contain soil Cr(VI) concentrations higher than industrial screening limits in soils (0 –152cm) from the previous industrial use for electrical manufacturing, metal-plating and polishing, wood and furniture finishing, and vehicle maintenance and repair. The field soil was sieved to remove large rocks (5 mm), collected in buckets, and thoroughly homogenized in large totes.

AMF spores were extracted from 10 x 25-gram soil samples with 6 replicates of each using the sucrose method (Allen et al., 1979; Ianson & Allen, 1986) and counted under a dissecting microscope at 30 to 50x (Allen et al. 1979; Brundrett et al., 1996). Soil sterilization was deemed unnecessary because less than 2 spores were observed in the 250 grams of soil tested. These low spore counts are likely because soils were beneath 5 feet of concrete for >60 years with few nearby plant hosts or other sources of inoculum. Soil sterilization can minimize the functional complexity of the soil biological community and therefore not be representative of their activity in the natural environment (Jacques et al., 2008; Koide & Li, 1989; Van Der Heijden et al., 2008). Additionally,

soils from field sites are typically mixed with sand at a 1:1 ratio to achieve a 70% sand content for use in pot experiments to increase infiltration (e.g., Hassan et al., 2013); however, since the soils used in this study are composed of ~72% sand, additional sand amendment was deemed unnecessary.

To ensure soil concentrations of Cr were roughly uniform in all pots at the three Cr concentration treatments (uncontaminated, low, and high), highly contaminated soils (>2000 μg/g Cr) from Slauson and Wall were diluted with Cr-uncontaminated soil (< 100 μg/g Cr) from the same site to reach the desired concentration of Cr. Chromium concentrations in corrected soil were confirmed in each pot using X-ray fluorescent (XRF) spectroscopy. Average concentrations of other metals of concern were below unrestricted screening limits: As (0.78 μg/g), Cd (0.71 μg/g), Cu (89.3 μg/g), Pb (56.5 μg/g) and Zn (184.05 μg/g).

Soil characteristics were assessed (Table 2; Appendix 1) and methods for analysis are summarized below in Section 4.3.2. 100%, 75% and 35% soil water holding capacity (WHC) were measured as the gravimetric water content of field soil saturated and allowed to drain over 6 hours in a filter funnel and drip irrigation was set up in pots to supply the desired 75% and 35% WHC's.

4.2 Plant seed and AMF inoculum selection, collection, and preparation

Medicago sativa (Alfalfa) was selected as the plant type because it is a known Cr (and other metal) phytoremediator plant known to form arbuscular mycorrhizal fungal associations; additionally, it has multiple uses including as a biofuel and feed crop. CUF

101 variety was used because it is adapted to the climatic conditions present in greater LA County. Seeds were supplied by Top Notch Seeds (Brawley, CA). Seeds were sterilized in sodium hypochlorite and rinsed three times with DI water before sowing. Seeds were started in pots containing 100 g of metal-contaminated soil and grown until seedlings emerged (~3 weeks) before transplanting. The supplier for commercial arbuscular mycorrhizal inoculum blend was Mycorrhizal Applications (product= MycoApply Endo, OR). The inoculum contains the AMF species *Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum* and *Glomus etunicatum*.

4.3 Experimental set-up

The experiment was conducted at the UCR Greenhouse facilities (PR1). Greenhouse conditions were maintained at 28/20°C Day/night temperature (with thermostat and fans), and 60% relative air humidity (with a dual-humidistat/humidifier-dehumidifier). Irrigation volumes were supplied as double-deionized water (DDI) via drip irrigation to each pot with either one (35% WHC; 0.476 L/day) or two (75% WHC; 1.02 L/day) drip spikes.

New plastic pots (7-gallon) (Greenhouse Supply, Sacramento, CA) were filled with soil based on their Cr contamination level and amended prior to planting with a field-equivalent rate of 80 kg N ha^{-1} as urea (28.15 g/pot) to remove N limitations to plant growth (Di Tomassi et al., 2021). Two seedlings were transplanted per pot and each seedling was inoculated with 68 g of commercial powdered inoculum (MycoApply Endo, OR). Non-mycorrhizal plants received 10 ml of just clay carrier (no spores) from the

commercial inoculum supplier. Each planter pot had a catchment tray placed beneath it for leachate collection.

4.4 Pot experiment solid phase analyses

4.5 Plant growth evaluation and plant tissue analyses

Plant growth parameters such as plant height and flowering status were measured and recorded on a weekly basis. At the end of the experiment (once the plants started flowering), plants were harvested, differentiating between aboveground (stems and leaves) and belowground (roots) parts of the plant. Roots were separated from shoots, washed with deionized water to remove soil particles, and weighed separately from the shoots. Length of shoots and roots were measured. After the fresh weight of shoots and roots were measured, they were oven dried for 48 hours at 60°C and weighed to determine dry weight. Three samples from each plant's roots and shoots were sampled at random, combined and analyzed for metal concentrations following EPA method 3052 (microwave acid digestion) and measured by inductively coupled plasma mass spectrometry (Agilent 7700, Santa Clara, CA). Metal accumulation by plant shoots and roots was estimated by multiplying the plant metal concentration (μg/L) by the dilution factor and dividing by the tissue dry weight (g). Biological concentration factor (BCF) was calculated for each metal as the ratio of metal concentration in plant tissue to metal concentration in soil (Marchiol et al., 2013).

4.6 Soil analyses

pH was measured in all pots using a field soil pH meter (Hannah instruments, Smithfield, RI) prior to experiment beginning. Soil moisture and temperature in each pot were taken using TM5 soil temperature and moisture probes and recorded with a EM50 data logger (Meter Corp, San Francisco, CA). Soil texture was determined using the LS-13-320 Grain Size Analyzer (Beckman-Coulter, Brea, CA) after hydrogen peroxide digestion of organic carbon and deflocculation with SHMP in each pot. Soil organic C, N and C/N isotopes were assessed using elemental analyzer (Costech ECS 4010-Delta V Plus IRMS). Water-extractable organic carbon (WEOC) was extracted in DI water using the method outlined in (Homyak et al., 2018) by shaking soil samples for three hours followed by centrifugation and quantified using the TOC-VCPH Total Organic Carbon Analyzer (Shimadzu, Columbia, MD). Soil phosphate (PO_4) concentrations were measured using the Olsen sodium bicarbonate extraction method (Olsen & Sommers, 1982; Olsen, 1954), nitrate (NO_3^-) and ammonium (NH_4^+) were extracted from soils with 2.0 M KCl solution (Maynard et al., 1993). PO_4, NO_3^- and NH_4^+ concentrations were measured using the Flow Solution IV Automated wet chemistry system (Alpkem, College Station TX).

Total soil elemental concentrations (specifically total Cr) were determined using X-ray fluorescence (XRF) spectrometry (SPECTRO, Model XEPOS03HE) in dry soils sieved to 2 mm and ground to homogenize prior to treatment and at the end of the experiment. Cr(VI) concentrations were determined using EPA method 3060A “Alkaline

digestion method" and diphenyl carbazide colorimetric procedure (EPA Method 7199) in pre- and post-treatment soils.

4.7 Arbuscular mycorrhizal fungal analyses

Dried roots were placed in cassettes, cleared in 2.5% KOH for 20 minutes at 90ºC, acidified in 1% HCl, and stained in 0.5% trypan blue for 20 minutes at 90ºC, and de-stained (rinsed) in tap water. Fine roots were observed for AM infection at 100x using a Leitz Labotlux II microscope for infection structures including hyphae, arbuscules, or vesicles. 50 intercepts per sample were examined as per Giovannetti and Mosse (1980) for quantification.

4.8 Aqueous phase analyses

Leachate was collected from trays beneath pots 1 h after watering on a weekly basis, filtered, and stored at −20°C until analysis. Leached Cr concentrations were determined by ICP-MS. Trays were rinsed with DDI water after collecting leachate to prevent any collection of Cr on trays during the study from influencing subsequent measurements.

4.9 Statistical analyses

All statistical analyses were performed using R version 4.3.1 (R, 2023). The effects of Cr concentration, mycorrhizal inoculation, soil moisture and organic carbon amendment on plant dry mass, mycorrhizal root colonization percentages, shoot and root metal concentrations, soil metal content, and BCF values were analyzed by two-way analysis of

variance (2-way ANOVA). Data was log transformed as needed to meet normality requirements for ANOVA. Repeated measures ANOVA was used to compare Cr(VI) in soils between pre-treatment and the end of the experiment. Post hoc comparisons of means were done using the Tukey's HSD test ($p \leq 0.05$). Simple linear regression was used to determine how variables contribute to Cr immobilization or uptake into plants. When a significant interaction between the two factors was found, the effect of one factor was analyzed within each level of the second factor. Because the uptake of metals into plants could be influenced by multiple factors, principal components analysis (PCA) was used to assess the relative importance of each of these factors.

5. Results

5.1 Soil properties, total Cr, and Cr(VI) Concentrations

Table 2 and Appendix 1 and 2 show soil chemical composition and physical characteristics of soils prior to experimental treatments. Total soil Cr concentration in each pot was correlated with the correct Cr range for each level (uncontaminated, low, and high Cr contamination) ($p = <0.0001$), meaning our soil mixing during experimental setup achieved

Table 2. Mean Soil Properties with Standard Deviation (n = 144)

Soil property	Mean
Mean Soil Temp (°C)	37.37±5.23
% Clay	3.009±1.21
% Silt	25.06±6.36
% Sand	71.922±7.37
Texture	sandy loam
% Soil OC	0.49±11.66
WEOC (mg/L)	19.50±5.41
C/N	21.14±1.97
NH_4 (∝g/g)	1.08±165.60
NO_3 (∝g/g)	73.12±0.37
pH	8.42±2.79
CEC* (meq/100 g)	9.52±1.04
PO_4 (∝g/g)	1.78±6.12
Total P (%)	0.1595±0.02
Total S (∝g/g)	610.44±324.6

*CEC was determined in 10 random samples; all other analyses were completed for all 144 pots.

desired Cr levels. Soil Cr(VI) concentrations in each pot were not correlated with total Cr concentrations ($p = 0.1189$). Analyses proceeded using both total Cr and Cr(VI) concentrations for each pot.

5.2 Confirmation of AMF inoculation success

Although initial testing suggested a minimal AMF spore load in the field soils, root staining at the end of the study for each plant ($n = 144$) revealed that more than 10% of plants from both inoculated and uninoculated treatments were ultimately colonized by AMF (Figure 1). Mean AMF root colonization was lower in uninoculated pots (10%) than inoculated ones (23.29%), but the difference in AMF root colonization percentage between inoculated and uninoculated treatments was not statistically significant ($p = 0.4445$; $n = 77$ per treatment). AMF spore presence in uninoculated treatments likely occurred because of AMF spore transported into pots by insects, via previously existing spores in the greenhouse, or in the unsterilized field soils (Salomon, M., 2022). Therefore, all analyses used actual AMF colonization percentages in each plant instead of inoculation status (i.e., AMF inoculated versus uninoculated). AMF colonization percentage was significantly higher in uncontaminated soils ($p = < 0.0001$) than in low and high Cr contamination treatments, consistent with

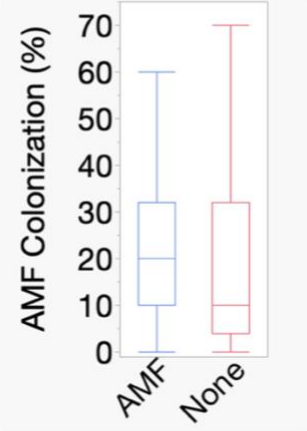

Figure 1. Effect of AMF inoculation on actual plant root AMF colonization. AMF = inoculated with commercial inoculum; None = no inoculation with AMF.

results from similar studies that investigated AMF colonization rates in Cr-contaminated soils (Davies et al., 2007).

5.3 Effects of Treatments on *Medicago sativa* Growth and Biomass

Medicago sativa biomass (dry weight) and height were significantly correlated with Cr level, carbon amendment and percentage AMF root colonization (Table 3). Soil texture class, clay, silt and sand content, soil moisture content, pH, soil organic carbon content, total carbon (μg), soil Cr(VI) concentration, irrigation level and water-holding capacity (WHC) were not significantly correlated with *Medicago sativa* weight or height.

Table 3. Effects of Treatments on *M. sativa* Growth and Biomass (n = 144)

Dependent variable	Factor	r	p-value
Plant height (inches)	Total Cr level	0.5616	<2.2 e-16
	Carbon amendment	0.05138	1.78e-06
	% AMF root colonization	-0.007083	0.0379
Plant weight (g)	Cr level	0.2161	1.298 e-08
	Carbon amendment	0.02403	0.03523
	% AMF root colonization	0.002199	0.04089

Total Cr level had the strongest correlation with *M. sativa* heights and weights. Higher *Medicago sativa* heights were correlated with low Cr levels (p = 6.36e-05) and lower heights with high Cr levels (p = 8.55e-13). *Medicago sativa* weight was only significantly correlated with uncontaminated soil (p = 0.00106). Phosphate concentration was significantly positively correlated with *Medicago sativa* height (p=0.03636) in the full factorial model, but when a reduced model was run with only significant factors to

control for interaction effects between variables, the statistical significance of phosphate was lost.

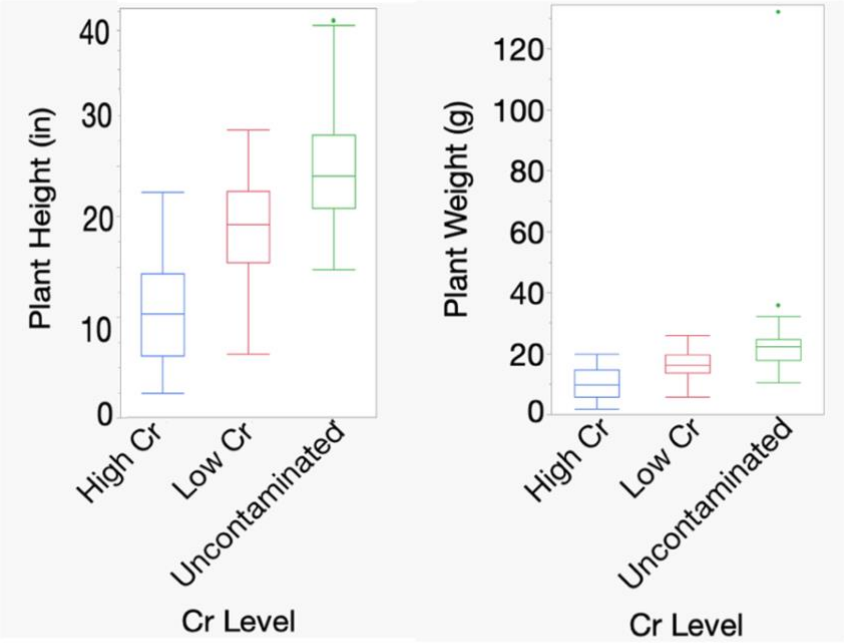

Figure 2. *Medicago sativa* height and weight by soil chromium contamination level.

5.4 Effects of Treatments on *Medicago sativa* Cr Uptake

Medicago sativa root and shoot Cr concentrations were positively correlated with total Cr concentrations, and higher soil Cr concentrations were strongly correlated with higher root and shoot Cr concentrations (Table 4). Root BCF was also correlated with Cr(VI) concentrations. Shoot Cr content, which indicates Cr translocation, was strongly correlated with increased soil moisture content, and with soil organic carbon content. Shoot BCF was also strongly correlated with soil moisture (explaining 24 % of the variation) and soil organic carbon content (explaining 11% of the variation). Higher moisture contents were correlated with higher shoot Cr concentrations and shoot BCF's.

Variable effects of carbon-related factors were observed on shoot Cr content and BCF: total C and organic C content increased (marginally) shoot Cr uptake.

Table 4. Regression Results for *M. sativa* Cr Accumulation (n = 144)			
Plant Part	**Factor**	**r**	**p-value**
Shoot BCF	Soil moisture	0.2444	0.000784
	Carbon: no amendment	0.002986	0.000784
	SOC %	0.1136	2.152e-05
	Total carbon concentration	0.06784	0.009439
Shoot Cr	Soil moisture	0.5018	< 2.2e-16
	% SOC	0.06695	0.001016
	Cr level	0.1928	1.03e-07
Root BCF	Cr(VI) concentration	0.02883	0.0243
	Cr level	0.03018	0.04403
Root Cr	Cr level	0.02056	0.0008
	Total Cr concentration	0.08316	0.0486

Fungal treatments had variable effects on *M. sativa* Cr uptake and less significance than expected. The addition of the fungus *P. ostreatus* was correlated with reductions in shoot Cr. Percentage AMF colonization was not strongly correlated with Cr uptake into *M. sativa*; however, shoot Cr content was near significance ($p = 0.104$). For root and shoot Cr content and BCF, the highest Cr uptake occurred at the lowest AMF colonization percentages. In sum, AMF colonization reduced Cr accumulation and translocation into *M. sativa*, although not in a statistically significant way.

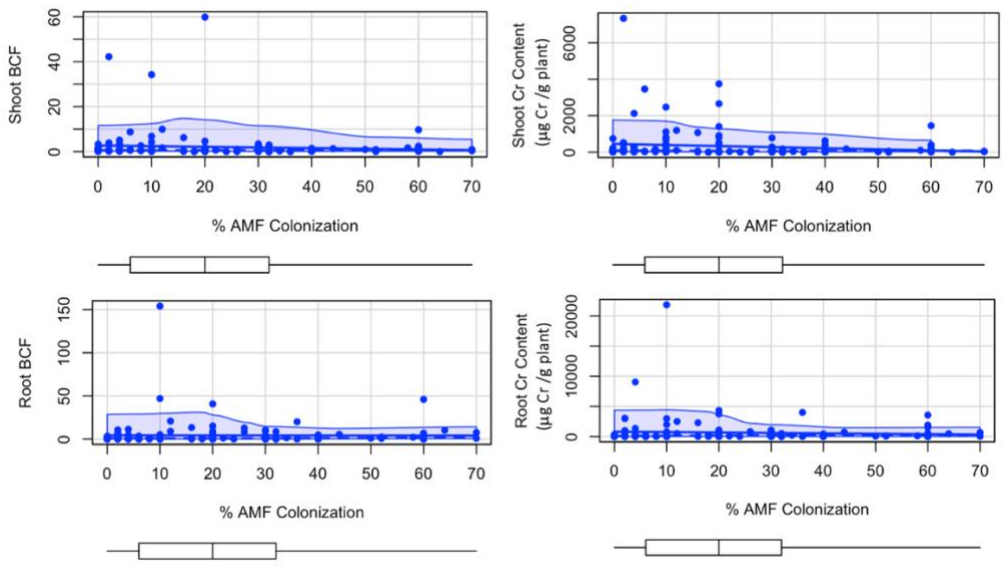

Figure 3. *M. sativa* root and shoot Cr content and BCF by percentage AMF root colonization (n = 144). Individual values are shown as data points in the scatter plot; the solid blue line represents the fitted regression; the shaded blue area shows the data that falls within the 75% cut-off and the box plot shows the median.

When principal components analysis was used to understand the effects of multiple soil biotic and abiotic variables on root and shoot Cr uptake in a reduced dimensional space, AMF root colonization was determined to be highly correlated with Cr uptake (although negatively correlated) (Figure 4 and 5). Percentage AMF root colonization explained 31 % of variance in shoot Cr content and 27 % of variance in shoot Cr uptake. Shoot Cr was strongly negatively influenced by % AMF colonization, silt and sand contents, pH, and phosphate concentration. Root Cr accumulation was strongly negatively influenced by % AMF, sand content, pH, and phosphate concentration.

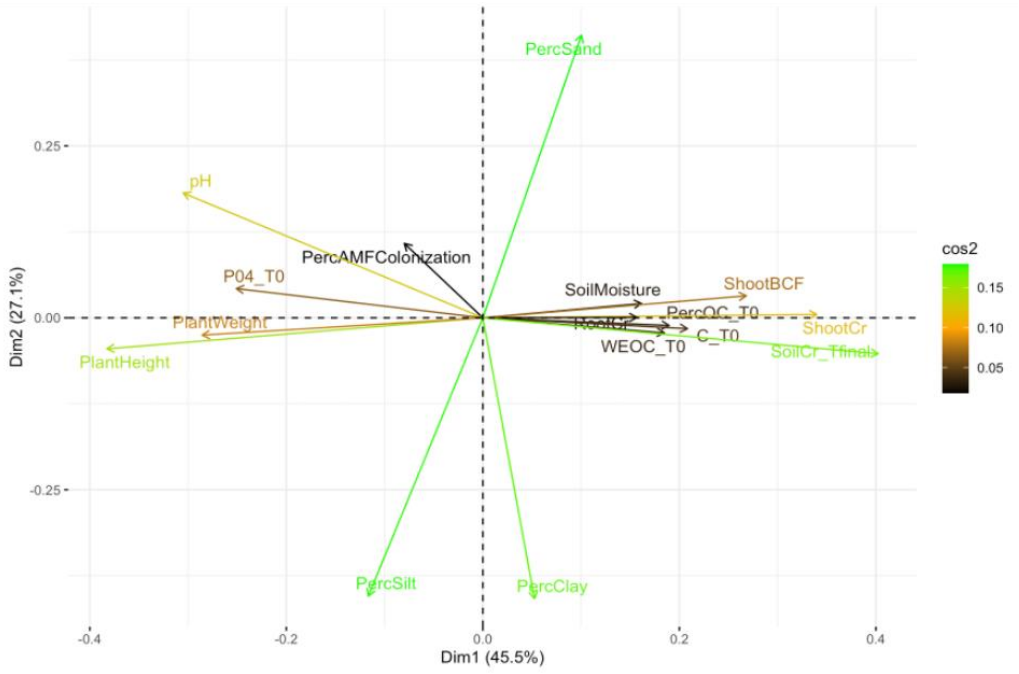

Figure 4. PCA plot of variables correlated with *M. sativa* shoot Cr contents. Variables in green have high cos scores, those in orange have mid cos scores and those in black have low cos scores.

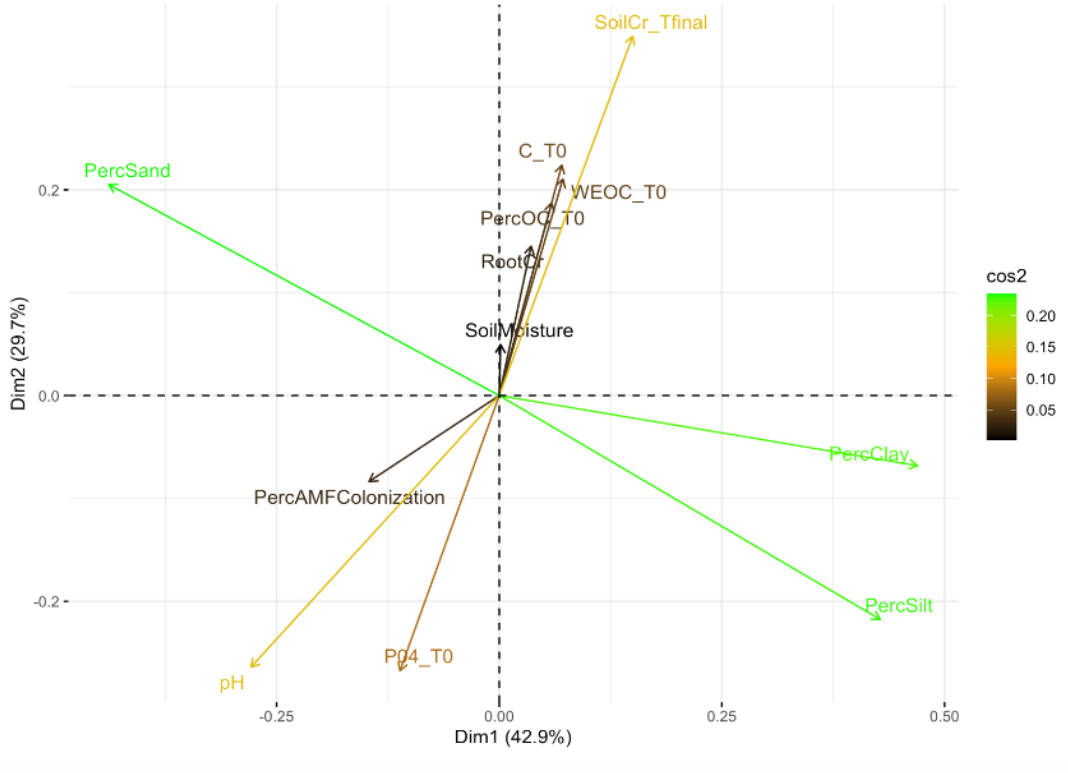

Figure 5. PCA plot of variables correlated with *M. sativa* root Cr contents. Variables in green have high cos scores, those in orange have mid cos scores and those in black have low cos scores.

5.5 Changes in Soil Chromium Concentrations

Significant reductions in both total Cr and Cr(VI) soil concentrations were observed in treated soils. There was an average 46% reduction in total Cr concentrations in soils from pre- to post-treatment ($p = 0.001623$) (Figure 6). The change in soil Cr(VI) concentrations was more variable: there was an average 61% *increase* in Cr(VI) concentrations between pre- and post-treatment soils ($p = 2.2e\text{-}16$) (Figure 7). Change in Cr and Cr(VI) concentrations and statistics by treatment group are reported in Appendix 4.

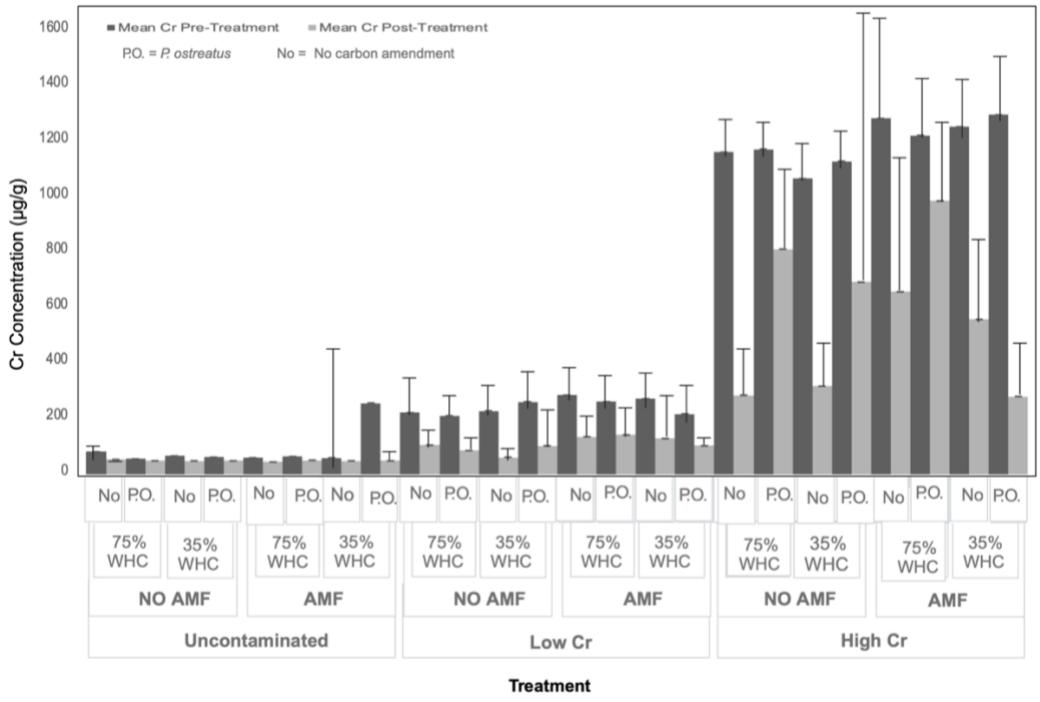

Figure 6. Change in mean total soil Cr concentrations by treatment (6 replicates per treatment group). "P.O." represents carbon treatment with *Pleurotus ostreatus* mulch and "No" represents no carbon amendment.

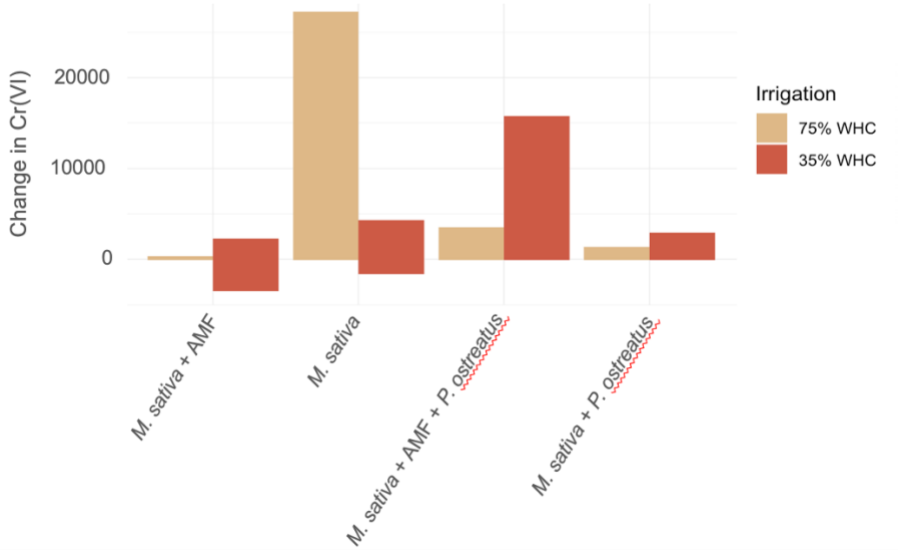

Figure 7. Change in mean soil Cr(VI) concentrations by treatment (6 replicates per treatment group): AMF and no AMF, *P. ostreatus* and no *P. ostreatus*, both AMF and *P. ostreatus*, by irrigation level (75% WHC and 35% WHC).

5.6 Effects of Treatments on Soil Cr and Cr(VI) Concentration Changes

Change in soil Cr concentrations (from pre- to post-treatment) were correlated with AMF inoculation, total soil carbon concentrations, and irrigation level (increased change with drought conditions). Soil Cr(VI) concentrations were correlated with AMF inoculation (reduced soil Cr with no AMF inoculum and at low AMF colonization percentages) and soil organic carbon (Cr(VI) concentrations significantly increased with SOC % and near significant increase with *P. ostreatus* mulch ($p = 0.0505$)) (Table 5).

Table 5. Regression Results for Changes in Soil Cr and Cr(VI) Concentrations
(n = 144)

Analyte	Factor	r	P value
Total Cr	Fungal treatment (AMF)	0.3851	0.02198
	Total carbon concentration	0.2939	0.000784
	WHC (35%)	0.1036	0.07642
Cr(VI)	SOC %	0.03324	0.01154
	Fungal treatment (AMF)	0.06695	0.001016

6. Discussion

6.1 AMF increased *M. sativa* biomass and growth parameters under Cr and Cr(VI) stress

Our results showed that higher concentrations of soil Cr and Cr(VI) were correlated in reduced *Medicago sativa* biomass and height in a concentration-dependent manner, with significantly lower plant weights and heights at higher soil Cr and Cr(VI) concentrations. Similar results have been shown in other studies of *Medicago sativa* (Christou et al., 2020) and for other plants (Shahid et al., 2017) because Cr(VI) is extremely phytotoxic (Christou et al., 2020; Shanker et al., 2005; Singh et al., 2013). In

the present study, *Medicago sativa* biomass and growth was increased significantly by AMF colonization. While differing effects of AMF inoculation on plant growth and biomass have been reported, in contaminated soils in general and Cr(VI)-contaminated soils specifically, AMF inoculation tends to increase plant growth and biomass (Li et al., 2023; Chen et al, 2022; Davies et al., 2007). This may result from indirect pathways such as AMF enhancement of nutrient uptake and/or increased physiological activities diluting plant Cr concentrations, and directly by AMF inhibition of plant metal uptake and translocation to plant shoots and/or alleviation of metal phytotoxicity (Li et al., 2023; Wu et al., 2019). For example, transcriptional levels of genes involved in ROS and proline metabolisms were found to be upregulated in plants by AM fungal colonization (Li et al, 2023).

6.2 AMF reduced *M. sativa* root and shoot Cr but soil moisture and organic carbon were more important to Cr uptake

Chromium uptake by *Medicago sativa* roots was most significantly correlated with soil Cr and Cr(VI) concentrations, with higher uptake at higher concentrations, possibly as a result of the high concentrations overwhelming plant defenses against Cr stress, especially at low AMF colonization levels (Li et al., 2023). It was not possible to compare AMF-infected with non-AMF-infected plants in our study, since most plants were colonized by AMF to some extent, regardless of inoculation status. Instead, we compared within levels of AMF colonization from low to high. Chromium accumulation was greatest in the roots of *M. sativa* at lower percentages of AMF colonization. Root to

shoot Cr transfer was also highest at lower percentages of AMF percentage colonization of roots. This is consistent with other studies that observed that plant AMF colonization reduced shoot Cr transfer (Wu et al, 2015; Chen et al., 2022; Davies et al., 2007; Li et al., 2023). Wu et al. (2015 and 2016) demonstrated several mechanisms by which AMF can inhibit Cr translocation to plant shoots via the reduction of Cr(VI) to Cr(III) followed by Cr(III) immobilization in mycorrhizal mycelium by complexing with phosphate or histidine analogs. In the present study, phosphate concentrations explained the variation in both root and shoot Cr uptake. This may be due to the interactions between phosphate and Cr(III) that have been found to increase plant Cr tolerance (Arshad et al., 2016, Du et al., 2014, Singh et al., 2015) and the changes to root and shoot Cr uptake observed at low phosphate contents in AMF-inoculated plants (Wu et al., 2018). Another consideration is that 'uninoculated' pots contained strains of AMF present in, and thus tolerant or resistant to, the Cr-contaminated field soils. Mycorrhizal fungal species isolated from metal contaminated sites have been found to confer metal tolerance or resistance to their plant partner, whereas AMF strains without previous exposure to metals (such as those within the commercial AMF inoculum used in the inoculated pots) may not (Gadd, 1994). For example, Diaz et al. (1996) observed lower Pb concentration in the shoots of plants inoculated with an AM isolate from contaminated soil (*G. mosseae*) than in the shoots of plants inoculated with an isolate from noncontaminated soil (*G. macrocarpum*). These strain differences in tolerance to Cr may have explained some of the effects seen.

Chromium translocation from roots to shoots was most strongly correlated with soil moisture and soil organic carbon percentage, with increased shoot Cr concentrations

at higher soil moistures and higher SOC percentages. Other studies have found that plants grown under Cr contamination and drought stress resulted in reduced Cr accumulation in plant shoots compared with those grown under Cr stress alone (Vishnupradeep et al., 2022; Ashraf et al., 2023). Higher soil moisture increases pore channel connectivity which enhances microbial access to metabolites that are otherwise isolated under dry conditions (e.g., Smith et al., 2017); similarly Cr(VI) access by plants and microbes is likely increased as more soil pores are water-filled and connected. Additionally, soil moisture affects the availability and transport of plant nutrients because nutrient transporters are water-dependent, which may also explain the significance of higher soil moisture and Cr(VI) uptake observed in the present study. In soil, Cr in the pore water solution or exchangeable fractions would be most available to plants, and increased soil moisture could potentially increase Cr uptake into plants via increased plant nutrient transport processes. Although this has not been tested for Cr specifically, it has been observed for other metals (Audet & Charest, 2007; Joner & Leyval, 1997; Lasat, 2002; Toler et al., 2005).

The addition of a carbon-rich amendment (*Pleurotus ostreatus* fungal mycelium grown on sawdust) was significantly, although weakly, correlated with reduced shoot Cr and shoot Cr BCF as expected. Inversely, without the carbon-amendment, shoot Cr uptake increased significantly. Surface adsorption processes (ion exchange, complexation, precipitation) can occur between Cr and the carbon substrate (sawdust) as well as the fungal biomass cell wall components (Gadd, 1990). Fungal mycelium has a large surface area with an overall negative charge, a variety of functional groups for Cr

ions to bind to and high redox potential (Turnau, 2006, Smith and Read 1997). Organic carbon from the *P. ostreatus* amendment may have also contributed to the reduction of Cr(VI) to insoluble Cr(III), making it unavailable for plant uptake.

In contrast to the inhibitory effect of carbon-rich amendment addition on Cr uptake, we found that shoot Cr uptake increased with higher SOC content. This is surprising given SOC has been shown to adsorbs Cr in soils, reducing Cr availability to plants (Giasson et al., 2008). However, the SOC content in the field soils being very low (< 0.5%) which may not have been present in high enough quantities influence the mobility of Cr. Further, soil pH was relatively high (mean = 8.42; range 7.93 – 9.35) and poor immobilization of Cr(VI) has been seen under alkaline conditions, particularly in less weathered, sandier soils (Dong et al., 2011; Viti et al., 2014). Cr(VI) is easily solubilized and mobilized in both acid and alkaline soils and may have been mobilized into shoots even at relatively higher SOC contents (Kabata-Pendias, 2010).

6.3 AMF contributed to significant soil reductions in Cr under drought conditions

Soil Cr concentrations were reduced significantly compared to pre-treatment concentrations and correlated with AMF inoculation, irrigation level (drought condition), and soil carbon concentration. AM fungal inoculation was correlated with a 45.8% reduction in soil Cr concentration. This is consistent with past findings that showed AMF inoculation with biochar reduced soil Cr concentrations in *Medicago sp*. by up to 40 % (Li et al., 2023), and that AMF inoculation decreased both total Cr and Cr(VI) soil concentrations significantly in *Helianthus sp*. (Chen et al., 2022). The correlation

between drought and SOC and total Cr reductions has several possible explanations. Cr(III) is often adsorbed to soil organic carbon. Low soil moisture conditions simulating drought (35% WHC) alters redox conditions and increases oxygen diffusion into soils which can contribute to Cr(III) oxidation to soluble Cr(VI) (Bartlett and James 1988; Bartlett 1997; Kim et al., 2002). It is possible that drought conditions resulted in Cr(VI) transport from soils via leaching (out of the pots) or enhanced transport (and thus its removal from soils) into *Medicago sativa*, mediated by AMF and immobilized in fungal structures within plant roots.

A significant increase in soil Cr(VI) concentrations (123% on average) occurred during the study period. The most significant increases in Cr(VI) occurred in the absence of AMF and at low AMF colonization percentages (<20%) as well as at higher SOC contents. Chromium(III) oxidation rate in soils is generally fast (Bartlett 1991) and is plausible within the time period of the present study (9 weeks). This increase in Cr(VI) concentrations in the absence of AMF is consistent with the demonstrated capacity for AMF to reduce Cr(VI) to Cr(III) and immobilize within fungal mycelium (Wu et al., 2016). The redox fluctuating conditions present in the pots being watered once per day and the oxic conditions within droughted pots could explain the increase in Cr(VI) via oxidation processes.

7. Summary and Conclusions

The present work investigated the interactions between AM fungi and Cr in *Medicago sativa* under droughted and optimal soil moisture regimes in unsterilized field soils

collected from a contaminated brownfield. Our findings provide insight into the soil parameters relevant to AMF-assisted phytoremediation of Cr and Cr(VI) and how drought might affect these dynamics. *M. sativa* biomass and height was significantly increased by AMF colonization, especially under drought and Cr stress. Chromium accumulation and translocation to *Medicago sativa* shoots was reduced by AMF inoculation and under drought stress, suggesting a protective effect of the AMF. Soil total Cr concentrations were reduced significantly (47% on average) with AMF inoculation and under drought conditions. Inversely, significant increases in Cr(VI) concentrations (123% on average) occurred in the absence of AMF inoculation and at low AMF colonization percentages (<20%), likely due to the decreased AMF-mediated reductions of Cr(VI) to Cr(III). These results suggest it may be beneficial to inoculate with AMF for soil Cr and Cr(VI) phytoremediation.

These results have relevance for the phytoremediation of Cr-contaminated soils in arid/semi-arid regions, where similar drought or redox-fluctuating conditions may exist. Phytotoxicity and risk of remediation failure may be alleviated by AMF inoculation. These findings may also be applicable in agricultural contexts where soil Cr(VI) contamination poses a risk to crop survival and growth and crop contamination with Cr(VI) could present a human health concern. AMF-assisted phytoremediation is a less expensive, long-lasting, solar-energy dependent and eco-friendly strategy for decontaminating Cr-polluted soils (Ali et al., 2013; Dhala et al., 2013; Ferrol et al., 2016; Vidal et al., 2018).

Although the present study utilized unsterilized field soils and large pots to mimic the field environment, our results determined under controlled greenhouse conditions may differ from those determined under natural field conditions (Dietterich et al., 2017). Therefore, further experimental studies on AMF-assisted phytoremediation of Cr should be carried out under field conditions so that the results will have better practical application. Future work should further experimentally determine the ranges of Cr and Cr(VI) concentrations at which uptake versus inhibition are observed in additional phytoremediator plants and test the significance of AMF strain and species differences on Cr uptake under different soil moisture and other edaphic conditions.

8. References

Audet, P., & Charest, C. (2006). Effects of AM colonization on “wild tobacco” plants grown in zinc-contaminated soil. *Mycorrhiza*, *16*(4), 277-283.

Audet, P., & Charest, C. (2007). Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives. *Environmental pollution*, *147*(3), 609-614.

Ballen, K. G., & Graham, P. H. (2002). The role of acid pH in symbiosis between plants and soil organisms. In *Handbook of Plant Growth pH as the Master Variable* (pp. 371-390). CRC Press.

Bartlett RJ, James BR (1988) in Chromium in the Natural and HumanEnvironments, eds Nriagu JO, Nieboer E (Wiley, New York), pp 267–303

Chen, B., Li, X., Tao, H., Christie, P., & Wong, M. H. (2003). The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. *Chemosphere*, *50*(6), 839-846.

Chen, B., Zhu, Y.-G., Duan, J., Xiao, X., & Smith, S. (2007). Effects of the arbuscular mycorrhizal fungus Glomus mosseae on growth and metal uptake by four plant species in copper mine tailings. *Environmental pollution*, *147*(2), 374-380.

Christie, P., Li, X., & Chen, B. (2004). Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. *Plant and soil*, *261*(1), 209-217.

Crowley, D. E., & Alvey, S. A. (2002). Regulation of microbial processes by soil pH. In *Handbook of Plant Growth pH as the Master Variable* (pp. 343-370). CRC Press.

Davies Jr, F., Puryear, J., Newton, R., Egilla, J., & Saraiva Grossi, J. (2002). Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth, and gas exchange. *Journal of Plant Nutrition*, *25*(11), 2389-2407.

Davies Jr, F. T., Puryear, J. D., Newton, R. J., Egilla, J. N., & Grossi, J. A. S. (2001). Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (Helianthus annuus). *Journal of Plant Physiology*, *158*(6), 777-786.

Davis, M. R., Zhao, F. J., & McGrath, S. P. (2004). Pollution-induced community tolerance of soil microbes in response to a zinc gradient. *Environmental Toxicology and Chemistry: An International Journal*, *23*(11), 2665-2672.

del Val, C., Barea, J., & Azcón-Aguilar, C. (1999). Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. *Applied Soil Ecology*, *11*(2-3), 261-269.

Deram, A., Languereau-Leman, F., Howsam, M., Petit, D., & Van Haluwyn, C. (2008). Seasonal patterns of cadmium accumulation in Arrhenatherum elatius (Poaceae): Influence of mycorrhizal and endophytic fungal colonisation. *Soil Biology and Biochemistry*, *40*(3), 845-848.

Diaz, G., Azcón-Aguilar, C., & Honrubia, M. (1996). Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of Lygeum spartum and Anthyllis cytisoides. *Plant and soil*, *180*(2), 241-249.

Gadd, G. M. (1994). Interactions of fungi with toxic metals. *The Genus Aspergillus*, 361-374.

Giasson, P., Karam, A., & Jaouich, A. (2008). Arbuscular mycorrhizae and alleviation of soil stresses on plant growth. *Mycorrhizae: Sustainable agriculture and forestry*, 99-134.

Giovannetti, M., and Mosse, B. (1980). An Evaluation of Techniques for Measuring Vesicular ArbuscularMycorrhizal Infection in Roots. *The New Phytologist* 84(3)**,** 489-500.

Glassman, S. I., & Casper, B. B. (2012). Biotic contexts alter metal sequestration and AMF effects on plant growth in soils polluted with heavy metals. *Ecology*, *93*(7), 1550-1559.

Hassan, S. E., Hijri, M., & St-Arnaud, M. (2013). Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil. *New biotechnology*, *30*(6), 780-787.

Homyak, P. M., Blankinship, J. C., Slessarev, E. W., Schaeffer, S. M., Manzoni, S., & Schimel, J. P. (2018). Effects of altered dry season length and plant inputs on soluble soil carbon. *Ecology*, *99*(10), 2348-2362.

Hovsepyan, A., & Greipsson, S. (2004). Effect of arbuscular mycorrhizal fungi on phytoextraction by corn (Zea mays) of lead-contaminated soil. *International Journal of Phytoremediation*, *6*(4), 305-321.

Hutchinson, S., Schwab, A., & Banks, M. (2003). Biodegradation of petroleum hydrocarbons in the rhizosphere. *Phytoremediation: transformation and control of contaminants*, 355-386.

Jacques, R. J., Okeke, B. C., Bento, F. M., Teixeira, A. S., Peralba, M. C., & Camargo, F. A. (2008). Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. *Bioresource Technology*, *99*(7), 2637-2643.

Jamal, A., Ayub, N., Usman, M., & Khan, A. G. (2002). Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil. *International Journal of Phytoremediation*, *4*(3), 205-221.

Jansa, J., Smith, F. A., & Smith, S. E. (2008). Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist*, *177*(3), 779-789.

Joner, E., & Leyval, C. (1997). Uptake of 109Cd by roots and hyphae of a Glomus mosseae/Trifolium subterraneum mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytologist*, *135*(2), 353-360.

Joner, E., & Leyval, C. (2001). Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. *Biology and Fertility of Soils*, *33*(5), 351-357.

Joner, E. J., Briones, R., & Leyval, C. (2000). Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and soil*, *226*(2), 227-234.

Kim, J. G., Dixon, J. B., Chusuei, C. C., & Deng, Y. (2002). Oxidation of chromium (III) to (VI) by manganese oxides. *Soil Science Society of America Journal*, *66*(1), 306-315.

Koide, R. T., & Li, M. (1989). Appropriate controls for vesicular–arbuscular mycorrhiza research. *New Phytologist*, *111*(1), 35-44.

Lasat, M. M. (2002). Phytoextraction of toxic metals: a review of biological mechanisms. *Journal of Environmental Quality*, *31*(1), 109-120.

Leyval, C., Berthelin, J., Schontz, D., Weissenhorn, I., & Morel, J. (1991). Influence of endomycorrhizas on maize uptake of Pb, Cu, Zn, and Cd applied as mineral salts or sewage sludge. *Heavy metals in the environment, CEP Consultants, Edinburgh, UK*, 204-207.

Li, X.-L., Marschner, H., & George, E. (1991). Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. *Plant and soil*, *136*(1), 49-57.

Marschner, P., Jentschke, G., & Godbold, D. L. (1998). Cation exchange capacity and lead sorption in ectomycorrhizal fungi. *Plant and soil*, *205*(1), 93-98.

Martin, F. M., Perotto, S., & Bonfante, P. (2000). Mycorrhizal fungi: a fungal community at the interface between soil and roots. In *The Rhizosphere* (pp. 279-312). CRC Press.

Meharg, A., Bailey, J., Breadmore, K., & Macnair, M. (1994). Biomass allocation, phosphorus nutrition and vesicular-arbuscular mycorrhizal infection in clones of Yorkshire Fog, Holcus lanatus L.(Poaceae) that differ in their phosphate uptake kinetics and tolerance to arsenate. *Plant and soil*, *160*(1), 11-20.

Morley, G. F., & Gadd, G. M. (1995). Sorption of toxic metals by fungi and clay minerals. *Mycological research*, *99*(12), 1429-1438.

Pawlowska, T. E., Błaszkowski, J., & Rühling, Å. (1997). The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. *Mycorrhiza*, *6*(6), 499-505.

Roesti, D., Ineichen, K., Braissant, O., Redecker, D., Wiemken, A., & Aragno, M. (2005).

Bacteria associated with spores of the arbuscular mycorrhizal fungi Glomus geosporum and Glomus constrictum. *Applied and Environmental Microbiology*, *71*(11), 6673-6679.

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Academic press.

Smith, A. P., Bond-Lamberty, B., Benscoter, B. W., Tfaily, M. M., Hinkle, C. R., Liu, C., & Bailey, V. L. (2017). Shifts in pore connectivity from precipitation versus groundwater rewetting increases soil carbon loss after drought. Nature Communications, 8(1), 1335.

Toler, H., Morton, J., & Cumming, J. (2005). Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. *Water, Air, and Soil Pollution*, *164*(1), 155-172.

Toljander, J. (2006). *Interactions between soil bacteria and arbuscular mycorrhizal fungi* (Vol. 2006).

Turnau, K., & Haselwandter, K. (2002). Arbuscular mycorrhizal fungi, an essential component of soil microflora in ecosystem restoration. In *Mycorrhizal technology in agriculture* (pp. 137-149). Springer.

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, *11*(3), 296-310.

Wang, F., Lin, X., & Yin, R. (2005). Heavy metal uptake by arbuscular mycorrhizas of Elsholtzia splendens and the potential for phytoremediation of contaminated soil. *Plant and soil*, *269*(1), 225-232.

Wang, Y. T. (2000). Microbial reduction of chromate. *Environmental Microbe-Metal Interactions*, 225-235.

Wu, S. L., Chen, B. D., Sun, Y. Q., Ren, B. H., Zhang, X., & Wang, Y. S. (2014). Chromium resistance of dandelion (Taraxacum platypecidum Diels.) and bermudagrass (Cynodon dactylon [Linn.] Pers.) is enhanced by arbuscular mycorrhiza in Cr (VI)-contaminated soils. *Environmental toxicology and chemistry*, *33*(9), 2105-2113.

Wu, S., Zhang, X., Sun, Y., Wu, Z., Li, T., Hu, Y., ... & Chen, B. (2015). Transformation and immobilization of chromium by arbuscular mycorrhizal fungi as revealed by SEM–EDS, TEM–EDS, and XAFS. *Environmental Science & Technology*, *49*(24), 14036-14047.

Wu, S., Zhang, X., Chen, B., Wu, Z., Li, T., Hu, Y., ... & Wang, Y. (2016). Chromium immobilization by extraradical mycelium of arbuscular mycorrhiza contributes to plant chromium tolerance. *Environmental and Experimental Botany*, *122*, 10-18.

Wu, S., Zhang, X., Sun, Y., Wu, Z., Li, T., Hu, Y., ... & Chen, B. (2016). Chromium immobilization by extra-and intraradical fungal structures of arbuscular mycorrhizal symbioses. *Journal of Hazardous Materials*, *316*, 34-42.

Weissenhorn, I., Glashoff, A., Leyval, C., & Berthelin, J. (1994). Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. *Plant and soil*, *167*(2), 189-196.

Wielinga, B., Mizuba, M. M., Hansel, C. M., & Fendorf, S. (2001). Iron promoted reduction of chromate by dissimilatory iron-reducing bacteria. *Environmental Science & Technology*, *35*(3), 522-527.

Zhang, H., Powell, J. R., Plett, J. M., Churchill, A. C., Power, S. A., Macdonald, C. A., . . . Tissue, D. (2021). Climate warming negates arbuscular mycorrhizal fungal reductions in soil phosphorus leaching with tall fescue but not lucerne. *Soil Biology and Biochemistry*, *152*, 108075.

S1 Table 1. Biological Data: Percentage AMF Colonization and Plant Biomass and Height by Treatment							
Pot #	**Fungal Treatment**	**Carbon**	**Cr Level**	**WHC**	**% AMF Coloniz-ation**	**Plant Height (in)**	**Plant Weight (g)**
1	AMF	No Addition	Low	75%	16	6.5	5.75
2	AMF	No Addition	Low	75%	30	14.5	12.25
3	AMF	No Addition	Low	75%	52	16	13.75
4	AMF	No Addition	Low	75%	18	14.5	18.25
5	AMF	No Addition	Low	75%	20	25	10.25
6	AMF	No Addition	Low	75%	2	20	16.25
7	AMF	*P. ostreatus* Mulch	Low	75%	26	19	7
8	AMF	*P. ostreatus* Mulch	Low	75%	10	18	13
9	AMF	*P. ostreatus* Mulch	Low	75%	2	13	14.25
10	AMF	*P. ostreatus* Mulch	Low	75%	0	29.75	25.75
11	AMF	*P. ostreatus* Mulch	Low	75%	44	19.25	14
12	AMF	*P. ostreatus* Mulch	Low	75%	0	23.5	19.5
13	AMF	No Addition	Low	35%	30	12	9.35
14	AMF	No Addition	Low	35%	12	17	13.5
15	AMF	No Addition	Low	35%	60	10	8.25
16	AMF	No Addition	Low	35%	10	24.5	20.5
17	AMF	No Addition	Low	35%		21	16.75
18	AMF	No Addition	Low	35%	24	17.5	15.75
19	AMF	No Addition	Low	35%	30	21	16.25
20	AMF	*P. ostreatus* Mulch	Low	35%	30	24.5	18.75
21	AMF	*P. ostreatus* Mulch	Low	35%	32	23	19
22	AMF	*P. ostreatus* Mulch	Low	35%	32	21.75	22.5
23	AMF	*P. ostreatus* Mulch	Low	35%	32	26.25	22.25
24	AMF	*P. ostreatus* Mulch	Low	35%	16	20.5	17.25
25	None	No Addition	Low	75%	36	20.75	17.75
26	None	No Addition	Low	75%	36	13.25	11.65
27	None	No Addition	Low	75%	70	20	18.25
28	None	No Addition	Low	75%	18	19.25	16
29	None	No Addition	Low	75%	16	18.75	16
30	None	No Addition	Low	75%	2	25.5	24
31	None	*P. ostreatus* Mulch	Low	75%	32	25.5	19.75
32	None	*P. ostreatus* Mulch	Low	75%	32	16.25	12
33	None	*P. ostreatus* Mulch	Low	75%	40	18	14.75
34	None	*P. ostreatus* Mulch	Low	75%	20	23.5	19.5
35	None	*P. ostreatus* Mulch	Low	75%	10	25.5	17.5

36	None	*P. ostreatus* Mulch	Low	75%	34	22.75	16
37	None	No Addition	Low	35%	60	22.5	20.25
38	None	No Addition	Low	35%	64	28.5	26
39	None	No Addition	Low	35%	58	15.75	22.85
40	None	No Addition	Low	35%	10	13.5	11.25
41	None	No Addition	Low	35%	6	21.75	19.75
42	None	No Addition	Low	35%	52	14.75	12.5
43	None	*P. ostreatus* Mulch	Low	35%	30	18.75	14.25
44	None	*P. ostreatus* Mulch	Low	35%	26	17	13.5
45	None	*P. ostreatus* Mulch	Low	35%	32	21	14.75
46	None	*P. ostreatus* Mulch	Low	35%	20	16	15.5
47	None	*P. ostreatus* Mulch	Low	35%	22	20.5	16.75
48	None	*P. ostreatus* Mulch	Low	35%	8	24.25	22.25
49	AMF	No Addition	High	75%	12	3.75	3.25
50	AMF	No Addition	High	75%	10	3	1.75
51	AMF	No Addition	High	75%	4	2.75	2.25
52	AMF	No Addition	High	75%	10	10.75	9.75
53	AMF	No Addition	High	75%	20	14.25	14
54	AMF	No Addition	High	75%	0	12.25	12.5
55	AMF	*P. ostreatus* Mulch	High	75%	10	17.25	15.5
56	AMF	*P. ostreatus* Mulch	High	75%	30	9.5	9.5
57	AMF	*P. ostreatus* Mulch	High	75%	10	4.5	4.25
58	AMF	*P. ostreatus* Mulch	High	75%	60	14	13.85
59	AMF	*P. ostreatus* Mulch	High	75%	40	9.25	9
60	AMF	*P. ostreatus* Mulch	High	75%	20	14.5	14.25
61	AMF	No Addition	High	35%	20	6.25	6.5
62	AMF	No Addition	High	35%	20	5	4.75
63	AMF	No Addition	High	35%	20	9.25	9
64	AMF	No Addition	High	35%	60	7	4
65	AMF	No Addition	High	35%	4	10.75	10.75
66	AMF	No Addition	High	35%	40	6	6.5
67	AMF	*P. ostreatus* Mulch	High	35%	40	21	20
68	AMF	*P. ostreatus* Mulch	High	35%	60	13.5	11.5
69	AMF	*P. ostreatus* Mulch	High	35%	60	10.75	9.5
70	AMF	*P. ostreatus* Mulch	High	35%	4	10	9.75
71	AMF	*P. ostreatus* Mulch	High	35%	10	15.17	15.25
72	AMF	*P. ostreatus* Mulch	High	35%	30	23.25	19
73	None	No Addition	High	75%	6	3	3.25
74	None	No Addition	High	75%	30	6.75	5.5

75	None	No Addition	High	75%	20	7.5	7
76	None	No Addition	High	75%	2	15	15
77	None	No Addition	High	75%	4	15.75	15.75
78	None	No Addition	High	75%	20	12.25	10.25
79	None	*P. ostreatus* Mulch	High	75%	2	11.5	11.5
80	None	*P. ostreatus* Mulch	High	75%	30	12	10.5
81	None	*P. ostreatus* Mulch	High	75%	2	8.75	6.25
82	None	*P. ostreatus* Mulch	High	75%	10	15.75	15
83	None	*P. ostreatus* Mulch	High	75%	2	14	11.5
84	None	*P. ostreatus* Mulch	High	75%	4	13.5	11
85	None	No Addition	High	35%	4	2.5	2.5
86	None	No Addition	High	35%	10	5.75	5.5
87	None	No Addition	High	35%	0	8.5	8.5
88	None	No Addition	High	35%	20	10	9.75
89	None	No Addition	High	35%	10	17.25	14.75
90	None	No Addition	High	35%	0	5.5	5.5
91	None	*P. ostreatus* Mulch	High	35%	2	19	19.25
92	None	*P. ostreatus* Mulch	High	35%	10	3.25	1.75
93	None	*P. ostreatus* Mulch	High	35%	10	8.5	8
94	None	*P. ostreatus* Mulch	High	35%	30	20	19.5
95	None	*P. ostreatus* Mulch	High	35%	10	19	17.75
96	None	*P. ostreatus* Mulch	High	35%	10	21.75	19.25
97	AMF	No Addition	Uncontaminated	75%	40	22.5	14.75
98	AMF	No Addition	Uncontaminated	75%	50	20	19.25
99	AMF	No Addition	Uncontaminated	75%	60	19	19.15
100	AMF	No Addition	Uncontaminated	75%	10	23.5	15
101	AMF	No Addition	Uncontaminated	75%	0	21.5	19.25
102	AMF	No Addition	Uncontaminated	75%	0	25.5	23
103	AMF	*P. ostreatus* Mulch	Uncontaminated	75%	30	28	23
104	AMF	*P. ostreatus* Mulch	Uncontaminated	75%	20	26.5	21.25
105	AMF	*P. ostreatus* Mulch	Uncontaminated	75%	30	31.25	22.5
106	AMF	*P. ostreatus* Mulch	Uncontaminated	75%	30	29.5	24.5
107	AMF	*P. ostreatus* Mulch	Uncontaminated	75%	40	24	24.75
108	AMF	*P. ostreatus* Mulch	Uncontaminated	75%	10	26.5	21.5

109	AMF	No Addition	Uncontaminated	35%	20	19	16.25
110	AMF	No Addition	Uncontaminated	35%	10	20.5	17.5
111	AMF	No Addition	Uncontaminated	35%	10	40.5	31.75
112	AMF	No Addition	Uncontaminated	35%	0	23	19.75
113	AMF	No Addition	Uncontaminated	35%	10	31.25	25.75
114	AMF	No Addition	Uncontaminated	35%	10	22	19.75
115	AMF	*P. ostreatus* Mulch	Uncontaminated	35%	60	25	15.5
116	AMF	*P. ostreatus* Mulch	Uncontaminated	35%	40	24.75	17.75
117	AMF	*P. ostreatus* Mulch	Uncontaminated	35%	30	19	14.25
118	AMF	*P. ostreatus* Mulch	Uncontaminated	35%	20	41	32.25
119	AMF	*P. ostreatus* Mulch	Uncontaminated	35%	0	31.25	26
120	AMF	*P. ostreatus* Mulch	Uncontaminated	35%	2	26.25	22
121	None	No Addition	Uncontaminated	75%	40	20.75	15.75
122	None	No Addition	Uncontaminated	75%	70	22.5	18
123	None	No Addition	Uncontaminated	75%	40	24	20
124	None	No Addition	Uncontaminated	75%	6	15.25	14.75
125	None	No Addition	Uncontaminated	75%	2	37	31
126	None	No Addition	Uncontaminated	75%	20	26.25	22.5
127	None	*P. ostreatus* Mulch	Uncontaminated	75%	60	27.75	22.75
128	None	*P. ostreatus* Mulch	Uncontaminated	75%	60	21	17.75
129	None	*P. ostreatus* Mulch	Uncontaminated	75%	4	25	17.5
130	None	*P. ostreatus* Mulch	Uncontaminated	75%	4	33.5	28.75
131	None	*P. ostreatus* Mulch	Uncontaminated	75%	6	32.25	27
132	None	*P. ostreatus* Mulch	Uncontaminated	75%	0	26.75	132
133	None	No Addition	Uncontaminated	35%	8	21	23.75
134	None	No Addition	Uncontaminated	35%	30	18.75	17.25
135	None	No Addition	Uncontaminated	35%	60	17.5	10.5
136	None	No Addition	Uncontaminated	35%	0	28.25	22.25

137	None	No Addition	Uncontaminated	35%	2	34	35.75
138	None	No Addition	Uncontaminated	35%	10	28.25	25.5
139	None	*P. ostreatus* Mulch	Uncontaminated	35%	10	22	22.5
140	None	*P. ostreatus* Mulch	Uncontaminated	35%	4	31.5	23.75
141	None	*P. ostreatus* Mulch	Uncontaminated	35%	4	24.25	23.75
142	None	*P. ostreatus* Mulch	Uncontaminated	35%	0	25	20.25
143	None	*P. ostreatus* Mulch	Uncontaminated	35%	40	28.25	22.75
144	None	*P. ostreatus* Mulch	Uncontaminated	35%	40	31.25	27.5

S1 Table 2. Soil Physical Properties for each pot

Pot #	Soil Moisture	Soil Temp	Irrigation Level	% Clay	% Silt	% Sand	Texture Class
1	0.243	21.3	1	2.34	26.045	71.618	sandy loam
2	0.244	22.4	2	1.87	21.454	76.673	loamy sand
3	0.26	19.9	2	1.78	18.814	79.403	loamy sand
4	0.132	21.3	2	1.95	21.883	76.167	loamy sand
5	0.213	21.9	1	1.83	21.901	76.274	loamy sand
6	0.241	20.9	2	2.66	26.259	71.083	sandy loam
7	0.198	20.2	2	1.745	19.434	78.821	loamy sand
8	0.236	21.9	1	1.837	21.846	76.317	loamy sand
9	0.201	21.1	2	2.028	21.603	76.369	loamy sand
10	0.263	20.8	2	1.62	23.267	75.117	loamy sand
11	0.239	20.3	2	1.755	19.763	78.482	loamy sand
12	0.241	23.8	2	1.897	20.954	77.148	loamy sand
13	0.2	20.3	2	1.88	19.936	78.186	loamy sand
14	0.201	21.6	1	1.75	24.024	74.227	loamy sand
15	0.29	21.3	1	1.8	20.117	78.086	loamy sand
16	0.2	19.3	1	1.65	18.262	80.093	loamy sand
17	0.166	20.8	1	2.41	25.034	72.551	loamy sand
18	0.178	22.4	1	2.29	21.743	75.97	loamy sand

19	0.172	21.4	1	1.72	19.365	78.919	loamy sand
20	0.148	21.4	1	2.2	22.622	75.181	loamy sand
21	0.138	19.9	1	1.55	18.702	79.748	loamy sand
22	0.217	19.9	1	1.96	22.032	76.008	loamy sand
23	0.182	20.3	1	1.58	18.578	79.84	loamy sand
24	0.202	21.3	1	1.7	19.295	79.001	loamy sand
25	0.269	21.9	2	1.67	18.801	79.528	loamy sand
26	0.212	19.9	2	1.69	19.533	78.775	loamy sand
27	0.18	22.7	2	1.7	22.159	76.137	loamy sand
28	0.206	20.6	2	1.73	18.718	79.556	loamy sand
29	0.258	22.3	2	1.49	17.192	81.316	loamy sand
30	0.215	21.8	2	2.16	21.108	76.731	loamy sand
31	0.22	21.8	2	1.62	19.295	79.086	loamy sand
32	0.282	20.2	2	1.84	20.137	78.026	loamy sand
33	0.205	21.8	2	1.58	16.904	81.512	loamy sand
34	0.179	20.4	2	1.77	21.323	76.91	loamy sand
35	0.19	21.9	2	1.61	18.718	79.672	loamy sand
36	0.227	22.6	2	1.84	20.203	77.961	loamy sand
37	0.154	21.1	1	1.59	19.881	78.529	loamy sand
38	0.226	22.6	1	1.72	20.238	78.041	loamy sand
39	0.198	20.7	1	1.46	18.234	80.309	loamy sand
40	0.207	20.4	1	1.87	19.891	78.235	loamy sand
41	0.202	21	1	2.07	24.02	73.91	loamy sand
42	0.184	21.1	1	1.87	21.191	76.939	loamy sand
43	0.279	22.4	1	1.99	23.884	74.124	loamy sand
44	0.193	21.4	1	2.04	23.047	74.914	loamy sand
45	0.209	21.1	1	2.05	22.583	75.367	loamy sand
46	0.19	19.6	1	3.39	33.541	63.067	sandy loam

47	0.153	21	1	1.81	20.464	77.729	loamy sand
48	0.284	22.2	2	1.97	23.031	75.003	loamy sand
49	0.192	21.8	2	2.43	23.703	73.87	loamy sand
50	0.261	21.8	2	2.73	27.142	70.13	sandy loam
51	0.221	21.8	2	2.75	24.548	72.703	sandy loam
52	0.182	21.5	2	2.5	23.923	73.576	loamy sand
53	0.263	20.6	2	1.6	14.748	83.648	loamy sand
54	0.255	21.1	2	2.59	22.891	74.518	loamy sand
55	0.291	20.2	2	2.22	20.577	77.201	loamy sand
56	0.279	20.7	2	2.77	23.118	74.114	loamy sand
57	0.264	20	2	2.45	22.43	75.12	loamy sand
58	0.258	20.8	2	1.91	21.153	76.932	loamy sand
59	0.28	21.3	2	2.67	25.34	71.99	sandy laom
60	0.259	21.6	1	2.86	31.8	65.3	sandy loam
61	0.191	21.8	1	2.47	22.348	75.186	loamy sand
62	0.286	20.9	1	2.53	24.273	73.196	loamy sand
63	0.238	22.3	1	2.71	24.313	72.98	loamy sand
64	0.278	20.1	1	2.56	24.355	73.089	loamy sand
65	0.221	21.1	1	2.23	20.916	76.859	loamy sand
66	0.239	21.2	1	2.45	20.453	77.094	loamy sand
67	0.251	22	1	2.39	23.959	73.649	loamy sand
68	0.241	21.3	2	2.65	22.888	74.458	loamy sand
69	0.234	19.9	1	2.62	22.542	74.839	loamy sand
70	0.234	22.5	1	2.39	22.335	75.271	loamy sand
71	0.229	19.9	1	2.48	21.837	75.686	loany sand
72	0.296	20.4	2	2.54	23.065	74.392	loamy sand
73	0.294	19.3	2	2.52	22.732	74.753	loamy sand
74	0.266	22.4	2	2.81	19.577	77.611	loamy sand

75	0.282	23.4	2	2.1	19.425	78.47	loamy sand
76	0.273	21.5	2	2.21	19.949	77.843	loamy sand
77	0.269	21.4	2	2.47	21.643	75.892	loamy sand
78	0.227	21.4	2	2.2	19.942	77.855	loamy sand
79	246	19.5	2	2.41	21.144	76.446	loamy sand
80	0.28	20.9	2	2.54	24.436	73.025	loamy sand
81	0.184	21.7	2	2.5	24.554	72.942	loamy sand
82	0.241	21.1	2	2.81	29.262	67.925	sandy loam
83	0.245	21.1	2	2.52	21.346	76.131	loamy sand
84	0.197	20.7	1	2.19	22.165	75.65	loamy sand
85	0.153	20.8	1	2.14	19.593	78.263	loamy sand
86	0.294	21.9	1	2.48	22.804	74.712	loamy sand
87	0.281	19.7	1	2.52	24.67	72.808	loamy sand
88	0.192	22.4	1	2.2	21.092	76.708	loamy sand
89	0.237	21.1	1	2.19	24.893	72.921	loamy sand
90	0.235	21.7	1	2.5	21.546	75.955	loamy sand
91	0.185	22.3	1	2.08	18.739	79.182	loamy sand
92	0.282	21.7	1	2.88	26.458	70.66	sandy loam
93	0.273	22.3	1	2.39	21.239	76.372	loamy sand
94	0.21	20.9	1	2.61	25.31	72.084	sandy loam
95	0.243	20.9	1	2.32	22.139	75.538	loamy sand
96	0.234	20.1	2	2.96	28.909	68.132	sandy loam
97	0.264	19.6	2	2.93	29.928	67.139	sandy loam
98	0.256	20.8	2	2	20.97	77.033	loamy sand
99	0.265	22.3	2	2.2	21.853	75.943	loamy sand
100	0.233	20.9	2	2.47	23.507	74.022	loamy sand
101	0.19	19.9	2	1.31	15.942	82.753	loamy sand
102	0.268	22.2	2	2.61	26.027	71.363	sandy loam

103	0.155	21.1	2	2.66	25.95	71.391	sandy loam
104	0.197	22.4	2	3.34	30.756	65.9	sandy laom
105	0.255	21.9	2	1.46	17.544	80.991	loamy sand
106	0.233	22.6	2	2.9	30.883	66.221	sandy loam
107	0.117	21.4	1	2.58	25.084	72.338	sandy loam
108	0.1009	21.3	1	2.48	29.163	68.361	sandy loam
109	0.149	21.8	1	3.78	40.3	55.922	sandy loam
110	0.259	22.1	1	2.18	21.391	76.43	loamy sand
111	0.177	21.7	1	3.74	41.675	54.582	sandy loam
112	0.194	19.1	1	3.26	29.286	67.459	sandy loam
113	0.182	19.3	1	2.12	22.571	75.313	loamy sand
114	0.157	20.2	1	2	27.35	70.647	sandy loam
115	0.137	21.7	1	2.33	25.077	72.593	loamy sand
116	0.111	21.7	1	2.56	28.798	68.645	sandy loam
117	0.135	20.5	1	2.38	23.062	74.558	loamy sand
118	0.18	21.1	1	2.41	26.688	70.901	sandy loam
119	0.185	19.5	2	2.59	24.345	73.069	loamy sand
120	0.291	22.6	2	1.45	19.512	79.035	loamy sand
121	0.201	21.1	2	2.8	28.188	69.015	sandy loam
122	0.022	21.3	2	2.9	28.427	68.67	sandy loam
123	0.21	21.8	2	2.24	25.412	72.347	loamy sand
124	0.3	21.5	2	2.2	23.377	74.425	loamy sand
125	0.163	21.7	2	2.62	26.584	70.8	sandy loam
126	0.231	20.3	2	2.24	25.946	71.812	sandy loam
127	0.137	19.9	2	2.47	26.742	70.785	sadny loam
128	0.175	21.4	2	1.85	22.942	75.209	loamy sand
129	0.236	22.5	2	2.19	23.991	73.817	loamy sand
130	0.209	21.5	2	3.18	30.125	66.696	sandy loam

131	0.125	21.4	1	2.68	27.313	70.002	sandy loam
132	0.268	22.3	1	1.98	22.813	75.21	loamy sand
133	0.259	20	1	1.66	23.652	74.69	loamy sand
134	0.194	22.5	1	2.49	24.1	73.408	loamy sand
135	0.18	19.9	1	2.45	27.371	70.179	sandy loam
136	0.153	20.6	1	2.61	28.341	69.049	sandy loam
137	0.227	19.8	1	2.61	26.609	70.785	sandy loam
138	0.203	21.8	1	1.94	22.073	75.983	loamy sand
139	0.225	20.7	1	2.91	32.599	64.49	sandy loam
140	0.17	20.8	1	2.16	22.701	75.135	loamy sand
141	0.15	21.5	1	3.95	36.814	59.238	sandy loam
142	0.199	24	1	2.95	30.193	66.857	sandy loam
143	0.199	24	1	2.31	25.817	71.876	sandy loam
144	0.199	24	1	2.77	28.711	68.521	sandy loam

S1 Table 3. Soil Chemical Properties and Changes Over Time

Pot #	WEOC T0	WEOC Tfinal	pH	C (µg) T0	C (µg) Tfinal	Change in C	% OC T0	% OC Tfinal	Change in OC	P04 T0	P04 Tfinal
1	13.630	4.060	8.820	202.221	182.414	-9.795	0.650	0.577	-11.269	1.164	3.012
2	12.560	5.860	8.630	202.221	182.414	-9.795	0.650	0.577	-11.269	1.584	0.919
3	12.690	5.210	9.140	202.221	182.414	-9.795	0.650	0.577	-11.269	1.672	1.236
4	13.680	6.200	9.060	202.221	182.414	-9.795	0.650	0.577	-11.269	2.412	1.311
5	13.110	5.410	8.640	202.221	182.414	-9.795	0.650	0.577	-11.269	1.552	2.080
6	13.700	5.130	8.890	163.607	128.422	-21.505	0.535	0.433	-19.029	1.809	1.835
7	13.470	4.870	8.470	163.607	128.422	-21.505	0.535	0.433	-19.029	1.099	1.842
8	11.540	10.840	9.160	163.607	128.422	-21.505	0.535	0.433	-19.029	1.223	0.685
9	13.000	6.330	8.960	163.607	128.422	-21.505	0.535	0.433	-19.029	1.320	1.164
10	15.990	4.990	8.970	163.607	128.422	-21.505	0.535	0.433	-19.029	1.206	1.017
11	14.110	7.970	8.790	163.607	128.422	-21.505	0.535	0.433	-19.029	1.089	1.134
12	19.150	7.080	9.020	131.542	228.591	73.778	0.444	0.753	69.761	0.698	4.166
13	15.090	4.440	8.880	131.542	228.591	73.778	0.444	0.753	69.761	1.927	0.822
14	16.970	5.130	8.800	131.542	228.591	73.778	0.444	0.753	69.761	1.017	0.929
15	16.700	5.810	8.750	131.542	228.591	73.778	0.444	0.753	69.761	1.177	2.517
16	17.810	5.140	9.030	131.542	228.591	73.778	0.444	0.753	69.761	1.359	2.210

17	16.290	3.480	8.870	131.542	228.591	73.778	0.444	0.753	69.761	1.513	0.789
18	17.090	4.250	8.360	153.680	124.829	-18.773	0.524	0.407	-22.366	3.501	1.386
19	15.290	5.290	8.800	153.680	124.829	-18.773	0.524	0.407	-22.366	0.870	1.591
20	18.920	8.930	9.110	153.680	124.829	-18.773	0.524	0.407	-22.366	0.903	0.492
21	18.130	9.600	9.350	153.680	124.829	-18.773	0.524	0.407	-22.366	0.769	2.024
22	16.670	6.520	8.740	153.680	124.829	-18.773	0.524	0.407	-22.366	1.121	1.813
23	17.730	6.360	9.030	153.680	124.829	-18.773	0.524	0.407	-22.366	1.503	1.884
24	19.040	5.830	9.040	120.690	335.663	178.120	0.393	1.067	171.398	0.985	2.905
25	25.790	5.260	8.900	120.690	335.663	178.120	0.393	1.067	171.398	1.076	1.304
26	16.980	6.090	8.840	120.690	335.663	178.120	0.393	1.067	171.398	1.024	1.095
27	17.780	5.930	9.170	120.690	335.663	178.120	0.393	1.067	171.398	0.822	0.962
28	15.760	5.630	8.550	120.690	335.663	178.120	0.393	1.067	171.398	1.102	0.753
29	15.160	6.230	8.700	120.690	335.663	178.120	0.393	1.067	171.398	0.929	0.854
30	18.870	6.250	9.040	146.311	143.016	-2.252	0.495	0.467	-5.684	1.209	1.363
31	11.470	6.040	8.710	146.311	143.016	-2.252	0.495	0.467	-5.684	0.861	1.141
32	17.230	5.930	8.890	146.311	143.016	-2.252	0.495	0.467	-5.684	0.942	0.883
33	15.110	8.160	8.840	146.311	143.016	-2.252	0.495	0.467	-5.684	1.121	1.800
34	11.630	12.560	8.580	146.311	143.016	-2.252	0.495	0.467	-5.684	1.288	1.007
35	12.300	11.430	9.030	146.311	143.016	-2.252	0.495	0.467	-5.684	0.831	0.883
36	14.300	9.340	8.980	133.291	160.253	20.228	0.412	0.530	28.511	0.994	1.167
37	11.150	7.070	9.070	133.291	160.253	20.228	0.412	0.530	28.511	1.011	0.802
38	18.050	7.130	9.030	133.291	160.253	20.228	0.412	0.530	28.511	1.219	1.138
39	9.330	5.390	8.720	133.291	160.253	20.228	0.412	0.530	28.511	0.786	0.870
40	8.240	4.820	8.970	133.291	160.253	20.228	0.412	0.530	28.511	0.988	1.249
41	10.890	7.400	8.860	133.291	160.253	20.228	0.412	0.530	28.511	1.343	1.141
42	13.320	4.570	8.970	155.735	128.471	-17.507	0.503	0.417	-17.219	0.949	0.789
43	10.110	8.260	9.250	155.735	128.471	-17.507	0.503	0.417	-17.219	0.874	2.279
44	10.800	6.860	8.870	155.735	128.471	-17.507	0.503	0.417	-17.219	0.861	1.757
45	9.970	10.330	8.840	155.735	128.471	-17.507	0.503	0.417	-17.219	1.275	1.200
46	11.450	8.670	8.700	155.735	128.471	-17.507	0.503	0.417	-17.219	0.701	2.895
47	10.830	11.610	8.750	163.086	164.294	0.741	0.503	0.417	-17.219	1.637	0.844
48	9.230	11.710	9.150	163.086	164.294	0.741	0.541	0.527	-2.584	0.913	0.952
49	8.770	7.360	8.080	163.086	164.294	0.741	0.541	0.527	-2.584	1.148	1.164
50	13.970	5.220	8.140	163.086	164.294	0.741	0.541	0.527	-2.584	1.121	1.063
51	12.940	2.950	8.170	163.086	164.294	0.741	0.541	0.527	-2.584	1.682	0.854
52	13.220	5.650	8.060	163.086	164.294	0.741	0.541	0.527	-2.584	0.509	1.105
53	13.520	5.240	8.080	132.627	137.197	3.446	0.541	0.527	-2.584	0.499	0.528
54	16.090	5.600	8.030	132.627	137.197	3.446	0.437	0.463	6.107	0.704	1.063
55	17.690	4.780	8.120	132.627	137.197	3.446	0.437	0.463	6.107	0.424	0.737

56	14.220	4.380	8.090	132.627	137.197	3.446	0.437	0.463	6.107	0.372	0.776
57	24.570	11.100	7.930	132.627	137.197	3.446	0.437	0.463	6.107	0.293	1.138
58	27.540	6.240	8.030	132.627	137.197	3.446	0.437	0.463	6.107	0.434	0.952
59	25.650	5.670	8.160	117.424	166.569	41.852	0.437	0.463	6.107	0.241	0.815
60	24.100	3.780	8.040	117.424	166.569	41.852	0.420	0.540	28.571	0.975	0.818
61	26.270	16.400	8.220	117.424	166.569	41.852	0.420	0.540	28.571	0.482	1.428
62	31.710	6.510	8.060	117.424	166.569	41.852	0.420	0.540	28.571	0.313	0.887
63	26.170	7.330	8.100	117.424	166.569	41.852	0.420	0.540	28.571	0.668	0.694
64	35.180	5.530	8.000	117.424	166.569	41.852	0.420	0.540	28.571	0.417	0.838
65	17.960	4.060	8.200	215.291	154.568	-28.205	0.420	0.540	28.571	0.887	0.597
66	18.960	7.980	8.040	215.291	154.568	-28.205	0.390	0.517	32.569	0.430	1.451
67	17.420	7.580	8.000	215.291	154.568	-28.205	0.390	0.517	32.569	0.597	1.102
68	20.680	9.840	8.240	215.291	154.568	-28.205	0.390	0.517	32.569	0.525	1.007
69	24.220	11.050	8.380	215.291	154.568	-28.205	0.390	0.517	32.569	0.456	1.597
70	24.890	4.300	7.990	215.291	154.568	-28.205	0.390	0.517	32.569	0.584	0.792
71	26.350	14.300	8.280	280.134	231.865	-17.231	0.390	0.517	32.569	0.489	1.594
72	24.150	12.340	8.130	280.134	231.865	-17.231	1.058	0.767	-27.563	0.528	1.086
73	23.120	13.030	8.200	280.134	231.865	-17.231	1.058	0.767	-27.563	0.502	0.971
74	19.620	4.710	8.140	280.134	231.865	-17.231	1.058	0.767	-27.563	0.874	0.975
75	20.840	5.550	8.300	280.134	231.865	-17.231	1.058	0.767	-27.563	0.584	0.942
76	16.050	5.550	8.360	280.134	231.865	-17.231	1.058	0.767	-27.563	1.578	0.795
77	15.010	5.100	8.110	189.918	172.008	-9.431	1.058	0.767	-27.563	0.414	0.681
78	12.950	6.060	7.870	189.918	172.008	-9.431	0.540	0.567	4.860	0.414	1.203
79	11.730	6.590	8.300	189.918	172.008	-9.431	0.540	0.567	4.860	0.584	1.307
80	12.680	8.000	8.010	189.918	172.008	-9.431	0.540	0.567	4.860	0.398	0.864
81	20.290	7.900	8.250	189.918	172.008	-9.431	0.540	0.567	4.860	0.339	1.340
82	17.860	8.280	8.400	189.918	172.008	-9.431	0.540	0.567	4.860	0.456	1.086
83	14.600	9.010	8.010	141.429	178.469	26.190	0.540	0.567	4.860	0.528	1.099
84	7.140	9.470	8.160	141.429	178.469	26.190	0.670	0.623	-6.965	0.173	2.484
85	9.450	9.120	8.010	141.429	178.469	26.190	0.670	0.623	-6.965	1.242	1.063
86	14.200	20.540	8.090	141.429	178.469	26.190	0.670	0.623	-6.965	0.551	1.174
87	14.850	4.910	8.370	141.429	178.469	26.190	0.670	0.623	-6.965	0.414	0.743
88	15.530	4.640	7.850	141.429	178.469	26.190	0.670	0.623	-6.965	0.632	1.112
89	13.190	6.290	8.030	121.371	222.427	83.263	0.670	0.623	-6.965	0.401	1.372
90	14.750	5.490	8.280	121.371	222.427	83.263	0.444	0.727	63.771	0.844	0.737
91	12.840	4.010	8.080	121.371	222.427	83.263	0.444	0.727	63.771	0.463	1.046
92	15.700	5.140	8.370	121.371	222.427	83.263	0.444	0.727	63.771	0.606	0.854
93	14.800	11.640	8.080	121.371	222.427	83.263	0.444	0.727	63.771	0.577	0.724
94	14.410	6.160	8.420	121.371	222.427	83.263	0.444	0.727	63.771	0.538	1.187

95	15.700	8.130	7.920	135.136	160.849	19.028	0.444	0.727	63.771	0.538	0.675
96	14.770	28.230	8.160	135.136	160.849	19.028	0.400	0.530	32.507	0.479	0.877
97	11.520	32.770	8.680	135.136	160.849	19.028	0.400	0.530	32.507	0.518	1.441
98	14.840	4.700	8.590	135.136	160.849	19.028	0.400	0.530	32.507	0.502	0.874
99	15.000	3.874	8.910	135.136	160.849	19.028	0.400	0.530	32.507	0.851	0.890
100	17.650	5.230	9.290	135.136	160.849	19.028	0.400	0.530	32.507	0.564	0.421
101	14.800	4.760	8.720	188.097	113.667	-39.570	0.400	0.530	32.507	-0.196	0.593
102	21.250	4.910	8.750	188.097	113.667	-39.570	0.680	0.520	-23.572	0.505	0.606
103	21.560	4.690	8.580	188.097	113.667	-39.570	0.680	0.520	-23.572	0.698	0.763
104	14.340	8.520	8.370	188.097	113.667	-39.570	0.680	0.520	-23.572	0.531	0.652
105	21.240	8.620	8.840	188.097	113.667	-39.570	0.680	0.520	-23.572	0.645	0.447
106	21.100	12.510	8.500	188.097	113.667	-39.570	0.680	0.520	-23.572	0.075	0.427
107	13.850	6.650	8.810	174.456	144.453	-17.198	0.680	0.520	-23.572	0.336	0.998
108	19.660	9.960	8.490	174.456	144.453	-17.198	0.447	0.697	55.970	0.225	0.528
109	13.560	6.760	8.450	174.456	144.453	-17.198	0.447	0.697	55.970	0.189	1.115
110	19.260	20.290	8.590	174.456	144.453	-17.198	0.447	0.697	55.970	0.303	0.799
111	20.060	7.130	8.030	174.456	144.453	-17.198	0.447	0.697	55.970	0.652	0.906
112	12.980	6.940	8.010	174.456	144.453	-17.198	0.447	0.697	55.970	0.629	0.789
113	24.570	10.700	8.500	174.456	144.453	-17.198	0.447	0.697	55.970	1.460	1.281
114	21.200	3.490	8.540	180.169	156.088	-13.366	0.460	0.447	-2.830	0.717	2.350
115	12.150	6.990	8.730	122.597	156.088	27.318	0.460	0.447	-2.830	0.574	0.945
116	16.730	34.730	8.650	122.597	156.088	27.318	0.460	0.447	-2.830	0.786	1.258
117	15.350	28.210	8.740	122.597	156.088	27.318	0.460	0.447	-2.830	2.028	1.007
118	11.640	35.160	8.380	122.597	156.088	27.318	0.460	0.447	-2.830	0.460	0.730
119	23.950	13.400	8.150	122.597	156.088	27.318	0.460	0.447	-2.830	1.255	0.864
120	11.030	17.050	8.300	122.597	156.088	27.318	0.408	0.437	7.104	0.482	1.174
121	19.720	15.020	8.600	100.852	213.701	111.896	0.408	0.437	7.104	0.590	0.769
122	16.300	15.330	8.400	100.852	213.701	111.896	0.408	0.437	7.104	1.806	1.359
123	10.290	7.510	8.300	100.852	213.701	111.896	0.408	0.437	7.104	4.192	6.582
124	11.980	3.470	8.740	100.852	213.701	111.896	0.408	0.437	7.104	0.818	1.027
125	11.350	28.720	9.300	100.852	213.701	111.896	0.408	0.437	7.104	6.119	1.526
126	10.020	6.350	8.980	100.852	213.701	111.896	0.430	0.553	28.682	2.800	1.643
127	14.330	21.060	8.960	149.327	137.586	-7.863	0.430	0.553	28.682	2.830	2.350
128	7.720	13.570	8.640	149.327	137.586	-7.863	0.430	0.553	28.682	3.104	2.373
129	9.960	21.220	8.880	149.327	137.586	-7.863	0.430	0.553	28.682	1.848	2.624
130	8.440	10.270	8.300	149.327	137.586	-7.863	0.430	0.553	28.682	1.963	2.820
131	10.240	17.720	8.450	149.327	137.586	-7.863	0.430	0.553	28.682	0.691	0.085
132	9.030	7.120	8.440	149.327	137.586	-7.863	0.507	0.359	-29.050	2.810	2.755
133	9.920	8.600	8.380	130.948	125.025	-4.524	0.507	0.359	-29.050	3.511	2.588

134	10.500	5.210	8.940	130.948	125.025	-4.524	0.507	0.359	-29.050	1.324	1.307
135	8.640	8.080	8.530	130.948	125.025	-4.524	0.507	0.359	-29.050	3.439	1.692
136	9.050	6.630	8.430	130.948	125.025	-4.524	0.507	0.359	-29.050	2.641	1.496
137	10.960	4.230	8.670	130.948	125.025	-4.524	0.507	0.359	-29.050	2.031	0.714
138	7.600	25.020	8.120	130.948	125.025	-4.524	0.585	0.425	-27.470	1.314	1.888
139	8.420	3.640	8.530	146.378	168.831	15.339	0.585	0.425	-27.470	1.203	1.330
140	12.110	3.960	8.930	146.378	168.831	15.339	0.585	0.425	-27.470	0.841	0.988
141	10.200	3.640	8.590	146.378	168.831	15.339	0.585	0.425	-27.470	0.841	2.452
142	12.900	6.750	8.730	146.378	168.831	15.339	0.585	0.425	-27.470	1.017	1.467
143	10.300	9.850	8.740	146.378	168.831	15.339	0.585	0.425	-27.470	1.832	2.386
144	12.480	7.100	8.730	146.378	168.831	15.339	0.417	0.548	31.353	2.018	0.401

S1 Table 4. Mean and Standard Deviation for Change in Soil Cr and Cr(VI) by Treatment *

*Replicates of 24 by treatment group were averaged to get the means shown.

Treatment	**Cr Mean ± Stdev**	**Cr(VI) Mean ± Stdev**
AMF inoculum	-45.79 ± 21.32	-1.658 ± 48.37
No AMF inoculum	-46.16 ± 21.81	123.32 ± 341.70
35% WHC	-39.43 ± 38.59	14.12 ± 49.91
75% WHC	-0.2 ± 108.59	107.54 ± 346.95
No carbon amendment	-48.77 ± 19.11	118.48 ± 342.26
P. ostreatus mulch	-3.22 ± 110.49	3.19 ± 56.84

Chapter 4. Field Testing of Phyto-Mycoremediation on Contaminated Sites in LA County

1. Abstract

The present study evaluated a novel biological soil metal remediation approach utilizing California native plants with different fungal treatments including arbuscular mycorrhizal fungi (AMF) inoculum and *Pleurotus ostreatus* inoculum in the form of spent mushroom blocks on three brownfields with different levels of organic and inorganic contaminants in Los Angeles under irrigated and unirrigated water regimes. To our knowledge, it was the first field study of phyto-mycoremediation in Southern California, and was carried out in partnership with community groups, city, and regulatory agencies. Results show that treatment with plants and fungi reduced soil concentrations of all metals of concern, and produced statistically significant reductions for Pb, Cu and As, when compared with control plots. Although the native plants tested were not as performant in metal accumulation and extraction into aboveground parts as the control plant, *Chrysnopogon zizanioides* (dryland remediator Vetiver), they had much greater survival and biomass production in both irrigated and unirrigated plots. *Heterotheca grandiflora* had the highest mean Pb uptake of the native plants tested, but was not significantly different than *Erigonium fasciculatum*, suggesting both may be suitable native plant options for phytoremediation efforts in Southern California. Irrigation and inoculation with AMF significantly increased metal accumulation in all plants tested, although AMF-mediated effects on Pb, Cr and As translocation from plant roots to shoots was plant species dependent. Unexpectedly, significant increases to plant

germination, survival, biomass, and metal uptake were observed with *Pleurotus ostreatus* inoculation. Study results suggest that longer timelines are necessary for Pb reductions in highly contaminated brownfield soils using plant-fungi combinations. Regional pilot-scale studies of the best plant-fungal treatment combinations could be explored over longer timeframes for in-situ brownfields bioremediation.

2. Introduction

Contaminated sites are sites at which hazardous substances occur at concentrations above background levels and where assessment indicates it poses or is likely to pose an immediate or long-term hazard to human health or the environment (USEPA, 2017). Brownfields are a subset of contaminated sites defined as "properties, the expansion, redevelopment, or reuse of which may be complicated by the presence or potential presence of a hazardous substance, pollutant, or contaminant." (EPA, 2017). Brownfields usually contain complex mixtures of organic and inorganic compounds, rather than a single metal or type of contaminant (Wuana, 2011; Ajmone-Marsan, 2010). There are at least 1,319,100 contaminated sites across the United States covering an estimated 22 million acres nationally (USEPA, 2017; Kiaghadi, 2021; Simons, 1998). California is thought to host ~150,000 contaminated sites covering 96,000 acres, a startling $1/10^{th}$ of the state's total land acreage, making it the US state with the second highest number of contaminated sites, after New Jersey (USEPA, 2017; Brodsky, 2022). The prevalence of brownfields is a public health hazard because people living near contaminated sites can be exposed to hazardous contaminants through entrained dust, air, surface water, and groundwater (USEPA,2017; Brender et al, 2011).

Chronic, low-dose exposure to common brownfields contaminants can lead to decreased immunological function, increased risk of certain cancers, kidney damage and endocrine disruption (Järup, 2003; Jaishankar, 2014; Järup, 2009; Humans, 1993). A recent study found that living within one mile of a contaminated site is correlated with decreased life expectancy by as much as 1.22 years in areas that are highly

socioeconomically disadvantaged, and that ~17% of the U.S. population, including 18% of all children younger than five live within that range (Kighadi, 2021). Both racial and income disparities have been documented with respect to the communities living in proximity to brownfields: Black and Latina/o/x people and residents of low-income households are disproportionately burdened (Aelion et al., 2013; Jones et al., 2009; McClintock, 2012; Mielke et al., 2017; Rothenberg et al., 1996; White et al., 2016). A recent scoping review found that ~34% of Superfund sites are categorized under 'Native American Interest' by the US Environmental Protection Agency (USEPA), while Native American people make up only 2.9% of the total population (Chong, 2022).

The goal of any remediation effort is to reduce concentrations of the contaminants of concern to below screening limits for the intended future land use and in doing so, mitigate risk of human exposures. The types of contaminants present on a brownfield depend on the industry that previously operated on the site. The EPA's Brownfields Case Study report (1999) cited petroleum hydrocarbons, lead, construction debris (lead paint or asbestos containing materials), polychlorinated biphenyls (PCBs), treated wood (creosote, cadmium/chromium/arsenic), industrial chemicals, and diesel fuel as the most common. Organic contaminants (carbon-based, i.e., diesel, solvents, etc.) can be degraded through microbial metabolism, and potentially have their concentrations or toxicity reduced, whereas most metal contaminants (i.e., Pb) cannot be degraded and must be removed from or stabilized in soils.

The rate of remediation of contaminated sites is slowing down and on average one contaminated site is cleaned up per year in the entire US (since 2016), as compared to

eight per year between 2006-2015 and 24 per year between 1995-2005 (EPA, 2021). The cost of remediation is a commonly cited reason for the persistence of these sites (Elias, 2003). The EPA suggests that soil remediation can cost between $300-$3,000/cubic yard on average, depending on the scale, type and extent of the contamination and other site-specific factors (CLU-IN, 2000). As a result of this high cost, many brownfields within urban, residential areas can remain untreated for decades, creating a public health risk and exacerbating environmental injustice (CalEnviroScreen, 2021). Excavation and disposal ("dig and dump") is the most common method of remediation which involves physical removal and replacement or isolation via capping and covering of contaminated soil (Sharma, 2018; USEPA, 2021); on average, this method of soil remediation costs $504 USD/cubic yard, however, soils containing contaminant concentrations higher than industrial screening limits can cost 10 times more to dispose of (USEPA, 2017; Mielke, 2016; Lee et al, 2004). In addition to the financial costs of excavation and disposal, there are significant environmental and social costs (considered "externalities" in economics) to this method (Sparks, 2003; Khan, 2000). Excavated contaminated soils from California are typically trucked to Utah, Nevada and Arizona and become a major contributor to landfills (DTSC, 2017). Many of these contaminated soil and hazardous waste landfills are located on tribal reserves due to loopholes in the Resource and Conservation Recovery Act of 1967 (Sitkowski et al., 1991; Stetson et al., 1993; Wilson et al., 1990).

Bioremediation utilizes fungi, bacteria, and/or plants to extract or stabilize metals and degrade organic contaminants can serve as alternative strategies to excavation and disposal remediation methods. Bioremediation is a promising alternative to conventional

remediation which can be done *in situ*, removing the need for soil excavation thus decreasing exposure to contaminated soils during excavation and transportation, providing significant cost savings, as well as additional environmental and social co-benefits (Sparks, 2003; USEPA, 1996; Jabeen, 2009). For example, bioremediation can provide 50-80% cost savings compared to excavation and disposal methods and include environmental benefits such as the creation of soil on site (Jabeen, 2009; USEPA, 2001; Farraji, 2016).

Phytoremediation utilizes plants to accumulate metals (phytoextraction), stabilize metals (phytostabilization) or degrade organic contaminants in the soil (Henry, 2013; Banks, 2003; Khan, 2006; Garbisu, 2001). Plants capable of hyperaccumulating the metals Zn, Cd, Ni and As have demonstrated success in the field, whereas few plants capable of hyperaccumulating the metals Pb, Cu and chromium (Cr) have had variable success in the field (Van der Ent 2013). Some limitations of phytoremediation include remediation zone restricted to rooting depth, long time scales for thorough remediation (depending on the initial concentration), and the need to dispose of the contaminated plant material (Wuana, 2011; USEPA, 2001; Farraji, 2016). The most effective depths for phytoremediation are within the top three feet of soil, although for some plant species remediation of up to 10 feet below surface has been reported (Kennen, 2015; ITRC, 2009). The removal of metals from soil via phytoextraction can take multiple growing seasons or years depending on the starting concentrations, type of metal, and other factors (USEPA, 2001).

Most bioremediation research has focused on a single contaminant at a time, but most contaminated sites contain multiple contaminants. The remediation of mixed-contaminated soils, with both metal and organic contaminants, presents challenges such as the inhibition of plant and microbial growth at elevated metal concentrations (Dong et al., 2013) or the need for sequential treatment (Lu et al., 2010). Research to date has not systematically considered the potential benefits of native plants for phytoremediation (GOA, 1999). Use of native plants growing on contaminated sites may be effective due to being acclimated to local environmental conditions. Drought tolerance and dense, deep root systems adapted to the seasonal rainfall of the Mediterranean climate make native plants in southern California potentially effective candidates for local phytoremediation (Hellmers, 1955; Negri 2003), however, California native plant remediators had not previously been identified nor tested in field conditions.

Mycoremediation uses fungi to degrade organic contaminants, facilitate metals stabilization or enhance phytoremediation. Although fungi play major roles in ecosystem processes and biogeochemical cycles relevant to remediation of organic and inorganic contaminants in soils, the potential for employing fungi in remediation remains unknown (Harms, 2011). Several decomposer fungi which metabolize lignocellulolytic materials for their carbon source are also capable of enzymatically degrading complex organic contaminants. For example, *Pleurotus ostreatus* (Oyster mushroom) has been shown to efficiently degrade diesel, solvents such as trichloroethylene and perchloroethylene, and other organic contaminants (Harms, 2011; Thomas, 2004). Some of the challenges of utilizing decomposer fungi for remediation include difficulties accessing or producing

inoculum, competition with native soil microbes, and the need to maintain conditions suitable for fungal growth (e.g., suitable moisture content, additional substrates, etc.) (Akhtar, 2020).

Arbuscular mycorrhizal fungi (AMF) are a type of plant-root associated fungi that may enhance metal phytoremediation. AMF associate with ~80% of all plants worldwide in obligate, symbiotic relationships (Turnau, 2002; Giasson, 2008). Mycorrhizal colonization improves plant establishment and survival particularly in adverse conditions such as in low fertility and arid soils and increases plant tolerance to stresses (Allen, 1996; Allen, 1991). Contaminated sites are generally poor in nutrients and contain a highly altered soil structure, thus mycorrhizal fungi can play an important role in plant establishment for phytoremediation (Giasson, 2008; Turnau, 2002). In addition, these mycorrhizae have been shown to protect plants from harmful effects of excess metals (Diaz, 1996; Khan, 2000; Khan, 2006; Shi, 2019; Killham, 1983). Although a vast number of publications on the effect of AMF on the uptake of metals by plants is available (Joner, 2000; Gonzalez-Chavez, 2002; Diaz, 1996; Li, 2018; Khan, 2006; Khan, 2000; Krishnamoorthy, 2015; Tan, 2015; Turnau, 2002; Yang, 2015; Xavier, 2002; Chaudhry, 2002; Chen, 2007; Chen, 2003; Gildon, 1983; Wu et al., 2010 and others), the majority of studies were carried out in a laboratory or greenhouse setting in agricultural crops. The effect of AMF on metal uptake in plants under field, phytoremediation conditions is currently poorly understood and AMF impact on plant uptake of metals under drought conditions has not been investigated.

The joint implementation of bioremediation methods using plants (phytoremediation) and fungi (mycoremediation) has been shown to be advantageous (Camenzuli & Freidman, 2015; Harms et al., 2011; Juwarkar et al., 2010; Robichaud et al., 2019; Thomas et al., 2009). For example, synergistic bioremediation mechanisms have been established between plants and fungi to accelerate petroleum-hydrocarbon degradation, while reducing soil metal concentrations (Asemoloye et al., 2017; Robichaud et al., 2019). Combining phyto- and myco-remediation is termed phyto-mycoremediation. These joint methods of biological remediation of mixed contaminants are promising, but few have been tested in field conditions in semi-arid and arid regions, and no field studies of phyto-mycoremediation had been undertaken in Southern California. Soil bioremediation in hot and arid environments is particularly challenging because water is a limiting resource, which can lead to slow production of biomass for phytoextraction of metals or degradation of organic contaminants.

This study evaluates a novel bioremediation approach using California native plants with fungal treatments including commercial AMF inoculum and *Pleurotus ostreatus* inoculum on three brownfields containing elevated concentrations of organic and inorganic contaminants in Los Angeles under irrigated and unirrigated water regimes. Specifically, we investigated the introduction of AM and decomposer fungi inoculum on Pb, As, Cr and Cd uptake in four California native plants under droughted and irrigated field conditions on three contaminated sites (i.e., brownfields) in a semi-arid climate zone. Organic contaminant (diesel and gasoline-range organics, solvents, and polycyclic aromatic hydrocarbon) degradation was also monitored. To our knowledge, it is the first

study of phyto-mycoremediation in Southern California carried out on brownfields that incorporates fungi and native plants in partnership with community groups, city, and regulatory agencies.

We hypothesized that combined plant-fungal treatments would have higher uptake of metals than individually and that native plants would have relatively higher uptake of metals than non-native plants when grown in mixed-metal contaminated soils under droughted (unirrigated) field conditions. We also hypothesized that translocation of Cr would be increased under drought (non-irrigated) conditions, whereas AMF-mediated As translocation into plants will increase under irrigated conditions and that uptake of divalent cationic metals (Pb and Cd) will have variable response to alterations in soil moisture.

3. Methods

This study was carried out as a community-academic-governmental research partnership with guidance from the Health Disparities Research (HDR) Center at UC Riverside. The research questions and study design were informed by community, tribal, regulatory, and governmental agency stakeholders to address gaps in remediation methods for urban brownfields. Methods of community and stakeholder engagement and research partnership development, including regulatory (Department of Toxic Substances Control (DTSC)) and local governmental requirements, are outlined in an addendum at the end of this chapter.

3.1 Field Site Characterization

3.1.1 Initial Site Characterization and Field Site Descriptions

All sites had been characterized through EPA Phase I and II Assessments. This data provided a starting point in the identification of contaminants of concern, their depth, and extrapolated concentrations across each site (Ninyo and Moore, 2010; CLA BOE, 2019; EPA, 2017).

Three brownfields in the greater LA area were included in the study: two City-owned sites (Taylor Yard and SW) and one community-land-trust-owned site (LA Ecovillage). The density of residential housing, schools, and community spaces proximal to these long-standing contaminated sites, and the planned redevelopment of these brownfields necessitates remediation that reduces exposure to soil contaminants to below screening limits. These sites are all located in neighborhoods with demographics containing majority Latino/a/x, Black, and other historically disadvantaged groups, and are simultaneously low-income neighborhoods (California EPA, 2018).

Taylor Yard, G2 Parcel (34.0990 -118.2375) soils contain high levels of lead (320 - 25,000 mg/kg), and arsenic (12 -16 mg/kg); as well as high levels of diesel (>25,000 mg/kg) and solvent co-contamination. The site is 100 acres in size along the LA River in Northeast LA and is a former Union Pacific railyard. It is planned to be developed into a park (City of LA, Bureau of Engineers, 2021). Remediation activities are regulated by DTSC.

LA Ecovillage, Songs Property (34.072651507669214, -118.29065692967363) soils contain low levels of lead (85 - 300 mg/kg), arsenic (6 - 19 mg/kg) and cadmium (10 - 15 mg/kg), high levels of copper (6,600 mg/kg) and low levels of diesel and PAH co-contamination (EPA, 2017). This site is ~ ¼ acre in size located in Koreatown. It was formerly an autobody shop and is planned to be developed into a community hub with tiny houses and urban agriculture (USTU board, personal communication). On site remediation activities are regulated by the LA Fire Department.

Slauson and Wall (SW) (33.98762527296143, -118.27117757601722) soils contain high levels of hexavalent chromium (Cr(VI)) (1,100 mg/kg) and lead (1,200 mg/kg), as well as low levels of diesel and high levels of solvent co-contamination. It is a former site of the U.S. Electrical Manufacturing Company and has been occupied for manufacturing, metal plating and polishing, furniture finishing and vehicle repair (AECOM, 2019). The site is 7 acres in size and located in south central LA. It is planned to be redeveloped into housing with green space, a new community center, and a shopping center (LASAN Brownfields Program, personal communication).

3.1.2 Fungal identification in plots and inoculum collection for study

Indigenous fungi present in plots prior to treatment were determined amplification and sequencing targeting the ITS2 region of the rRNA gene at ZymoBIOMICS (Irvine, CA). The supplier for commercial arbuscular mycorrhizal inoculum blend was

Mycorrhizal Applications (MycoApply Endo, OR). Smallhold (Vernon, CA) provided the spent oyster mushroom (*Pleurotus ostreatus*) blocks for the decomposer fungal treatment.

3.1.3 Plant seed selection, collection, and preparation

Phytoremediator plants were chosen based on the following criteria: 1) is a native plant found growing on field sites that can uptake metals of concern and 2) plants known to form arbuscular mycorrhizal associations. Native seeds were purchased from S & S Seeds (Carpentaria, CA). Seeds were used rather than transplants because of the reduced cost, applicability, and scalability to larger sites, and because sowing seeds avoids possible dilution of contaminated soil or introduction of microbial populations. The known dryland remediator plant, *Chrysnopogon zizanioides* (Vetiver grass), was purchased as slips from Drylands Farming Company (Carpentaria, CA). In addition to the three main plants tested in a full factorial design, two other California native plants were tested: *Baccaris salsifolia* (n = 18) only at Taylor Yard, and *Encelia californica* (n = 18) only at the SW site; transplanted as 1-gallon seedlings from Theodore Payne Foundation (Los Angeles, CA).

3.2 Experimental design

Field plots were laid out in a factorial, completely randomized design at each of the three study sites, with treatments completely randomly assigned across plots. Factors include four plant treatments, five fungal treatments, and two irrigation regimes (Figure 1). Six replicates of each treatment were installed per site, resulting in 144 plots per study site. The SW site also had a Cr(VI)-impacted area in which 44 plots were installed.

Within the Cr(VI)-impacted area, all treatment combination were included but only with irrigation and no drought treatment was tested. This resulted in a total of 188 plots at the SW site. Taylor Yard had two additional treatment types separate from the other plots: *Salix sp.* with AMF and *P. ostreatus* mulch.

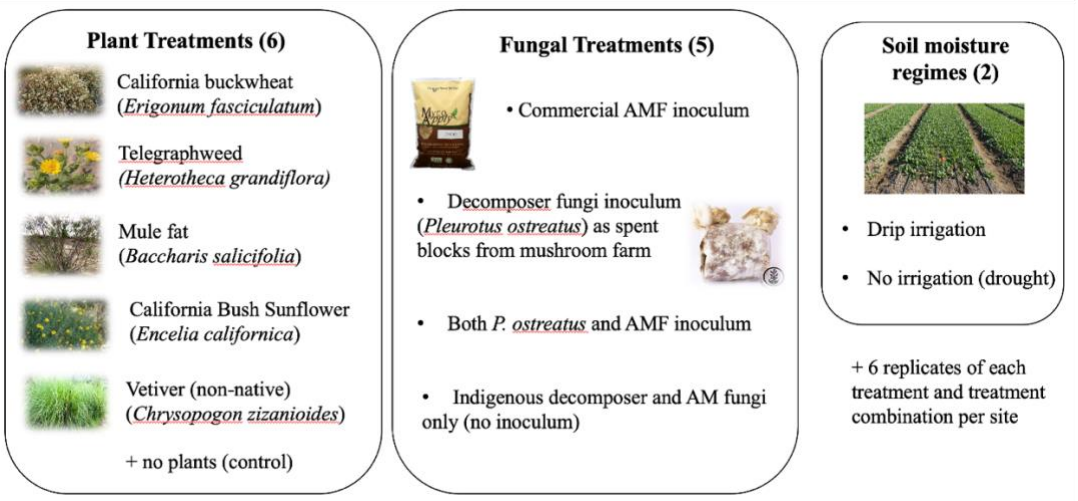

Figure 1. Experiment design for the phyto-mycoremediation study. Was run as a full factorial design at three field sites.

3.3 Installation of field plots

Study plots were installed between December 15, 2021, and March 15, 2022, and were maintained until de-installation and final sampling in May and early June 2023. Plots at Taylor Yard and SW were 3 ft x 3 ft in size, and plots at LA Ecovillage were 2 ft x 2 ft in size. Plots were marked with flags in each corner and tagged with their plot number (corresponding to a treatment). 2-3 feet wide aisles were established between plots to minimize the possibility of inoculum spreading between plots and contaminating them and to provide pathways to walk and sample in the study area. Aisles were treated with several inches of woodchips to prevent contaminated dust from being entrained in

wind and blown from the study area. Drip-line irrigation was installed at each site with connectors to pipe without holes used for plots without irrigation. At LA Ecovillage, the irrigation system was connected to a tap on site and at SW the irrigation system was connected to large cisterns filled by a pipe and pump to a nearby fire hydrant. The irrigation system at these two sites was run by a timer three times per week for two hours to supply irrigated plots. At Taylor Yard, irrigation was set up in the same way except was water was supplied by a water truck provided by the Korean Youth Community Centre twice per week. The drip irrigation system delivered 2.5 cm irrigation weekly to irrigated plots.

Seeds were sown at three times the recommended sowing rate for each species since seedling germination can be inhibited in highly contaminated soils: *Eriogonum fasciculatum* was sown at 0.104 g/ft^2 and *Heterotheca grandiflora* at 0.052 g/ft^2. Two *C. zizanioides* slips were transplanted per plot, one *E. californica* seedling per plot, and two *B. salsifolia* seedlings per plot. AMF inoculum treatments were implemented by mixing seeds and inoculum together in pre-weighed seed packets before sowing or poured in the bottom of the hole for transplants, at three times the recommended inoculation rate (1.229 g/ft^2) to increase chances of inoculation success in toxic soils. 10 lbs of *P. ostreatus* mycelium was added per 4 ft^2 of soil by mulching on the soil surface (when in combination with a plant treatment) or layered into the soil with woodchips.

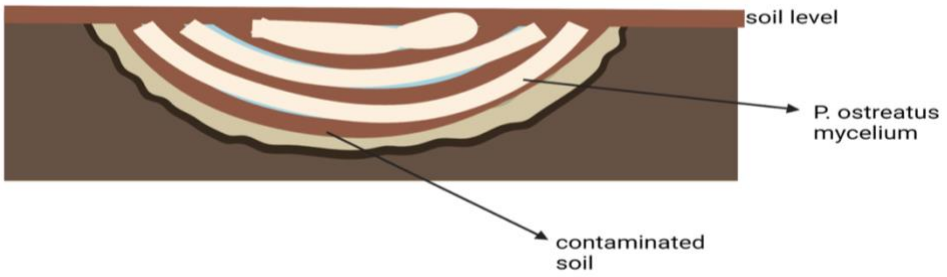

Figure 2. Cross section of mycoremediation treatment. *Pleurotus ostreatus* mycelium was layered with contaminated soil and thin layers of woodchips at a ratio of 1:10 mycelium to contaminated soil by mass. This was one of two methods for inoculating using the decomposer fungal treatment which involved digging the soil trench-style.

3.4 Methods for Monitoring, Sampling and Analysis

3.4.1 Solid phase analyses

3.4.1.1 Plant Growth Evaluation, Sampling, and Plant Tissue Analyses

Seed germination, survival, height was measured, and plant coverage of each plot was visually estimated monthly throughout the study period. Spontaneous growth of other plants and fungi on the treatment plots was visually assessed with percentage coverage of each plot noted, identified, and sampled for metals concentrations before removing. Every three months, small samples were taken at random from plants above-ground parts in each plot, labeled by plot, and dried for storage. *H. grandiflora* was harvested whole once its lifecycle was completed (~ 6 months) and began to dry so that plant materials would not fall over and blow away.

At the end of the experiment (~12 months), all plants were harvested completely, including the roots. Plant height and the length of roots and shoots were measured and recorded. Plants were washed to remove dirt particles; roots were scrubbed as needed to

remove soil. Plants were then weighed, differentiating between aboveground (stems and leaves) and belowground (roots) parts of plants, and biomass determined. After measurement, small subsamples of the roots and shoots were taken from each plant into labeled paper bags and brought back to the lab. After the fresh weight of shoots and roots was recorded, they were labeled by plot number and dried under sunlight for 72 hours and re-weighed to determine dry weight. Dried plants were then bagged and stored. After being oven dried at 60^{o}C for 3 days, plant samples were ground using a Geno/Grinder plant tissue homogenizer using stainless steel balls, and microwave digested following EPA method 3052. Briefly, 0.45 g of dried ground plant and 5 ml HNO_3, 3 ml H_2O_2, and 2 ml HCl was added to the microwave vessel. Digestate (~8.5 ml) was then poured into 50 ml polypropylene tubes and diluted to 50 ml with DDI and filtered using 0.22-μm filters. 6 ml of digestate was then acidified with 2% with trace metal grade HNO_3 and further diluted to reduce total dissolved solids before analysis using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700, Carpinteria, CA).

Metal accumulation by plant shoots and roots was estimated by multiplying tissue metal concentration (μg/g) by tissue dry weight (g). Biological concentration factor (BCF) was calculated for each metal(loid) of concern as the ratio of metal concentration in plant tissue (total metal concentration for each metal in the roots and shoots) to metal concentration in soil for each plot (Marchiol et al., 2013). Translocation factor was calculated as the ratio of the total concentration of elements in the shoot tissues or aerial parts of the plant to the concentration in the root tissues (Quihang et al, 2011). Translocation factor for each plant was calculated because of its use in phytoextraction

where metals are translocated from soil > root > shoot aboveground and can then be removed from the soil.

3.4.1.2 Soil Sampling and Analyses

Surface soil (0-30 cm) was sampled in each plot prior to treatment using a steel telescoping auger. Three soil samples within each plot were taken and composited. Soils were extremely compacted, and it was not possible to sample deeper than 30 cm at any of the sites. At Taylor Yard, a generator and hammer drill were needed to sample at a depth of 30 cm due to compaction. Before each sampling, auger, buckets, and sampling tools were decontaminated with deionized water and 70% ethanol and cleaned with soap and water between sites. Three types of samples were taken in each plot including 1) samples for microbial sequencing which were deposited into sealed bags (Whirl Pak) and stored on dry ice immediately until storage at -80^{o}C until DNA extraction; 2) bulk soil samples for metals concentration measurement which were deposited into Ziplock bags, homogenized within the sealed bag, and stored at 4^{o}C until analysis; and 3) organic contaminant samples which deposited into amber glass vials with PTFE caps and stored at 4^{o}C until shipment to CLS Laboratories (Grand Terrace, CA) for analysis.

Soil samples were taken from each plot every three months during the study period for organic contaminant and metal concentrations and other chemical and physical properties. At the end of study when treatments were removed, soils in each plot were sampled with the same method as initial sampling for microbial community analysis, organic contaminants, and bulk soils for soil physical and chemical analyses.

3.4.1.3 Methods for Soil Contaminant Analyses

All analyses were completed at UCR laboratories unless otherwise stated. EPA Method 3060A (Alkaline Digestion for Hexavalent Chromium) was used to solubilize Cr(VI) from soil samples stored at 4^{o}C until processing, followed by analysis using diphenyl carbazide colorimetric procedure (EPA Method 7196). A $MgCl_2$ phosphate buffer was added to suppress oxidation due to the expected higher than LOD Cr(VI) concentrations. Samples were run in triplicate, and data was only used if spike recovery was greater than 85%. EPA Method 6200 was used to determine soil total elemental concentrations using a benchtop X-ray fluorescence (XRF) spectrometry (SPECTRO, Model XEPOS03HE) in air-dried soils sieved to 2 mm and ground. Analysis of organic contaminants in soil (diesel range and gasoline range organics, polycyclic aromatic hydrocarbons (PAHs) and solvents) were carried out at outside laboratories (Torrent Labs for samples at 3, 6 and 9 months (Milpitas, CA) and CLS Labs for T0 and Tfinal samples (Grand Terrace, CA) using EPA Methods 3510B, 5030 and LUFT-HPLC for sample preparation and EPA Method 8260M, 8260B, and 8310 for analysis.

3.4.1.4 Methods for Soil Physical and Chemical Analyses

Soil moisture and temperature in each pot were taken using TM5 soil temperature and moisture probes and recorded with a EM50 data logger (Meter Corp, San Francisco, CA) at a depth of 6 inches. Soil texture was determined using the LS-13-320 Grain Size Analyzer (Beckman-Coulter) after hydrogen peroxide digestion of organic carbon and deflocculation with SHMP in each pot. Soil organic C, N and C/N isotopes were assessed

using elemental analyzer (Costech ECS 4010-Delta V Plus IRMS, Valencia, CA). Water-extractable organic carbon (WEOC) was extracted in DI water using the method outlined in (Homyak et al., 2018) by shaking soil samples for three hours followed by centrifugation and quantified using the TOC-VCPH Total Organic Carbon Analyzer (Shimadzu Instruments, Columbia, MD). Soil phosphate (PO_4) concentrations were measured using the Olsen sodium bicarbonate extraction method (Olsen & Sommers, 1982; Olsen, 1954), nitrate (NO_3^-), and ammonium (NH_4^+) were extracted from soils with 2.0 M KCl solution (Maynard et al., 1993). PO_4, NO_3^-, and NH_4^+ concentrations were measured using the Alpkem Flow Solution IV Automated wet chemistry system.

3.4.1.5 Methods for Arbuscular Mycorrhizal Fungal Analyses

Mycorrhizae were assessed within all planted plots (including uninoculated controls) by collecting a small sample of the roots adjacent to the base of the plant in each plot, effort was taken to not disturb the plant. Root samples were collected at month six. Dried roots were placed in cassettes, cleared in 2.5% KOH for 20 minutes at 90ºC, acidified in 1% HCl, and stained in 0.5% trypan blue for 20 minutes at 90ºC, and de-stained (rinsed) in tap water (Kormanik & McGraw, 1982). The fine roots capable of supporting arbuscular mycorrhizal infection were highly variable between samples. Many roots were suberized, and in others, only a single or a very few fine roots were found in many samples. A variation of the root intercept method described by (Giovannetti and Mosse, 1980) was used. The entire fine root system was examined at 40x using a Leitz Labotlux II microscope to determine if the roots were suberized and identify fine roots

capable of hosting AM infection. Fine roots were then examined at 100x for infection structures, hyphae, arbuscules, or vesicles. Because of the limited fine root length in many samples, 50 intercepts were scored, but organized into three groups: 0 = no infection observed, 1 = trace infection (<10% of fine root intercepts), 2 = moderate infection (10-50% of fine root intercepts), and 3 = highly infected (>50% of fine root intercepts). Notes on other fungi observed such as *Pythium*, *Phytophthora*, *Olpidium*, and *Rhizoctonia*, were recorded.

3.4.1.6 Methods for Fungal Community Analyses

The PowerSoil Powerlyzer extraction kit (MO BIO Laboratories, Inc., Carlsbad, California) was used to extract DNA from three samples (~0.4 g/sample) (biological replicates) per treatment per to account for soil heterogeneity. The manufacturer's extraction protocol was followed, with the following modifications: a heated lysis step was added (25 minutes at 65ºC) before homogenization (Rubin et al., 2014). Samples were purified from residual contaminants with the PEG-bead protocol using a 1:2 sample: bead ratio (Rohland & Reich, 2012). DNA concentrations were determined by fluorescence using the DS-11 nanodrop (DeNovix, Wilmington, DE, USA) and each sample was standardized to ~10 ng/μL. After extraction, DNA was stored at -20 ºC until further analysis. DNA extracts were then delivered on dry ice to ZymboBIOMICS (Irvine, CA) for amplification and sequencing. The fungal library was prepared by targeting the ITS2 subregion using custom fungal primers in which PCR reactions were performed in real-time PCR machines to control cycles and therefore limit PCR chimera

formation. The final PCR products were quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned with the Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), then quantified with TapeStation® (Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA). The ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research, Irvine, CA) was used as a positive control for each targeted library preparation. Negative controls (i.e. blank extraction control, blank library preparation control) were included to assess the level of bioburden carried by the wet-lab process. The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with 10% PhiX spike-in. Unique amplicon sequences variants were inferred from raw reads using the DADA2 pipeline (Callahan et al, 2016).

To measure the biomass of the viable microbial community, chloroform fumigation extraction method (Horwath & Paul, 1994) was used with modifications (Fierer, 2003). TOC and NPOC were then determined using a TOC-V Analyzer (Shimadzu). Microbial biomass (μg C/g) was calculated as the difference between extracted organic C from fumigated and non-fumigated soil samples.

3.4.1.7 Aqueous phase analyses

Sequential extractions following the method outlined in (Tessier et al., 1979) was used to quantify metals (Pb, As, Cd, Cr, Zn, Cu) associated with three fractions: water-extractable, exchangeable, and weak-acid extractable. Dissolved metals in extracts were analyzed using ICP-MS (Agilent 7700, La Jolla, CA).

3.5 Statistical Methods

All statistical analyses were performed in R Version 4.1.0 (R Core Team et al., 2023). Independence was met by randomizing plots at field sites. Levene's test for variance found that fungal treatment had unequal variance, but other treatments had equal variance. Analyses of covariance (ANCOVA) were used to test the differences between irrigated and non-irrigated soil moisture content on plant metal concentrations in plant species, differences between plant species in metal uptake, and differences between sites. When normality assumptions were not met due to several outliers, data was log transformed to reach the normality assumptions required by ANCOVA. A linear mixed-effects model fitted by the log transformed metal uptake data was used to compare differences in metal uptake between treatments and identify interactions between them. No interactions between treatments were seen, except between AMF alone and AMF plus *P. ostreatus* fungal treatment, which is expected. If a treatment had a significant effect, Tukey's HSD multiple comparison of means (Type III) was used for post-hoc determination of which treatment within each treatment category explained the results. Kruskal-Wallis's rank sum (non-parametric) tests were used to test the effects of fungal treatments on plant metal uptake because of the unequal variance observed for those treatments. Post-hoc tests were performed using a Wilcoxon test for each treatment pair, with a Bonferroni correction for multiple hypothesis testing. An alpha level of 0.05 was used, however, observances with p-values near 0.05 were noted. Differences in metal concentrations between T0 (before treatment) and at the end of the study (Tfinal) were tested using two-way ANOVA's and post-hoc analyses using Tukey's HSD. Soil

properties relevant to explaining variation seen between sites in soil metal concentration changes were analyzed using principal components analysis (PCA).

4. Results

4.1 Site Characteristics and Soil Physical and Chemical Properties

Soils at study sites were generally hot (19 - 40 °C) and dry (< 0.00001 - 1.49 m^3/m^3) on average, with seasonal variation. Taylor Yard was the hottest and driest site, SW was similarly hot and dry, whereas LA Ecovillage was cooler and wetter on average and experienced partial shading from buildings surrounding the property. While there were differences in mean soil moisture and temperature between sites, the difference was not statistically significant (p(temp) = 0.0982; p(moisture) = 0.4135). Soils at study sites had different texture classes: Taylor Yard is sandy; LA Ecovillage is loamy, and SW is a sandy loam. Mean clay content was variable (3 – 7.77 %) with LA Ecovillage soils having the highest clay content. Silt content was variable (16.42 – 39.80 %) with LA Ecovillage soils containing the highest concentrations of silt and SW and Wall site containing higher percentages of silt (25 %). Taylor Yard (79 %) contained the sandiest soils, although all sites were dominated by sand. Soil physical properties for each site are shown in Appendix 1.

The soil chemical properties with the highest variation between study sites were pH, soil organic carbon (SOC), and nutrient contents. Mean pH across at LA Ecovillage and Taylor Yard was circumneutral (6.53 – 7.49) but at SW was calcareous at 8.42. Mean SOC content at Taylor Yard and SW was low with an average of 1.71 and 1.63 %

respectively, as compared to LA Ecovillage which had a higher SOC of 6.6 %. Taylor Yard had a higher average extractable phosphate concentration in soils (12.73µg/g) relative to LA Ecovillage (5.10µg/g) and SW (1.78µg/g). Mean exchangeable ammonium concentrations were low (1.85µg/g) and mean exchangeable nitrate concentration was variable between sites: highest at SW (73.12µg/g) and LA Ecovillage (51.58µg/g) and very low at Taylor Yard (0.87µg/g).

Water-extractable organic carbon (WEOC) refers to the fraction of organic carbon compounds in soil that can be dissolved in water and is the carbon pool most available to microbes (Homyak et al., 2018). Mean WEOC was low, but similar, at all study (18.71-20.91 mg/L). The average C/N ratio at all sites was ~21, suggesting an equilibrium state between mineralization and immobilization of N. Mean CEC was highest at LA Ecovillage (10.77 meq/100 g), and lowest at SW and Taylor Yard (7.82 and 8.19 meq/100 g) although there was not a significant difference between sites. Soils had a wide range of salinity (determined by electrical conductivity, EC) and a mean EC of 218.94, although tended to be highly saline. Soil chemical properties for each site are shown in Appendix 1.

4.2 Starting Soil Metal(loid) Concentrations at Each Site

Mean soil metal concentrations at a depth of 1 foot prior to treatment for the study area of each site are shown in Table 1. LA Ecovillage study plots contained mean soil Pb (127.45 µg/g avg concentration; 539.7 µg/g maximum) and As concentrations (4.3 avg concentration; 14.4 µg/g maximum) elevated beyond the unrestricted screening

limits. SW and Taylor Yard study soils contained mean As, Cd, Cr and Pb concentrations higher than screening limits for unrestricted land use and upper ranges for those metals higher than industrial screening limits for soil. SW shallow soils contained concentrations of Pb as high as 1096 μg/g, As as high as 107.3 μg/g, Cd as high as 43.9 μg/g and total Cr as high as 109,200 μg/g plus hexavalent Cr (Cr(VI) as high as 23,500 μg/g. Taylor Yard shallow soils contained Pb as high as 713.9 μg/g, As as high as 38.2 μg/g, Cd as high as 28.9 μg/g and Cr up to 84,150 μg/g.

Table 1. Mean Pre-Treatment Soil Metal Concentrations in Surface Soils (0-1 ft deep)

Site	Cr (μg/g)	Cu (μg/g)	Zn (μg/g)	As (μg/g)	Cd (μg/g)	Pb (μg/g)
SW	1830.58	97.48	553.48	4.49	1.63	152.62
LA Ecovillage	49.57	86.86	255.59	3.32	0.86	127.45
Taylor Yard	1419.68	104.10	222.19	8.68	2.03	127.98

4.3 Soil Metal Availability

None of the metals of concern (Pb, As, Cd or Cr) were readily available based on sequential extraction results (Appendix 2); however, all metals tested can potentially be mobilized by weak acids (such as those excreted by plants and fungi) or if the soil acidity increases. Arsenic was particularly immobile relative to other metals of concern and was predominantly found in the weak acid fraction. Relative to other study sites, LA Ecovillage soils contained the most exchangeable Pb, weakly-adsorbed and can potentially be released by ion exchange (Filgueiras et al., 2002). Lead was most water-soluble at Taylor Yard, likely due to the low carbon content and sandy soils at that site

limiting adsorption potential and increasing soil water infiltration. SW contained the most available Cr relative to the other study sites: soil Cr was water-soluble and exchangeable (weakly adsorbed), probably because the Cr species at that site is Cr(VI) which is more mobile and available than Cr(III), the other form commonly found in the environment.

4.4 AMF Inoculation Success

Roots of all plants in inoculated as well as uninoculated plots were checked for AMF colonization after 6 months of growth. Although inoculated plots had a higher percentage of colonized roots (89.4% as compared to 78.5% in uninoculated plots), the difference was not statistically significant ($p = 0.838$). High rates of AMF root colonization observed in uninoculated plots is likely due to AMF spores (i.e., native AMF) that were already present in study plots. Statistical analysis proceeded with assessing the effects of AMF fungal treatment (i.e., inoculated or not inoculated) as well as the actual AMF colonization level measured in each plant's roots.

4.5 Plant Shoot Lead Concentrations after 6 Months of Growth

Mean Pb content in the plant shoots tested 6 months after sowing was as follows, from lowest to highest: *E. californica* 10.6 μg/g, *B. salsifolia* 12.38 μg/g, *C. zizanioides* 19.70 μg/g, *E. fasciculatum* 20.82 μg/g and *H. grandiflora* 65.80 μg/g. There was large variability seen in the Pb concentrations within plant species; although there were differences in mean shoot Pb concentrations between plant species tested, plant species was not a significant predictor of Pb uptake at 6 months ($p = 0.07622$).

Regression analysis determined that fungal treatment and irrigation significantly affected plant Pb uptake. Without irrigation, Pb content in plants decreases by an average of 4.972 μg Pb/g plant (dry weight). Of the fungal treatments, AMF inoculum significantly increased Pb uptake by an average of 8.1137 μg Pb/g plant, whereas no fungal inoculation (without AMF nor *P. ostreatus*) significantly decreased Pb uptake by an average of 6.9138 μg Pb/g plant.

Table 2. Regression results for *H. grandiflora* and *E. fasciculatum* shoot Pb after 6 months of growth (n = 64)

Treatment	*r*	p-value
Irrigation	0.1391	0.002701
Fungal treatment	0.1953	0.002465
Plant type	0.0396	0.07622

4.6 Results After 12 Months

4.6.1 Change in Soil Metal Concentrations

Despite biotic, abiotic, and environmental differences between the three brownfield sites, treated plots (with combinations of plants and fungi) exhibited reductions on average in the metals of concern (Pb, Cd, As, Cr, Cu and Zn) during the full study period (12 months) when compared to control (untreated) plots. These reductions were statistically significant for Pb, As and Cu (Table 3).

Table 3. Results for Change in Soil Metal Concentrations Before and After Treatment		
Metal(loid)	**Mean Change**	**p-value**
Pb	- 16.73 %	0.000661*
As	- 39.77 %	0.00011*
Cu	- 19.73 %	6.04 e-06*
Cr	- 60.13 %	0.168
Cd	- 33.95 %	0.451
Zn	- 19.78 %	0.168
Ni	- 30.99 %	0.837
All sites were pooled together for this analysis; n=260 treated and 260 control plots. *p=<0.05		

4.6.2 Soil Metal Concentration Changes by Treatment

When treatments with plants and/or fungi were grouped together (treated) and compared with un-treated plots (control), an average reduction in all metal concentrations was seen as compared to the controls, which did not change (or increased). These are especially pronounced for Pb and Zn, as seen in Figure 3. Irrigation significantly affected soil metal concentration changes: all treated and irrigated plots experienced reductions on average in the seven metals of concern, whereas treated but unirrigated plots experienced minimal changes in concentrations or small increases in average metal concentrations. The greatest mean reduction in soil Pb in treated soils occurred at LA Ecovillage (~50%), which also had the lowest mean starting concentrations of Pb compared to other sites. Instead, there was low (~1%) reduction in mean soil Pb concentrations at SW and Taylor Yard, which had higher average initial soil Pb concentrations than LA Ecovillage soils. This does not mean that Pb concentrations were not reduced in soils at these two sites, as statistically significant reductions occurred during the study period, however, these were not large concentration changes when averaged across 144 – 200 samples per site. The higher Pb reductions at LA Ecovillage may be explained by the more available Pb, where

extraction results showed relatively higher exchangeable Pb than other sites, along with slightly acidic soil pH (~6.4) which can enhance Pb desorption due to surface charge repulsion. LA Ecovillage also had the highest mean Cu (~33%) and Zn (~49%) reductions, and high As reductions (~45%). SW and Taylor Yard had much higher mean Cr reductions (92 % and 84% respectively) than LA Ecovillage. These sites also much higher exchangeable Cr as compared to LA Ecovillage, which may have contributed to its removal by plants.

Soil from control plots on average showed no change or increase in all metals of concern in both irrigated and unirrigated plots.

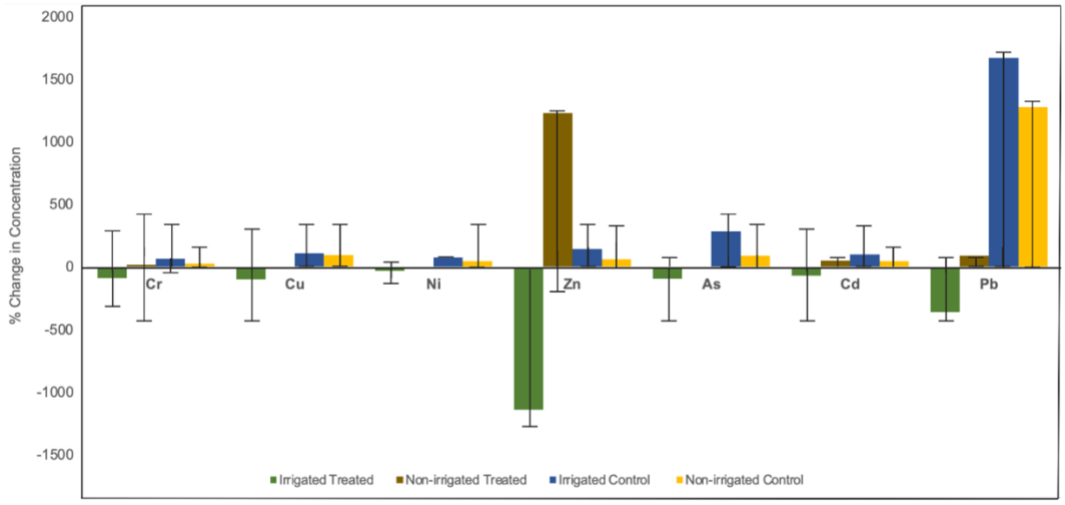

Figure 3. Average change (%) in concentrations of the metals of concern: Cr, Zn, Cu, As, Cd and Pb from pre- and post-treatment soils. Treated plots are grouped together "Treatment" and compared with untreated plots "Control" and within each treatment group, "Irrigated" and "Unirrigated" are compared. There were significant differences between treated and control plots for Zn, Cu, Cd and Pb but not for Cr and As and significant differences between irrigated and unirrigated plots for changes in metals concentrations.

4.6.3 Effects of Plant, Fungal and Irrigation Treatments on Soil Metal Concentration Changes

Plant treatment as significantly correlated with soil concentration changes for Pb and Cr, but not for As, except for the plant *E. californica*. Significant differences in metal concentration changes between sites were seen for all metals of concern, Pb, Cr and As (Table 4). Fungal treatment was not significantly correlated with soil As concentration changes, but was significantly correlated with soil Pb and Cr changes. Irrigation was significantly correlated with changes in Pb, Cr, and As soil concentrations with treatment. Regression results are shown in Table 4.

Table 4. Regression Results for Soil Pb, As and Cr Concentration Changes with Treatment (n = 430)

Metal	Treatment	*r*	p-value
Lead (Pb)	Irrigation	0.04767	<2 e-16
	Plant	0.06873	0.0199
	Site	0.1843	5.27 e-07
	Fungal treatment	0.01766	0.0757
Arsenic (As)	No irrigation	-0.415	0.018154
	Irrigation	-0.005601	0.000128
	Site	0.004464	0.01853
Chromium (Cr)	Fungal treatment	0.01766	<2 e-16
	Plant	0.6873	0.01452
	Site	0.1494	2.879 e-06
	Irrigation	0.04767	<2 e-16
	No irrigation	-0.7153	0.0000289

4.6.3.1 Soil Lead Concentration Changes

Soil Pb concentration reductions were observed at all sites (Figure 4). However, study sites were significantly different from one another in terms of soil Pb concentration

changes, which explained 18.5% of the variation observed (Figure 4). Fungal treatment was not correlated with soil Pb concentration changes overall, but AMF inoculation was positively correlated with soil Pb concentration changes ($p < 2e\text{-}06$), though not strongly correlated. No plant (control) plots were significantly different ($p = 0.00735$) from planted plots and experienced insignificant changes in soil Pb concentrations. Irrigation was negatively correlated with soil Pb concentrations, and significant differences were observed between irrigated and unirrigated plots in soil Pb changes.

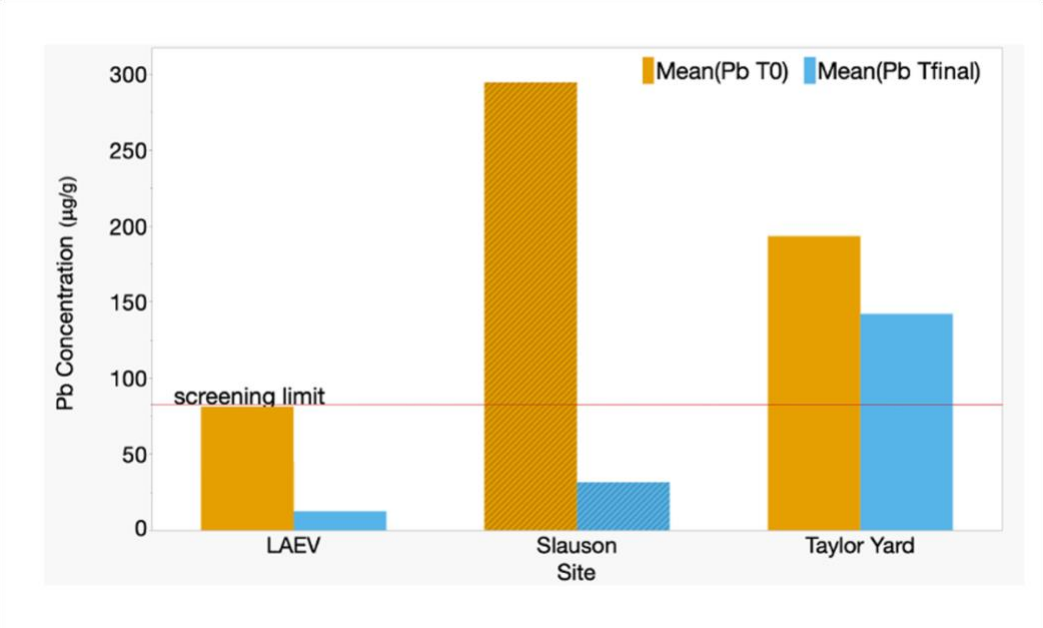

Figure 4. Mean soil Pb concentrations (n = 430) between 0 – 30.48 cm depth at T0 (pre-treatment) and Tfinal (post-treatment) for each study site. The screening limit shown is for unrestricted land use (80 µg/g).

4.6.3.2 Soil Arsenic Concentration Changes

Study sites were significantly different from one another with regards to mean soil As concentration changes (Figure 5). Non-irrigated plots had significantly lower

reductions in soil As concentrations as compared to irrigated plots. Plots with no plants (controls) had significantly lower reductions in soil As concentrations.

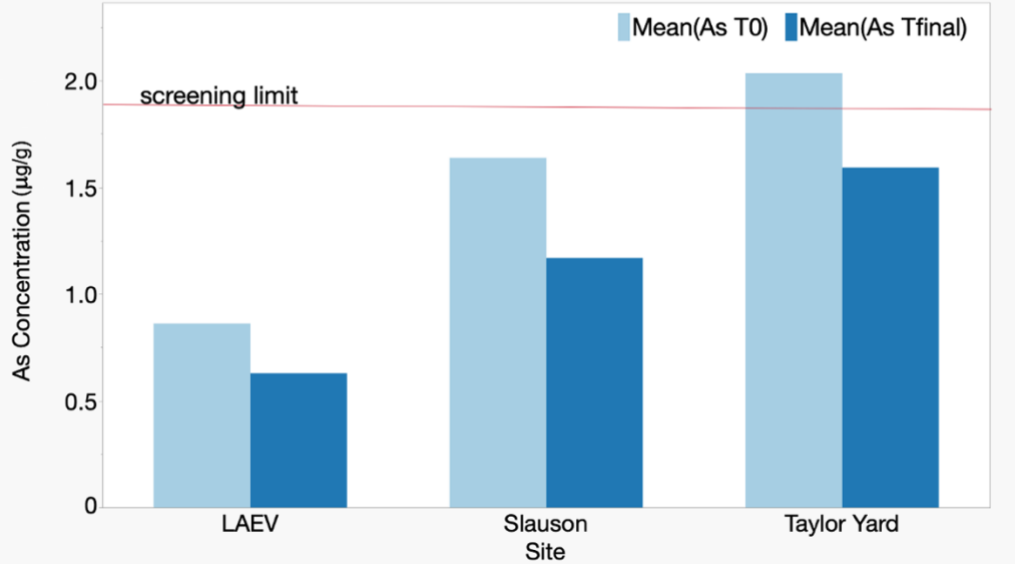

Figure 5. Soil As concentrations (n = 430) between 0 – 30.48 cm epth at T0 (pre-treatment) and Tfinal (post-treatment) for each study site. Screening limit shown is for unrestricted land use (1.9 µg/g) (DTSC, 2023). LAEV = LA EcoVillage, Slauson = SW.

4.6.3.3 Soil Cr Concentration Changes

Study sites significantly differed from one another in soil Cr concentration changes, and site explained ~15% of the variation observed (Figure 6). Taylor Yard experienced the least change in soil Cr concentrations during the study period. The SW site contained Cr in the hexavalent form and Cr(VI) concentration changes were analyzed separately for that site (Section 4.6.3.4). Fungal treatment, specifically AMF inoculation, significantly reduced soil Cr but was not strongly correlated with soil Cr changes. Irrigation significantly affected soil Cr concentrations, however, non-irrigated plots

experienced significantly greater reductions in Cr concentrations and was strongly correlated with soil Cr concentration changes, explaining 71.5% of the changes observed.

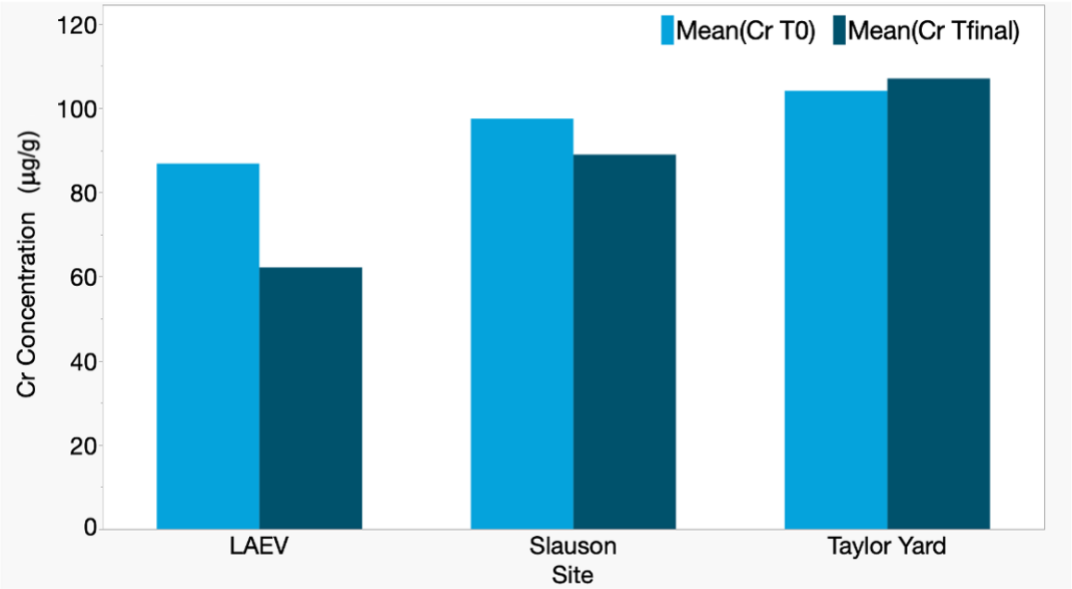

Figure 6. Mean soil Cr concentrations between 0 – 30.48 cm depth at TO (pre-treatment) and Tfinal (post-treatment) for each study site. The unrestricted screening limit is not shown because it is higher than the concentrations observed (DTSC, 2017).

4.6.3.4 Soil Hexavalent Chromium Changes at SW

SW was the only study site with known Cr(VI) contamination. Previous reports showed concentrations as high as 23,500 mg/kg (AECOM, 2017); exceedingly higher than the residential (0.29 mg/kg) and industrial (240 mg/kg) screening limits for Cr(VI) (DTSC, 2021). Significant reductions in soil Cr(VI) concentrations were observed after 12-months of treatment ($p = < 0.0001$) (Appendix 3), however, no significant differences between treatments (fungal treatments or plant types) were detected with regards to Cr(VI) removal from soils. California native plants grew very well in the chromium area, but non-native weed species did not. The native plants tested in the Cr(VI) area of the

SW site had a high bioconcentration factor (BCF) for chromium (Table 8), likely due the higher solubility and therefore accessibility of Cr(VI) to plants. There was an overall reduction in Cr(VI) concentrations throughout the study period, however, reductions were seen in controls (no treatment) as well as treated plots, likely via leaching due to the higher mobility and availability of Cr(VI) as compared to Cr(III).

4.7 Relative importance of soil properties within sites to explaining differences in soil Pb, As and Cr concentration changes between sites

Since soil metal concentration changes were significantly different between sites, principal components analysis was used to determine which soil physical and chemical variables within sites explained most of the variation seen between sites with changes in soil metal concentrations (Figures 7 - 9). Soil texture variables (sand and clay content) and carbon variables (total soil C and organic carbon content) explained most of the variation seen in soil Pb, As and Cr concentration changes.

Percentage sand explained 31.2 % and carbon content 27.8 % of the variance seen and positively influenced soil Pb concentration changes (Figure 8). Percentage clay explained 17 % and pH explained 6 % of the variance seen and negatively influenced soil Pb concentration changes. Soil Pb changes were strongly negatively influenced by weak-acid extractable Pb content, pH, and ammonium (NH_4) content. Soil Pb changes were strongly positively influenced by sand and silt content, organic carbon, total carbon, and nitrogen content.

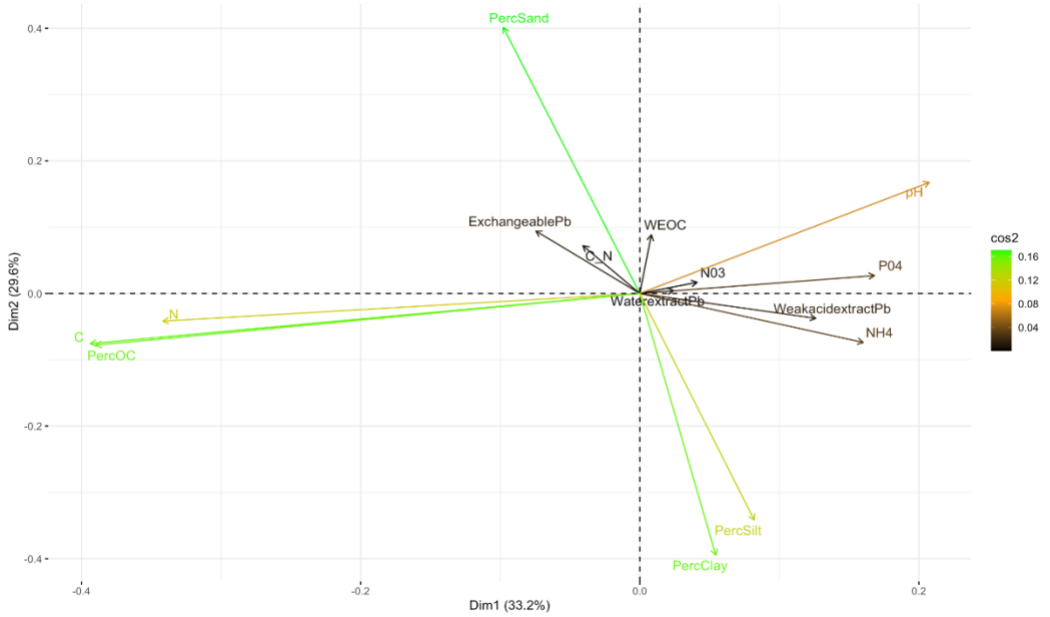

Figure 7. PCA plot of soil variables correlated with change in soil Pb concentrations within sites. Variables in green have high cos scores, those in orange have mid cos scores and those in black have low cos scores.

Percentage sand explained 36.5 % of the variance seen and negatively influenced soil As concentration changes (Figure 9). Percentage clay explained 26 % and carbon content 14.3 % of the variance seen and positively influenced soil As concentration changes. Soil As changes were strongly negatively influenced by weak-acid extractable As content, pH, and phosphate ($P0_4$) content. Soil As changes were strongly positively influenced by clay content, organic carbon, total carbon, and nitrogen content.

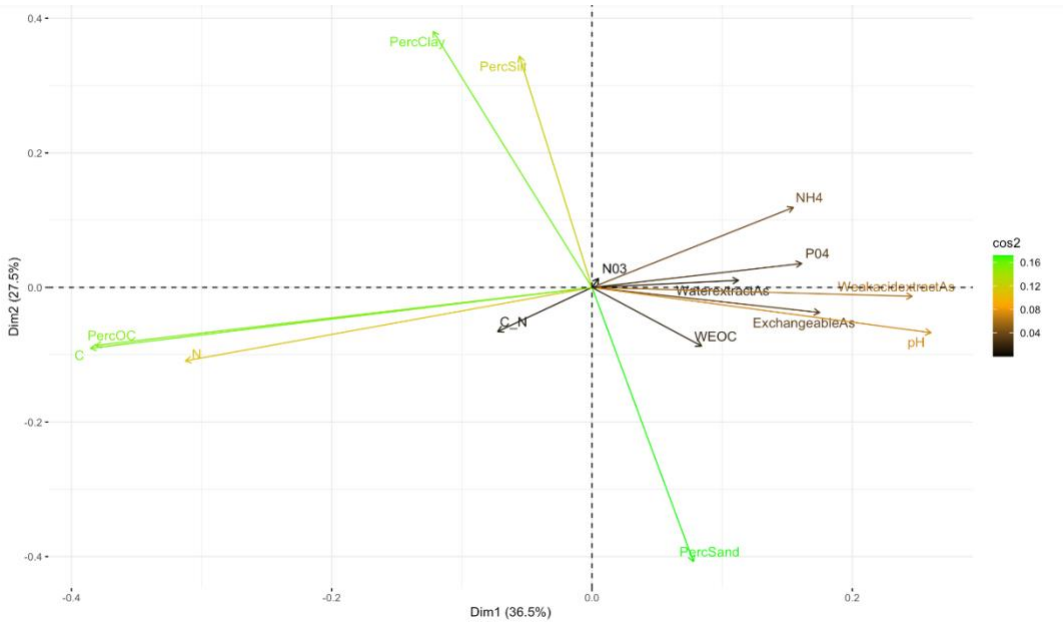

Figure 9. PCA plot of soil variables correlated with change in soil As concentrations within sites. Variables in green have high cos scores, those in orange have mid cos scores and those in black have low cos scores.

Soil carbon content explained 33.1 % and organic carbon content 15.7 % of the variance seen and positively influenced soil Cr concentration changes (Figure 10). Percentage sand explained 26.9 % of the variation seen and negatively influenced soil Cr concentration changes. Soil Cr changes were strongly negatively influenced by weak-acid extractable Cr content, pH, and sand content. Soil Cr changes were strongly positively influenced by clay and silt content, organic carbon, total carbon, and nitrogen content.

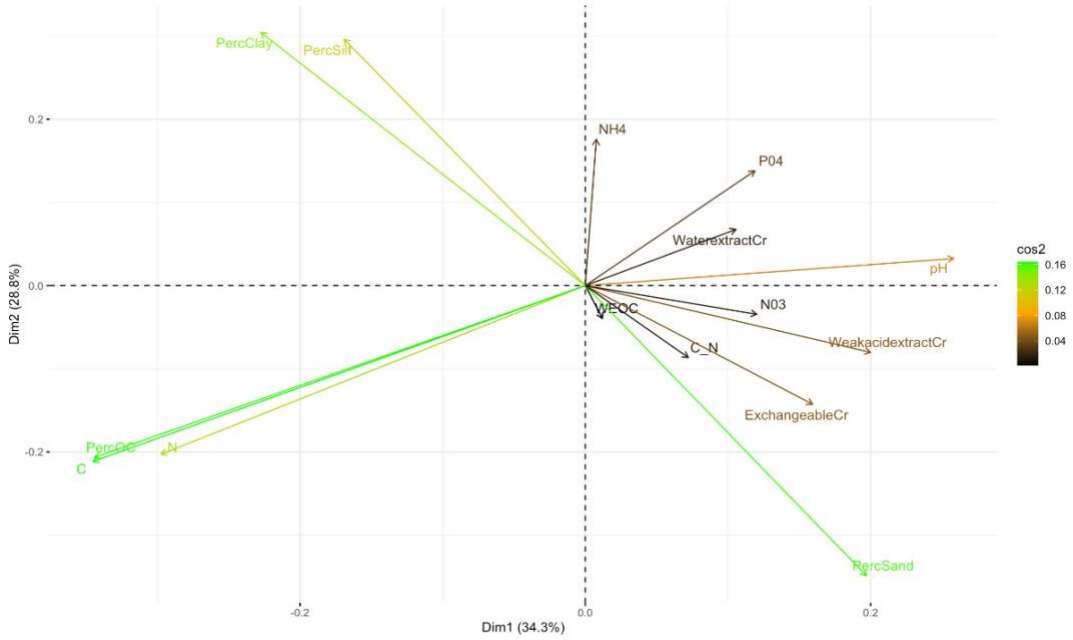

Figure 9. PCA plot of soil variables correlated with change in soil Cr concentrations within sites. Variables in green have high cos scores, those in orange have mid cos scores and those in black have low cos scores.

4.8 Plant Survival and Metal(loid) Uptake

4.8.1 Plant germination, survival, and biomass

Native plants in general had a much higher survival rate than non-native plants tested, and native plants that had been found growing on contaminated sites regionally (Chapter 2) had a much higher survival rate than those that were not found growing naturally in contaminated soils. Survival rate was measured as the number of plants (per species) that grew to harvest out of the number that were planted or sown. Survival rate can be affected by insect and rabbit predation, inadequate water or nutrients, soil compaction limiting rooting and inability to grow in the specific soil and environmental conditions of a site (i.e., toxicity, texture, etc.), and other factors. Vetiver grass, *C.*

zizanioides, transplanted as slips, is a drought-tolerant species with low nutrient requirements (Truong, P., 1999), however, it had a very low survival rate, even though it was planted at the ideal time of year and was irrigated during and after planting. Of the 146 vetiver slips planted, only 33 survived across all study sites, and at Taylor Yard, the hottest and driest site, zero survived of 60 planted; only irrigated vetiver survived. California buckwheat (*E. fasciculatum)* had the highest survival rate for a native plant across sites and irrigation regimes. It germinated and had a 100% survival rate at two of three study sites, and a 60% survival rate at the most challenging site, Taylor Yard. The agricultural species *Trifolium ciliotum*, *Helianthus annulus* and *Hordeum californica prostate* were sown and none of them survived (0% survival rate). This may indicate that these species are unable to tolerate the toxicity, soil moisture, low nutrient soils or other environmental conditions found on these brownfields. At Taylor Yard, some plots for each species germinated, however, none survived after germination, through a combination of heat waves and being eaten by rabbits. Mulefat (*B. salsifolia*) was only planted at Taylor Yard due to the site's proximity to the LA River. Mulefat and Willow are phreatophytes which typically grow in riparian areas and have at least part of their root system constantly in touch with water; thus, these plants were only tested at Taylor Yard, the site along the river (Robinson, 1958 and McCutcheon 2003). Mulefat had the highest survival rate of all the transplants (100%) (Table 5).

Table 5. Plant Survival Rate by Site ***Calculated by averaging 48-60 plots per site, a total of 144 -180 plots per plant				
Plant	**Planting Type**	**Site**	**Survival Rate**	**Mean Survival Rate**
C. zizanioides * (non-native known remediator)	Slips	SW	41%	26%
		LA Ecovillage	36%	
		Taylor Yard	0%	
E. californica	Seedlings (1-gallon)	SW	67%	67%
B. salsifolia	Seedlings (1-gallon)	Taylor Yard	100%	100%
E. fasciculatum *	Seeds	SW	100%	87%
		LA Ecovillage	100%	
		Taylor Yard	60%	
H. grandiflora *	Seeds	SW	82%	39%
		LA Ecovillage	25%	
		Taylor Yard	10%	

California bush sunflower (*E. californica*) produced the highest biomass on average per plant, more than double or triple the other plants tested, including Vetiver, known for being able to produce high biomass within one growing season (Truong, P., 1999). Rapid and high biomass production is desirable in a phytoremediation plant since it results in more rapid removal of metals from soils into the plant biomass. ANCOVA of log-transformed data revealed that plant type ($p = 0.0408$), site ($p = 0.0346$) and level of AMF root colonization ($p = 0.0543$) significantly

Table 6. Mean Biomass per Plant	
Plant	**Average weight (kg)**
Encelia californica	5.35
Erigonium fasciculatum	3.81
Heterotheca grandiflora	2.01
Baccharis salsifolia	2.69
Chrysnopogon zizinaides	1.41

affected plant biomass. No AMF root colonization resulted in lower biomass on average, whereas between 10-50% AMF root colonization resulted in the highest plant biomass (Appendix 4).

Table 7. Plant Biomass Produced by Plant and Site						
Site	***Heterotheca grandiflora***	***Erigonium fasciculatum***	***Chrysnopogon zizinaides***	***Encelia californica***	***Baccharis salsifolia****	**Total (kg)**
SW	127.61	184.50	453.64	101.65	268.6	461.81
LAEV	132.21	257.21	205.80	0	0	410.00
Taylor Yard**	400.00	812.60	0	0	537.20	135.38
All Sites	141.23	291.48	474.22	101.65	564.06	**638.19**
**Baccharis salisifolia* was only grown at Taylor Yard and was not harvested yet, so this is an estimate based on the one plant harvested						
*** *Salix sp*. were also grown at Taylor Yard but were not harvested during the study time period so are not included in this biomass calculation						

Phytoremediation is limited to the rooting depth of the plants used; therefore, the root length for potential phytoremediator plants is important. In the present study, *E. fasciculatum* (California Buckwheat) had the longest mean root length, at 23.4 inches, followed by *Baccharis salsifolia* (Mulefat) and *Encelia californica* (California bush sunflower), *H. grandiflora* (Telegraphweed) of 11.1 inches and lastly, *C. zizanioides* (Vetiver) at 9 inches.

4.8.2 Plant Accumulation of Soil Metals and Translocation to Shoots

The bioconcentration factor (BCF) indicates a plant's capacity to accumulate metals from the soil (Qihang et al., 2011), whereas a plant's ability to translocate metals from the roots to the shoots is measured using the translocation factor (TF). Both BCF and TF are important for assessing the feasibility of a plant species for phytoremediation

purposes: plants with a BCF>1 and TF <1 have the potential for phytostabilization, plants with a BCF and TF > 1 have the potential for phytoextraction (van der Ent, 1999). BCF and TF values for each plant tested are given in Table 8.

The present study showed that *C. zizanioides* has the potential for Pb phytoextraction, and the California native plants *E. fasciculatum* and *H. grandiflora* have the potential for Pb phytostabilization (on average). *H. grandiflora* had a higher average Pb BCF than the other plants tested, suggesting that it accumulates more Pb from soil but stores the Pb in roots rather than translocating it aboveground. *E. californica* had the highest translocation factor for As, and the three California native plants tested all met requirements for As phytoextraction, whereas the non-native plant *C. zizanioides* did not qualify as an As phytoextractor, but did as an As phytostabilizer. None of the plants tested had both a mean TF and BCF > 1 for Cr, but all plants tested had a mean BCF > 1 for Cr, suggesting that these plants are potential Cr phytostabilizers.

Table 8. Translocation Factor and Bioconcentration Factor by Plant

Plant *	Mean TF	TF Range	Mean BCF	BCF Range
Cr				
Erigonium fasciculatum **	0.526 ± 0.814	0.001 - 3.382	36.897 ± 162.52	0.119 – 1059.29
Heterotheca grandiflora **	0.522 ± 0.0306	0.029 - 0.085	20.122 ± 26.206	1.168 – 109.891
Encelia californica **	0.064 ± 0.803	0.005 - 3.632	11.732 ± 2.975	8.892 – 14.890
Chrysnopogon zizaides **	0.920 ± 0.389	0.483 - 1.227	17.208 ± 1.109	16.424 – 17.992
As				
Erigonium fasciculatum *	1.8950 ± 3.345	0.0858 - 19.34	1.1766 ± 2.755	0.135 – 11.948
Heterotheca grandiflora *	1.7103 ± 1.945	0.5219 - 4.367	2.639 ± 2.474	0.121 – 7.683
Encelia californica *	2.6175 ± 2.183	0.1037 - 7.881	1.146 ± 1.136	0.358 – 2.449

Chrysnopogon zizinaides **	0.5912 ± 0.599	0.2030 - 1.282	2.408 ± 0.732	1.890 – 2.926
Pb				
Erigonium fasciculatum **	0.550 ± 0.951	0.005 - 4.915	1.308 ± 1.228	0.136 – 4.418
Heterotheca grandiflora **	0.414 ± 0.062	0.014 - 0.135	6.3075 ± 19.753	0.204 – 87.624
Encelia californica	0.066 ± 0.736	0.0090 - 3.754	0.840 ± 0.603	0.265 – 1.468
Chrysnopogon zizinaides *	2.625 ± 4.107	0.191 - 7.368	4.319 ± 2.461	2.579 – 6.060
*Plants meeting both requirements for phyto-extraction for each metal are marked (BCF and TF > 1) ** Plants meeting requirements for phyto-stabilization for each metal are marked (BCF >1 and TF < 1)				

4.8.3 Effect of Fungal Treatments, Irrigation and Site Differences on Plant TF and BCF

While fungal treatment did not significantly affect overall BCF's, the level of AMF root colonization significantly affected the BCF for Pb, As and Cr. The level of AMF colonization was determined based on root staining and microscopic analysis of all plant roots in the study and is a more accurate measure of AMF activity than inoculation. No other treatment significantly affected the BCF of the metals tested. There were significant differences between sites for As but not Pb or Cr BCF. Fungal treatment and plant type significantly affected the TF for each of the metals of concern (Pb, As, Cr and Cd) but irrigation and level of AMF colonization did not. The only exception was that Cr TF was significantly affected by irrigation.

Table 9. Regression Results for Plant BCF and TF by Treatment
(n = 232)

Metal	Analysis	Treatment	*r*	p-value
Lead (Pb)	BCF	Level AMF colonization	0.315	7.188 e-06
	TF	Plant	0.05192	0.004302
	TF	Fungal treatment	0.02788	0.02389

Arsenic (As)	BCF	Level AMF colonization	0.04191	0.01312
	BCF	Site	0.1087	0.009953
	TF	Plant	0.1813	0.005334
	TF	Fungal treatment	0.02863	0.02204
Chromium (Cr)	BCF	Level of AMF colonization	-0.007867	0.04819
	TF	Fungal treatment	0.01698	0.07517
	TF	Irrigation	0.01306	0.08203
	TF	Plant	0.1076	9.908 e-06

4.8.3.1 Treatment Effects on Lead Bioconcentration and Translocation Factor

The most significant differences in Pb BCF were between plant roots with 0 and those with greater than 50% AMF colonization ($p = <1e-05$) (i.e., none and highest) and the highest level of AMF colonization and all lower levels (level 1= trace, 1-10%, $p = <1e-05$; level 2= 11-50%, $p = <1e-05$). Fungal treatment significantly affected Pb BCF ($p = 0.686$), with *P. ostreatus* inoculation increasing Pb BCF ($p = 0.0686$). Although plant type was not significant overall in metal BCF for the metals tested, despite their different concentrations of metal accumulation, *Heterotheca grandiflora* was nearly significantly higher ($p = 0.096$) for Pb BCF. Post-hoc analysis revealed that the highest level of root colonization by AMF (>50%) significantly increased the BCF for Pb ($p = 1.26e-06$) and explains ~35% of the variation seen.

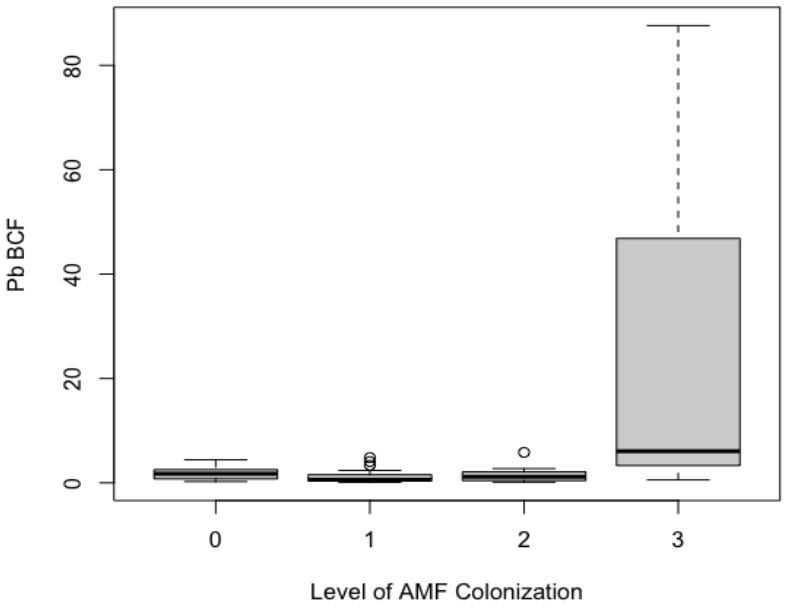

Figure 10. Levels of AMF root colonization and Pb BCF. Level 0 corresponds to zero AMF colonization, 1 to trace levels between 1-10 %, 2 to levels between 10-50% and 3 to colonization > 50%.

Plant type and fungal treatment but not site, irrigation or level of AMF colonization explained differences in plant Pb translocation to shoots. *C. zizanioides* (non-native) and two California native plants *H. grandiflora* and *E. fasciculatum*, had significantly higher Pb TF than *E. Californica* ($p = 0.01$), *B. salsifolia* ($p = 0.0253$) and *S. laseolepsis* ($p = 0.0324$). Mean Pb translocation to shoots in plants with no fungal treatment was significantly higher than AMF and *P. ostreatus*-inoculated plants. No fungal treatment ($p = 0.0292$) and AMF inoculation ($p = <2e\text{-}16$) had the greatest effect on Pb uptake to plant shoots for all plants at all irrigation levels at all sites. However, differences were seen between specific plants with regards to the effects of AMF inoculation: in most plants tested, no AMF inoculation increased Pb translocation into shoots, but for *H. grandiflora*, AMF inoculation resulted in higher Pb translocation into shoots than un-inoculated plants. This may be a result of the higher AMF infectivity of *H. grandiflora* than the other plants tested. *H. grandiflora* roots showed high levels of AMF

colonization (>30%) whereas *E. fasciculatum* tended to have trace or low levels of AMF colonization.

Mean Pb translocation into plant shoots was not significantly different between irrigation treatments overall, but contrary to our hypothesis, Tukey's HSD revealed that irrigated plots had significantly lower mean Pb uptake ($p = <2e\text{-}16$) than non-irrigated plots. This may be due to drought-stressed plants expending less energy on protection mechanisms against toxic metal movement into aboveground parts (Prasad et al., 1999; Barceló et al, 2008).

4.8.3.2 Treatment Effects on Arsenic Bioconcentration and Translocation Factor

AMF inoculation significantly affected As BCF: no AMF colonization of roots significantly increased As BCF ($p = 2.59e\text{-}06$) and even trace AMF colonization of roots significantly decreased As BCF ($P = 0.0389$). Plant BCF for As was significantly higher at SW and LA Ecovillage than Taylor Yard. Arsenic translocation to plant shoots was significantly affected by the plant type and fungal treatment. Post hoc analysis revealed that no fungal treatment and AMF + *P. ostreatus* had the most significant effects on As uptake into shoots. In terms of differences between plants, *E. californica* and *C. zizanioides* ($p = 0.01$) had significantly higher As translocation into shoots than the other plants tested.

4.8.3.3 Treatment Effects on Chromium Bioconcentration and Translocation Factor

AMF colonization of roots was most correlated with As BCF in the plants tested: no (zero) AMF colonization of roots significantly increased Cr BCF ($p = 0.0224$)

whereas any level of AMF colonization, even a trace amount, decreased Cr BCF. Cr translocation into plant shoots was significantly affected by plant type: *E. californica* had significantly higher Cr translocation into shoots than the other plants tested ($p = 0.001116$). All study sites were dissimilar to each other with regards to Cr translocation in the plants tested.

4.8.3.4 Treatment Effects on Cadmium Translocation Factor

Cadmium uptake into plants was significantly affected by site ($p = 0.002109$), plant ($p = 0.003534$), irrigation ($p = 0.03878$) and fungal treatment ($p = 0.03534$). *S. laseolepsis* had significantly higher Cd translocation into shoots than the other plants tested. Un-irrigated plots had decreased Cd translocation in plants tested. The sites SW and LA Ecovillage were significantly different from one another in terms of Cd uptake into plant shoots ($p = 0.0469$). There was a significant difference between *P. ostreatus* inoculated plants and no fungal treatment ($p = 0.0219$), and a near significant difference between AMF-inoculated and uninoculated plants.

4.9 Estimates of plant removal of Pb, As and Cr from study site soils

Although we were not able to calculate a mass balance for each metal for each plot at each site, we attempted to estimate whether Pb, As and Cr accumulated in plant biomass that was removed from the sites accounts for the reductions in soil Pb and Cr concentrations seen through treatment during the study period. We calculated the mass of Pb and Cr removed by each plant for each site using the total weight of each plant

removed from each site and the average Pb/As/Cr shoot concentration for each plant. These values were determined after ~6 months for *H. grandiflora*, an annual, and after ~12 months for *E. fasciculatum, C. zizanioides*, *B. salsifolia* and *E. californica*, perennial plants.

At the SW site, 302.99 kg of Pb was removed by plants (not considering that removed by the roots). At LA Ecovillage, 211.69 kg of Pb was removed by plants. At Taylor Yard, 480.917 kg Pb was removed by plants. Overall, combining all plants and study sites, 995.6 kg of Pb was removed from soils into plant biomass.

In hexavalent-chromium-contaminated soils at SW, *Heterotheca grandiflora* took up significantly more Cr (2.667 μg Cr/g dry plant) on average than *Erigonium fasciculatus* (1.585 μg Cr/g dry plant). *Chrysnopogon zizanioides* took up the most Cr on average (3.307 μg Cr/g dry plant). For just the hexavalent-chromium-contaminated portion of the SW study area, this resulted in the estimated removal of ~314 kg Cr from soils. Chromium, like Pb, is thought to be difficult to extract using plants (Kennen, 2015), in part due to its phytotoxicity (Unnikannan et al., 2013), and the discovery that several California native plants can tolerate Cr toxicity and accumulate Cr from soils may contribute to further phytoremediation research using these species.

5. Discussion

5.1 Treatment with plants and fungi significantly reduced soil Pb concentrations relative to untreated plots

In the present study, Pb levels were reduced by a mean of 17% during the 12 months from sowing to harvest at all sites combined, but two of three study sites experienced ~ 1% reduction in soil Pb on average over the 12-month plant growth period (1 cropping cycle) and one site experienced ~50% reduction in soil Pb. Mean Pb levels are below unrestricted screening limits at two of three study sites after the study (LA Ecovillage and SW). Only one site, Taylor Yard, contained mean Pb levels higher than unrestricted screening limits as of final sampling in May 2023. This site produced the least plant biomass (about $1/4^{th}$ the other sites, 135 kg), likely due to highly compacted soil, concrete beneath soil, and added toxicity from the high levels of co-contamination with petrochemicals and solvents in soils limiting plant growth. Although soil Pb reductions at two sites, SW, and Taylor Yard, were minimal (~1% average reduction), the reductions were significant when compared to the control plots, for which higher concentrations were returned after final sampling. In other words, without treatment, soil Pb concentrations did not change or may have increased, on average; with treatment, soil Pb concentrations decreased, on average.

It is unclear what might account for the increased Pb concentrations observed within control plots. There are no obvious present-day sources of Pb deposition for all three study sites. During the present study, plants were harvested completely, including the root systems, so the metals contained therein were not deposited on surface soils. One

possible source of metals may have been irrigation water, which was not tested for metals content. There were significant differences in metal concentrations between irrigated and unirrigated plots. Another possibility is that increases in control plots may be the result of spatial heterogeneity in Pb concentrations in study soils (i.e., sampling in different areas of each plot, concentrations may vary greatly even inches away) resulting in different readings of soil concentrations for a given plot area (plots were 4 – 9 ft^2 depending on study site). Remediation practitioners have cited inadequate assessment methods as a major barrier to phytoremediation being considered feasible, because it can be difficult to demonstrate that contaminant decreases are occurring "despite the active remediation that maybe occurring" (Kennen, 2017; Gerhardt 2009). However, since increases in Pb concentrations were only seen in control plots and not in treated plots, and differences in Pb concentration changes between control and treated plots were statistically significant, this is unlikely. Further, measures were taken during sampling and sample analysis to reduce this source of error based on best practices for similar studies (Blaylock, 2013). Compositing of three samples per plot, homogenization, and the use of averages for reporting of changes in Pb results across replicates were employed to obtain more accurate readings of existing contamination and treatment effectiveness (Blaylock, 2013).

Lead phytoextraction is not considered feasible by some authors (Zia 2011; Egendorf, 2017) due to Pb's chemistry in soils limiting its bioavailability to plants. Brownfield soils within study sites had some soil properties suited to Pb phytoextraction (i.e., high Pb concentrations, low phosphate and organic matter content, relatively acidic pH), and differences in soil properties between sites, particularly with regards to soil pH,

may have explained differences in Pb uptake by plants and corresponding reductions in soil Pb (Chaney, 2013). LA Ecovillage had the highest Pb reductions (50%) and highest plant uptake of Pb, and soils at that site had the lowest soil pH of study sites. Low soil pH may have greatly enhanced the availability of Pb to plants corresponding with the higher Pb concentrations in plants roots and shoots (Yang et al., 2016). SW soils contained low phosphate and low organic carbon content as well as high Pb concentrations, but the calcareous pH (mean = 8.8) may have limited Pb mobility and availability for plant uptake. Taylor Yard had the lowest Pb uptake in plants, and although soils at that site contained high Pb concentrations and low carbon and phosphate concentrations, neutral pH may have contributed to Pb immobilization in the soil.

5.2 Several native plants accumulated Pb, As and Cr from contaminated soils

The non-native but proven plant *C. zizanioides* (Vetiver) tended to accumulate higher concentrations of metals for some metals (Pb and Cr, but not As and Cd) but also had a low survival rate compared to the native species, especially in unirrigated plots. It also required transplanting rather than sowing from seed, which adds significantly to labor, and successful establishment requires planting within a narrow time window (May in Southern California). In comparison, the two native plants *H. grandiflora* and *E. fasciculatum*, both had a high survival rate and produced significant biomass in both irrigated and unirrigated plots, grew rapidly from seed when planted at any time of year, accumulated Pb and Cr second only to Vetiver, and tolerated the range of conditions present during the study period (including flooding as well as heat waves).

California native plant *Encelia californica* was determined to be an As hyperaccumulator and phytoextractor with an As BCF and TF much higher than known remediator plant, *Chrysnopogon zizanioides*. Since As is present in background concentrations near or above the screening limit due to the underlying bedrock throughout much of Southern California (DTSC, 2017; USGS, 2005), this discovery could be of benefit to bioremediation of As-contaminated soils regionally. *E. californica* is a drought tolerant plant which produces a lot of aboveground biomass rapidly and could outcompete the commonly utilized As phytoextractor fern which has exceedingly high water requirements, limiting its usefulness in semi-arid Southern California (USEPA, 2010).

Of the native plants tested, *Heterotheca grandiflora* took up more Pb on average into the aboveground parts (0.375 μg Pb/g dry plant) than *Erigonium fasciculatum* (0.309 μg Pb/g dry plant), but the difference was not statistically significant. The other native plants tested: *Salix sp*., *Baccharis salsifolia* and *Encelia californica* and took up less Pb (0.279μg, 0.1486 μg and 0.066 μg Pb/g dry plant) than *H. grandiflora* and *E. fasciculatum*. The known remediator plant *Chrysnopogon zizanioides* had an average Pb uptake of 0.422 ug Pb/g dry plant, slightly higher than native plants *H. grandiflora* and *E. fasciculatum*.

Although the native plants tested in this study on average did not meet requirements to be considered Pb phytoextractors (based on translocation of Pb into aboveground parts), the California native plants *H. grandiflora* and *E. fasciculatum* did accumulate Pb (and Cr) in their biomass at concentrations high enough to reduce soil Pb

concentrations significantly as compared to controls during a 12-month period. However, these plants concentrated Pb within their roots at concentrations meeting requirements for phytostabilizers. These results suggest that both *H. grandiflora* and *E. fasiculatum* are worth further testing in Pb-contaminated soils regionally. Since they can be sown from seed, did not require irrigation and both perform equally well at Pb accumulation, sowing combinations of these native plants may, with further testing, prove a low cost and community-accessible means for Pb stabilization in soils. Further testing of these plants for phytoextraction purposes should be explored over longer time periods with successive rounds of sowing. In addition to inoculation with AMF and *P. ostreatus*, there may be other practices (i.e., chemical, or electrokinetic methods) that could further optimize Pb phyto-extraction that could be incorporated into future field testing (Jacobs, 2017; Koul et al., 2018).

It is difficult to determine a rate of Pb (and Cr and As) removal for each of the plants tested due to the short time frame of the present study, which only gathered data over a 12-month-period, the typical length of time for one-round of cropping in phytoremediation. Such estimates have limited accuracy because some authors argue assuming a linear decline in contamination with time overestimates remediation capacity (Van Nevel et al, 2007; Robinson et al., 2006). Different modeling approaches (such as logarithmic decay models for successive cropping) have been explored, but these models are difficult to validate because they do not account for spatial and temporal heterogeneity of metals in soil and uncertainties such as effects of nutrient depletion and other factors that may change plant uptake (Van Nevel et al, 2007; Robinson et al., 2006).

However, even if timeframes for Pb removal by plants can be better constrained, and further optimization can occur, the best estimates suggest that it will take decades for effective soil Pb remediation to occur using plants alone at sites containing high concentrations of Pb (i.e., Pb > industrial screening limits).

5.3 Effect of Irrigation on Bioremediation Success

Irrigation generally increased plant biomass but had variable effects on Pb, As and Cr accumulation in plants. Our results support our hypothesis that irrigation would alter the redox conditions and affect AMF-mediated metal(loid) translocation into plants based on redox-activity of each metal(loid). Translocation of Cr specifically was increased under drought (non-irrigated) conditions, which alters redox conditions and increases oxygen diffusion into soils which can contribute to Cr(III) oxidation to soluble Cr(VI) (Bartlett and James 1988; Bartlett 1997; Kim et al., 2002). On the other hand, AMF-mediated As translocation into plants increased under irrigated conditions, as is known to occur (e.g., Seyfferth et al., 2023; Fendorf et al., 2010). Drip irrigation, as was used in the present study, can create micro-pockets of saturation where redox conditions may change and arsenate (As(V)) may be reduced to arsenite (As(III)) and taken up by plants (Yamaguchi et al., 2014). Uptake of divalent cationic metals (Pb and Cd) had a variable response to alterations in soil moisture in field conditions because they are less controlled by redox processes, for e.g., on some sites increased Pb extraction by plants was observed in unirrigated plots, whereas irrigated plots in other cases experienced greater reductions in soil Pb. PCA's showed that percent sand and clay as well as total and organic carbon

content explained the variable responses seen between sites, and metals. The strong relationship between soil texture, carbon and water infiltration (i.e., sandier and lower carbon soils have greater and more rapid infiltration, whereas higher clay and carbon content increases water retention and reduces infiltration) and between soil texture, carbon content and metal adsorption (i.e., greater Pb, Cr and As adsorption at higher carbon and clay contents) likely explained these effects. For example, clay rich soil can minimize plant As and Pb bioavailability by adsorbing As and Pb in (Suriyagoda et al., 2018).

5.4 Inoculation with the fungi AMF and *P. ostreatus* enhanced plant survival, growth and had variable effects on plant Pb, As and Cr accumulation

The need to mitigate plant stress in contaminated soils has been cited as the most significant gap that needs to be addressed for phytoremediation to become an effective and viable remediation strategy because if plants cannot grow on a site, it is impossible for phytoremediation to be successful (Kennen, 2015). The present study also tested several types and combinations of fungal inoculum as a possible means to mitigate plant stress in contaminated soils. Our findings suggest that AMF inoculum enhanced plant germination, survival and biomass as compared to non-AMF inoculated soils. This is likely due to the protection from metal-stress AMF confer to their plant symbiont and possible increased access to phosphorus and water (Yang et al, 2016; Allen et al., 2022). Plant biomass production is closely correlated with N, P, S and Mg concentrations, thus our observation that AMF increased plant biomass may be attributed to the increases in

plant macronutrient access in mycorrhizal plants (Yang 2016). Soil Pb can block ion absorption at plant cell membranes and compete for ion binding sites, reducing plant nutrient uptake from soil, but AM fungi mitigate these effects by extending the root zone of plants as much as 10 cm and solubilizing nutrients such a phosphate that are poorly available to plants on their own (Yang et al., 2016).

Unexpectedly, inoculation with the decomposer fungus *Pleurotus ostreatus* also increased plant germination, survival, and biomass significantly, possibly due to increased soil moisture retention and buffering against temperature extremes afforded by the carbon-rich, mulch-like amendment. This has not been studied, so these hypotheses are based on observation of higher moisture content and lower soil temperatures in *P. ostreatus*-mulched plots (Appendix 1). Since spent *P. ostreatus* blocks are available in large quantities in most urban (and rural areas) from local mushroom farms, and otherwise may contribute to landfills, our findings suggest a beneficial use for these agricultural wastes in brownfield bioremediation.

Variable effects on metal uptake were observed in AMF-inoculated vs uninoculated plants. Uninoculated plants had significantly higher Cr and As accumulation and even trace amounts of AMF significantly reduced Cr and As accumulation in the plants tested. However, AMF and *P. ostreatus* inoculation significantly increased Cr translocation to plant shoots. For arsenic, uninoculated plants had the highest As translocation to shoots. For Pb, uninoculated plants had lower accumulation, and AMF-inoculated plants had significantly higher Pb accumulation of Pb. In fact, the greater the percentage of root colonization by AMF, the greater the Pb

accumulation in the plant. AMF inoculation explained 35% of the variation seen in Pb accumulation in plants. When it comes to Pb translocation to shoots, plants had differing responses to AMF inoculation: all plants tested except for *Heterotheca grandiflora* had reduced shoot translocation of Pb, but *H. grandiflora* had increased Pb translocation with AMF inoculation. This variability may be because of differences in plant mycorrhization responses since *H. grandiflora* had the greatest response to AMF inoculation. Another possible explanation is plant-specific differences in arbuscular mycorrhizal-assisted protective effects from Pb toxicity. AMF have been shown to restrict Pb translocation to prevent damage to above-ground plant parts (Diaz et al., 2014; Yang et al, 2016). Vesicles in the AMF hyphae are considered storage spaces for Pb (and other metals that are toxic to the fungus) complexed into less available forms (Göhre and Paszkowski, 2011). Root staining of plant roots in our study revealed vesicles in AMF structures, which may contribute to the higher Pb concentrations within mycorrhizal roots (Yang et al., 2016). Our findings that AMF increase Pb accumulation in plant roots while generally decreasing translocation of Pb to plant shoots are consistent with findings from other studies in different plants and environments (Yang et al., 2016; Diaz et al., 2014) and suggest that AMF inoculation may enhance phytoremediation, albeit phytostabilization rather than phytoextraction.

Chelators are known to speed up phytoextraction of metals (Evangelou, 2007), but adding chelators such as ethylenediaminetetraacetic acid (EDTA) to soils in field settings is illegal in the United States due to the risks of increased leaching from soils into groundwater and other unknowns (Chaney 2007 and 2014; Dickenson 2009). Our

findings suggest the addition of AMF and *P. ostreatus* inoculum may provide a natural and less risky means of accelerating phytoremediation of metals. Rhizospheric fungi such as AMF can act as chelators and mobilize weakly adsorbed and precipitated metals in soils, increasing the phytoavailable fraction via the release of organic acids, phenolic compounds and siderophores that act as chelating agents and acidify or influence redox changes in the rhizosphere (Lloyd et al., 2003; Glick 2010; Muehe et al, 2015). *P. ostreatus* also increased Pb translocation to shoots. *P. ostreatus*, although not root-associated, produces oxalic and carboxylic acids which may have explained the significant increase in Pb and Cr uptake into plants studied when inoculated with this fungus. These acids can leach complexed metals and provide protons for redox reactions to occur, making certain metals more available for plant uptake (Strasser et al 1994; Schmidt, 1999).

6. Limitations and Considerations for Phyto-Mycoremediation

Lead is commonly found on brownfields yet is one of the most difficult metals to extract from soils using plants. Generally, less than 1% of soil Pb is taken up by plants in a cropping cycle, and significant metal removal takes many years. Consistent with other studies (see Porębska and Ostrowska, 1999), two of three sites in the present study experienced a reduction in less than 1% of soil Pb over the 12-month period of the study. These long-time frames make phyto-mycoremediation of Pb-contaminated soils more suited to sites with less urgent remediation needs. Alternatively, phytostabilization using

these native plants and assisted by AMF and *P. ostreatus*, may be a more feasible approach.

Variation between sites, due to site-specific soil and environmental properties, can lead to differences in bioremediation success and is an important consideration with any biological remediation approach. The combinations of native plants and fungi tested on three sites in Los Angeles provide information about relative metals-removal specific to the soil and environmental properties found on these sites. Findings are likely applicable throughout LA County and parts of Southern California with similar climatic and soil types. However, soils and environmental variables should be evaluated at each field site prior to bioremediation efforts, and these specific combinations of native plants and fungi should be tested on brownfields with different soil properties to gain a better understanding of which soil properties are most suited to successful remediation using these approaches. In the present study, carbon content, soil texture and pH were the most significant soil properties influencing metals removal by the plants tested. While carbon can be added to soils and soil pH altered using agronomic practices, soil texture is difficult to alter. However, soils regionally tend to be dominated by sand due to soil-forming factors including climate, pointing to the likelihood of replicability of study findings in similar soils in the climatic region.

Irrigating large brownfield for bioremediation purposes (especially in a region experiencing a drought) adds significant costs and can be a logistical challenge, thus is a limitation of biological remediation approaches in semi-arid/arid regions. However,

higher levels of irrigation would have been required to maintain growth of the non-native species, pointing to the benefits of utilizing native species in brownfields bioremediation.

Disposal of metal-contaminated plant material is an important consideration for phytoremediation. Phytoextractor plants rich in accumulated metals are typically processed by controlled incineration (ashing) or contained composting (sludging). Harvesting typically happens annually of aboveground biomass, which then needs to be tested to see if it can be disposed of in a municipal landfill or hazardous waste facility. Regardless of the disposal method required, the plant biomass produced via metals phytoextraction for a given site is likely substantially smaller in volume and mass than the contaminated soils at that site and reduce the quantity (and cost) of metal contamination disposal. Using one of the study sites as an example, just for the study area portion of the site, 1112 bank cubic yards (>2,000,000 kgs) of contaminated soil were proposed to be excavated and disposed of versus 350 kgs of wet plant biomass, which, if incinerated or sludged would be reduced in volume to ~ 75 kg. Greenhouse gas emissions from soil excavation and disposal out of state are significant (and lesser discussed) and in-situ, nature-based remediation methods such as the ones tested in the present study have been estimated to reduce greenhouse gas emissions from remediation by ~50–80% (Hou et al., 2023).

Because disposal of plant material used in phytoextraction has an ecological footprint and cost, and is considered a potential source of secondary pollution, this problem should be addressed in future research to build a circular economy for remediation. Some extracted metals can also be reclaimed from ash or sludge, generating

recycling revenues (Corzo Remigio et al., 2020; van der Ent et al., 2015). In addition to reuse of precious metals in ash (i.e, phytomining / agromining), several other uses for phytoremediation biomass have been proposed, including: the use of biofuel plants in phytoremediation (i.e., Vetiver, tested in the present study is a biofuel plant) and subsequent transformation of plant matter into fuel, lumber or pulp, the use of extractor plants as animal fodder or feed, have been proposed (Erikson et al., 2021; Chaney et al., 2007; Conesa et al., 2012; Garbisu & Alkorta, 2001; Grispen et al., 2006; Mulligan et al., 2001; Sharma et al., 2018; van der Ent et al., 2015; Watanabe, 2001). Plant biomass would require testing for metals content to determine suitable re-use, and for some of the proposed uses, have metals extracted from the biomass prior to reuse. Other proposed uses for brownfields that could be paired with phyto-mycoremediation to increase circularity and sustainability include solar energy production and climate change mitigation via tree planting for sinking carbon (Erikson et al, 2021; Jensen et al., 2010; Hou et al, 2023; Ferrey et al., 2000).

7. Conclusion

In this first study testing myco-phytoremediation of mixed metal-contaminated brownfields in field conditions in Southern California, several California native plants demonstrated capacity for stabilization of Pb and extraction of Pb, As, Cd and Cr. For soils containing Pb exceeding industrial screening limits (especially those with neutral or high soil pH), Pb removal utilizing these native plant-fungal combinations is expected to take years or even decades. Enhanced metal accumulation and biomass generation (as well as plant survival) were observed in arbuscular-mycorrhizal fungi and *Pleurotus*

ostreatus inoculated plants. Irrigation significantly increased plant accumulation of all metals of concern, so although it was not required for survival by the native plant metal accumulators for survival, it is important for optimal metal removal into plants. The top plant-fungal combinations determined in this study demonstrate bioremediation potential that can be for further tested on California brownfields and those in similar climatic regions.

This type of remediation approach may be best suited to contaminated sites in the following scenarios: [1] where soil excavation is infeasible or inappropriate; [2] in combination with other remediation strategies, such as capping, partial excavation or chemical methods; [3] as a 'holding strategy' while sites are vacant awaiting other uses (Kennen et al., 2007); [4] integrated into landscape design of parks, recreational and green spaces utilized for stabilization of metals rather than extraction; or [5] sites with low (elevated) concentrations of metals of concern. Examples of suitable sites for this type of remediation include publicly or privately owned brownfields planned for parks and green spaces where soil must be restored to be able to support plant growth (Capuana et al., 2020), and sacred sites of importance to Indigenous Tribes, for which soil excavation would alter culturally important lands (i.e., ceremonial, and burial grounds).

The phyto-mycoremediation strategy tested in the present study is significantly more cost-effective than conventional remediation, and required only seeds, commercial inoculum and (free) spent mushroom blocks for supplies beyond water, labor, and sample analysis costs. It utilized methods known by farmers and restoration ecologists and could potentially be easily applicable by communities impacted by contaminated sites in their

neighborhoods. It demonstrated several advantages over soil excavation and disposal, including social (i.e., engagement of neighboring communities and workforce development through the local high school) and environmental (i.e., the improvement of soil on these sites and reduction in soil metal concentrations utilizing solar and human-powered technologies).

California, the state with the second-highest contaminated land acreage in the United States (EPA, 2021), a burgeoning population requiring housing development, a climate suited to year-round plant cultivation, and projected major impacts from climate change, is perhaps best suited for, and most in need of, more affordable and sustainable remediation strategies tailored to the state's climatic, geologic, and biological context, such as those tested in the present study. While some contaminated site owners may not want to invest the time required for remediation using biological methods, one of the best uses for phyto-mycoremediation could be as a holding strategy for brownfield sites awaiting remediation and redevelopment plans. Phyto-mycoremediation during that time may significantly reduce or eliminate the need for soil excavation and disposal and could be considered "safe to fail", i.e., if the low-cost installation (less than half the cost of conventional remediation) does not work, the risk (and cost) involved is minimal (Kennon et al, 2007; Fiorenza 1999; McCutheon 2004; Pivetz 2001). Given the public health risks and health disparities correlated with leaving vast expanses of contaminated sites unremediated, these biological, "nature-based" approaches are deserving of further field testing at pilot-scales. This strategy is potentially less detrimental than taking no action.

8.Acknowledgments

We are grateful to the generous support of several groups without whose support this ambitious study would not have been possible. Funding was provided through UC Riverside's Health Disparities Research Institute (Continuity Collaboratory Graduate Student Award) and the City of Los Angeles's Economic and Workforce Development Office care of LA Sanitation's Brownfields Sustainability Program and CD 9. Financial support for sequencing and bioinformatics came from CoRenewal. The Santa Monica Mountains Resource and Conservation Authority funded confirmation testing of 10% of soil samples for organic and metal contaminant concentrations at an accredited outside laboratory. Metabolic Studio donated farm lab labour and the rental of a generator and hammer drill for initial sampling. Friends of the LA River partnered with us to develop and launch a high school bioremediation program in schools neighboring the contaminated study sites, and funnelled Prop 1 grant funding to support the inclusion of these youth in the research project and pathways to STEM higher education and workforce development program. Lois Arkin at LA Ecovillage provided the water for irrigation of the study area at that site and arranged for asphalt removal from the study area. The City of LA provided water for the study at the SW site care of piping from a nearby fire hydrant and the required labor, permits and water costs, and paid for and arranged concrete removal from the study area and a storage locker for supplies. Yuval Bar-Zemer paid for irrigation water deliveries from the Korean Youth Community Centre for the Taylor Yard study. Bart Sexton from Sand County Environmental consulted about scaling phytoremediation, designed the irrigation system, and removed existing

vegetation from the study area at Taylor Yard. Smallhold Mushrooms donated spent Oyster Mushroom blocks for use in the study. Mycorrhizal Applications donated the arbuscular mycorrhizal inoculum and consulted on application rates.

This multidisciplinary study was supported through lab and instrument access, training, and guidance from numerous labs across UCR, including individual support from several PI's and graduate students. Mike and Edie Allen advised on mycorrhizal fungal and plant methodology and carried out root staining and microscopic analysis on all the plant root samples for this study. They also provided encouragement, statistics advice, and a place to stay during the last years of my PhD while I was carrying out this study. All DNA extraction for this study was carried out in Emma Aronson's lab in Microbiology with training and guidance from Mia Maltz, Linton Freund, and Talyssa Topio. UCR's Genomics Core was extremely helpful in troubleshooting sequencing. David Lyons at the Environmental Sciences Research Laboratory (ESRL) helped with TOC, NH4, PO4, microwave digestion and ICP-OES. Ying Lin at UCR's EDGE Institute ran isotopic C, N and C/N on the elemental analyzer. Cheng Tan in Dr. Li's group in Chemical Engineering ran sequential extracts and plant digests for metals concentrations on the ICP-MS. Charlie Diamond in Tim Lyons Geology lab ran thousands of plant digests, leachate samples, and soil extracts for metals concentration on ICP-MS on a short timeline.

Thank you to my lab mates in the Soil Biogeochemistry Group: Claudia Avila, Macon Abernathy, Ben Maki and Miranda Aiken who showed me the ropes in the lab and provided countless help with troubleshooting as well as moral support. Thank you to

Aral Greene and Johann Puespoek in the Homyak Lab for guidance and help troubleshooting microbial biomass and N and P methods. Site information, access and help with permitting was made possible through collaboration with the City of LA: LASAN's Brownfields Sustainability program (special thanks to Nuna and Collette) and Bureau of Engineers (special thanks to Jordan Henry for the initial connection, Bryan Powell, Evann Gonzales, Thomas Lang, Luz Rabelo), Deborah Weintraub, the Department of Toxic Substances Control (special thanks to Efrem Newirth for setting up the introduction) and LA Ecovillage (special thanks to Lois Arkin and the board of directors).

This massive undertaking would literally not have been possible without the hours put in in the lab and field by undergraduate research assistants and volunteers, including Seth Hankla, Cameron Daley, Andrew Tseng, and especially, Valeria Covarrubias-Santillan, who helped with every aspect of this study, and spent many long days with me in the lab and field doing tedious and physically taxing tasks with a smile and a little bit of dancing. Additionally, thank you to the community volunteers in the field – Huan Manton, Michelle Wong, Josh Chiang, Aaron Tupac, Zoe Rhenborg, Bettina, Anne Trumble, Liz Barry, Patrick Ballard and the many others who volunteered their time and labor to support this study!

An unexpected partnership arose in doing this study that led to the development of a youth Phyto-Mycoremediation Certificate program with Friends of the LA River. Sotomayor High School is located within eyesight of Taylor Yard, one of the study field sites (a brownfield). Together with FOLAR and the agriculture program at Sotomayor,

we taught a semester long bioremediation course for 10 high school students. They received their 40-hr HAZWOPER certification and learned about soil science, metals contamination and bioremediation, learned how to collect environmental samples and process them as well as analyze data and communicate about science. The program ended with the students presenting their findings to the neighborhood and school community. It was incredibly personally rewarding to engage youth in the community near one of the study sites in the research. The program is now in its second year.

There were numerous unexpected challenges that arose while carrying out this study which have affected the results and are worth mentioning. Access to the sites was delayed considerably due to pandemic-related restrictions, and when I finally received access, I was not permitted to have any help due to health and safety regulations. As a result, I completed most of the installation of these field studies at three sites myself; it took ~4 months of daily work from dawn until after dark to complete. Access to water for the irrigation system was very difficult to secure, and water was not available at SW and Taylor Yard until the end of May 2022 (planting occurred in January and February)- for SW, the water cisterns and fire hydrant access were vandalized, for Taylor Yard, there was no nearby water access. The lack of water after planting combined with successive early heat waves in spring of 2022 killed seedlings and resulted in the need to replant 2-4 times. Certain species of plants – notably only the non-native plants – were entirely consumed by rabbits at one of the field sites, despite fencing, cougar urine, a scarecrow, an owl, and every means of determent possible. During field work there were several instances in which there were threats to physical safety, including having to shelter in the

storage locker for most of a day during an active police shootout, threats of physical sabotage of one of the study sites, and other issues. The study site at Taylor Yard was affected by flooding covering 1/3rd study plots for ~ 3 months during winter of 2022/2023 and the need to drop 1/3rd of study plots due to hidden concrete about 1 foot deep preventing plant growth. At the end of the study period, after harvest, final soil sampling and de-installation was complete, the UCR fleet vehicle containing the plant root samples and many of the shoot samples, was stolen. After more than 30 phone calls to the police station that recovered the vehicle, we found out that all the samples were confiscated and destroyed by the LAPD. These have resulted in missing data points, and we have had to adapt the study analyses accordingly.

9. References

Ajmone-Marsan F, Biasioli M. Trace Elements in Soils of Urban Areas. Water, Air, & Soil Pollution. 2010;213(1):121-43.

Allen MF, Allen MF, Allen MF. The ecology of mycorrhizae: Cambridge University Press; 1991.

Augustsson A, Filipsson M, Öberg T, Bergbäck B. Climate change — An uncertainty factor in risk analysis of contaminated land. Science of The Total Environment. 2011;409(22):4693-700.

Aelion, C. M., Davis, H. T., Lawson, A. B., Cai, B., & McDermott, S. (2013). Associations between soil lead concentrations and populations by race/ethnicity and income-to-poverty ratio in urban and rural areas. *Environmental geochemistry and health*, *35*(1), 1-12.

Antoniadis, V., & Alloway, B. (2001). Availability of Cd, Ni and Zn to ryegrass in sewage sludge-treated soils at different temperatures. *Water, Air, and Soil Pollution, 132*(3), 201-214.

Asemoloye, M. D., Ahmad, R., & Jonathan, S. G. (2017). Synergistic action of rhizospheric fungi with Megathyrsus maximus root speeds up hydrocarbon degradation kinetics in oil polluted soil. *Chemosphere*, *187*, 1-10.

Baghour, M., Moreno, D., Hernandez, J., Castilla, N., & Romero, L. (2001). Influence of root temperature on phytoaccumulation of As, Ag, Cr, and Sb in potato plants (Solanum tuberosum L. var. Spunta). *Journal of Environmental Science and Health, Part A*, *36*(7), 1389-1401.

Berg, A., & Sheffield, J. (2018). Climate Change and Drought: the Soil Moisture Perspective. *Current Climate Change Reports*, *4*(2), 180-191. https://doi.org/10.1007/s40641-018-0095-0

Brender JD, Maantay JA, Chakraborty J. Residential proximity to environmental hazards and adverse health outcomes. Am J Public Health. 2011 Dec;101 Suppl 1(Suppl 1):S37-52. doi: 10.2105/AJPH.2011.300183. Epub 2011 Oct 25. PMID: 22028451; PMCID: PMC3222489.

Brodsky, C., Center for Creative Land Recycling, "How Many Brownfields Does California Have?", 2007, accessed 6/22/22, https://www.cclr.org/wp-content/uploads/2021/06/how-many-brownfields-in-ca.pdf.

California Environmental Protection Agency (CalEPA), O., CEJA. . (2018). *CalEnviroScreen3.0: A Critical Tool for Achieving Environmental Justice in California*

Camenzuli, D., & Freidman, B. L. (2015). On-site and in situ remediation technologies applicable to petroleum hydrocarbon contaminated sites in the Antarctic and Arctic. *Polar Research*, *34*(1), 24492.

Devinny, J., Longcore, T., Bina, A., Kitts, C., & Osborne, K. H. (2005). Phytoremediation with native plants. *University of Southern California, Los Angeles*.

Dong, Z.-Y., Huang, W.-H., Xing, D.-F., & Zhang, H.-F. (2013). Remediation of soil co-contaminated with petroleum and heavy metals by the integration of electrokinetics and biostimulation. *Journal of hazardous materials*, *260*, 399-408.

DTSC, "Community Protection and Hazardous Waste Reduction Initiative" (2017). Accessed at: https://dtsc.ca.gov/wp-content/uploads/sites/31/2021/05/FINAL-CPHWRI-REPORT_ADA_FINAL.pdf?emrc=0e9fe7

Ferrey, S. (2007). Converting brownfield environmental negatives into energy positives. *BC Envtl. Aff. L. Rev.*, *34*, 417.

Fendorf, S., Nico, P. S., Kocar, B. D., Masue, Y., & Tufano, K. J. (2010). Arsenic chemistry in soils and sediments. In *Developments in soil science* (Vol. 34, pp. 357-378). Elsevier.

Ferrol N, Tamayo E, Vargas P. The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. Journal of experimental botany. 2016:erw403.

Frew, A. (2021). Contrasting effects of commercial and native arbuscular mycorrhizal fungal inoculants on plant biomass allocation, nutrients, and phenolics. *PLANTS, PEOPLE, PLANET*, *3*(5), 536-540. https://doi.org/https://doi.org/10.1002/ppp3.10128

Garten, C. T., Classen, A. T., & Norby, R. J. (2009). Soil moisture surpasses elevated CO2 and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment. *Plant and soil*, *319*(1), 85-94. https://doi.org/10.1007/s11104-008-9851-6

Gaur A, Adholeya A. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Science. 2004:528-34.

Giovannetti, M., and Mosse, B. (1980). An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. The New Phytologist 84(3), 489-500.

Gonzalez-Chavez C, D'haen J, Vangronsveld J, Dodd J. Copper sorption and accumulation by the extraradical mycelium of different Glomus spp.(arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant and Soil. 2002;240(2):287-97.

Harms H, Schlosser D, Wick LY. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nature Reviews Microbiology. 2011;9(3):177-92.

Hart, M. M., Antunes, P. M., Chaudhary, V. B., & Abbott, L. K. (2018). Fungal inoculants in the field: Is the reward greater than the risk? *Functional Ecology*, *32*(1), 126-135. https://doi.org/https://doi.org/10.1111/1365-2435.12976

Hou, D., Al-Tabbaa, A., O'Connor, D. *et al.* Sustainable remediation and redevelopment of brownfield sites. *Nat Rev Earth Environ* **4**, 271–286 (2023). https://doi.org/10.1038/s43017-023-00404-1

Hu R, Cooper JA, Daroub SH, Kerl CF, Planer-Friedrich B, Seyfferth AL. Low levels of arsenic and cadmium in rice grown in southern Florida Histosols - Impacts of water management and soil thickness. Sci Total Environ. 2023 Apr 15;869:161712. doi: 10.1016/j.scitotenv.2023.161712. Epub 2023 Jan 20.PMID: 36682547

Homyak, P. M., Blankinship, J. C., Slessarev, E. W., Schaeffer, S. M., Manzoni, S., & Schimel, J. P. (2018). Effects of altered dry season length and plant inputs on soluble soil carbon. *Ecology*, *99*(10), 2348-2362.

Hooda, P., & Alloway, B. (1993). Effects of time and temperature on the bioavailability of Cd and Pb from sludge-amended soils. *Journal of Soil Science*, *44*(1), 97-110.

Jensen, B. B. (2010). *Brownfields to green energy: redeveloping contaminated lands with large-scale renewable energy facilities* (Doctoral dissertation, Massachusetts Institute of Technology).

Joner E, Leyval C. Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. Biology and Fertility of Soils. 2001;33(5):351-7.

Joner E, Leyval C. Uptake of 109Cd by roots and hyphae of a Glomus mosseae/Trifolium subterraneum mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytologist. 1997;135(2):353-60.

Joner EJ, Briones R, Leyval C. Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant and soil. 2000;226(2):227-34.

Jones, R. L., Homa, D. M., Meyer, P. A., Brody, D. J., Caldwell, K. L., Pirkle, J. L., & Brown, M. J. (2009). Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988–2004. *Pediatrics*, *123*(3), e376-e385.

Juwarkar, A. A., Singh, S. K., & Mudhoo, A. (2010). A comprehensive overview of elements in bioremediation. *Reviews in Environmental Science and bio/technology*, *9*(3), 215-288.

Kiaghadi, Amin, Hanadi S. Rifai, and Clint N. Dawson. "The presence of Superfund sites as a determinant of life expectancy in the United States." *Nature communications* 12.1 (2021): 1947.

Kormanik, P., & McGraw, A. (1982). Quantification of vesicular-arbuscular mycorrhizae in plant roots.

Landis WG, Rohr JR, Moe SJ, Balbus JM, Clements W, Fritz A, et al. Global climate change and contaminants, a call to arms not yet heard? : Wiley Online Library; 2014.

Lasat MM. Phytoextraction of toxic metals: a review of biological mechanisms. Journal of environmental quality. 2002;31(1):109-20.

Lima, Ana T., Zeinab Safar, and JP Gustav Loch. "Evaporation as the transport mechanism of metals in arid regions." *Chemosphere* 111 (2014): 638-647.

Lu, M., Zhang, Z., Qiao, W., Wei, X., Guan, Y., Ma, Q., & Guan, Y. (2010). Remediation of petroleum-contaminated soil after composting by sequential treatment with Fenton-like oxidation and biodegradation. *Bioresource Technology*, *101*(7), 2106-2113.

Maltz, M. R., & Treseder, K. K. (2015). Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. *Restoration Ecology*, *23*(5), 625-634.

Marchiol, L., Fellet, G., Boscutti, F., Montella, C., Mozzi, R., & Guarino, C. (2013). Gentle remediation at the former “Pertusola Sud” zinc smelter: evaluation of native species for phytoremediation purposes. *Ecological Engineering*, *53*, 343-353.

McClintock, N. (2012). Assessing soil lead contamination at multiple scales in Oakland, California: Implications for urban agriculture and environmental justice. *Applied Geography*, *35*(1-2), 460-473.

Mielke, H. W., Gonzales, C. R., & Powell, E. T. (2017). Soil lead and children’s blood lead disparities in pre-and post-Hurricane Katrina New Orleans (USA). *International journal of environmental research and public health*, *14*(4), 407.

Phillips, M. L., Aronson, E. L., Maltz, M. R., & Allen, E. B. (2020). Native and invasive inoculation sources modify fungal community assembly and biomass production of a chaparral shrub. *Applied Soil Ecology*, *147*, 103370. https://doi.org/https://doi.org/10.1016/j.apsoil.2019.103370

Pilon-Smits E. Phytoremediation. Annu Rev Plant Biol. 2005;56:15-39.

Robichaud, K., Stewart, K., Labrecque, M., Hijri, M., Cherewyk, J., & Amyot, M. (2019). An ecological microsystem to treat waste oil contaminated soil: Using phytoremediation assisted by fungi and local compost, on a mixed-contaminant site, in a cold climate. *Science of the total environment*, *672*, 732-742.

Rothenberg, S. J., Williams Jr, F. A., Delrahim, S., Khan, F., Kraft, M., Lu, M., . . . Wooten, D. J. (1996). Blood lead levels in children in south central Los Angeles. *Archives of Environmental Health: An International Journal*, *51*(5), 383-388.

Shukla P, Skea J, Calvo Buendia E, Masson-Delmotte V, Pörtner H, Roberts D, et al. IPCC, 2019: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. 2019.

Suriyagoda, Lalith DB, Klaus Dittert, and Hans Lambers. "Mechanism of arsenic uptake, translocation and plant resistance to accumulate arsenic in rice grains." *Agriculture, Ecosystems & Environment* 253 (2018): 23-37.

Simons RA. How Many Urban Brownfields are Out There?: An Economic Base Contraction Analysis of 31 U.S. Cities. Public Works Management & Policy. 1998;2(3):267-73.
Smith SE, Smith FA. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia. 2012;104(1):1-13.

State Water Resources Control Board's (SWRCB) GeoTracker database (https://geotracker.waterboards.ca.gov/)

Thomas S, Aston L, Woodruff D, Cullinan V. Field demonstration of mycoremediation for removal of fecal coliform bacteria and nutrients in the Dungeness watershed, Washington. Final Report. Pacific Northwest National Laboratory PNWD-4054-1. 2009.

USEPA. Brownfields Title VI Case Studies Summary Report. June 1999, EPA Document Number: EPA 500-R-99-003).

USEPA, Support EM. Phytoremediation resource guide: US Environmental Protection Agency, Office of Solid Waste and Emergency; 1999.

U.S. Environmental Protection Agency. 2017. Office of Land and Emergency Management (OLEM) Accomplishments Reports and Benefits.

White, B. M., Bonilha, H. S., & Ellis, C. (2016). Racial/ethnic differences in childhood blood lead levels among children< 72 months of age in the United States: a systematic review of the literature. *Journal of racial and ethnic health disparities*, *3*(1), 145-153.

Wu S, Hu Y, Zhang X, Sun Y, Wu Z, Li T, et al. Chromium detoxification in arbuscular mycorrhizal symbiosis mediated by sulfur uptake and metabolism. Environmental and Experimental Botany. 2018;147:43-52.

Wu S, Zhang X, Sun Y, Wu Z, Li T, Hu Y, et al. Transformation and immobilization of chromium by arbuscular mycorrhizal fungi as revealed by SEM–EDS, TEM–EDS, and XAFS. Environmental Science & Technology. 2015;49(24):14036-47.

Wuana RA, Okieimen FE. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology; 2011:402647.

Appendix 1. Soil Physical and Chemical Properties of Study Sites.

S1 Table 1. Soil Physical Properties by Study Site*
* Calculated by averaging 30 replicates per site from pre-treated soils.

Site	Mean Soil Temp (°C)	Mean Soil Moisture (m^3/m^3)	% Clay	% Silt	% Sand	Texture Class
Taylor Yard	38.07±8.09	0.0297±0.844	4.83±4.335	16.42±11.30	79.10±14.55	sandy
LA Ecovillage	33.82±4.27	0.0494±0.037	7.77±4.40	39.80±15.92	52.41±20.13	loam
SW & Wall	37.37±2.29	0.048±0.006	3.00±1.21	25.06±6.36	71.92±7.37	sandy loam

S1 Table 2. Soil Chemical Properties by Study Site*
* Calculated by averaging 30 replicates per site from pre-treated soils.

Site	Salinity (EC)	WEOC (mg/L)	C/N	SOC (%)	NO_3 (μg/g)	pH	CEC (cmolc/kg soil)	PO_4 (μg/g)
Taylor Yard	189.46	20.91±12.87	20.78±7.27	1.71±2.99	0.87±0.81	6.53±3.94	8.19±6.19	12.73±6.31
LA Ecovillage	336.19	18.71±10.65	21.01±6.42	6.60±2.56	51.58±0.39	7.49±3.73	10.77±6.56	5.10±5.11
SW	131.19	19.49±11.63	21.14±5.04	1.63±1.96	73.12±0.36	8.42±2.79	7.82±1.04	1.78±6.12

Appendix 2. Soil Metal Availability

S3. Mean Soil Metal Water-extractable, Exchangeable and Weak-Acid Extractable Fractions with Standard Deviation				
Site	**Metal(loid)**	**Exchangeable (ppb)**	**Water-extractable (ppb)**	**Weak-acid extractable (ppb)**
LAEV	**Pb**	5527.85 ± 6662.03	82.69 ± 686.13	692,999.86 ± 1,005,450.9
SW		4707.23 ± 3324.54	717.82 ± 2579.08	1,072,128.9 ± 1,575,144.5
Taylor Yard		4133.12 ± 2782.38	1222.97 ± 1493.45	1,013,739.5 ± 1,610,170.4
LAEV	**Cr**	3841.66 ± 4560.39	857.58 ± 2804.94	7,007.94 ± 4022.27
SW		10444.76 ± 25979.3	7103.87 ± 30877.01	54,361.50 ± 117,122.65
Taylor Yard		8600.37 ± 27189.57	455.55 ± 347.50	34,493.53 ± 26,947.09
LAEV	**As**	667.47 ± 562.12	144.94 ± 132.56	1899.61 ± 825.80
SW		745.63 ± 440.61	233.73 ± 115.93	3539.32 ± 2167.41
Taylor Yard		662.71 ± 447.19	169.23 ± 99.21	3384.93 ± 2294.01
LAEV	**Cd**	3277.34 ± 3803.4	16.77 ± 21.11	2177.91 ± 1698.50
SW		6191.87 ± 14726.61	24.68 ± 73.70	9312.01 ± 19584.86
Taylor Yard		4336.67 ± 2851.39	18.74 ± 14.27	6965.05 ± 5090.92

Appendix 3. Soil Cr(VI) Concentration Changes at SW by Treatment.

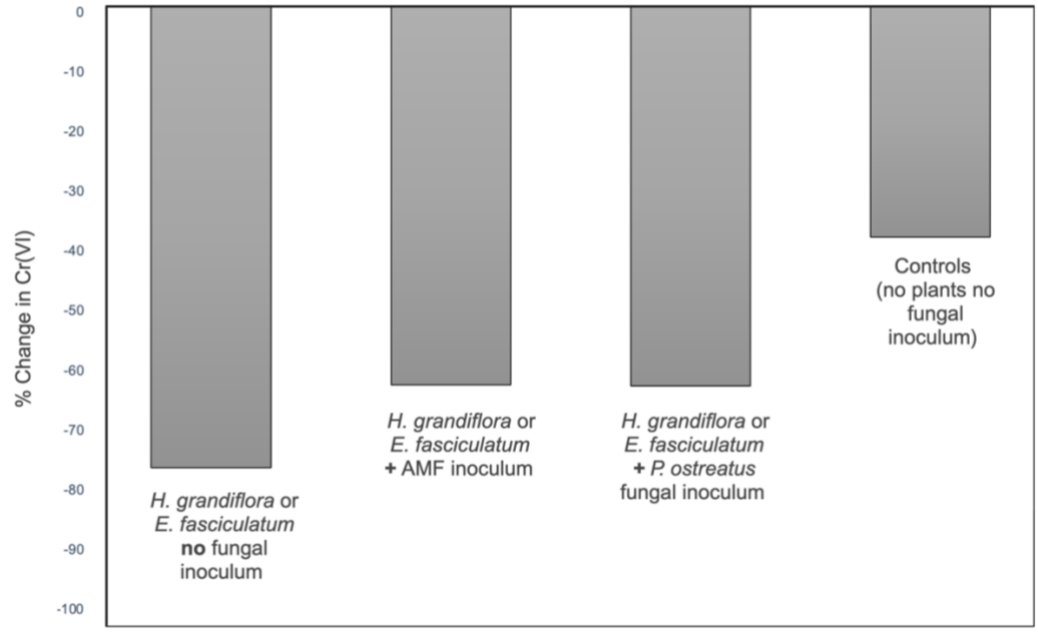

Figure S1. Percentage change in soil Cr(VI) in treated vs control plots (n = 18 per treatment group).

Appendix 4. Effect of Fungal Treatments on Plant Biomass

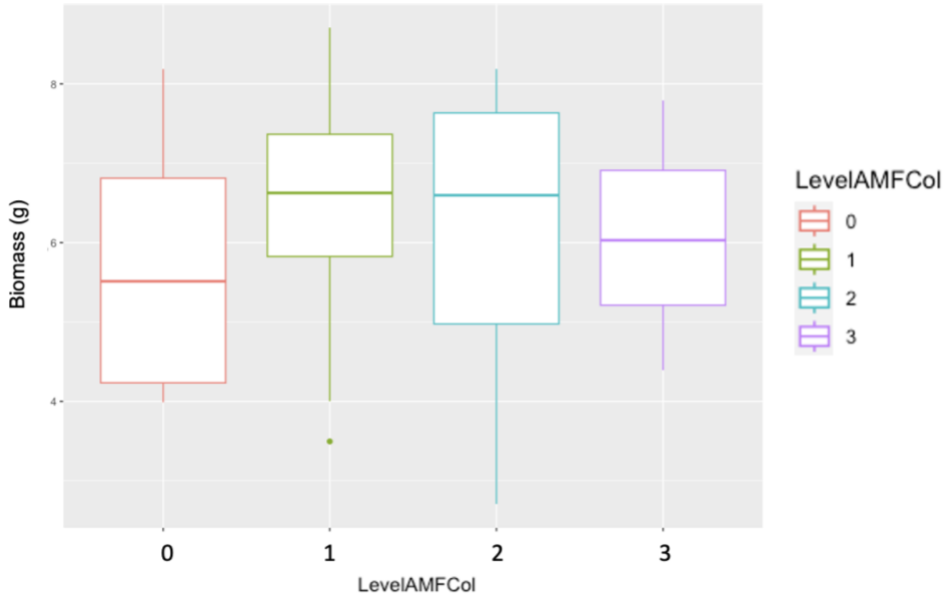

Figure S2. Level of root AMF colonization and plant biomass.

Addendum: Methods for Community-Governmental-Academic Remediation Research

1. Field site identification

LA Ecovillage

LA Ecovillage intended to implement bioremediation on their ¼ acre contaminated brownfield as a demonstration method and were looking for someone with the expertise to carry this out. I was referred to them through the Los Angeles Sanitation department. I performed a site visit in Fall of 2020 and held planning meetings with the Board of Directors, Director, and site manager for LA Ecovillage and gave presentations on bioremediation and the study plan for the neighborhood.

Taylor Yard

I was invited to a meeting about remediation options for Taylor Yard with City of LA agencies involved in Brownfields remediation, and the Department of Toxic Substances Control (DTSC), by an LA Sanitation Department staff, Jordan Henry, who had interviewed me for his thesis project on Mycoremediation after Committee Member Jason Stajich referred him to me. I provided information on biological remediation options based on my decade of experience in bioremediation and told them about my research objectives for this study. After numerous meetings and presentations starting in summer of 2020, I was subsequently invited to implement my field study at Taylor Yard.

Slauson & Wall

City of LA Brownfields Sustainability Program Director Nuna Tershibashian attended the Taylor Yard meetings and reached out about another brownfield in their office's ownership, the Slauson and Wall site. I was invited to present about my research plans at a stakeholder meeting for the site, and afterwards, to prepare a proposal and budget for the inclusion of this site in the Phyto-Mycoremediation study. Meetings with the City of LA were held with the teams of site managers, engineers, contractors, DTSC and City administrative staff to discuss logistics required to implement this study.

2. Requirements for Remediation Study Approval

2.1 Health and Safety

I was required to complete a 40-hr-HAZWOPER certification prior to being able to access the sites. We were required to develop a health and safety plan to ensure study activities protected the health and safety of researchers, volunteers, and nearby residents, in addition to the field safety plan required by UCR. This involved identifying potential hazards from working on a contaminated site and mitigating them, primarily through proper protective gear and safety planning. Initially, undergraduate researchers or volunteers were not permitted to help with field work unless they also had the 40-hr HAWOPER certification. I confirmed with the California Office of Environmental Health Hazard Assessment (OEHHA) that this is not a requirement for volunteers under the management of a supervisor with the certification.

2.2 Risk Reduction Requirements from Study Activities

Dust control. A surface layer of woodchips was applied 1-2 inches deep in aisles around plots to prevent contaminated dust from being entrained in wind and being blown from the study area. Woodchips were supplied by the City of Los Angeles Parks Department and were analyzed for elemental concentrations prior to installation. These were required by site regulators, DTSC.

Fencing. Each site (and study area) must be protected from human entry by tall, barbed-wire fencing and locked gates for restricted entry. Additional protective measures were not deemed necessary.

Disposal of contaminated plant matter. After the study was completed, and plant samples were analyzed, plant matter used in the study were disposed of through UCR's Environmental Health and Safety hazardous waste pickup.

The biggest concern of the regulatory agency was dust creation through soil sampling and planting. We needed to ensure that no dust was mobilized from the site and have a plan to mitigate dust entrainment; otherwise, California Air Resources Board would have needed to be engaged and dust monitoring measures would have needed to be taken. To prevent dust entrainment, we covered all bare soil with thick layers of woodchips and had water hoses set up for any activities in which soil was being turned. One of the major benefits of the approach tested in the study is that soil is not disturbed because seeds and inoculum (and drip irrigation) were all placed on top of the soil.

2.3 Agreements and Permits

Memorandums of Understanding were developed between UCR and the City of LA, and between UCR and LA Ecovillage and needed to be signed off on at the highest level (the City Commissioner). We needed to get 'right of entry' for myself and my research team for each site, as well as permits to engage in sampling and work in the soil. These access permits needed to go through several institutions and be ultimately signed off on by the City of LA's mayor's office. Getting these MOUs signed and right of entry took months to years, depending on the site. Permits were also required to access water from nearby fire hydrants, or have pipes run aboveground from a water source, or to have on-site water truck deliveries. We coordinated with city agencies including the General Services Division, Bureau of Engineers, Brownfields Program (part of LA Sanitation Department) as well as Parks and Rec, to get keys to sites, have woodchips and compost deliveries, etc..

2.4 Liability

Part of what was required for the MOU was to show proof of liability coverage (> $600 million) from UCR- this liability coverage was a major advantage of undertaking such a study through a large academic institution. In addition to this, all volunteers with study activities were required to sign a waiver.

2.5 Work Plan

I was required to develop a comprehensive work plan for the remediation study and have it approved by DTSC, which required several rounds of meetings and revisions. Initially, DTSC was wary of field testing of a 'new remediation method that hasn't been proven in numerous field trials', but since this was a study and not a pilot, we received approval. We altered our study plans based on their feedback. Their concern was primarily around sampling and sample analysis. For example, they required three soil samples per plot and for 10% of all 'before' and 'after' 1-year study soil samples to be analyzed at an outside, accredited laboratory for all contaminants of concern (COC's). This was so that the study findings would be considered seriously and provide insights into the feasibility, cost, and effectiveness of this alternative remedial strategy for contaminated sites within Southern California.

2.6 Community Engagement

Prior to commencing study activities, including site characterization, I was required to post notice of remediation activities (posters) in the neighborhoods around the sites, as well as present about the study at each neighborhood council meeting in the areas around each site. I gave presentations at 6 different neighborhood council meetings around Los Angeles and several other community events. I developed a web page for more information about the study (https://phyto-myco-remediation.ucr.edu) and signage that was posted on the fences around each site. One-page summaries of the research plan and goals were created in plain language targeting different groups of stakeholders for each

site: regulators, site managers (typically engineers), and community groups. Ongoing meetings were held with each group of stakeholders for each site to discuss the research proposal, criteria for site selection, clarify and respond to questions and concerns, identify logistical constraints and brainstorm solutions together, identify suitable plot locations on each site, agree upon timelines and ensure regulatory and other criteria for remedial pilot study were met.

3. Selection of Study Plot Locations Within Each Site

Size and area of study plots on each site was selected through communication with site partners, based on historical site characterization data and the following criteria: confirmed elevated soil metal concentrations for at least one metal of concern (Cd, Cr, As, Pb), confirmed access to the site and study location without interference to study plots from other site activities (e.g. construction) during the time period, access to soil (i.e., must not be under concrete or asphalt), access to water for irrigation and ease and safety of vehicular access for myself and my research helpers (i.e. undergraduates) with tools and equipment. This resulted in coordination to have asphalt (LA Ecovillage Songs property) and concrete (Slauson and Wall) removed from study areas so that soil could be accessed. This would have been required regardless of the study for any type of remediation to occur on these sites.

Conclusions

This dissertation research explored the role of plants and fungi in metals removal from contaminated soils across multiple brownfields in California, specifically examining the potential for native plants use in bioremediation. We looked at native plants because the semi-arid to arid climate found in Southern California is not formidable for known phytoremediators, but native plants are already acclimated. We tested several beneficial fungi in conjunction with native plants to assess their potential to enhance plant survival and remediation capacity under the stressful conditions found on contaminated sites in semi-arid Southern California.

Field and greenhouse-based methods were utilized to investigate plant-fungal dynamics in metals uptake in contaminated soils and multiple environmental factors, specifically drought vs irrigated conditions, were combined in our investigations. We also compared biostimulation and bioaugmentation by testing carbon amendments (woodchips) vs no carbon amendments, commercial vs native arbuscular mycorrhizal fungi (AMF) and decomposer fungal inoculum (known remediator *Pleurotus ostreatus* in the form of spent mycelium from local mushroom farms) vs native decomposer fungi.

The research approach, community-academic-governmental research partnership, also demonstrated the importance of community engagement and collaboration and coordination with public and regulatory agencies to provide applicable results.

1. **Native plants and fungi with metals remediation potential were identified in Southern California, and the soil properties driving their composition elucidated**

In the first study of the biology of contaminated sites in Southern California, seven contaminated brownfields and Superfund sites were surveyed for plant, bacterial and fungal volunteers. Several California native shrubs previously unidentified as plant hyper-accumulators of copper (Cu), including *Baccharis salcifolia* and *sarothroides*, *Eriogonum fasciculatum* and *Brickellia californica,* as well as one unstudied non-native Cu hyperaccumulator, *Helminthotheca echiodes,* were identified. Other California native accumulators of metals such as lead (Pb), chromium (Cr), and nickel (Ni), were identified. Fungal and bacterial communities were dominated by the Ascomycota and Proteobacteria phyla, and the Dothideomycetes, Sordariomycetes and Spizellomycetes fungal and Gammoproteobacterial and Planstomycetes classes. Several microbial taxa previously identified as contaminant remediators were seen at all study sites, as well as some yet untested taxa which may be candidates for use in bioremediation. Most of the variation in microbial community composition was explained by differences across sites. Water-extractable organic carbon, silt and sand content, and cationic exchange capacity were the most significant drivers of microbial community composition within sites. Our findings identified potential plant and fungal candidates for further exploration in bioremediation applications suited to Southern California and the most significant soil and environmental drivers influencing their communities. Future work should screen the fungi (and bacteria) that were identified on contaminated sites in Southern California for remediation capacity and explore bio stimulation potential in-situ. Further, the top plant accumulators (particularly native plants) of metals of concern should be tested in greenhouse and field experiments for phytoremediation capacity, with attention paid to

environmental factors relevant to Mediterranean climate found regionally and the significance of water provision for bioremediation in semi-arid/arid regions.

2. Arbuscular Mycorrhizal fungi and the fungus *Pleurotus ostreatus* enhance *Medicago sativa* growth and Cr(VI) reduction under drought in Cr-contaminated soils in a pot study

Arbuscular mycorrhizal fungi (AMF) have been applied in past studies to increase plant survival in chromium (Cr) contaminated soils, however, the conditions under which Cr uptake is inhibited versus enhanced in AMF-plant partners is unclear. The present work investigated the interactions between AMF and Cr in *Medicago sativa* under two different soil moisture regimes, drought and optimal moisture, in unsterilized field soils collected from a contaminated brownfield. Chromium translocation to *Medicago sativa* shoots was reduced by AMF inoculation and significantly increased by soil moisture and soil organic carbon content. *Medicago* plants grown under drought stress showed reduced Cr accumulation in plant shoots. AM fungal inoculation reduced soil Cr concentration by 45.8% and the greatest Cr reductions were seen in droughted soils with higher soil carbon concentrations and AMF inoculation. Inversely, significant increases in Cr(VI) concentrations (123% on average) occurred when there was no AMF inoculation and at low AMF colonization percentages (<20%) as well as at higher SOC percentages. These findings suggest it may be beneficial to apply AMF for the phytoremediation of Cr and Cr(VI) contaminated soils. These results have relevance in phytoremediation of Cr-contaminated soils in semi-arid regions, where similar drought

or redox-fluctuating conditions may exist. Future work should experimentally determine the ranges of Cr and Cr(VI) concentrations at which uptake versus inhibition are observed in additional phytoremediator plants and test the significance of AMF strain and species differences on Cr uptake under different soil moisture and other edaphic conditions. Additionally, further experimental studies on AMF-assisted phytoremediation of Cr should be carried out under field conditions so that the results will have better practical application.

3. **Native plants, AMF *Pleurotus ostreatus* fungi demonstrate potential for metals remediation in irrigated and unirrigated soils on three contaminated sites in LA**

We evaluated a novel biological soil metal remediation approach utilizing California native plants with different fungal treatments including arbuscular mycorrhizal fungi (AMF) inoculum and *Pleurotus ostreatus* inoculum in the form of spent mushroom blocks on three brownfields with different levels of organic and inorganic contaminants in Los Angeles under irrigated and unirrigated water regimes. This was the first field study of phyto-mycoremediation in Southern California, and was carried out in partnership with community groups, city, and regulatory agencies. Results show that treatment with plants and fungi reduced soil concentrations of all metals of concern, and produced statistically significant reductions for Pb, Cu and As, when compared with control plots. Although the native plants tested were not as performant in metal accumulation and extraction into aboveground parts as the control plant, *Chrysnopogon zizanioides* (dryland remediator Vetiver), they had much greater survival and biomass

production in both irrigated and unirrigated plots. *Heterotheca grandiflora* had the highest mean Pb uptake of the native plants tested, but was not significantly different than *Erigonium fasciculatum*, suggesting both may be suitable native plant options for phytoremediation efforts in Southern California. Irrigation and inoculation with AMF significantly increased metal accumulation in all plants tested, although AMF-mediated effects on Pb, Cr and As translocation from plant roots to shoots was plant species dependent. Unexpectedly, significant increases to plant germination, survival, biomass, and metal uptake were observed with *Pleurotus ostreatus* inoculation. Study results suggest that longer timelines are necessary for Pb reductions in highly contaminated brownfield soils using plant-fungi combinations. Regional pilot-scale studies of the best plant-fungal treatment combinations could be explored over longer timeframes for in-situ brownfields bioremediation; sites with different concentrations of metals contamination should continue to be explored to determine whether these approaches are more applicable for treating certain concentrations of a given metal. Additionally, future work could explore means of optimizing metals and organic contaminant remediation; proteomic and metabolomic assays may be useful in rapidly identifying fungal and plant needs for enhanced remediation efficacy and speed. Future work should explore options for recycling metals extracted via plants and the creation of circular alternatives to incineration and sludging for metals-contaminated plants used in phytoremediation.

4. **Limitations**

The field-based approaches utilized in this dissertation (even in the greenhouse study which used field soils), while having advantages, has many limitations. Foremost is the complexity and heterogeneity of soils. Although analysis of the major soil physical and chemical properties known to influence contaminant transport in soil-plant-fungal systems was undertaken, our results did not explain all the variation seen in study results for all studies, pointing to un-tested/analyzed factors that may been important explanatory variables (i.e., soil minerology and organic contaminant concentrations in each site, plot, or pot). Further, additional replicates per treatment group would have added confidence in our results. In the survey (Chapter 2), ten samples per site were analyzed; in the greenhouse study (Chapter 3), six replicates per treatment group were analyzed; and in the field remediation study (Chapter 4), six replicates were installed per treatment group per site (x 3 sites). Particularly for the field remediation study, a higher number of replicates within each site would have greatly enhanced confidence in the results, since not every replicate provided usable data (for example, if environmental or site factors resulted in a plot being dropped or not producing data due to lack of plant growth, etc.). Ultimately, the field remediation study results provide a starting point for further testing in the field (and in controlled conditions) to verify the results. Ultimately, the findings in this dissertation should point to areas of further study and replication. Plants identified as metals accumulators in the survey should be verified in experimental conditions. Treatment effectiveness as determined in the field remediation study should be tested again with reduced variables and verified in field conditions with different soil properties

within the region (or similar climes), as well as in more controlled conditions such as a pot study. Risk assessments of phyto-mycoremediation should be undertaken for neighboring communities, remediation workers, and potential transfer of contaminants to the food chain. These approaches will not be suitable or appropriate for any type of contamination and contaminated site, and site selection should carefully consider all potential benefits, risks and costs when weighing a remediation alternative. Depth, extent and type of contaminant, along with soil properties and environmental factors and community and regulatory needs are all important to consider and determine in remediation studies and efforts of any kind.