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Impact of Adding Natural and Simulated Root Exudates on the Functioning of Contaminated Soil

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Impact of Adding Natural and Simulated Root Exudates on the Functioning of Contaminated Soil

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DISSERTATION APPROVAL

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Dedication

Aditi, Gayatri, Pallavi, Prabhakar

For being my anchor and my kite

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List of Symbols and Abbreviations

LSP: Liberty State Park

HMF: Hutcheson Memorial Forest

PA: Phosphatase activity

LOI: Loss on Ignition

PAHs: Polycyclic Aromatic Hydrocarbons

SRE: Simulated /Artificial root exudate solution

CHAPTER 1

1. Introduction

1.1 Background

Soil is one of the essential resources that helps sustain life on earth. Soil consists of a complex interactive network that involves biological and physical-chemical processes (Nichols & Halvorson, 2013). Environmental factors are the key drivers of these intricate processes (Brussaard, 1997). The feedback loops between the biological components such as plants, micro- and macro-organisms of the soil with its abiotic components such as soil structure, minerals, moisture, and nutrient content make the soil composition unique to that region (Angers & Caron, 1998; Ehrenfeld et al., 2005; Hooper et al., 2000; Smith-Ramesh & Reynolds, 2017).

Anthropogenic industrial activities and rapid urbanization intensively impact worldwide soil quality rendering it dysfunctional to provide ecosystem services (Bastida et al., 2008). Many post-industrial sites have high contaminant (petroleum hydrocarbons, polycyclic aromatic hydrocarbons, pesticides, brines, metals) concentrations that persist in the soil (Li et al., 2017; Loures, 2015; Megharaj & Naidu, 2017). The resulting stresses on the soil biotic components, inhibit plant growth and degrade soil quality (Gallego et al., 2016; Krumins et al., 2015; Ling et al., 2007). Moreover, urban, post-industrial sites are a risk to human and environmental health due to their proximity to residential areas (Beriro et al., 2016; Wang et al., 2022). For example, urban soils from barren, post-industrial sites can transfer contaminants into the air and water bodies because of wind gusts and water runoff (Li et al., 2018). When plants grow in contaminated soil, then there is a concern about contaminant mobility in the food web (Soliman et al., 2022). For example, Soliman et al. (2022) reported bioaccumulation of heavy metals in an

agro-industrial food web with highest concentrations in apex predators (“a wolf spider”) compared to plants.

There is growing interest in protecting soil functionality in agricultural and non-agricultural soils for food security and public health for future generations (Kavamura & Esposito, 2010; Li et al., 2018; Oliver & Gregory, 2015). With rising demand for basic human needs such as quality air, food, water, and shelter, the research on soil quality for agriculture, and non-agriculture purposes has also escalated (Li et al., 2018; Maddela et al., 2022; Maikhuri & Rao, 2012). Currently, studies on urban soil contamination broadly encompass: 1) the life cycle of contaminants in soil, 2) environmental and human health risks, and 3) urban soil remediation and re-greening efforts for public use. The focus of my work is to understand the characteristics of barren, post-industrial soil, apply current soil remediation strategies to amend soil quality in the lab, and develop a strategy to revitalize barren, contaminated soil that can support vegetation.

1.2 Industrial barren landscapes

Increased population densities have resulted in urban development creeping within the vicinity of industrial sites raising massive concerns (Li et al., 2018; Loures, 2015; Semenkov & Koroleva, 2022; Wang et al., 2022). Persistent toxic chemicals at industrial sites degrade soil quality resulting in abandoned regions called “industrial barrens” that are resistant to natural restoration (Kozlov & Zvereva, 2007). Soil anthropogenic contaminants can limit enzymatic nutrient mineralization, either by direct regulation or via impacts on the microbial community, thus affecting plant growth in agricultural and non-agricultural soils (Zhang et al., 2006). Soils from contaminated sites typically display oxidative stress from the inorganic pollutants (Pb, As, Hg, Cu), limited nutrient availability, and low abundance of indigenous microbes resulting in

poor vegetation (Afegbua, 2014; Bardgett & Van Der Putten, 2014; Fayiga & Saha, 2016; Reeder et al., 2006). In addition, industrial barrens lack nutrient inputs to sustain the contaminant-resistant microbial communities (Grobelak et al., 2017; Ibraheem, 2007; Sun et al., 2010). Decreased soil enzymatic function is often the primary and fastest response to toxic chemicals (Bandick & Dick, 1999; Dick, 1994; Dick et al., 1996; Vepsäläinen, 2001).

Without vegetation, such sites cannot maintain soil structure to immobilize the pollutants and provide ecosystem services (Gallagher et al., 2018; Six et al., 2000). In addition, lack of moisture retention due to poor soil structure and organic matter content further restrict soluble nutrient mobility and cycling (Franzluebbers, 2002). Barren sites cannot capture toxic compounds to protect groundwater. Heavy rainfall in extreme weather may transfer contaminants beyond the site boundaries (Hartley et al., 2009).

One approach to ease the burden of meeting the urban land-use demands would be to restore the soil quality and regreen barren, inactive, contaminated sites. Various eco-friendly practices have been reported for the soil remediation post-industrial sites (Liao et al., 2021; Lynch & Moffat, 2005; Megharaj & Naidu, 2017; L. Wang et al., 2021). Among the prevalent soil remediation techniques, bioremediation and phytoremediation are the preferred “gentle-remediation options” (Cundy et al., 2013). An important aspect to consider while planning these remediation strategies would be to examine if the contaminated soil can support plant life despite the toxicity. Especially, when an abandoned, contaminated, barren spot is unable to support the wind deposited seeds, this could indicate a challenging interaction between the abiotic and biotic components in the soil. To address the remediation of such sites might require a combination of the conventional remediation methods that are discussed in the next section.

1.3 Current remediation strategies

A growing body of research indicates the development of "green remediation strategies" to revitalize contaminated sites (Awasthi et al., 2022; Cundy et al., 2016; Maddela et al., 2022; Pedron & Petruzzelli, 2011; Reddy & Adams, 2010). The United Nations recently released their "2030 Agenda for Sustainable Development," in which goal 15.3 stated the necessity to "restore degraded land and soil to achieve a land degradation-neutral world" (United Nations, 2022). Studies from Western European countries have reported over 2.5 million sites that risk human and ecological health due to the soil contaminant levels (Clausen et al., 2015; Drenning et al., 2022; Panagos et al., 2013; Swartjes, 2011; L. Wang et al., 2021). The US EPA has a "brownfields and land revitalization program" to promote the clean-up of more than 450,000 sites (United States Environmental Protection Agency, 2022). The New Jersey Department of Environmental Protection, Bureau of GIS, has listed 13,933 "known contaminated sites" in the state of New Jersey that require remediation (New Jersey Department of Environmental Protection, 2016). International and national concerns on degraded soil restoration have led scientists to develop several types of soil remediation technologies.

Various conventional methods have been applied to remediate contaminated soils from industrial sites and are currently well studied. These methods are categorized into physical, biological and chemical remediation technologies targeted to reduce soil toxicity (Megharaj & Naidu, 2017; Sharma et al., 2018; Song et al., 2022; L. Wang et al., 2021; Wu et al., 2022). The physical remediation methods include desorption of soil contaminants using thermal treatment and replacing contaminated layers of the soil (Song et al., 2022). For example, soil contaminated with organic contaminants can be heated using a "thermal desorber" machine for physical

volatilization of the contaminants as they separate from the soil particles (Wei et al., 2022; Zhao et al., 2019).

The chemical remediation techniques include “leaching, stabilization, electrokinetic remediation-permeable reactive barrier, and oxidation/reduction” (Dermont et al., 2008; Hashmi et al., 2022; Song et al., 2022; Wu et al., 2022; Zheng et al., 2022). In the chemical leaching technique, various chemical agents such as “acids, bases, salts, chelators, or surfactants” are used to aid extraction and dissolution of heavy metals in soil (Hashmi et al., 2022; Lomaglio et al., 2018). For example, Dermatas and Meng (2003) showed 90% immobilization efficiency in Cr contaminated soils by adding calcium oxide containing fly ash and cement as a stabilizing agent. However, the drawback of applying chelators or surfactants is that they alter the soil composition. In recent years, “electrokinetic technology with permeable reactive barrier” designed to immobilize heavy metals has proved to be a promising technique (Rahman, 2022; Jing Wang et al., 2021). Wang et al. (2021) fabricated an “aminated electrospun nanofiber membrane” that was applied as a permeable reactive barrier material to achieve 73% immobilization of in-situ soil Cr(VI).

Biological remediation methods are categorized into microbial remediation and phytoremediation (Byrd, 2019; Gavrilescu, 2022; Henry et al., 2013; Kumar et al., 2022; Ortiz-Hernández et al., 2018). In bioremediation, the use of genetically modified organisms designed to degrade soil contaminants has its pros and cons (Saranya Kuppusamy et al., 2016; Mrozik & Piotrowska-Seget, 2010; Nwankwegu et al., 2022). Jenkins (2008) cautions the use of this approach because native and genetically modified organisms (GMOs) compete for the same resources and between each other. Moreover, the system needs periodical, expensive, sustainable

seeding of the GMOs to achieve desirable results (Healey, 1989; Jenkins, 2008; Nwankwegu et al., 2022).

Biostimulation implies stimulation of native microbes using water, nutrients and/or altering oxygen to affect microbial growth (Balkrishna et al., 2022; Cunningham & Philp, 2000; Prada-VÁSquez et al., 2017; Song et al., 2022). Various studies have shown that microbial metabolic reactions produce enzymes responsible for catalytic transformation of inorganic and organic soil pollutants into less toxic products, which describes microbial remediation (Anyika et al., 2015; Gadd, 2010; Kotoky et al., 2018; Liu et al., 2010). Organic amendments such as industrial, agricultural, and municipal wastes, additives fabricated with green nano materials, and natural minerals and oxides have been studied to stimulate indigenous microbes (Awasthi et al., 2022; Dehnavi & Ebrahimipour, 2022; Tyagi et al., 2011). Studies on agricultural soils containing As have shown that compared to biochar, 5% rice straw application significantly increased As methylation and volatilization from paddy soils (Chen et al., 2017). Soil microbes normally survive on the root exudates of healthy and intact plants and thrive in the rhizosphere regions (Hartmann et al., 2009; Kotoky et al., 2018; Nannipieri et al., 2008; Randall et al., 2001; Rovira, 1969). Recent advances in interpreting the interactions of root exudates with the belowground organisms have gained substantial attention (Badri & Vivanco, 2009; Brimecombe et al., 2000; Haichar et al., 2014; Kunc & Macura, 1966). Given that an important part of my research is focused on the application of root exudates in soil remediation, I will discuss this in detail in section 1.5.

Soil microbial communities flourish in the presence of plants, which means the role of plants in soil remediation can be central (Afegbua, 2014; Emenike et al., 2018; Oladoye et al., 2022). Hence, phytoremediation considers plants as hyperaccumulators that capture and

accumulate contaminants in their aboveground parts (Gavrilescu, 2022; Qian et al., 2012). Studies have reported that leaving contaminated sites undisturbed allows natural attenuation through ecosystem services, though at a slower pace (Gallagher et al., 2018). Plants utilize solar energy to volatilize, trap, store, and convert organic and inorganic soil contaminants that depend on the plant species and environmental conditions (Ali et al., 2013; Awasthi et al., 2020; Balkrishna et al., 2022; Eskander & Saleh, 2017). For example, native plant species as well as fast growing grass species (winter rye or switchgrass) have been used to phytoextract, translocate, and stabilize organic contaminants (PAHs) in contaminated soils (Liu et al., 2015; Pradhan et al., 1998; Roy et al., 2005; Vamerali et al., 2010). Other plant species such as *Salix* and *Populus* have been commonly used in the phytostabilization and translocation of heavy metals such as Pb, Cu, and Cd to the above ground parts (Antoniadis et al., 2017; Balkrishna et al., 2022; Emenike et al., 2018).

Phytoremediation supports gentle clean-up of contaminants, biofuel crop production, and public acceptance of green spaces over barren landscapes (Kafle et al., 2022; S. Kuppusamy et al., 2016; Reddy & Adams, 2010). Kafle et al. (2022) reviewed the types of natural and synthetic amendments that have emerged to aid phytoremediation success. The organic materials that are more naturally produced such as sugar beet residue from fermentation processes (Wiszniewska et al., 2016), “composted sewage sludge” (Wang et al., 2019), “rice straw biochar” (Ghosh & Maiti, 2021), and simulated root exudates (Lu et al., 2017) were grouped as natural amendments. Other amendments include microbial, and genetically engineered plants and microbes (Kumar et al., 2022). Under chemical amendments, synthetic chelators such as “EDTA, ethylene glycol tetra acetic acid (EGTA), and sodium dodecyl sulfate (SDS)” were recommended for

phytoextraction of chelated contaminants to aid soil remediation (Kumar, 2019; Wiszniewska et al., 2016).

Among various soil remediation methods listed above, physicochemical methods were reported as expensive and the chances of creating additional secondary pollution were higher (Dehnavi & Ebrahimipour, 2022; Oladoye et al., 2022; Reddy & Adams, 2010). Many recent advances in soil remediation have suggested a combination of techniques for cost-effective and timely outcomes (Balkrishna et al., 2022; Oladoye et al., 2022; Song et al., 2022; L. Wang et al., 2021). Further, these studies have stated that the contaminant legacy of the site might reduce the positive effects of amendments, which then necessitate short-term or recurring interventions for sustainable effects (Bardos et al., 2017; Bonomo et al., 2002; Dermont et al., 2008).

Another aspect to consider would be the impact of one remediation strategy may further deteriorate the soil quality and even wipe out the previously restored soil ecosystem (Swartjes, 2011). Certainly, replacement of contaminated soils from post-industrial sites might be a preferred option (Song et al., 2022). However, it entails appropriate chemical treatment to remove the contaminants before final disposal, which can be tedious. This could be the reason why disposal of contaminated soils has been preferred over revitalization (Gerhardt et al., 2017; Marchand et al., 2016; Mench et al., 2010). A vast body of current research indicates that the advantages of combining chemical remediation with physical and biological methods have shown “large-scale application” potential with rapid results (Rinklebe & Shaheen, 2015; Song et al., 2022). Studies have suggested that while selecting a remediation strategy, it would be beneficial to assess the strengths and weaknesses of using a single technique over combined methods (Gavrilescu, 2022; Kafle et al., 2022; Song et al., 2022). Some drawbacks that the researchers presented were: chemical remediation inevitably resulted in secondary pollution and

time required to implement and reap the results of phytoremediation (Kafle et al., 2022; S. Kuppusamy et al., 2016; Song et al., 2022). In contrast, combined methods would provide environmental managers effective treatment efficiency in shorter time spans, more time efficient protocols, and economical benefits (Song et al., 2022).

Though natural attenuation and phytoremediation were reported as environmentally beneficial strategies, longer time was required to realize their positive impacts in restoring soil quality (Kafle et al., 2022; Kantola et al., 2022; Song et al., 2022). Nevertheless, recent reviews on biostimulation have considered it as an exceptionally favorable intervention that has showed shorter soil remedial time (Balkrishna et al., 2022; Oladoye et al., 2022; Song et al., 2022). Apparently, a combination of techniques might work for some sites that have poor vegetation. In some contaminated soils, despite high contaminant (organic and inorganic) concentrations, vegetation was supported while other sites remain barren (Gallagher et al., 2008; Kozlov & Zvereva, 2007; Salisbury et al., 2020; Zhang et al., 2006). The issues with barren, contaminated, inactive sites include poor soil structure, low microbial abundance, and negligible plant cover, so they may not support plant growth and a single remediation strategy may be inadequate (Kandeler et al., 2000; Sun et al., 2010).

To address these issues, I developed a combination of biostimulation and phytoremediation approaches with a novel technique to stimulate the native microbes using laboratory-prepared simulated root exudates solution. For this dissertation, I studied soils from an aged, contaminated site instead of spiking an inert soil material (sand) with various concentrations of organic and inorganic contaminants, which many studies have reported (Sun et al., 2010; L. Wang et al., 2021; Xie et al., 2012). Compared to spiked soils, my research provides a more realistic representation of how aged, contaminated soils respond to amendments (Vaidya

et al., 2020). Remediation interventions are aimed at revitalizing contaminated, poorly functioning soils (Rahman, 2022; Zheng et al., 2022). From the three projects that encompass my dissertation, I examined how effective the current remediation strategies were in revitalizing soil from an urban, barren, post-industrial site and how I could enhance these methods. To determine whether the interventions were effective, I measured soil enzymatic function by analyzing the soil enzymatic nutrient cycling turnover rate (Hagmann et al., 2015; Marx et al., 2001).

1.4 Soil function

Environmental disturbances and complex soil characteristics cause drastic variations in soil's abiotic and biotic components over short distances. Many soil quality indicators are used to assess soil functions (Doran & Parkin, 1994). For example, abiotic characteristics such as soil particle size distribution (Six et al., 2000), pH (Letey, 1958), available nutrients levels, salinity (Rietz & Haynes, 2003), and organic carbon content (Wiesmeier et al., 2019), influence the soil microbial abundance and diversity, consequently impacting the soil functioning (Burns, 1982; Verstraete & Voets, 1977). Soil micro- and macroscopic- organisms metabolically decompose soil organic matter and make nutrients available for the soil biota (Nannipieri et al., 1978; Sinsabaugh et al., 2002).

Research has shown that soil nutrient cycling enzymes secreted by the soil microbes mineralize the complex components of a dead animal or leaf litter (Rinkes et al., 2014; Sinsabaugh et al., 1991; Valášková et al., 2007). The metabolic reactions involving various nutrient cycling enzymes target the catalysis of complex natural polymers into simple compounds that can be assimilated by soil biota (Martinez, 1968). A large body of literature indicates that many soil nutrient cycling enzymes have been studied for assessing soil

functioning (Dick, 1994; Gianfreda & Rao, 2004; Skujiņš & Burns, 1976). These soil enzymes include phosphatase for phosphorus cycling, L- leucine amino peptidase for nitrogen cycling, and cellobiohydrolase for carbon cycling (Jian, 2016; Sinsabaugh et al., 2008).

Phosphorus is essential for plant and microbial cellular functioning (Cosgrove, 1967). For example, the phosphatase enzyme hydrolyzes the organophosphates in the soil organic matter to produce inorganic phosphorus, which is the preferred form for plant uptake and microbial cellular processes (Eivazi & Tabatabai, 1977). Interventions that can enhance soil nutrient cycling, whether in agricultural or contaminated soils, can be examined for their effectiveness through soil enzymatic function (Gul & Whalen, 2016; Shaw, 2013). Soil extracellular enzyme activities contribute to efficient nutrient cycling, enhance plant productivity, benefit soil-biota, and can serve as indicators of soil quality (Alkorta et al., 2003; Burns, 1982; Caldwell, 2005; Dick et al., 1996; Makoi & Ndakidemi, 2008). By determining the enzymatic functioning of the study soils in my projects, I observed the impact of phytoremediation and biostimulation on the soil functioning.

1.5 Simulated or artificial root exudates

The above- and below-ground parts of plants provide sustenance to the wildlife and soil organisms (Pascual et al., 2000). Plant roots secrete root exudates to support soil organisms and plants receive mineralized forms of nutrients in return (Hale et al., 1978; Rovira, 1969). The components of naturally secreted plant root exudates include primary metabolites, such as sugars, organic acids, amino acids, secondary metabolites, and proteins (Badri et al., 2009; Bais et al., 2006; Uren, 2000). Treating low functioning soils with low molecular weight substrates extracted from roots or a solution of low molecular weight compounds prepared in the laboratory

(artificial root exudates) has been shown to increase soil function (Balseiro-Romero et al., 2014; Kuzyakov, 2010; Meier et al., 2017; Nannipieri et al., 2008; Jiaolong Wang et al., 2021). Kuzyakov et al. (2000) defined these changes as "priming effects" where the soil function improved for a brief time with moderate additions of Carbon (C) and Nitrogen (N) containing substances that transformed the cycling of C and N in the soil. Primary metabolites also have been found to stimulate soil microbial functioning (Haichar et al., 2008; Kuzyakov et al., 2018; Lopez-Sangil et al., 2017; Xie et al., 2012) and to ameliorate metal toxicity (Bali et al., 2020; Vithanage et al., 2012).

Bais et al. (2004) described "an underground information superhighway" where plants "communicated" with soil microbes and other plants by exuding synchronized root exudates to modulate rhizosphere microbial communities for protection from pathogens, to enable symbiotic feedback, and cycle minerals in nutrient-poor environments (Bais et al., 2008; Bais et al., 2004; Bais et al., 2002). Moreover, the composition of root exudates has been shown to alter the microbial activity in Cd-contaminated soils (Renella et al., 2006). Additionally, root exudate composition has been shown to modulate soil microbial abundance and plant uptake exhibiting competition between them (Kim et al., 2010; Kirk et al., 2005). The quantity and diversity of root exudates have been shown to depend on the age and diversity of plants and soil microbial biomass (Eisenhauer et al., 2017; Steinauer et al., 2016).

Simulated root exudates are laboratory preparations of mixtures containing root exudate components (Kunc & Macura, 1966; Steinauer et al., 2016). Using simulated exudates to amend agricultural and polluted soils is a growing field in soil remediation (Gransee, 2001; Li et al., 1997; Luo et al., 2014; Shukla et al., 2011; Wang et al., 2020). Spohn and Kuzyakov (2013) reported that the addition of alanine showed a 6-fold increase in phosphatase activity in the top

and subsoil sections of an uncontaminated mixed deciduous forest. Montiel-Rozas et al., (2016) showed that three herbaceous species (*Poa annua* L., *Medicago polymorpha* L., *Malva sylvestris* L.) planted in washed sand mixed with increasing gradient concentrations of Cd, Cu, and Zn, controlled metal uptake by releasing specific root exudates (oxalic acid and citric acids) in high quantities to drive plant growth. These findings suggest that, in contaminated soils, some plant species facilitate higher shoot mass and metal uptake in the aerial regions of the plants by exuding specific root exudates (Montiel-Rozas et al., 2016).

On that note, studies using aged, contaminated soils with exceptional characteristics, such as our study site LSP, which supports a vibrant ecosystem, are rare. Phytoremediation strategies are not directly applicable to sites that do not support plants (Kuppusamy et al., 2017; Megharaj & Naidu, 2017; Uchimiya et al., 2020). Phytoremediation efforts yielded better plant productivity when soils were revitalized (Couic et al., 2018). Revitalizing barren, post-industrial soils with natural amendments to stimulate native microbial communities as shown in the second project of my thesis could lay the groundwork for effective phytoremediation intervention.

1.6 Study site

The soil studied through the work presented here is from an abandoned, barren, post-industrial rail yard located within the Liberty State Park (LSP) in Jersey City, New Jersey, USA. In the early 1900s, the construction debris and municipal waste from New York were collected and filled at this site for building the Central Railroad of New Jersey rail and cargo services station. This terminal transported many commodities such as coal, petroleum products, livestock, and passengers (Hagmann et al., 2015). A 104-ha section of the LSP site was fenced off from public access in 1969 after the closure of rail services because of high contaminant levels

(Gallagher et al., 2008). The peripheral sections of the area were reclaimed by replacing the topsoil with healthy soil and opened for public recreation (Hagmann et al., 2015). The section of LSP with restricted access is our study site, and it has some unique characteristics. Some areas within the former rail yard were naturally colonized by vegetation, while others have remained barren decades after the tracks were removed.

Previous studies of the LSP soils have detected polycyclic aromatic hydrocarbons and high concentrations of heavy metals (Gallagher et al., 2008; Gallagher et al., 2011). Hagmann et al. (2015) reported high extracellular phosphatase activity, an indicator of the phosphorus cycling in soils (Nannipieri et al., 2011), in site 146 soil within LSP despite high heavy metal contamination. This was an exceptional finding contrary to the many works stating negligible enzyme activity and soil quality in extreme environments (Dick et al., 1988; Kuperman & Carreiro, 1997; Wang et al., 2007). Some of the studies have reported that the presence of metal contaminants such as Zn, Cu, Cr, Cd, Pb, and As (same as those found in LSP soils), decreased phosphatase activity due to oxidative stress (Charlton, 2015; Doelman & Haanstra, 1989; Dumontet et al., 1992; Kandeler et al., 1996). Lee et al. (2002) studied the phosphatase enzyme activities at a shooting range in South Korea containing high concentrations of the metals listed above, and they reported that phosphatase activity at rich vegetation spots was higher compared to the barren spots.

In a recent study, Singh et al. (2019) reported that some LSP sites (146, 25F, and 43) showed high bacterial count and phosphatase activity, while site 25R displayed low bacterial cell count and below detection level phosphatase activity. The atypical characteristics of the LSP soils make them highly desirable for investigating soil function. In addition to the LSP study site, a reference site called Hutcheson Memorial Forest (HMF) in Somerset County, NJ, USA, owned

by Rutgers University, was used as a chronosequence reference site due to its piedmont landform and natural succession that matches with the timeline of LSP (Hagmann et al., 2015). LSP study site's unique ecosystem and soil characteristics provided a valuable opportunity to test the effectiveness of simulated root exudates on the native microbes and assess the application of biostimulation followed by phytostimulation on the functioning of the barren, post-industrial soil.

2. Research objectives

The LSP study site has natural forest growth formed over 5 decades that supports wildlife despite high concentrations of several heavy metals and organic contaminants. As the access to the study site is restricted and was left undisturbed for decades, LSP makes an exceptional site to study the interrelationships that have developed among the soil organisms, plants, and contaminants (Gallagher et al., 2018; Krumins et al., 2015; Singh et al., 2019; Vaidya et al., 2020). The soil enzymatic response to the high contaminant levels and other soil health indicators, provides a fantastic opportunity to study soil functioning in this multifaceted ecosystem (Hagmann et al., 2015; Hagmann et al., 2019). Insights on soil functioning of an undisturbed, industrial barren site such as LSP may help gain further understanding in mitigating soil restoration issues for other industrial barrens worldwide.

I worked on three projects to examine the practical application of current remediation practices (phytoremediation, bioremediation) and whether a modified approach of stimulating native soil microbes could be combined with the current bioremediation techniques to restore function in soil from a barren, contaminated, post-industrial site. The three experiments presented here were conducted using a climate-controlled growth chamber, and following

response variables were determined: extracellular phosphatase activity, soil moisture content, soil respiration rate, easily-extractable glomalin content in the soil, plant biomass, soil particle size distribution, and soil organic carbon content. Fresh soils from the top 10 cm were collected after the removal of surface gravel and leaf litter for these experiments. During soil collection, the sampling depth was selected corresponding to root concentration and biologically active zone of the soil (Gallagher et al., 2015; Gallagher et al., 2008). Within the LSP study site, I collected soils from sites: vegetated 146, vegetated 25F, and barren 25R. An uncontaminated soil from the HMF site was used as a reference.

The objective of my first project was to examine whether mixing a high functioning or active soil with a low functioning or inactive soil would increase the soil function of the low functioning soil. The goal was to assess the effect of adding soil microbial communities from the high functioning, vegetated 146 soil on barren, contaminated 25R soil by creating a soil mix gradient of the two soils with distinctly different characteristics. As a follow-up to this question, the next part of the first project examined the impact of plants on the low functioning soil. During this project, Jennifer Balacco collaborated with me on the watering schedule logistics. The first project consisted of two parts.

Part A examined:

- What would happen after mixing the high-functioning soil from vegetated 146 with the low-functioning soil from barren site 25R?
- Would the microbial communities from the contaminated but healthy 146 soil stimulate phosphorus cycling in the unhealthy, inactive 25R soil?

Then, in part B, using the same soil mixing strategy as before, I asked:

- Could plants germinate in 25R soil using growth chamber conditions?

- How would 25R soil phosphatase activity vary when plants were present?

The outcomes from the first project disclosed that plant growth was more beneficial than the physical mixing of the two active and inactive soils. Using this information, I hypothesized that plant roots exuded some beneficial compounds to stimulate the native microbes in the nutrient-limited, inactive 25R soil. By preparing a solution of some commonly found root exudate compounds in lab, I may be able to stimulate microbial functioning in 25R soil.

The objective of my second project was to examine whether a solution of simulated root exudate compounds could improve soil function of low functioning soil. I prepared a solution of root exudate compounds (mono and disaccharides, amino acids, organic acids) in the laboratory, using a recipe found in the literature. I added the exudate solution to the barren soil over 205 days to find whether feeding the contaminant-stressed microbes from 25R soil would stimulate nutrient cycling. The second project consisted of two experiments: the main experiment and a side experiment. The side experiment focused on determining an appropriate dose of the simulated root exudate compounds for the main experiment. During this project, Dr. Diane Hagmann collaborated with me on preparing simulated exudate solution and Jamila Haramuniz collaborated with me on statistical data analyses.

The main experiment focused on learning the effect of a laboratory-prepared solution of exudate compounds similar to those exuded by plant roots when added to the inactive 25R soil

- How would the phosphatase activity change with time?
- How long will it take to observe the effects of simulated root exudates?
- How effective is the exudates solution compared to plants?

In a side experiment, I determined the dose the simulated exudates solution that would be effective for increasing the phosphatase activity in the low functioning, barren 25R soil.

The outcomes from the second project suggested that the addition of simulated exudates showed a rapid increase in soil function and was more effective than growing plants. Using this information and thinking from a practical management perspective, it would be essential to determine whether soil function in inactive soil could be revived with a single dose of exudates solution.

The objective of my third project was to study the impact of single versus repeated additions of simulated exudate solution on the functioning of a contaminated, inactive soil. I tested whether a single addition of laboratory-prepared simulated root exudates solution was sufficient to stimulate native microbial metabolism and phosphorus cycling in three soil types: uncontaminated, vegetated HMF, contaminated, vegetated 25F, and contaminated, barren 25R soil. During this project, Sarah Krisak, collaborated with me on the watering schedule logistics, writing the manuscript, soil respiration measurements, and their data analyses. The questions I sought to answer were:

- Could a single addition of the exudate solution stimulate native microbial metabolism?
- How long would the effect of a single addition of simulated exudate solution last?
- Could simulated root exudate compounds stimulate native microbes in both contaminated and uncontaminated soils?
- What was the effect of exudate solution on phosphatase activity among the three soil types; was it similar or different?
- If seeds were added to the enriched 25R soil, would the plants growth improve?

I compared the single addition, where the exudates solution was added only once, with a repeated additions treatment, where the exudates solution was added three times per week over thirty days, for a total of 14 additions. The total amount of exudate compounds added was

similar; only the time frame was different. During the nine months of the experiment, I analyzed soil carbon dioxide respiration rate and phosphatase activity. Then, I added winter rye seeds to compare the plant biomass and shoot height differences between plants from enriched and unenriched barren 25R soils.

In this dissertation, chapter 1 begins by introducing the soil quality of urban post-industrial landscapes. It then summarizes current soil remediation approaches, benefits, and limitations of implementing these technologies. After discussing the techniques, the importance of soil function assessment using nutrient cycling enzymes followed by root exudates and their relevance to this work is explained. The next part of the introduction presents the research objectives of the three projects conducted and acknowledges the collaborators involved. The detailed studies conducted for the three projects are presented in chapters 2, 3, and 4, stating the experimental design, methods, results, and conclusions. Finally, chapter 5 reflects on the conclusions from each project and proposes a remediation strategy that may have the potential for field application. Then, it states the limitations of transferring the proposed strategy to a field deployment, followed by suggestions for field scale experiments. Overall, the work presented through this dissertation examines the effectiveness of natural and simulated root exudates on the functioning of barren, post-industrial soil.

Through these three projects, I evaluated the implementation of an environmentally-friendly technique to restore soil function in a barren, contaminated, inactive, post-industrial soil. With insights into the complex mechanisms occurring among plants, contaminants, and native soil microorganisms, environmental managers may develop “green” strategies to restore soil function in barren industrial sites. By stimulating contaminant-resistant microbes, it may be

possible to revive ecosystem services at the post-industrial barren sites and eventually redevelop these sites into green spaces for public recreation and renewable energy farms.

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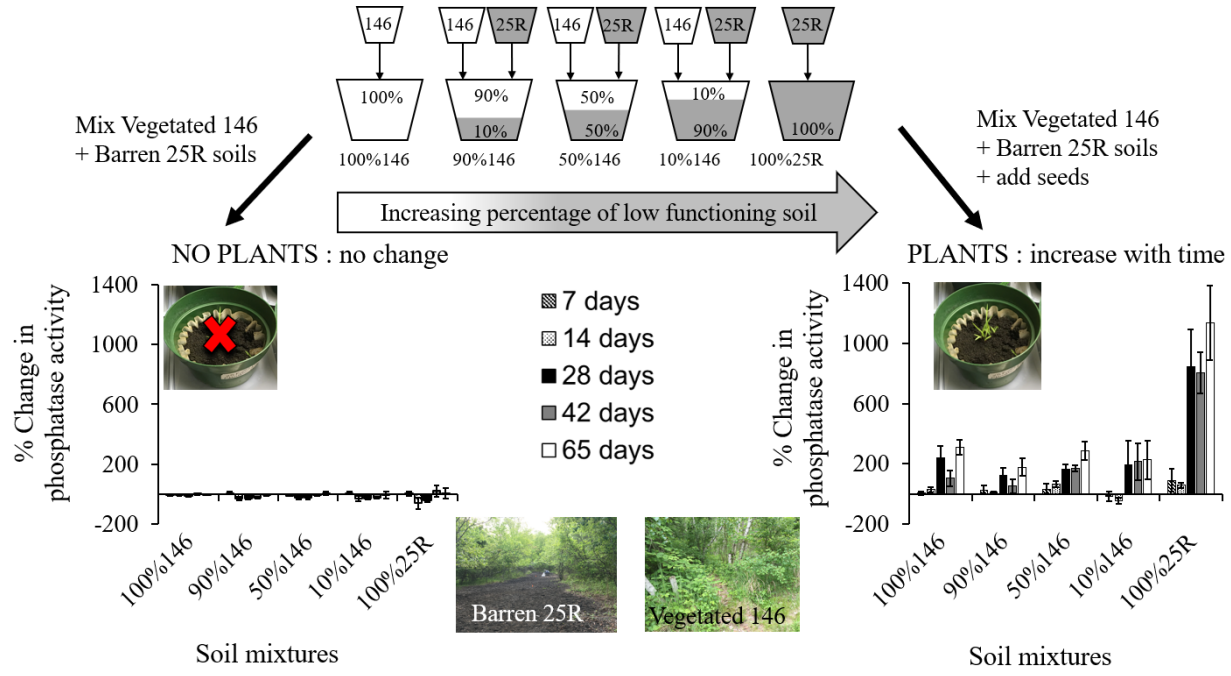
CHAPTER 2

Project 1: *Plants mitigate restrictions to phosphatase activity in metal contaminated soils*

Graphical abstract



Article 1. *Plants mitigate restrictions to phosphatase activity in metal contaminated soils*

Abstract

Soil anthropogenic contaminants can limit enzymatic nutrient mineralization, either by direct regulation or via impacts on the microbial community, thus affecting plant growth in agricultural and non-agricultural soils. The impact on phosphatase activity of mixing two contaminated, post-industrial rail yard soils was investigated; one was vegetated and had high phosphatase function, the other was barren and had low enzymatic function. The two soils had different abiotic properties, including contaminant load, vegetation cover, soil aggregate size distribution, and phosphatase potential. An experimental gradient was established between the two soils to systematically vary the abiotic properties and microbial community composition of the two soils, creating a gradient of novel ecosystems. The time dependence of extracellular phosphatase activity, soil moisture, and organic matter content was assessed along this gradient in the presence and absence of plants. Initially, mixtures with higher percentages of functional, vegetated soil had higher phosphatase activities. Phosphatase activity remained unchanged through time (65 days) in all soil mixtures in unplanted pots, but it increased in planted pots. For example, in the presence of plants, phosphatase activity increased from 0.6 ± 0.1 to 2.4 ± 0.3 $\mu mol \bullet h^{-1} \bullet g_{dry\ soil}^{-1}$ from day 1 to day 65 in the 1:1 functional to barren soil mixture. The presence of plants also promoted moisture retention. Inoculation of poorly functioning soil with 10% of the functional soil with its microbial community did not, over 65 days, revitalize the poorly functioning soil. The findings showed that abiotic limitations to enzymatic activity in barren brownfield soils could be mitigated by establishing primary production but not by the addition of enzymatically active microbial communities alone.

Keywords: soil heavy metals, soil phosphatase activity, soil abiotic conditions, phytostimulation, brownfield

1. Introduction

Anthropogenic toxic chemicals can restrict soil function in contaminated sites (Bandick & Dick, 1999; Dick, 1994; Dick et al., 1996; Vepsäläinen, 2001). Soil extracellular enzyme activities have been used as indicators of soil quality because they contribute to efficient nutrient cycling, plant productivity, and healthy soil-biota (Kim et al., 2010; Li et al., 2015; Roldan et al., 2003; Schimel et al., 2007; Zhang et al., 2015). Decreased soil enzymatic function can in extreme cases, result in abandoned spots called "industrial barrens or extreme environments" that do not support plant life and are resistant to natural restoration (Kandeler et al., 2000; Kozlov & Zvereva, 2007; Kuperman & Carreiro, 1997). Industrial barrens are undesirable because they do not support agriculture, provide ecosystem services to wildlife, or capture toxic compounds to protect groundwater.

Novel phytostimulation approaches based on biomaterial additives, plants, and microorganisms can be used to restore unproductive, contaminated soils (Awasthi et al., 2020; Kumar, 2019; Megharaj & Naidu, 2017; Nayak et al., 2018; Pedron & Petruzzelli, 2011; Wang et al., 2019). Application of both phytoremediation (Gaur & Adholeya, 2004) and rhizoremediation strategies (Gadd, 2010; Harris, 2003; Korade, 2009; Nakamura, 2004; Yu, 2003) requires soils to be vegetated. To support plants, soils must have a steady source of mineralized nutrients and thus extracellular enzyme activity. Previous studies have reported the mixing of biotic and/or abiotic biomaterial additives into contaminated soils to improve soil enzymatic function (Grobelak et al., 2017; Kumpiene et al., 2009; Rinklebe & Shaheen, 2015;

Rocco et al., 2018). These additives include compost, biochar, fly ash, lime, activated charcoal, soil slurries, microorganisms, and many others (Pichtel & Hayes, 1990; Shaheen et al., 2017a; Shaheen et al., 2017b; Zhang et al., 2016; Zhang et al., 2019).

Addition of soil slurries contributes microbes and microfauna and can provide insights specifically into the impact of biotic factors on soil function (Kuppusamy et al., 2016; Van Nevel et al., 2007). Conversely, adding whole soils contributes both the biotic and abiotic soil components (Smith-Ramesh & Reynolds, 2017). Here we created a systematic whole-soil gradient (100%, 90%, 50%, 10%, 0%) between two aged contaminated soils from Liberty State Park (LSP), a post-industrial brownfield in Northern New Jersey with unique properties; one vegetated, highly functioning and the other barren and poorly-functioning. When a small amount of soil A is added to soil B (for example, 10%) to create soil C, any differences in soil function between soils B and C are mostly due to the biotic factors such as soil microorganisms and microfauna. On the other hand, when a larger amount of soil A is added (for example, 50%), the differences between soils B and C are due to both the abiotic and biotic factors because a large amount of soil A added, significantly changed both the abiotic and biotic composition of the resulting soil C relative to soil B (Bennett & Klironomos, 2019; Bever, 2002, 2003; Ehrenfeld et al., 2005; Smith-Ramesh & Reynolds, 2017). The experimental gradient thus simultaneously provided information on the role of abiotic and biotic factors involved in the establishment of natural habitats in the presence of persistent anthropogenic soil pollutants. Moreover, the gradient approach did not rely on a microbial inoculum that might not capture the entire microbial community but rather used the whole soil to introduce microorganisms. Finally, the design did not require soil sterilizations to eliminate the biotic components, for example autoclaving, which may alter soil properties including structure.

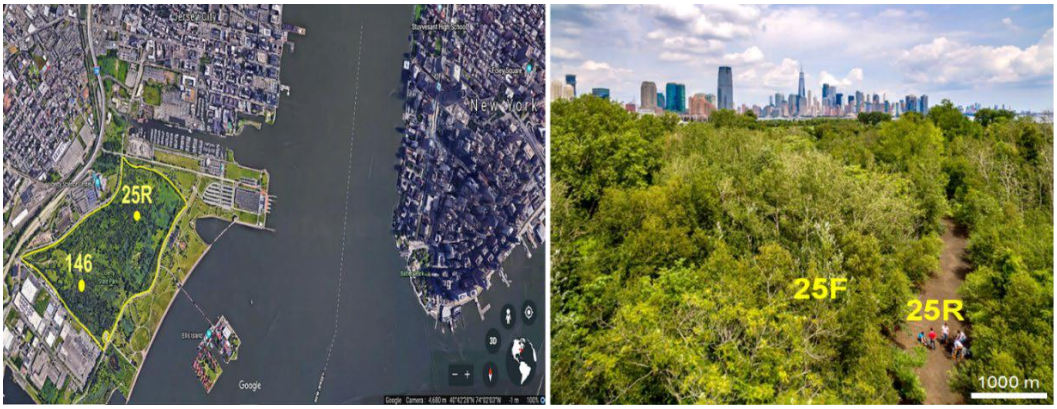


Figure 1. The location of study sites at Liberty State Park, Jersey City, New Jersey, (left). An aerial view of the site 25R lacking vegetation surrounded by abundant plant cover denoted as site 25F; the white bar denotes 1000 m (right) (photo credit: Mike Peters).

Some heavily contaminated soils at LSP were previously found to have unnaturally and atypically high extracellular enzymatic activities (Figure 1) (Hagmann et al., 2015). Previous findings suggest that, in the absence of plants, abiotic rather than microbial factors limit phosphatase activity (PA) in LSP soils (Singh, Ojinnaka, et al., 2019). There was no correlation found between the microbial community and PA (unpublished data). On the contrary, a strong correlation was found between Cu, Zn, and Pb concentrations and PA as well as organic matter with microbial community composition in LSP soils (Singh, Ojinnaka, et al., 2019).

Here we build upon these observations. It is possible that active LSP soils are a source of contaminant-resistant microbial communities that can be introduced to contaminated but poorly functioning LSP soils to improve soil function (Benjamin et al., 2003; Borggaard et al., 2019; Evans et al., 2015; Glæsner et al., 2019; Li et al., 2015; Pogrzeba et al., 2017). Alternatively, it is possible that microbial inoculation does not improve soil function because the abiotic factors limit microbial function (Clemente et al., 2008; Clemente et al., 2010; van Herwijnen, Hutchings, et al., 2007; van Herwijnen, Laverye, et al., 2007). To distinguish

between these two possibilities, we mixed contaminated but high-functioning soil into low-functioning and contaminated soil to inoculate one soil with the microbial community of the other (Figure 1). We measured the extracellular PA at six time points over 65 days and conducted these experiments both with and without plants. This study provides insights into the relative roles of plants and contaminant resistant microbial communities in functional revitalization of heavy metal contaminated soils (Gallagher et al., 2018; Gallagher et al., 2008; Krumins et al., 2015; Salisbury et al., 2017; Teng et al., 2011; Teng et al., 2015; Tripathi et al., 2015).

2. Materials and methods

2.1 Study site and soil sampling

The soil samples for our research were collected from a site located within LSP, Jersey City, NJ (40˚42’16N, 74˚03’06W). Our LSP study sites 146 and 25R are located inside a section of the park that is fenced off from the public. Site 146 has high extracellular soil PA despite the presence of high heavy metal concentrations (Hagmann et al., 2015). Site 25R is not vegetated and has low PA. Previous studies on soils from sites 146 and 25R noted correspondingly high and low phosphatase activities (PAs) that varied with soil collection season (Hagmann et al., 2015; Hagmann et al., 2019; Singh, Ojinnaka, et al., 2019; Singh, Vaidya, et al., 2019). Soil samples were collected from the top 10 cm along a 20 m transect at 5 m intervals, in the fall and winter for experiments with and without plants, respectively. The collected soil samples were stored at 4 °C until the experiment establishment. Soils from LSP sites 146 and 25R were sieved through a large mesh sieve (5 - 10 mm) and then through 2 mm to remove rocks, pebbles, and plant debris before setting the experimental pots.

2.2 Experimental design

The experimental design consisted of three independent variables on soil properties: The first was the proportion of soil 146 relative to soil 25R in a pot. The second variable was time as we measured soil properties at six-time points over 65 days. The third variable was the presence versus absence of plants. Each experiment was replicated four times in four individual pots for the soil proportion mixture. The dependent variables that we measured include soil PA, soil moisture, metal concentrations, soil organic matter, soil aggregate size, and plant biomass. Hagmann et al. (2015) reported three enzyme activities (L-leucine-amino-peptidase, cellobiohydrolase, and phosphatase) at five LSP sites to follow the same trends for all five sites; for example, if one enzymatic activity was low at a given site, it was also low at the other four sites (Hagmann et al., 2015). As such, we here selected PA as a proxy for the three enzyme activities that represent nutrient cycling of N, C, and P in the soils (Adetunji et al., 2017; Martinez, 1968; Nagajyoti et al., 2010; Nannipieri et al., 2011; Narendrula-Kotha & Nkongolo, 2017; Tarafdar & Jungk, 1987).

The soil samples were categorized into five soil mixtures by mixing the soils from the two sites (LSP sites 146 and 25R) in different proportions using polypropylene bags, and the mixtures were labeled based on the percentages (by weight) of each soil in the mixture. For example, the soil mixture in a pot labeled 50%146 consisted of 50 percent soil (by weight) from site 25R mixed with 50 percent soil from site 146 and the pot labeled 90%146 consisted of 10 percent of soil from site 25R mixed with 90 percent soil from site 146 (Figure 2). After mixing, soils were potted in plastic pots with dimensions 10.2 cm × 13.5 cm × 3.6 cm (height × rim diameter × base diameter) filled to 8 cm pot height, lined on the bottom with one coffee filter, and placed atop a plastic weigh boat to prevent water spillage. Each soil type and its

mixtures were potted into four pots (replicates a, b, c, and d), resulting in 20 pots per experiment. The experimental pots were kept in a climate-controlled chamber (growth chamber) maintaining a photo-period of 10.5/13.5 hour day/night cycle. The relative humidity was set at 65%, and the day-night temperatures at 24 °C and 16 °C, respectively. This experiment was conducted twice, once without plants and again with plants. In the second experiment, to assess the effect of plants on contaminated soils, we added 15 Ryegrass seeds (*Lolium perenne*) and 15 Switchgrass seeds (*Panicum virgatum*) to all replicates on the first day.

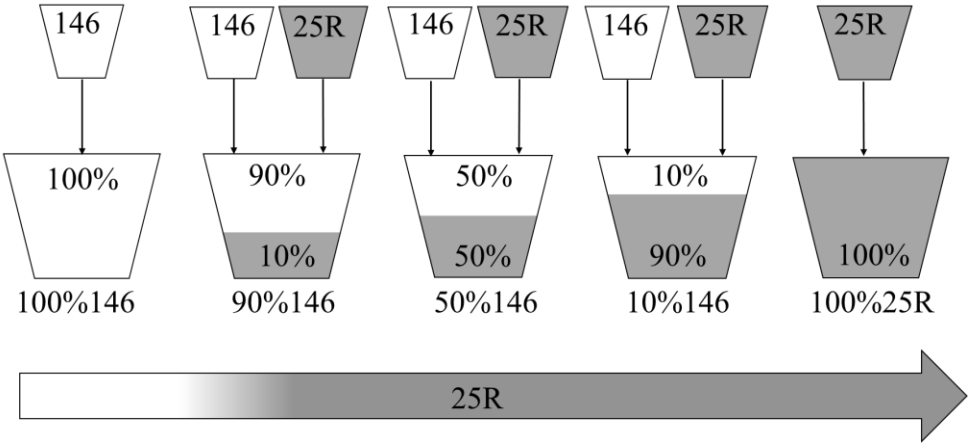


Figure 2. Experimental set-up diagram showing the gradient of LSP site 25R soil relative to site 146 soil (n = 4) in experimental soil mixtures.

2.3 Soil analysis

Phosphatase activity, which is necessary for the cycling of phosphorus, an essential nutrient in cellular metabolic processes, is here used as a proxy for soil enzymatic function (Dick et al., 1996; Nannipieri et al., 2011; Nannipieri et al., 1979). The PA for each replicate was measured on day 1 when the soils were mixed, and the pots were set with and without the seeds to track the change in the quality of soil over 65 days. All the pots were watered three times a week on alternate days with sterilized distilled water. The amount of water added to

each pot was calculated on the mass of soil in each pot. For example, if the mass of soil in one pot was 300 g, then 30.0 mL sterilized distilled water was added to that pot. Soil samples from the experimental pots were collected repeatedly at six different time points over 65 days. At each time point, 6.0 g soil was removed at random from three locations within each pot. Fluorometric assay protocol described by Hagmann et al. (2015), was used to measure the amount of 4-Methylumbelliferone product formed by the phosphatase enzymes present in the soil at each time point.

Moisture content in the soil samples was determined after drying approximately 2 g of the soil samples from each replicate in a conventional oven at 100 °C for 24 hours by the gravimetric method. Soil organic carbon in all samples was measured by loss on ignition. Dry ground soil (1.5 – 2 g) was heated to 550 °C for 4 hours. The percent organic matter was determined gravimetrically for each soil mix and its replicates.

The concentrations of certain elements (V, Cr, Cu, Zn, As, and Pb) were determined using Thermo ICAP Qc inductively coupled plasma mass spectrometry (ICP-MS). Metal concentrations were analyzed by following a modified protocol of EPA method 3050B. Dry homogenized soil (0.5 g) was digested using 1:1 HNO_3 (5 mL) for 10 minutes under reflux at 95 ± 5 °C. To the cooled sample, 2.5 mL concentrated HNO_3 was added. This step was repeated over 30 minutes of reflux at 95 ± 5 °C if brown fumes appeared in the previous step. The total volume of 7.5 mL was reduced to 2.5 mL by heating at 95 ± 5 °C. Next, deionized water (1 mL) followed by 30% H_2O_2 (1.5 mL) was added dropwise to the 2.5 mL leftover solution. Further, 30% of H_2O_2 was added dropwise until minimal effervescence was attained. The solution was reduced to 2.5 mL by heating at 95 ± 5 °C. Deionized water (DI) was added to the reduced solution until the volume reached 50 mL. The samples were analyzed on the

ICP-MS with solutions further diluted to appropriate concentrations to minimize the matrix effect. Standard solutions of V, Cr, Cu, Zn, As, and Pb were used to prepare a standard curve for each metal and to determine the concentration of respective metal in the prepared samples.

The aggregate size distribution of soil mixtures was measured using a standard operating protocol with refractive index 1.544 (quartz), absorption 0.9, and rotor speed 2200 rpm was followed on a Malvern Mastersizer 2000 with a Hydro 2000 MU wet sample dispersion unit. We did not treat our soil samples with a chemical dispersant or ultrasonic bath, and thus, our samples can contain aggregates that are > 2 mm (soil was sieved as described in section 2.2). The organic matter from the soil mixtures was removed by following previously established protocols, 1 g of moist soil was treated with 2 mL of DI water along with dropwise addition of 30% H_2O_2 at 85 °C until effervescence was minimal (Day, 1965).

Plant biomass was measured in the subsequent experiment, where 15 winter rye (*Lolium perenne*) and switchgrass (*Panicum virgatum)* seeds were added to each replicate on the seventh day after soil potting. In the soil mixtures in which seeds were added, germination of rye and switchgrass was noted seven days post experimental set up in all soil mixtures. At day 65, plants were harvested by separating roots and shoots, washing the roots with tap water to remove excess soil, and placing all separated plant material in the drying chamber at 70 °C for 13 days. Dry weights of roots and shoots were measured for each treatment, and total plant mass (grams) was calculated by adding dry root mass (grams) to dry shoot mass (grams). Root to shoot ratio was determined using these dry weights.

2.4 Data analysis

Differences in plant biomass and metal concentrations for different soil mixtures were analyzed using a one-way analysis of variance (ANOVA) in which soil treatment was the fixed

factor. Repeated measures (six-time points) two-way ANOVA was conducted such that soil treatment and the presence or absence of plants were the fixed factors and PA, percent moisture, and percent organic matter the response variables. Tukey's HSD post hoc test was used to separate the means among the soil mixtures. All data were analyzed using SPSS (IBM, Version 25). Spearman's correlation and follow-up statistical analyses were performed using R version 3.6.1, R Core Team (2019). We used the *tidyverse* package for data structuring (Wickham & Wickham, 2017), *Hmisc* (Harrell Jr et al., 2020), for statistical operations, *corrplot* (Wei & Simko, 2013), and *ggcorrplot* for data visualization (Kassambara & Mundt, 2017).

3. Results

3.1 Characterization of bulk soils and mixtures

LSP soils 146 and 25R have different properties, allowing us to create a gradient of new contaminated soil environments. We characterized the two bulk soils before mixing to establish the characteristics expected for each mixture in the gradient. We measured percent moisture, percent organic matter content, extracellular PA, aggregate size distribution, water holding capacity, pH, and metal concentrations (Table 1). Field site 146 is vegetated, while site 25R is barren (Gallagher et al., 2011; Hagmann et al., 2019). Soils from sites 146 and 25R have high (3.8 ± 0.1 SE) and low (0.1 ± 0.02 SE μmol h^{-1} $g_{dry\ soil}^{-1}$) extracellular PAs, respectively. Soil 146 has a higher organic matter content (47 ± 0.3 SE %) compared to soil 25R (24 ± 0.7 SE %) (Table 1). Concentrations of Cu, Zn, As, and Pb are 15, 40, 8, and 13 times higher at site 25R compared to 146, respectively (Table 1). Soil aggregates size distribution data showed that soil 146 consists predominantly of smaller soil aggregates (d (0.5) = 105 ± 4 SE μm) compared to 25R soil (d (0.5) = 443 ± 30 SE μm). Soil 146, (133 ± 0.9 SE % w/w) retained more water than

soil 25R (29 ± 0.8 SE % w/w) (Table 1).

Table 1. Abiotic bulk soil characteristics of sites 146 and 25R without plants.

Property[b]	**Site 146**	**Site 25R**
Vegetation	Vegetated	Barren
Moisture (%)	38 ± 0.3	18 ± 0.2
Organic matter (%)	43 ± 0.5	23 ± 0.2
Phosphatase activity ($\mu mol \bullet h^{-1} \bullet g_{dry\ soil}^{-1}$)	3.8 ± 0.1	0.13 ± 0.02
Aggregate size d (0.5) (µm)	105 ± 4	443 ± 30
Water holding capacity (% w/w)	133 ± 0.9	29 ± 0.8
pH[a]	5.20[a]	4.95
Vanadium ($\mu g/g_{dry\ soil}$)	183 ± 6	59 ± 4
Chromium ($\mu g/g_{dry\ soil}$)	110 ± 4	68 ± 5
Copper ($\mu g/g_{dry\ soil}$)	108 ± 2	1,469 ± 75
Zinc ($\mu g/g_{dry\ soil}$)	98 ± 2	3,994 ± 293
Arsenic ($\mu g/g_{dry\ soil}$)	41 ± 2	263 ± 24
Lead ($\mu g/g_{dry\ soil}$)	316 ± 9	4,249 ± 197

[a] Values from (Hagmann et al., 2015)
[b] Standard errors of the mean are shown, n = 4

After mixing 146 and 25R soils in different proportions (Figure 2), we investigated whether the soil mixtures had the expected abiotic properties based on the mixing proportions. Soil metal concentrations, organic matter content, extracellular PA, aggregate size distribution, and water holding capacity were found consistent with the proportions and characteristics of the two constituent soils 146 and 25R. These data showed that we successfully experimentally created the gradient of soil components as planned. For instance, mixtures with more soil 25R showed higher metal concentrations (supplementary material, Table S1). For example, Pb concentrations in 100%146 and 100%25R soils were 316 ± 9 SE $\mu g/g_{dry\ soil}$ and 4249 ± 197 SE $\mu g/g_{dry\ soil}$, respectively. SE here represents the standard error of the mean of four replicates. The Pb concentrations in soil mixtures 90%146, 50%146, and 10%146 were 988 ± 183 SE $\mu g/g_{dry\ soil}$, 2703 ± 198 SE $\mu g/g_{dry\ soil}$, and 3737 ± 790 SE $\mu g/g_{dry\ soil}$, respectively.

Mixtures with more soil 146 contained more organic matter. Soil mixtures 90%146,

50%146, and 10%146 contained 40 ± 0.3 SE %; 30 ± 0.3 SE %; 25 ± 0.2 SE % organic matter, compared to soils 100%146 and 100%25R (43 ± 0.52 SE % and 23 ± 0.2 SE %) respectively (Figure 3). Moreover, the aggregate size distributions of soil mixtures 90%146 and 10%146 were similar to those of 100%146 (d (0.5) =105 ± 4 SE μm) and 100%25R (d (0.5) = 443 ± 30 SE μm), respectively, while the particle size distribution of mixture 50%146 was found to be intermediate between 100%146 and 100%25R (Figure 4).

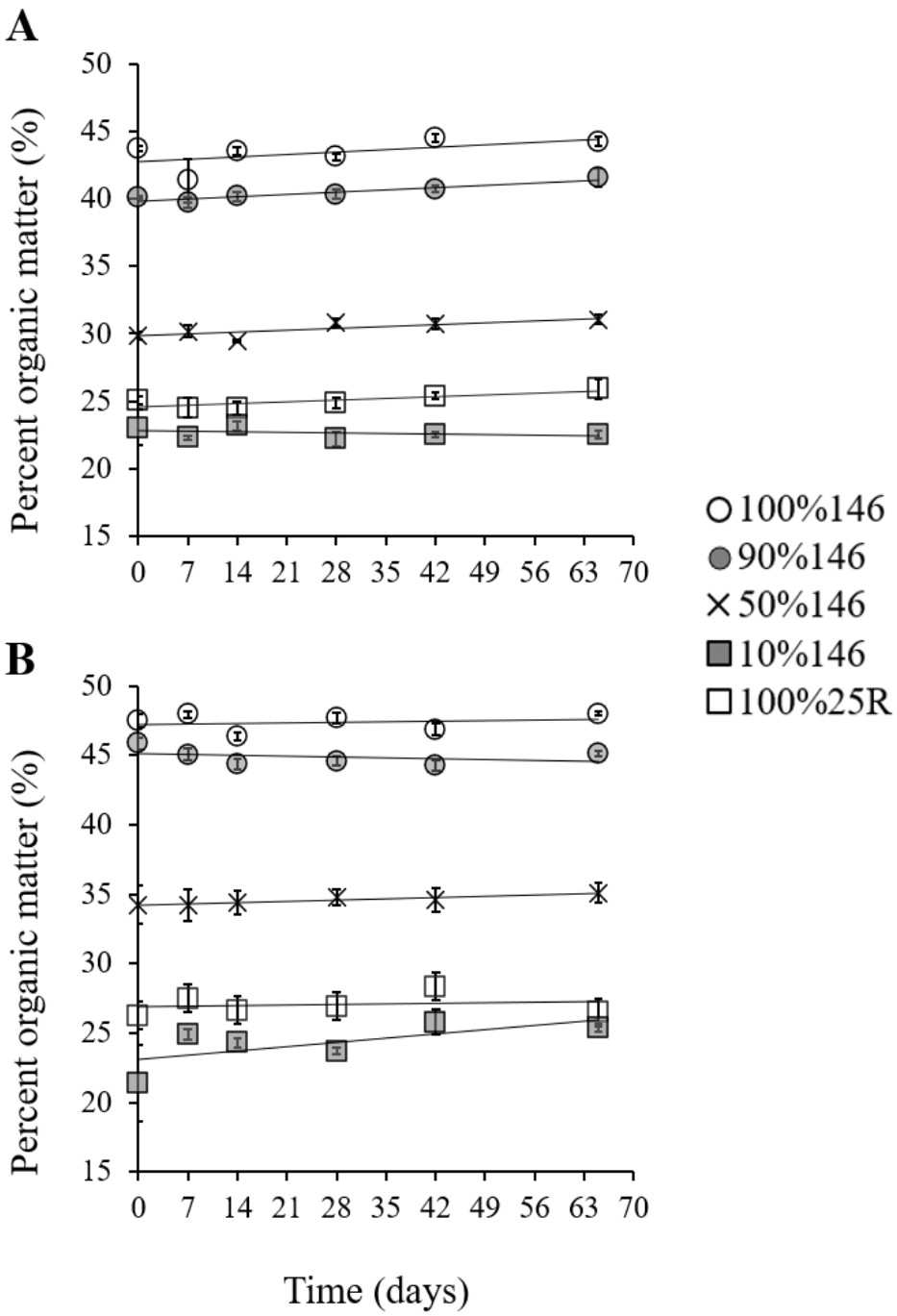


Figure 3. Percent organic matter was measured as loss on ignition of soil samples (100%146, 90%146, 50%146, 10%146, and 100%25R) in experiments without plants (A) and with plants (B) (n = 4 per experiment for each soil mixture). From the data analyzed for all time points (days 1, 7, 14, 28, 42, 65), the organic matter did not vary significantly with time. Error bars show the standard error (SE) of the mean (n = 4, ± 1 SE).

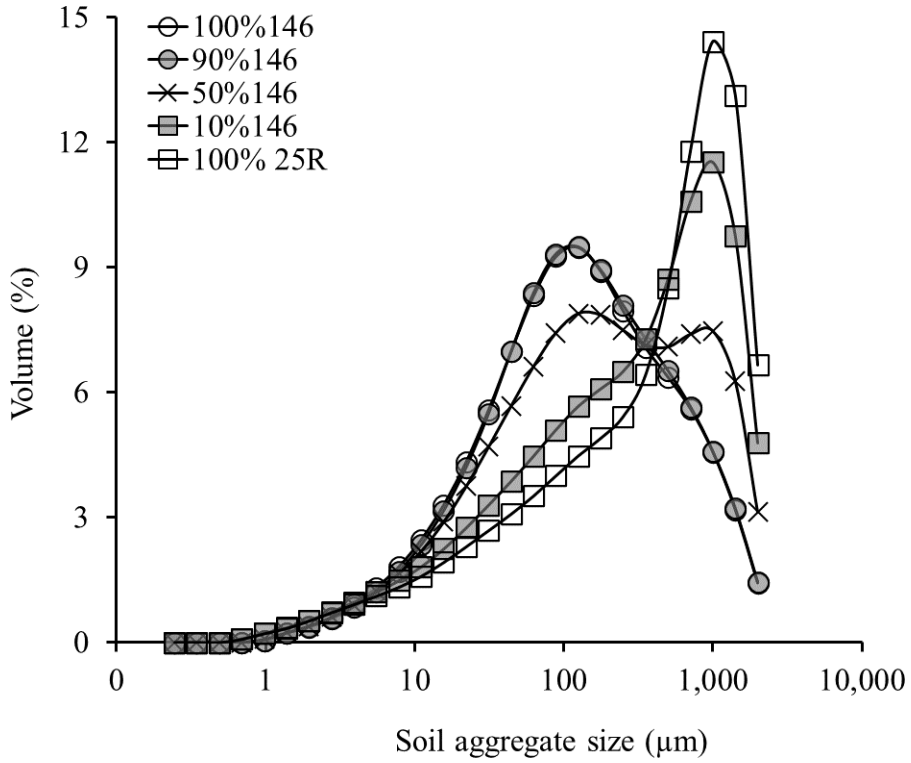


Figure 4. Average aggregate size (µm) distributions of each soil mixture (100%146, 90%146, 50%146, 10%146, and 100%25R) are shown (n = 4). The distributions were determined using a Malvern Mastersizer 2000 with a Hydro 2000 MU wet sample dispersion unit. Soil mixtures with a higher percentage of 25R by weight have larger aggregates compared to mixtures with a higher percentage of 146 soil.

3.2 Time-dependent changes in phosphatase activity with no plants

To determine whether PA changed over time in the soil mixtures when plants were absent, we measured soil extracellular PA at six-time points (Figure 5A). Immediately after mixing the soils (day 1), the activities were consistent with the proportions of the two constituent soils (Figure 5A). For example, day 1 PAs for 100%25R, 50%146, and 100%146 were 0.1 ± 0.02 SE, 2.0 ± 0.1 SE, and 3.8 ± 0.1 SE µmol h^{-1} $g_{dry\ soil}^{-1}$, respectively. PA in the experiment without plants did not change with time (Figure 5A).

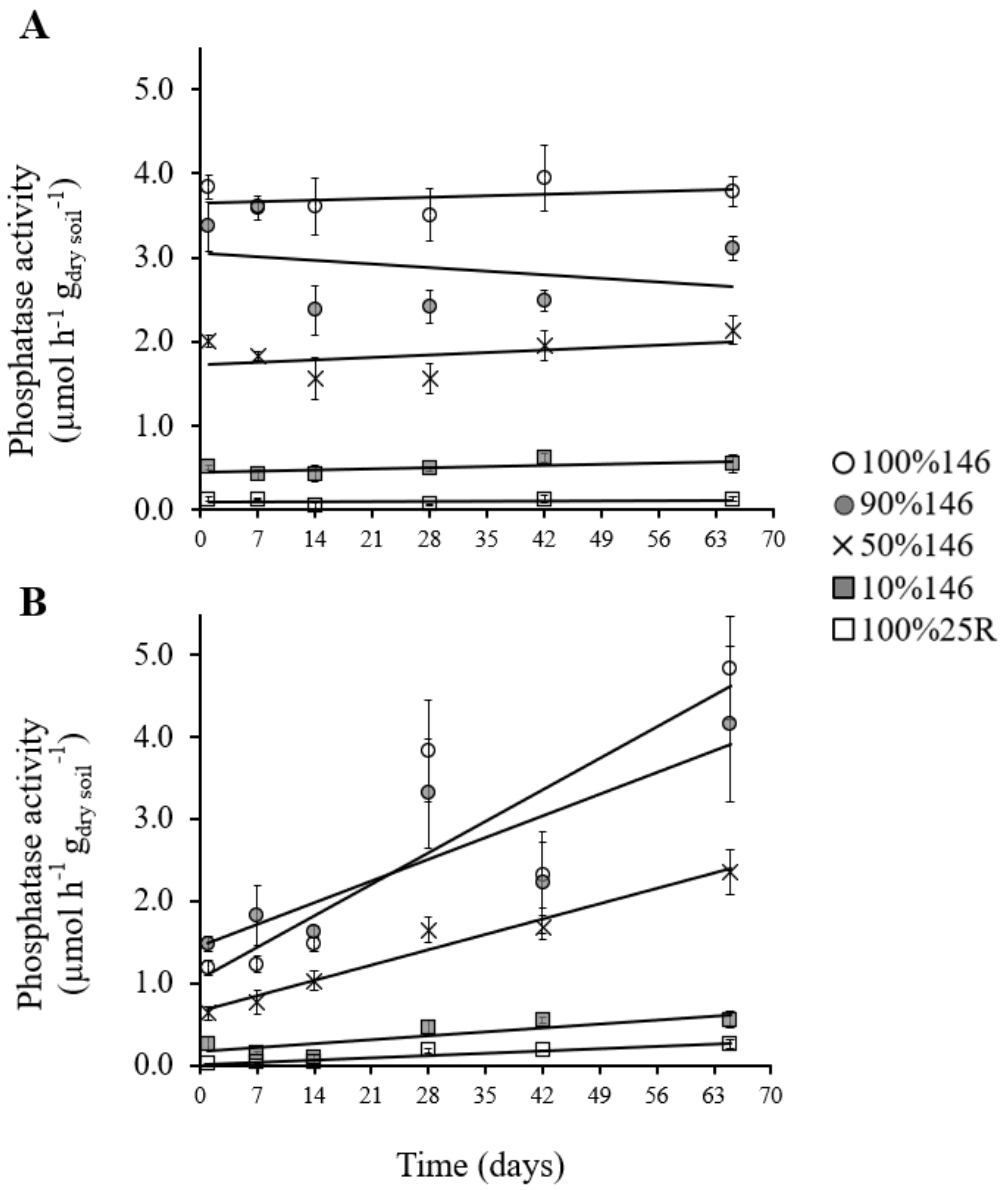


Figure 5. Phosphatase activities in experiments without plants (A) and with plants (B) are shown for five soil mixtures (100%146, 90%146 soil, 50%146, 10%146, 100%25R) at six-time points. The phosphatase activity in the unplanted soil mixtures did not show significant differences over 65 days, as shown in (A), although, among the gradient soil mixtures, it was significantly different ($F_{4,15} = 259.321$, $P < 0.0005$). Only the planted experiment showed significant differences in phosphatase activity over time ($F_{1,15} = 75.239$, $P < 0.0005$ as well as among the gradient soil mixtures ($F_{4,15} = 35.094$, $P < 0.0005$) as shown in (B). Each data point represents the average phosphatase activity of four experimental replicates and error bars show the standard error (SE) of the mean (n = 4, ± 1 SE).

3.3 Time-dependent changes in phosphatase activity with plants present

In the presence of plants (Figure 5B), all soil mixtures showed a significant increase in PA over 65 days ($F_{1,15}$ = 75.239, P < 0.0005, Figure 5B), as well as significant differences among the gradient soil mixtures ($F_{4,15}$ = 35.094, P < 0.0005). These results were significantly different from what was found in the absence of plants (Figure 5A), where statistically no change in PA with time was observed although the significant differences among the gradient soil mixtures remained ($F_{4,15}$ = 259.321, P < 0.0005). For all soil mixtures, the average percent change in PA from day 1 to day 65 was significantly higher for the soil mixtures with plants (428.5 ± 97.6 SE %) compared to the those without plants (3.6 ± 8.1 SE %) ($F_{1, 30}$ = 50.397, P < 0.0005, supplementary material, Figure S2). In the presence of plants, PAs of different soil mixtures changed by different percentages ($F_{4,15}$ = 9.211, P = 0.001, supplementary material, Figure S2); the PA of the least active soil (100%25R) increased proportionally the most relative to day 1. For example, the PA of the 100%25R soil increased by 1,139 ± 248 SE % from day one to day 65 while it only increased by 311 ± 49 SE % for soil 100%146 over the same period.

3.4 Plant biomass

Winter rye and switchgrass grew in all soil mixtures, including 100%25R, in the growth chamber. We found no significant differences in harvested root mass, shoot mass, root-to-shoot ratio, or total plant biomass for plants that grew in different soil mixtures (supplementary material, Figure S1).

3.5 Soil moisture and organic matter

In the absence of plants, soil moisture correlated with the amount of 146 relative to 25R: Mixtures with more 146 contained significantly more moisture, ranging from 24.3 ± 1.0 SE % moisture for 100%146 to 10.9 ± 1.2 SE % for 100%25R (Figure 6). Conversely, in the presence of plants, the moisture levels did not correlate with the amount of soil 146 relative to soil 25R. As stated in section 3.1, the initial organic matter content increased with an increasing proportion of 100%146 soil. Organic matter stayed relatively constant with time. There was a small increase with time in soil organic matter in 100%25R with plants, but this increase was not statistically significant (Figure 3).

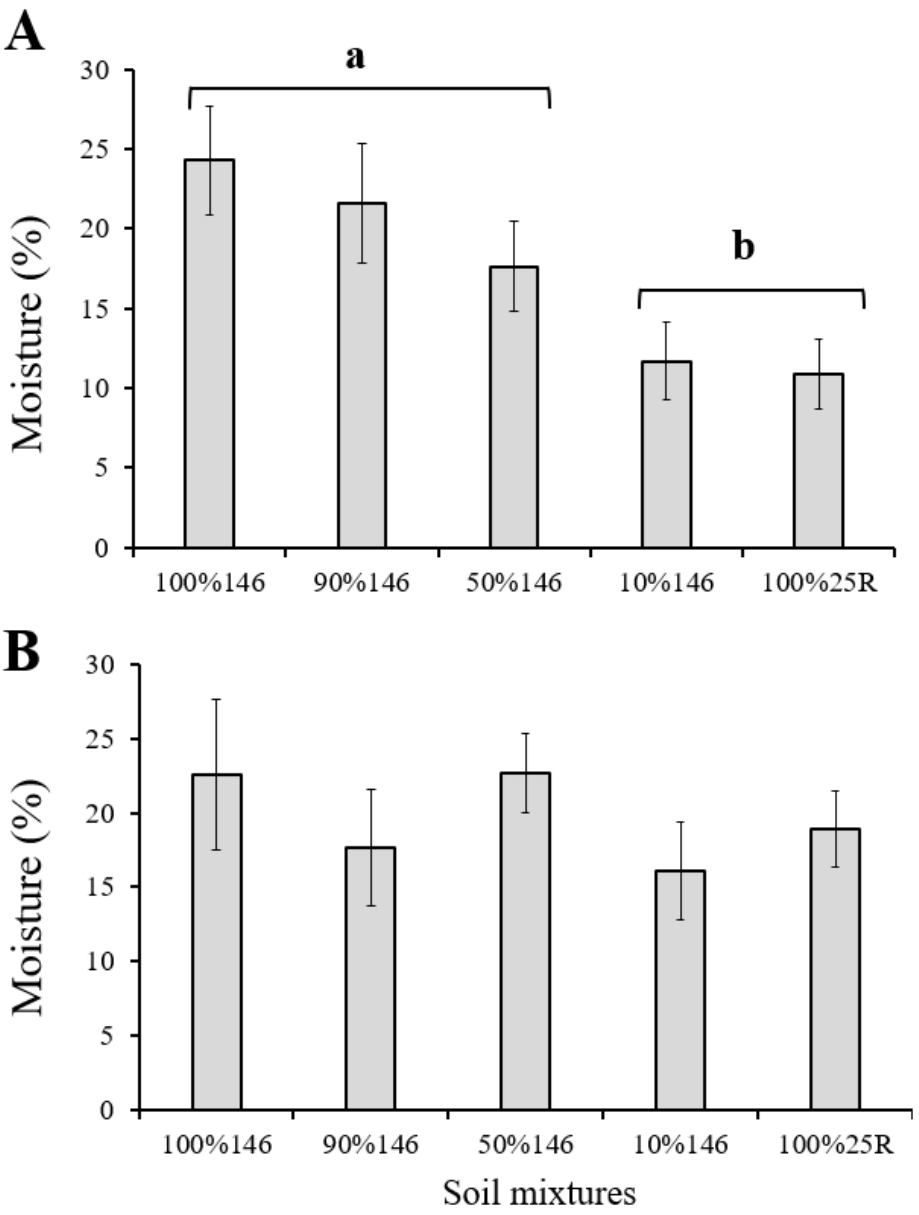


Figure 6. The percent moisture without plants (A) and with plants (B) are shown as averages of six-time points for five soil mixtures. In the experiment without plants, data averaged across six-time points for each soil mixture displayed statistically significant differences ($P < 0.01$) in soil moisture means between soil mixtures with higher gradient of site 146 soil (100%146, 90%146, 50%146) and lower gradient of site 146 soil (10%146, 100%25R). In the presence of plants, soil percent moisture did not display significant differences among the five soil mixtures. The error bars show the standard error (SE) of the mean ($n = 4$, ± 1 SE).

4. Discussion

4.1 Bulk soils 146 and 25R had different abiotic properties

Barren soil 25R, with low enzymatic function, was found to have several properties associated with poor soil quality. Metal concentrations of soil from site 25R was above the EPA clean-up criteria (New Jersey Department Environmental Protection, 2019), which can be attributed to the historical use of the area as an industrial rail yard (Table 1 and Table S1) (Hagmann et al., 2019). Soil 25R has higher heavy metal (Cu, Zn, As, and Pb) concentrations and lower organic matter content than soil 146. Further, the relatively small amount of organic matter in barren soil 25R consists of anthropogenic contaminants, including coal, rather than plant debris (Hagmann et al., 2019). Moreover, soil 25R consists of larger aggregates compared to soil 146, which can result in lower moisture retention due to larger drainage capacity of coarser soils (Arya & Paris, 1981). Soil aggregate formation controls the carbon storage capacity in uncontaminated soils by physically restricting the enzymatic and microbial interaction with the soil organic matter (Six, 2002). Larger aggregates may also contribute to a lower microbial activity due to reduced diffusion of oxygen into aggregates (Arya & Paris, 1981; Rabot et al., 2018; Six et al., 2000). Several of these factors likely contribute to the low function of soil 25R.

4.2 Microbial inoculation with functional soil failed to revitalize poorly-functioning soil

Our hypothesis was that adding high-functioning soil 146 into the poorly functioning barren soil 25R would improve soil function over time as contaminant-resistant microorganisms colonize the soil. Conversely to our hypothesis, results showed that (Figure 5A) inoculating soil 25R with the microbial community from soil 146 did not result in a time-dependent increase in

soil phosphatase function in the absence of plants. We note that this inability to revitalize soil 25R by the introduction of a microbial community from site 146 is consistent with our field observations: Site 25R has very low enzyme activity and is barren despite its location adjacent to a vegetated and high-functioning site 25F. This coexistence suggests that microbial communities from 25F either have not successfully colonized into site 25R, or they colonize but fail to increase PA in the 25R soil environment. To ensure that all microbes were transferred in this study, we used whole soil inoculations. These ensure that all microbes were transferred to the new environment.

In the experiment without plants, we approximate an environment where abiotic factors and PA remained stable over the time course of the experiment, consistent with stable community functioning in all soil mixtures (Carrillo et al., 2012). The PA was higher in mixtures with larger proportions of functional soil 146 and constant through time. Inoculation did not gradually increase PA over time. This observation suggests that the functional 146 microbial community could not successfully establish and exude active enzymes into the mixed soils. Moreover, these results indicate that abiotic factors limited microbial community establishment or functioning and PA (Fraser, 1999; Singh et al., 2019a).

The relative importance of abiotic and biotic soil factors on various soil properties has been discussed in the literature (Barto et al., 2010; Bennett & Klironomos, 2019; Blankinship & Schimel, 2018; Criquet et al., 2004; Ma, 2018). The relatively higher importance of abiotic factors in the functional outcome of LSP soils was previously reported by Singh et al. (2019a), where the authors cross-inoculated sterilized LSP soils with microbial inocula from LSP sites 146 and 43. Their results showed that abiotic factors were more important than microbial factors in the resulting enzymatic activities in the cross-inoculated LSP soils, and our findings

are consistent with these results (Singh et al., 2019a). Here we found a significantly positive relationship among PA, V, and Cr, not observed for other metals (Cu, Zn, As, Pb), which showed a strong negative relationship with PA in all soil mixtures that were not planted (supplementary material, Figure S3A).

4.3 Plants increased soil phosphatase activity in contaminated soil mixtures

The fact that plants grew in "barren" soil 25R in the growth chamber allowed us to measure the effect of plants on soil PA for all soil mixture proportions. Plants altered the contaminated soil environment, resulting in a time-dependent increase in PA in mixtures of two contaminated soils. During the 65-day time scale of our experiment, plant growth was found to be a primary driver of PA (Figure 5B). This could be due to an increased presence of arbuscular mycorrhizal fungi, increased moisture retention, redistribution of contaminants, and/or soil aeration by roots (Landis & Fraser, 2008; Nazeri et al., 2016; Tahat & Sijam, 2012). Moreover, plant growth and soil microbes can affect the species distribution of heavy metals, hence alter metal toxicity, and impact soil enzyme activity. (Awasthi et al., 2020; Emenike et al., 2018; Glick, 2003, 2010; Qian et al., 2012; Wang et al., 2019). Plants may have facilitated the microbial transformation of soil characteristics of the poorly functioning 25R soil (Antoniadis et al., 2017).

4.4 Increased phosphatase activity did not correlate with increased plant biomass

Increased PA is often viewed as an indicator of healthy soil function and nutrient mineralization, which can promote plant growth (Singh et al., 2004; Singh et al., 2019a; Singh et al., 2019b). A beneficial bidirectional relationship is thus often assumed between plants and extracellular enzymatic activity (Kuzyakov et al., 2018; Zhu et al., 2014). On the contrary, in

the LSP soils during the timescale of our experiment, the benefits appear unidirectional: plants increased PA, but higher PA did not result in bigger plants (supplementary material, Figure S3B).

4.5 Plants influenced phosphatase activity via soil moisture and organic matter content

It is known that the presence of plants can modulate moisture levels and possibly organic matter content, especially in low organic matter soils (Alkorta et al., 2003; Angers & Caron, 1998; Bauer et al., 2015). In the absence of plants, initial soil moisture levels were higher in mixtures with more soil 146, which has higher water retention (Figure 6A and Table 1). In the presence of plants, soil moisture did not correlate with the relative percentages of soils 146 and 25R (Figure 6B), indicating that plants may help the contaminated soils retain moisture and "override" the impact of poorly functioning soil 25R on moisture levels (Passioura, 2002; Pedron & Petruzzelli, 2011; Todd, 1972; Van der Putten et al., 2013; Young, 1995). When we correlated PA and moisture in the unplanted soil, we found a strong, positive, and significant relationship (supplementary material, Figure S3A, $P < 0.0001$). However, when we correlated PA and moisture in planted soil, we found a weakly positive, but non-significant relationship for all soil mixtures (Figure S3B). Plant size and PA did not correlate. Therefore, we conclude that the introduction of plants likely may have changed the soil matrix leading to increased moisture retention.

There was a small but statistically insignificant increase with time in soil organic matter in 100%25R with plants present, which could reflect the addition of a small amount of root exudates from the plants to this low organic matter environment (Blagodatskaya & Kuzyakov, 2008; Kuzyakov et al., 2000). Enzyme activity has been reported to vary with moisture and organic matter content (Bandick & Dick, 1999; Burns et al., 2013; Hinojosa et al., 2004;

Hudson, 1994; Kotroczó et al., 2014). These findings indicate that altered moisture and organic matter content may be critical in the specific mechanisms by which plants increase enzymatic activity in contaminated, poorly functioning soils.

5. Conclusions

Understanding the interplay between toxic substances, plants, and soil function in specific contaminated soils can be leveraged to facilitate the revitalization, restoration, re-greening, and remediation of poorly functioning post-industrial soils. In this study, we altered both the abiotic and biotic soil environment by mixing two soils with different properties in varying proportions. Our findings show that plants "nudged" the LSP soil to increase enzymatic function and address the "needs" of poorly functioning soil 25R in ways that microbial inoculation with functional soil 146 microbes did not. In fact, growing plants in the least functional, naturally barren 100%25R soil resulted in the largest percent increase in soil PA. Data show that plants may have increased moisture retention in contaminated soils and thus "overridden" the impact of poorly functioning soil 25R. The findings show that the mechanisms by which contaminants reduce soil enzymatic function are not simply a factor of microbial community composition, but they demonstrate the profound influence of abiotic factors in limiting - and plants in boosting - soil microbial function.

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Appendix: Supplementary material

Table S1. Heavy metal concentrations ($\mu g/g_{dry\ soil}$) in the soil mixtures from sites 146 and 25R. Each data point is an average of four experimental replicates. The metal concentration values measured are - the mean of four experimental replicates (n = 4) ± standard error of the mean. Soil cleanup criteria (µg/g) in non-residential direct contact soil as per the New Jersey Department of Environmental Protection guidelines (NJDEP, 2020).

Metals ($\mu g/g_{dry\ soil}$)	NJDEP (µg/g)	Soil mixtures				
		100%146	90%146	50%146	10%146	100%25R
V	370	183 ± 6	161 ± 2	110 ± 2	67 ± 2	59 ± 4
Cr	240	110 ± 4	104 ± 1	84 ± 2	68 ± 4	68 ± 5
Cu	600	108 ± 2	376 ± 48	922 ± 56	1,275 ± 262	1,469 ± 75
Zn	1500	98 ± 2	635 ± 112	3,351 ± 1458	3,422 ± 709	3,994 ± 293
As	20	41 ± 2	65 ± 6	138 ± 8	242 ± 21	263 ± 24
Pb	400	316 ± 9	988 ± 183	2,703 ± 198	3,737 ± 790	4,249 ± 197

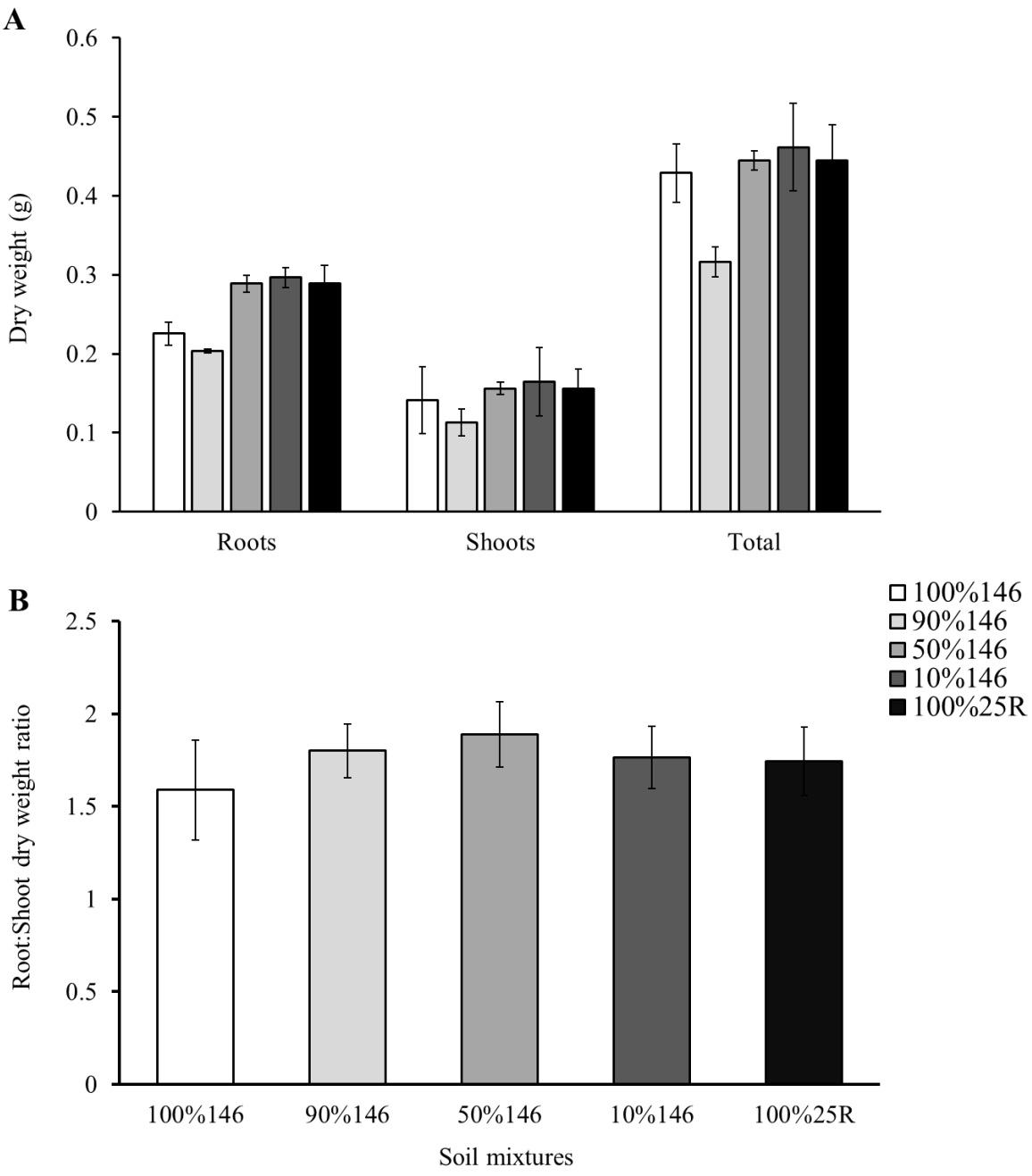


Figure S1. Average root mass, shoot mass, and total mass of plants grown (A) and average root to shoot ratio of plants grown (B) in each soil mixture. One-way ANOVA analysis of the data from (A) and (B) showed no significant differences in each of the three mass categories across soil mixtures as well as in root shoot ratio measurements.

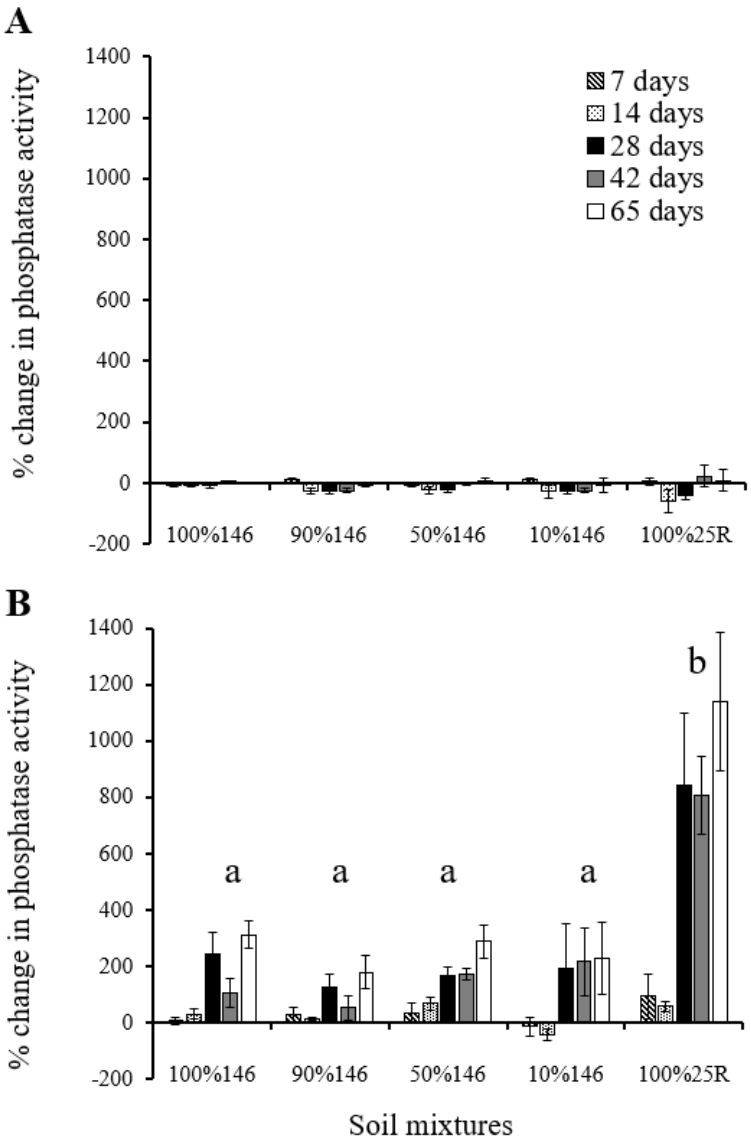


Figure S2. Percent changes relative to Day 1 in phosphatase activity at different time points for experiments without plants (A) and with plants (B) are shown. Bars represent an average of four experimental replicates ± the standard error of the mean (n=4, ± 1 SE). It should be noted that day 1 phosphatase activities in Figures 5A and 5B, without and with plants, are different because soils were obtained for the two experiments during different seasons and therefore the data without plants cannot be directly compared to the data with plants. We can, however, compare the changes in phosphatase activity with the time between the two experiments to make direct comparisons between the experiments with and without plants. To do so, we calculated the percent change in phosphatase activity for each soil mixture and each time point relative to Day 1 (Supplementary material, Figure S2A). Day 1 to Day 65 (428.5 ± 97.6 SE % for the pots with plants compared to the pots without plants 3.6 ± 8.1 SE %) was significantly higher ($F_{1, 30} = 50.397$, $P < 0.0005$), underscoring the impact the plants have on soil function in these contaminated soil mixtures. We conclude that the most dysfunctional soil mixture (100%25R) benefited the most from the plants.

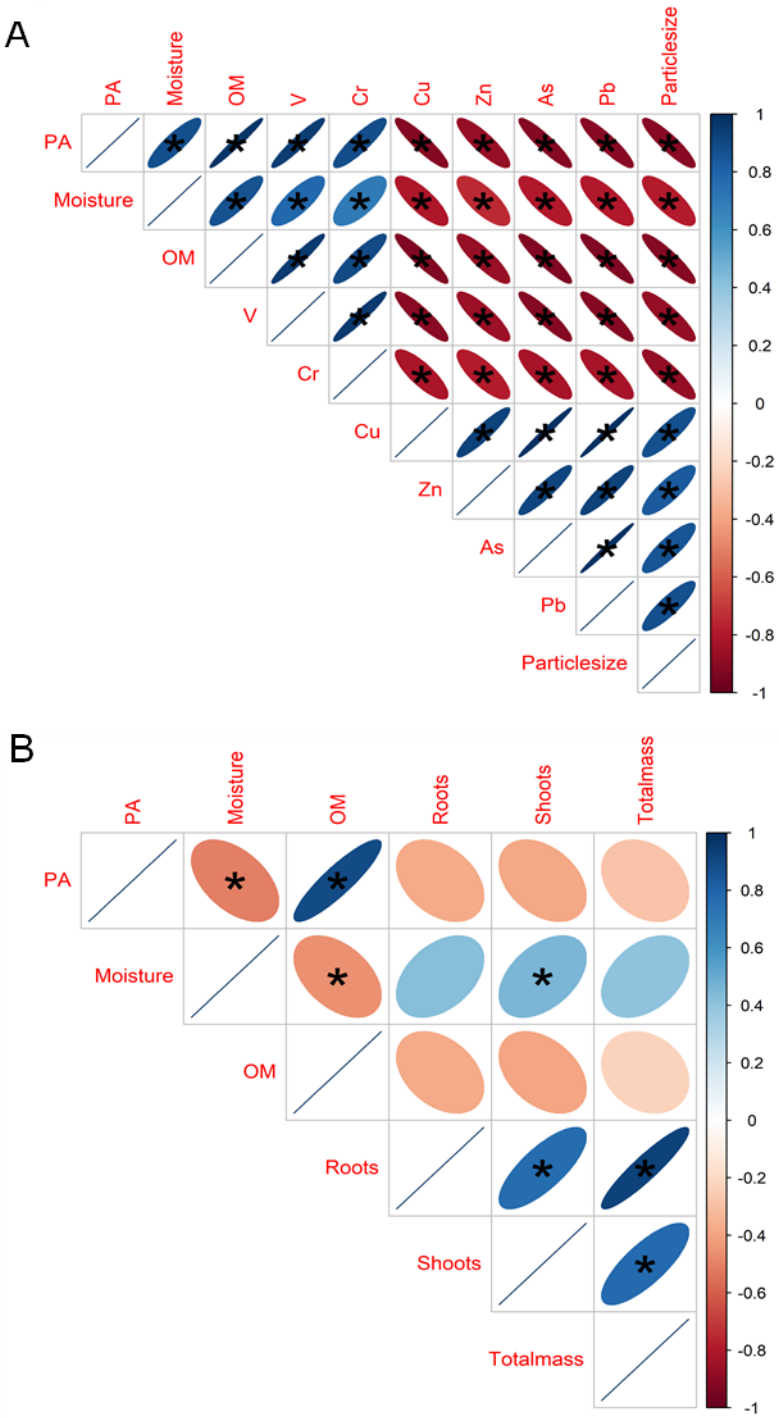
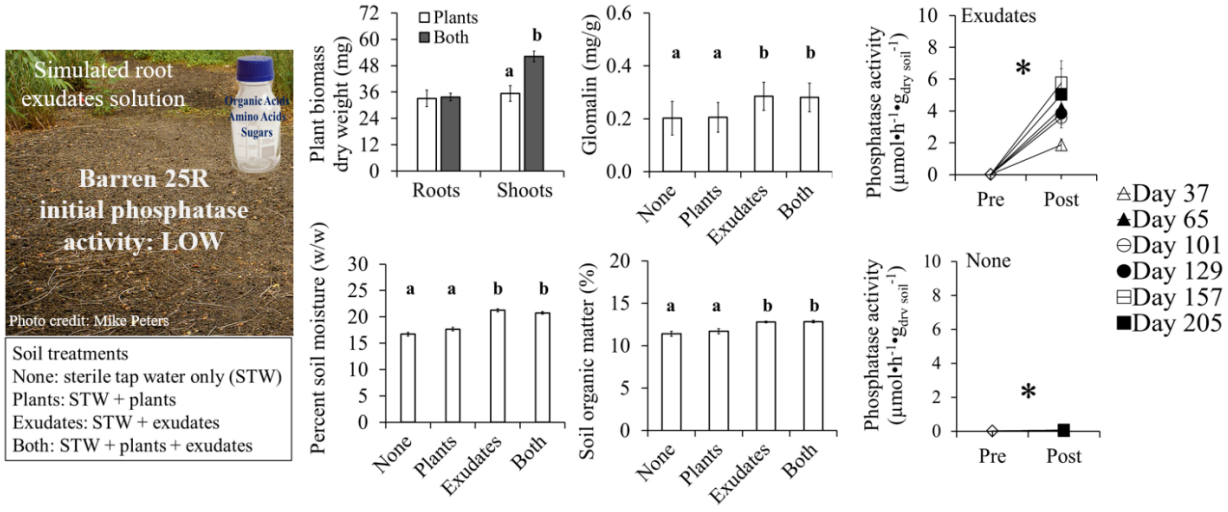


Figure S3. Spearman's rank correlation matrix for the experiment without plants (A) among phosphatase activity (PA), percent moisture, percent organic matter (OM), concentration of heavy metals, and particle size, and with plants (B) among phosphatase activity, moisture, organic matter, root mass, shoot mass, and total mass at data collected on day 65 using data for five soil mixtures (100%146, 90%146, 50%146, 10%146, 100%25R) (n = 4). The correlation coefficient (rho) is represented by the colored shading, as seen in the legend. Stars denote statistical significance at $P < 0.05$ or less.

CHAPTER 3

Project 2. ***Artificial root exudates restore microbial functioning in a metal contaminated, barren, inactive soil***

Graphical abstract



Article 2. *Artificial root exudates restore microbial functioning in a metal contaminated, barren, inactive soil*

Abstract

Restoring enzyme function in barren, brownfield soils using green strategies can improve microbial functioning and enable phytoremediation. It is known that adding simple, readily metabolized substrates secreted by growing plant roots (root exudates) or a laboratory-prepared solution of root exudate constituents (artificial root exudates) can stimulate soil microbial function. It is not known whether and how well this strategy works in a contaminated, low functioning soil from an industrial barren site because contaminants in the barren soil might inhibit microbial survival and functioning, or the microbial community might not be adapted to functionally benefit from root exudates. The objective of this study was to determine whether artificial root exudates stimulate microbial function in a barren soil. We collected soils from a barren brownfield (25R) site and an adjacent vegetated brownfield site (25F), with low and high enzyme activities, respectively. We subjected both soils to three treatments: switchgrass, (native to the site), artificial root exudates, and a combination of switchgrass and artificial root exudates. We measured enzymatic activity, plant growth, soil moisture, organic matter content, and easily extractable glomalin content over 205 days. By day 157, artificial root exudates increased the phosphatase activity by 9-fold in previously vegetated brownfield soil and by 351-fold in barren brownfield soil. When exudates were added to the barren soil, the plant shoot mass was higher (52.2 ± 2.5 mg) than when they were not (35.4 ± 3.6 mg). In both soils, adding artificial root exudates significantly increased the percent moisture, organic matter, and glomalin content. Treating contaminated, barren soil with artificial root exudates resulted in increased soil microbial function and improved soil properties that might promote a hospitable habitat to support vegetation in such extreme environments.

Keywords: root exudates, soil enzymes, heavy metals, phosphatase activity, industrial barren, glomalin

1. Introduction

Today, many contaminated, post-industrial sites lie barren and unused. Strategies to re-green these sites can reinstate soil ecosystem services and promote recreational use (Mathey et al., 2015). The United States Environmental Protection Agency has called for research into sustainable and green strategies for the bioremediation of brownfield sites (Lynch & Moffat, 2005; Pedron & Petruzzelli, 2011). Phytoremediation strategies, while promising, are not directly applicable to sites that do not support plants (Kuppusamy et al., 2017; Megharaj & Naidu, 2017; Uchimiya et al., 2020). The application of phytoremediation strategies is only possible after a barren soil has been revitalized to enable primary production (Couic et al., 2018). We previously found that planting ryegrass (*L. perenne)* and switchgrass *(P. virgatum)* seeds into previously barren, inactive brownfield soil increased phosphatase activity (PA), organic matter content, and percent moisture (Vaidya et al., 2020). Here we examined whether these beneficial effects of plants could be mimicked by adding laboratory-prepared artificial root exudates.

Root exudates are solutions secreted by plant roots and include sugars, organic acids, amino acids, proteins, and secondary metabolites (Badri et al., 2009; Bais et al., 2006; Nannipieri et al., 2008; Uren, 2000). In 2004, Bais and colleagues described “an underground information superhighway” where plants “communicate” with soil microbes and other plants by exuding synchronized root exudates that modulate rhizosphere microbial community composition to protect from pathogens, to enable symbiotic feedbacks, and to mineralize nutrients in nutrient-poor environments (Bais et al., 2008; Bais et al., 2004; Bais et al., 2002). Kuzyakov et al. (2000)

defined the effects of root exudates as “priming effects” where the soil function improved for a brief time with root exudate inputs. To study the effects of root exudates on soil function, scientists have not only extracted root exudates from natural roots but also prepared mixtures of root exudate compounds in the laboratory. Such artificial root exudate solutions are prepared to mimic the composition of natural root exudates. Adding either extracted solutions or artificial root exudates in soils spiked with contaminants have been shown to result in an increased soil microbial function (Balseiro-Romero et al., 2014; Dakora & Phillips, 2002; Maseko & Dakora, 2013). Meier et al. (2017) studied the effects of adding artificial root exudates to uncontaminated, unfertilized, and fertilized (NH_4NO_3) forest soils and concluded that exudates stimulated the soil microbes to decompose labile soil organic matter faster. Bali et al. (2020) and Vithanage et al. (2012) reported that in Cd-contaminated soils, plants secreted organic acids, which chelated with Cd ions to restrict their entry into the plant.

Soil organic matter consists of a prevalent, recalcitrant glycoprotein called “glomalin-related soil protein”, or glomalin. It is produced metabolically by arbuscular mycorrhizal fungi. Glomalin content in soil has been found to modulate soil aggregation in the rhizosphere region to support root growth and soil microbiota and sequester heavy metal contaminants (Holátko et al., 2021; Treseder & Turner, 2007; Wright & Upadhyaya, 1998). Soils amended with manure, compost, and biochar have been shown to exhibit glomalin production (Gispert et al., 2013; Singh, 2012; Walley et al., 2014). Glomalin promotes the amalgamation of soil particles. Soil aggregate formation helps maintain soil structure, reduces erosion, and traps pollutants (Singh et al., 2022).

This work focused on the effects of artificial root exudates on a barren, metal contaminated (As 1,325 ± 269 µg/g, Pb 21,965 ± 4,486 µg/g, Cu 7,460 ± 1,659 µg/g, Zn 20,736

± 4,690 µg/g) soil and sought to answer the following question: Can artificial root exudates increase the microbial functioning in a barren soil that is heavily contaminated and enzymatically nearly inactive? To our knowledge there is only one report on adding artificial root exudates to a barren soil: Adamczyk et al. (2021) showed that adding artificial root exudates to uncontaminated, barren, alpine soils changed the soil microbial community structure and raised the soil respiration rate. Likewise, our study site 25R is barren but in contrast to their study site, 25R is heavily contaminated and represents an industrial barren (Kozlov & Zvereva, 2007). We considered the possibility that adding artificial root exudates to 25R might not stimulate the microbially-mediated, extracellular soil acidic PA because 25R soil contains high concentrations of contaminants (Hagmann et al., 2019) that could inhibit soil enzyme function or reduce the abundance of the soil microbes as has been shown in other studies (Kuperman & Carreiro, 1997; Narendrula-Kotha & Nkongolo, 2017). Alternatively, the low enzymatic activity of barren 25R soil could be primarily caused by the lack of root exudate compounds in the soil and be restored by adding artificial root exudates.

To distinguish between these possibilities, we implemented four experimental conditions: 1) sterilized tap water represents control (None), 2) plants, 3) artificial root exudates, and 4) both plants and artificial root exudates. We measured soil properties (PA, plant biomass, easily extractable glomalin, soil organic matter, and moisture content) at six time points after adding these treatments for 205 days. This is the first observation of an interaction between exudate addition and glomalin content. These data were analyzed to determine whether artificial root exudates primed and restored enzymatic function, and plant productivity in a barren, poorly functioning, toxic soil.

2. Materials and methods

2.1 Site description and soil collection

The two study sites (25F and 25R) for our research are situated within a 100-ha section of the Liberty State Park (LSP), Jersey City, NJ (40˚42’16N, 74˚03’ 06W). These sites are located next to each other, fenced from human access, and have been left un-remediated for over 50 years. Contamination of these soils has been carefully mapped and studied (Gallagher et al., 2008; Qian et al., 2012; Salisbury et al., 2017). Site 25F is heavily forested (Gallagher et al., 2018; Gallagher et al., 2015; Gallagher et al., 2011) with plants and soil organisms adapted to polyaromatic organic contaminants and high metal concentrations, and displays high PA (Hagmann et al., 2015). In contrast, site 25R is barren and has low PA (Hagmann et al., 2019; Singh, Vaidya, et al., 2019; Vaidya et al., 2020). Fresh soil samples were collected from five markers, 5 m apart, along three parallel transects 10 m apart at each site with a field grid of 16 by 20 m. In total, fifteen samples from each site were combined to form a composite sample of that site. The composite fresh soil samples were sieved (2 mm) and stored at 4 °C for 24 h till the experimental set-up day or day 1. We collected soil samples from each pot before adding any treatments to the soils. These samples are referred to as “pre-treatment” samples or day 1 samples throughout this document.

2.2 Bulk soil characteristics

The bulk soil characteristics of the soils that were used in this study: vegetated 25F and barren 25R from LSP are listed in Table S1. PA was high in vegetated 25F soil and low in barren 25R soil. The pH was low in both soils while moisture content, organic matter content, and water holding capacity were lower in barren 25R soil than in vegetated 25F soil (Vaidya et al., 2020).

The heavy metal concentrations in 25F and 25R soils were high and above the threshold limits specified by the New Jersey Department of Environmental Protection guidelines (NJDEP, 2020). The concentrations of Cu, As, Pb, Zn at barren 25R site were two times higher than those of vegetated 25F soil (Table S1) (Singh, Vaidya, et al., 2019).

2.3 Experimental design

The main experiment was divided into two blocks based on site designation and vegetation (vegetated 25F and barren 25R). The first block consisted of 16 pots filled with 320 g of site 25F soil and the second block consisted of 16 pots filled with 350 g of site 25R soil such that both fresh soils occupied the same volume in the experimental pots.

A total of 32 pots were incubated in a climate-controlled growth chamber and watered three times weekly with sterilized tap water from day 1 to day 205. The relative humidity was set to 65%, and the photo-period of the day/night cycle was 10.5/13.5 h, the day/night temperatures were 24 °C and 16 °C, respectively. To maintain the same percent soil moisture as that measured on day 1 in vegetated 25F soil (30% soil moisture content by weight), we added the volume of sterile tap water needed to bring the weight of the pot to the desired value. The required volume ranged from 18 to 46 mL. The pots were watered three times per week from day 1 to day 205. Similarly, in barren 25R soil (13% soil moisture by weight), we added 9 to 50 mL sterile tap water three times a week over 205 days. By adding sterilized tap water, we mimicked the conditions of naturally available water as it can contain small quantities of dissolved salts, minerals, and nutrients. Since all pots were watered consistently three times per week over 205 days, each pot received the same amount of naturally occurring tap water nutrients, which were nominal compared to the concentration of the exudate compounds added. We used the tap water

from the same lab faucet throughout the experiment, thereby reducing the variation in tap water composition. The small amounts of dissolved nutrients from tap water possibly supported the native microflora and fauna in the potted soil. We autoclaved the tap water to minimize the introduction of any microbes to the native microbial consortia in the pots.

Each soil type underwent four treatments with four replicated pots for each treatment. The four treatments were None, Plants, Exudates, and Both. A brief description of each treatment: 1) None was the control treatment where only sterile tap water was added three times per week starting on day 1. 2) Plants was the treatment where each pot was sown with 20 switchgrass (*P. virgatum*) seeds and watered three times per week with sterile tap water starting on day 1. 3) Exudates was the treatment where 20 mL of artificial root exudate solution (described in section 2.5, composition in Table S2) was added three times a week starting on day 7 and sterile tap water was added to each pot starting on day 1. We began adding exudate solution on day 7 because in treatments Plants and Both seeds germinated by day 7. 4) Both was the treatment where 20 switchgrass seeds were added on the experiment set-up day and sterile tap water was added three times a week starting on day 1. In treatment Both, 20 mL of the artificial root exudate solution was added to each pot three times per week starting on day 7 in addition to watering. We selected *P. virgatum* because it is native to the LSP site (Salisbury et al., 2020). *P. virgatum* is also recognized as a promising biofuel, perennial crop with high photosynthetic efficiency and biomass accumulation, and widely used in phytoremediation of mining and brownfield sites (Kafle et al., 2022; Kantola et al., 2022; McLaughlin & Kszos, 2005). Moreover, Pradhan et al. (1998) demonstrated that *P. virgatum and M. sativa* aided desorption of polycyclic aromatic hydrocarbons by 57% from soils of an industrial site in Newark, NJ, that previously used coal and other petroleum products to manufacture gas. We

established a factorial design and tested three independent variables: soil type, soil treatment, and time. To study the effect of our treatments through time on the two soil types, we measured phosphatase activity, soil organic matter content, moisture content, glomalin content, and plant biomass at harvest.

2.4 Soil quality indicators

Phosphatase activity

The extracellular phosphatase activity (PA) in soil was measured using fluorometric assay protocol developed by Marx et al. (2001) and adapted to analyze LSP soils in 2015 by Hagmann et al. (2015). The fluorescence emitted by 4-methylumbelliferone product formed by the reaction of extracellular phosphatase enzymes and the substrate methylumbelliferyl phosphate in each soil sample was measured. Soil samples from three random locations in each pot were collected and analyzed at seven time points (day 1, 37, 65, 101, 129, 157, 205) over the course of the experiment. PA was expressed as micro moles of reaction product per hour per gram of dry soil.

Plant biomass

At day seven, 7 – 15 switchgrass (*P. virgatum*) seeds germinated in each planted treatment replicate. The number of plants in each planted pot were culled to seven, such that a similar overall capacity was maintained. On day 205, plants were harvested and separated into roots and shoots using tweezers and scissors. Roots were washed with tap water to remove excess soil, and placed in the conventional oven for drying at 70 °C for 13 days. Dry weights of

roots and shoots were measured to then calculate the total plant mass (grams) and root weight to shoot weight ratio for each planted treatment.

Moisture content

Soil moisture content was determined as percent dry soil weight ($w/w_{(dry\ soil)}$) measured after drying approximately 2.0 g of soil scooped from each pot and kept at 100 °C in a conventional oven for 24 h (Schmugge et al., 1980).

Organic matter

Soil organic matter in all samples was measured by loss on ignition. In brief, dry ground soil (2.0 ± 0.5 g) was heated to 550 °C for 4 h. The percent organic matter was determined gravimetrically for each soil sample (Davies, 1974).

Glomalin

Approximately 1.0 g of soil sample in 8.0 mL of 20 mM sodium citrate adjusted to pH 7.0 was autoclaved at 121 °C and 18 psi for 30 min. The samples were centrifuged and the supernatant was analyzed for glomalin (easily extractable) using the Bradford dye-binding assay with bovine serum albumin as the standard (Wright & Reddy, 2001; Wright, 2000).

2.5 Artificial root exudate solution dose and preparation

The artificial root exudate solution was prepared by combining primary metabolites such as sugars, organic acids, and amino acids in concentrations as shown in Table S2, and based on the method developed by Steinauer, Chatzinotas, and Eisenhauer in 2016 (Steinauer et al., 2016).

We chose 2.5x as the concentration of artificial root exudate solution that could invigorate the contaminated, barren soil for this study based on a separate experiment referred to here as the dose-response experiment (Fig. S1). This means that the concentrations we used were 2.5 times higher than those reported by Steinauer and coworkers. The concentrations in the 2.5x artificial root exudate solution were 25 mM of glucose, 25 mM of fructose, 25 mM of sucrose, 25 mM of maltose, 10 mM of acetic acid, 10 mM of citric acid, 10 mM of lactic acid, 10 mM of malic acid, 10 mM of succinic acid, 3 mM of alanine, 3 mM of arginine, 3 mM of asparagine, 3 mM of glutamic acid, 3 mM of glycine, 3 mM of histidine, 3 mM of leucine, and 3 mM of tyrosine.

2.6 Data analysis

The goal of this study was to determine the effects of plants and artificial root exudates on soil functioning. We measured this across two soil types that were similar in abiotic character with respect to contamination, but different in their biotic character with respect to vegetation history, 25F (vegetated) and 25R (barren). All statistical analyses were carried out in RStudio using R version 3.6.1. (R Core Team, 2019). Initially, treatment effects were analyzed separately for different treatments and soils to test whether there was a difference in PA before and after the treatments were applied. We used a paired *t*-test to compare phosphatase activities measured at the first time point to all post treatment measurements (observed at the six other time points). We also calculated the fold change of PA over time with the goal to understand changes in the PA. To analyze differences in the response variable, trends over time, as well as obtain parameter estimates, a linear mixed effects model was used. A natural log transformation was made on the PA (response variable) to reduce heteroscedasticity. The treatments crossed within soil types (25F, 25R) were treated as a random effect, which captured how treatments affected the PA

within each soil type. Within the mixed model, a Tukey HSD test was then performed to compare the main effect of the four treatments, as well as the interaction effect with soil type. To address differences in soil properties i.e., moisture, organic matter, and glomalin content, we again used mixed effects model followed by a Tukey HSD analysis to compare the effect of treatments on soil properties. The plant data was analyzed using general linear model followed by paired *t*-test to compare the effect of artificial root exudates on plant biomass for the two soil types separately. Glomalin data displayed heteroscedasticity even after performing a natural log transformation. In order to correct for the heteroscedasticity, we used the car package in R and with the hccm function calculated corrected covariance for linear models fit by least squares. These are also called "White-corrected" or "White-Huber" covariance matrices (White, 1980). For the dose-response experiment (see supplementary material), significant differences between the vegetated (146) and barren (25R) soils and the effect of different artificial root exudate concentrations added to these soils were examined by one-way analysis of variance (ANOVA). The interactions among the five exudate concentrations (0.1x, 0.5x, 1.0x, 2.0x, and 2.5x, see section 2.5 and Table S2) within a soil type were determined by two-way ANOVA followed by subsequent analysis of individual contrasts using Tukeys HSD post hoc test ($P < 0.05$). A square root transformation was made on the PA (response variable).

3. Results and discussion

3.1 Effect of treatments on soil phosphatase activity

Before treatments, vegetated 25F soil had 32 times higher PA (0.53 ± 0.027 μmol h^{-1} $g_{dry\ soil}^{-1}$) than the barren 25R soil (0.016 ± 0.014 μmol h^{-1} $g_{dry\ soil}^{-1}$), in agreement with previous findings (Fig. 1A, E) (Hagmann et al., 2015; Hagmann et al., 2019; Vaidya et al., 2020).

Phosphatase activity serves as an indicator of soil phosphorus cycling and soil health (Alkorta et al., 2003; Bardina et al., 2017; Eivazi & Tabatabai, 1977). Possible reasons for the low activity of 25R may be high contaminant loads, absence of vegetation, and/or dormant or limited microbial communities (Hagmann et al., 2019; Vaidya et al., 2020).

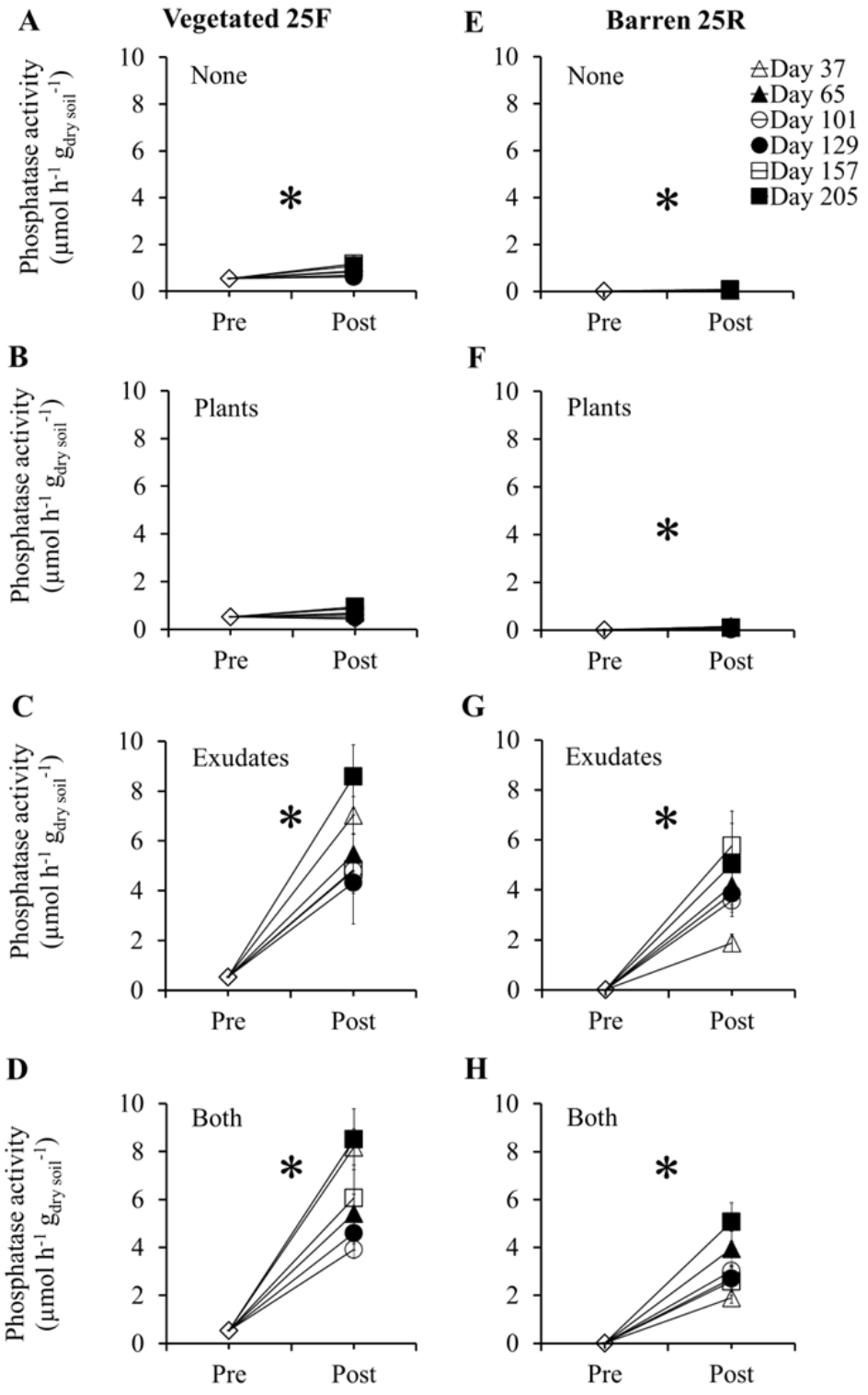


Figure 1. Phosphatase activity of vegetated 25F (left) and barren 25R (right) soils Pre- and Post-treatment. The treatments shown are None (A, E), Plants (*P. virgatum* seeds) (B, F), Exudates (for composition, see Table S2) (C, G), and Both (D, H). Data labeled 'Pre' represent the mean

phosphatase activity on day 1 before adding the treatments. Data labeled 'Post' represent the mean of phosphatase activities at six time points after adding the treatments; symbols used for different time points are indicated in the legend. Error bars indicate standard errors of the mean, n = 4. Symbol * indicates statistically significant increases in phosphatase activity from pre- to post-treatment at $P < 0.05$.

In Figure 1, we defined "Pre" treatment as the initial PA measured before treatments and "Post" treatment as the average PA measured over six time points after the treatments. When pre- and post-treatment phosphatase activities were compared, the addition of artificial root exudates to barren 25R soil resulted in the greatest increase in PA compared to the other treatments. For treatment exudates, PA increased significantly both in 25F (from 0.53 ± 0.027 pre to 5.84 ± 0.97 μmol h^{-1} $g_{dry\ soil}^{-1}$ post, t = - 10.23, df = 23, $P < 0.0001$) and 25R (from 0.016 ± 0.014 pre to 4.04 ± 0.94 μmol h^{-1} $g_{dry\ soil}^{-1}$ post, t = - 8.87, df = 23, $P < 0.0001$) (Fig. 1C, G, respectively). For treatment plants, the 25F post-treatment PA did not increase significantly (from 0.534 ± 0.027 pre to 0.677 ± 0.122 μmol $h^{-1}g_{dry\ soil}^{-1}$ post) but 25R activity did increase significantly (from 0.016 ± 0.014 pre to 0.110 ± 0.028 μmol h^{-1} $g_{dry\ soil}^{-1}$ post, t = - 8.49, df = 23, $P < 0.0001$) (Fig. 1B, F). Phosphatase activity increased more under treatment exudates than treatment plants. Spohn and Kuzyakov (2013) reported that the addition of alanine (12 mg C per gram of soil organic carbon) resulted in a 6-fold increase in PA in the top and subsoil sections of an uncontaminated mixed, deciduous forest. Our results indicated a 11-fold increase in PA in vegetated 25F when artificial root exudates (a more diverse composition, Table S2) were added, qualitatively similar to Spohn's and Kuzyakov's finding.

After artificial root exudates were added, the initially inactive, barren 25R soil (0.016 ± 0.014 pre to 4.04 ± 0.94 μmol h^{-1} $g_{dry\ soil}^{-1}$ post) reached nearly similar phosphatase activity as shown by the vegetated 25F soil (0.53 ± 0.027 pre to 5.84 ± 0.97 μmol h^{-1} $g_{dry\ soil}^{-1}$ post) after exudates treatment (Fig. 1C, G). This is notable because the result indicates the revitalization of a

mostly inactive and barren soil to an enzymatically functional environment. In 2019, Hagmann and coworkers reported that the bacterial density at site 25R was low but measurable, indicating that there may be dormant microbes present in this soil (Hagmann et al., 2019). The mechanisms by which exudate additions result in the large increases in PA are not known. Possibilities include exudate additions supplied metabolites that were required to override the nutrient limitation and toxicity in the inactive, barren 25R soil, (Balseiro-Romero et al., 2014; Blagodatskaya & Kuzyakov, 2008; Brimecombe et al., 2000; Kuzyakov & Blagodatskaya, 2015; Meier et al., 2017; Shukla et al., 2011) and/or impacted the microbial diversity or abundance of the environment (Kreyling et al., 2008; Steinauer et al., 2016).

Phosphatase activity increased by about the same amount regardless of whether only exudates or both exudates and plants were added. In the pots with treatment Both, PA increased significantly in both 25F (from 0.53 ± 0.03 pre to 6.12 ± 0.96 μmol h^{-1} $g_{dry\ soil}^{-1}$ post, t = - 10.43, df = 23, $P < 0.0001$) and 25R (from 0.016 ± 0.014 pre to 3.20 ± 0.61 μmol h^{-1} $g_{dry\ soil}^{-1}$ post, t = - 10.13, df = 23, $P < 0.0001$) (Fig. 1D, H, respectively). The amount of artificial root exudates that we added to the soils was higher than what would likely be secreted by plants (Hobbie & Hobbie, 2013; Strickland et al., 2015). Over the course of this experiment, it is possible that the labile nutrients added in the exudate treatment were realized by the microbial community faster and to a greater extent than the lower amounts of naturally occurring root exudates from growing seedlings.

Phosphatase activity increased by a greater factor in 25R than in 25F ($P < 0.0001$, Tukey HSD) (Fig. 2A, B). For example, the PA increased by 351-fold from day 1 to day 157 in 25R and only by 9-fold in 25F over the same time period. Because site 25F is heavily vegetated, it has a legacy of receiving naturally occurring root exudates (Salisbury et al., 2020). This may explain

why addition of artificial root exudates had a smaller impact on soil from site 25F than on soil from barren site 25R. In vegetated 25F soil, PA had already reached a substantially higher value by day 37, while in barren 25R soil, PA took longer to peak, reaching a maximum value by day 65 (Fig. 2B). This slower increase may also reflect the lower soil nutrient content and lower microbial counts originally present in 25R soil (Hagmann et al., 2019; Singh, Ojinnaka, et al., 2019). In a side experiment (dose-response) described in supplementary material, data showed that a higher exudate concentration was required to reach maximum PA in 25R compared to a vegetated but contaminated soil from the same park (Fig. S1).

Barren, inactive 25R soil appears to have a higher requirement for artificial root exudates to reach phosphatase activities that are comparable to the vegetated 25F soil. For example, the effects of artificial root exudates treatment on a low functioning clay loam soil, spiked with phenanthrene and pyrene, showed higher consumption of exudates than the treatment with planted ryegrass root exudates (Sun et al., 2010). Increased soil enzyme activity may indicate increased nutrient cycling and might also serve as an indication of microbial revival (Gunina & Kuzyakov, 2015; Zhang et al., 2019). Future experiments will investigate the microbial revival potential in barren soil that may result from addition of artificial root exudates.

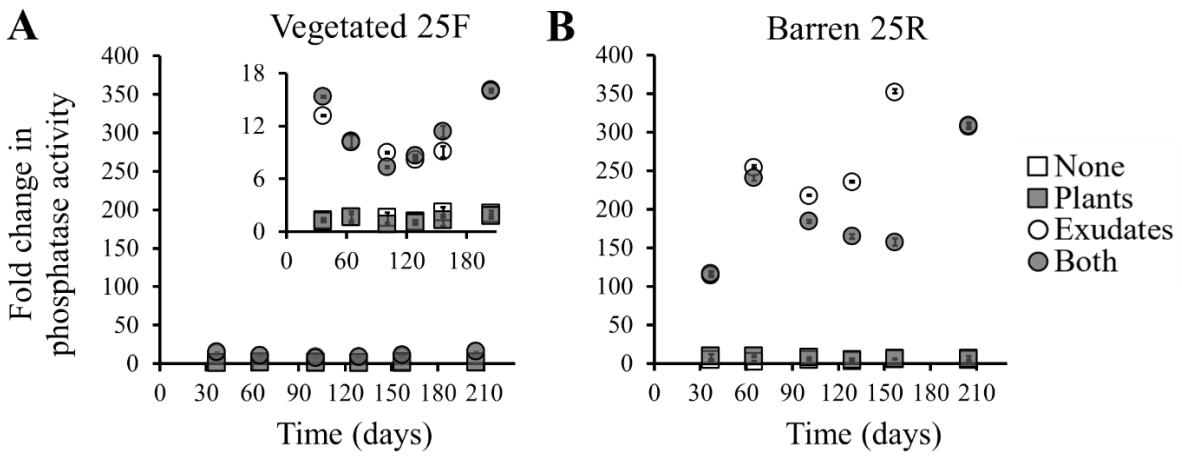


Figure 2. Fold changes in phosphatase activity relative to day 1 over time for vegetated 25F (A) and barren 25R (B) soils are shown. Fold changes are shown for six time points from 37 to 205 days with four treatments (legend): None, Plants, Exudates, and Both. Addition of root exudates over 205 days increased phosphatase activity in barren 25R soil by 246-fold (B) compared to the vegetated 25F soil (11-fold) (A) ($P < 0.0001$, Tukey HSD). Error bars show the standard error of the mean, (n = 4).

3.2 Effect of treatments on soil properties

3.2.1 Plant biomass

Plant root exudates nourish the soil microbial communities, which in turn exude enzymes that can mineralize organic matter and other soil components to make nutrients bioavailable, supporting plant growth (Montiel-Rozas et al., 2016). Plants grown in 25R were smaller than those grown in 25F (Fig. 3 and Fig. 4A, E). Exudate treatment did not significantly affect the shoot mass of plants harvested from 25F soil (Fig. 4A). In contrast, in barren 25R soil, adding artificial root exudates resulted in significantly larger shoots (52.2 ± 2.5 mg) compared to the untreated Plants treatment (35.4 ± 3.6 mg) ($t(12) = -2.778$, $P < 0.05$) (Fig. 4E). Similar work by Montiel-Rozas et al. (2016) showed that three herbaceous species (*P. annua, M. polymorpha, M. sylvestris*) planted in washed sand mixed with increasing gradient concentrations of Cd, Cu, and Zn, modulated metal uptake by releasing specific root exudates (oxalic acid and citric acids) in high quantities to drive plant growth. These findings suggest that in contaminated soils, some

plant species facilitate higher shoot mass and metal uptake in the aerial regions of the plants by exuding specific root exudates (Montiel-Rozas et al., 2016).

Exudate treatment did not change root mass for either soil when compared to their respective untreated Plants. By adding artificial root exudates, we may have overcome nutrient limitation for shoot growth in the barren 25R soil (Edayilam et al., 2018; Garcia et al., 2005; Meier et al., 2017). Concordantly, in the vegetated 25F soil, the addition of artificial root exudates had little effect on either the root or shoot mass (Fig. 4A). Perhaps the plants at the 25F field site had "saturated" the soil with root exudates, and further addition of artificial root exudates was ineffective with regards to plant productivity. In barren 25R, the added artificial root exudates may have facilitated shoot growth, possibly due to increased microbial soil enzyme activity and nutrient cycling that made nutrients available for plants. The added artificial root exudates may have demotivated the roots to spread and grow in search of nutrients, perhaps explaining why there was no corresponding increase in 25R soil root mass.

Figure 3. In barren 25R soil, switchgrass plants growing in treatment Both (seven plants and exudate solution; column one from left) were visually bigger compared to those growing in treatment Plants (seven plants; column three from left). In all pots, the plants were culled to a total number of seven plants.

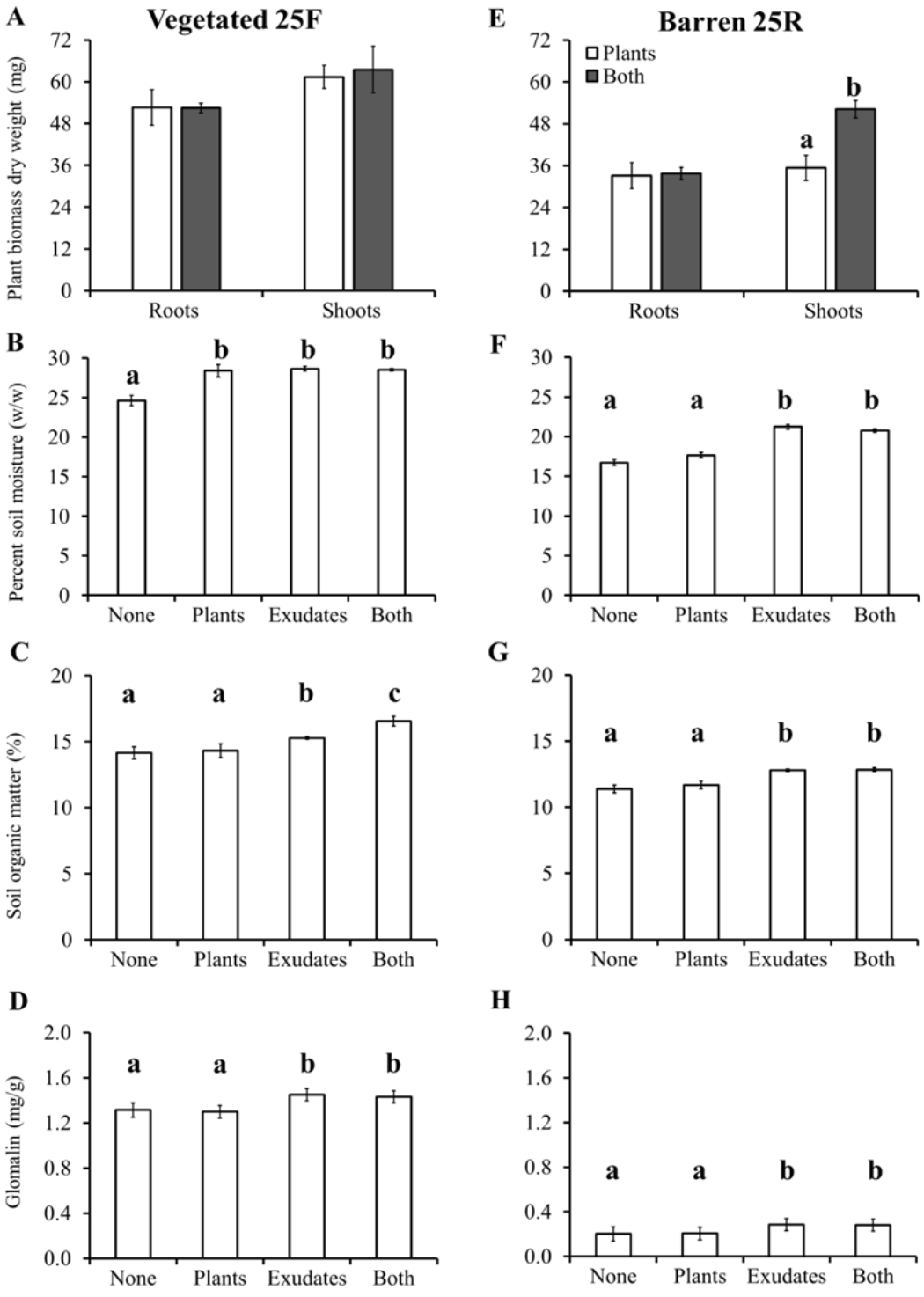


Figure 4. The effect of treatments (None, Plants, Exudates, and Both) on plant biomass dry weight (mg) (A, E), percent soil moisture ($w/w_{(dry\ soil)}$) (B, F), soil organic matter content (%) (C, G), and glomalin content ($mg/g_{(wet\ soil)}$) (D, H) are shown for 25F (left) and 25R (right). Same

number of plants was maintained in all planted pots by culling the germinated seeds to seven plants in each replicate. The data represent mean values of four replicate pots and six time points (on days 37, 65, 101, 129, 157, 205) (total data points = 96, n = 4, ± standard error of the mean). The treatments include None, Plants, Exudates, and Both. One-way analysis of variance among treatments was performed followed by Tukey HSD test. Lower case letters indicate statistically significant differences at $P < 0.05$.

3.2.2 Soil moisture and organic matter content

The vegetated site 25F had higher moisture (18.9 ± 0.1 %) than the barren soil 25R (15.6 ± 0.2 %). Percent moisture was significantly higher in both soils when they were treated with artificial root exudates than when they were not, even though all pots received the same amount of water (Fig. 4B, F). The moisture content in soil 25F, averaged over 205 days including day 1, was 24.6 ± 0.7 % when no exudates were added and 28.7 ± 0.3 % when exudates were added *(P* < 0.001, Tukey HSD) (Fig. 4B). The averaged moisture content in the barren 25R soil also increased significantly as a result of artificial root exudates (16.7 ± 0.4 % no exudates added; 21.2 ± 0.3 % exudates added, *P* < 0.0001, Tukey HSD) (Fig. 4F). The increased moisture retention in treated soils may be due to an altered soil structure, potentially caused by increased microbial activity that resulted from assimilation of artificial root exudates (Arya & Paris, 1981; Hinojosa et al., 2004; Yao et al., 2009). Soil moisture acts as a mobile medium through which labile nutrients, microbes, and other organic materials travel in polluted and unpolluted soil environments. Previous studies have shown low nutrient cycling in soils with low moisture content with a caveat that microbial activity may increase in the spots holding moisture that gather concentrated enzymes and substrates (Burns et al., 2013; Fierer & Schimel, 2002).

The initial soil organic matter content was higher in 25F (15.3 ± 0.3 %) compared to 25R (12.6 ± 0.4 %). As a barren soil, site 25R with the lower organic matter content likely receives less plant litter input. Soil organic matter contains essential nutrients necessary for the

biochemical functioning in plants and soil organisms (Nelson & Sommers, 1996). Organic matter and soil nutrients are needed for soil biota to flourish in the presence of pollutants (Bhadha et al., 2017; Hudson, 1994; Six et al., 2000). When the soil organic matter content was averaged over the course of the experiment (205 days), we found that soils treated with exudates had significantly more organic matter. For example, in the vegetated 25F soil, the organic matter content was significantly lower at 14.2 ± 0.5 % with no exudates compared to 15.3 ± 0.1 % when exudates were added, ($P < 0.001$, Tukey HSD) (Fig. 4C). In the barren 25R soil, the organic matter content was also significantly lower at 11.4 ± 0.3 % when no exudates were added compared to 12.8 ± 0.1 % with exudates added ($P < 0.0001$, Tukey HSD) (Fig. 4G). In general, exudates emitted by plant roots contribute to the soil organic matter content, in turn enhancing microbial biomass and enzyme production (Chander et al., 1997; Kögel-Knabner, 2002; Poirier et al., 2018; Yao & Shi, 2010). Caution is necessary when interpreting these results because addition of exudate solution in treatments Exudates and Both introduces additional organic matter compared to treatment None and Plants and the increase shown in this study may or may not reflect increased microbial biomass or enzyme production.

3.2.3 Easily-extractable glomalin content

In general, the presence of plants increases glomalin concentrations (Nichols & Wright, 2005; Purin & Rillig, 2007; Treseder & Turner, 2007). It was not surprising that the initial glomalin concentrations of easily-extractable glomalin were larger (1.5 ± 0.03 mg/g) at the vegetated study site 25F than the barren soil 25R (0.21 ± 0.01 mg/g) on day 1 without treatment. On the other hand, we were surprised to find an increased concentration of glomalin in both vegetated 25F and barren 25R soils when artificial root exudates were added (Fig. 4D, H).

To our knowledge, this is the first report of the effect of artificial root exudates on soil glomalin content. The average glomalin content over 205 days including day 1, in vegetated soil 25F, was significantly lower (1.3 ± 0.06 mg/g) with no exudates compared to when exudates were added (1.5 ± 0.03 mg/g, $P < 0.05$, Tukey HSD). In the barren 25R soil, the glomalin content was significantly different with no exudates added (0.20 ± 0.01 mg/g) compared to when exudates were added (0.30 ± 0.03 mg/g, $P < 0.0001$, Tukey HSD). Exudates either increased the rate of glomalin production or decreased its decomposition rate. In this study, we detected an increase in glomalin concentrations on the relatively short time scale of this experiment (205 days) despite the reported slow turnover rates of glomalin (Wang et al., 2011). Soil glomalin has been reported to modify a suboptimal microbial growth environment by increasing soil aggregate adhesion, moisture content, and nutrient retention (Hammer & Rillig, 2011; Kumar et al., 2018; Lovelock et al., 2004; Singh et al., 2018). Increased glomalin concentrations may have contributed to the mechanisms by which artificial root exudates increased PA, moisture retention, and organic matter content in our experimental soils (Fig. 4D, H). However, we do not know which came first, increased glomalin concentrations or other improved soil properties.

4. Conclusions

Environmentally safe and affordable remediation strategies to revitalize and re-green barren, contaminated post-industrial sites, are necessary. The data presented here showed that repeated additions of artificial root exudates to barren, metal contaminated, low functioning soil significantly increased soil PA in only 37 days of treatment. In barren 25R soil, the effect of adding artificial root exudates was realized by day 65 when the PA reached a maximum. In addition to the increased phosphatase cycling, artificial root exudates increased plant

productivity. Moreover, we found other indicators of improved soil quality, including significantly higher soil moisture, organic matter content, and easily extractable glomalin content in soils that were treated with artificial root exudates compared to controls with no addition. Increased glomalin content due to the addition of artificial root exudates has not been previously reported and further investigation of this phenomenon is expected. Notably, glomalin content in soil has been positively correlated with soil-aggregate formation, moisture retention, and aeration, creating a soil structure favorable for plant growth (Kumar et al., 2018; Wright & Upadhyaya, 1998).The changes in soil properties were likely interdependent, and some remediation efforts are expected to be most successful when they employ a combination of strategies that include soil microbial stimulation. The application of this approach will be repeated in the future at other contaminated, barren sites to evaluate the effectiveness of artificial root exudates in soil remediation. The results here showed that adding artificial root exudates increased primary production, improved soil quality indicators, enhanced soil enzyme function, and nutrient cycling in a previously nearly inactive, barren, contaminated soil.

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Appendix: Supplementary material

Table S1. Bulk soil properties of the sites at Liberty State Park.

Soil properties	Vegetated 25F	Barren 25R[a]	Vegetated 146[a]
Vanadium ($\mu g/g_{dry\ soil}$)	114 ± 13[b]	106 ± 17	183 ± 6
Chromium ($\mu g/g_{dry\ soil}$)	120 ± 3[b]	216 ± 32	110 ± 4
Copper ($\mu g/g_{dry\ soil}$)	2,714 ± 470[b]	7,460 ± 1,659	108 ± 2
Zinc ($\mu g/g_{dry\ soil}$)	8,451 ± 1971[b]	20,736 ± 4,690	98 ± 2
Arsenic ($\mu g/g_{dry\ soil}$)	850 ± 265[b]	1325 ± 269	41 ± 2
Lead ($\mu g/g_{dry\ soil}$)	8,824 ± 1817[b]	21,965 ± 4,486	316 ± 9
pH	5.06	4.95	5.20
Moisture (% w/w)	19 ± 0.1[c]	16 ± 0.2	38 ± 0.3
Organic matter (%)	15 ± 0.3[c]	13 ± 0.4	43 ± 0.5
Water holding capacity (%)	49 ± 2.8[c]	29 ± 0.8	133 ± 0.9
Phosphatase activity ($\mu mol\ h^{-1}\ g_{dry\ soil}^{-1}$)	0.53 ± 0.027[d]	0.016 ± 0.014	3.67 ± 0.18

[a] Soil properties of site barren 25R compared with data reported by Vaidya et al. (2020).

[b] Heavy metal concentrations ($\mu g/g_{dry\ soil}$) in the soils from the study sites with standard error of the mean shown (n = 3) (Singh, Vaidya, et al., 2019).

[c] Moisture content, organic matter content and water holding capacity in the soils from the study sites with standard error of the mean shown (n = 4).

[d] Phosphatase activity in the soils from study sites with standard error of the mean shown (n = 4).

Table S2. Composition of the artificial root exudates solution to study the effect of different concentrations in the dose-response side experiment.

Artificial root exudates	Amount (mmol) of each compound added per week	0.1x	0.5x	1.0x[a]	2.0x	2.5x
Sugars	Glucose, Fructose, Sucrose, Maltose	0.02	0.1	0.2	0.4	0.5
Organic acids	Acetic acid, Citric acid, Lactic acid, Malic acid, Succinic acid	0.008	0.04	0.08	0.2	0.2
Amino acids	Alanine, Arginine, Asparagine, Glutamic acid, Glycine, Histidine, Leucine, Tyrosine	0.003	0.01	0.03	0.05	0.06

[a] The concentration of sugars, organic acids, and amino acids in 1.0x artificial root exudates solution was prepared from the work by Steinauer et al. (2016). For example, in a 20 mL 1.0x artificial root exudate solution that was added to each pot weekly, the four sugar compounds were 0.2 mmol each, the five organic acids were 0.08 mmol each, and the eight amino acids were 0.03 mmol each. The different concentrations (0.1x to 2.5x) were prepared by calculating the appropriate concentration and quantities of artificial root exudates based on the 1.0x solution.

Effect of artificial root exudate solution concentration (side experiment)

Methods

In the dose-response experiment, different concentrations of the low molecular weight metabolites were added to soils from vegetated and barren LSP sites and phosphatase activities were measured on days 1, 45 and 90. Soil from another vegetated LSP site 146 was used instead of vegetated 25F soil due to limited availability of resources at the time of the dose response experiment. The bulk properties of the LSP soils 146, 25F, and 25R are listed in Table S1. Briefly, the soils from vegetated sites 146 and 25F had high phosphatase activities, whereas

barren 25R soil had a low phosphatase activity; soils from all sites had high metal concentrations (Hagmann et al., 2015; Hagmann et al., 2019; Singh, Vaidya, et al., 2019; Vaidya et al., 2020).

The experimental design of the dose-response experiment was similar to the main experiment. The vegetated 146 and barren 25R soils were treated as two separate blocks based on soil bulk densities, site designation, and vegetation. The block with vegetated 146 soil included 20 pots based on the five exudate concentrations (0.1x, 0.5x, 1.0x, 2.0x, 2.5x) plus four pots labeled as 0.0x, treated with only sterile tap water (control treatment). Each pot was filled with 55 g vegetated 146 soil. All pots were incubated in a growth chamber, settings maintained identical to the main experiment, over 90 days and watered three times weekly, including the exudate solution. Similarly, the second block consisted of 24 pots, each filled with 137 g barren 25R soil. We used different soil weights because 55 g of vegetated 146 soil occupied the same volume in the same size pot as the volume occupied by 137 g of barren 25R soil.

To vary the concentrations of the artificial root exudates (0.1x, 0.5x, 1.0x, 2.0x, 2.5x), we prepared five different concentrations of artificial root exudate solution. The concentration of exudates in the 1.0x solution was identical to the composition of the solution used by Steinauer et al. (2016). For the dose response experiment, we prepared four additional concentrations (0.1x, 0.5x, 2.0x, 2.5x) of exudate solutions (see details in Table S2).

Results

Effect of artificial root exudate solution concentration

We examined the effect of exudate solution concentration on phosphatase activity in a separate experiment. We added five concentrations of exudate solutions to LSP soils vegetated 146 (higher enzymatic function) and barren 25R (low enzymatic function). The data showed that the 2.5x concentration yielded a 47-fold increase in phosphatase activity (2.28 ± 0.21 μmol h^{-1} $g_{dry\ soil}^{-1}$) in soil 25R, averaged over 90 days, compared to the control (only sterile tap water) (0.049 ± 0.011 μmol h^{-1} $g_{dry\ soil}^{-1}$) (Fig. S1B).

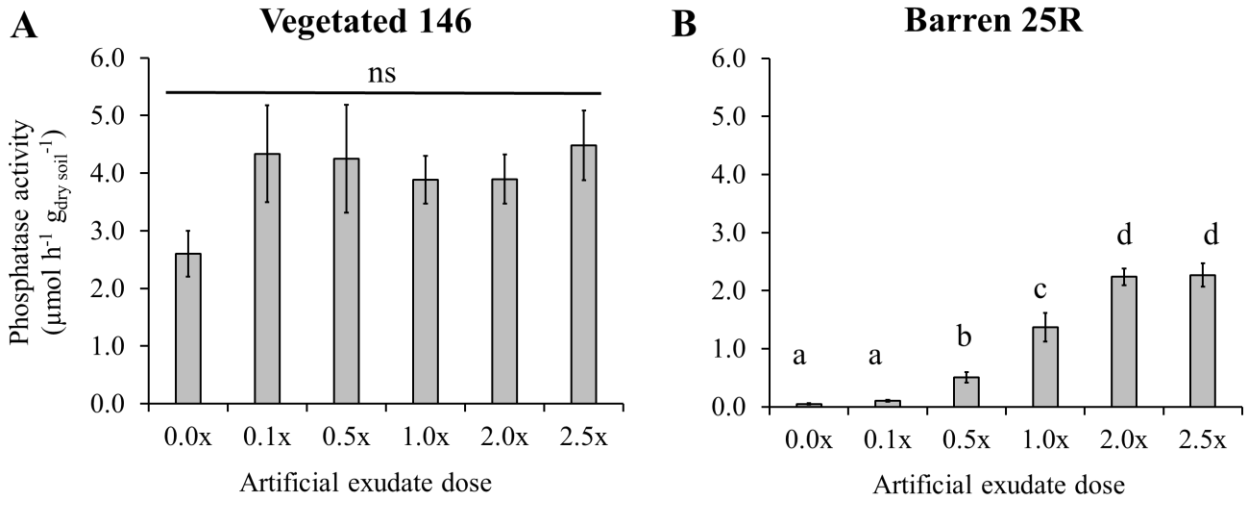


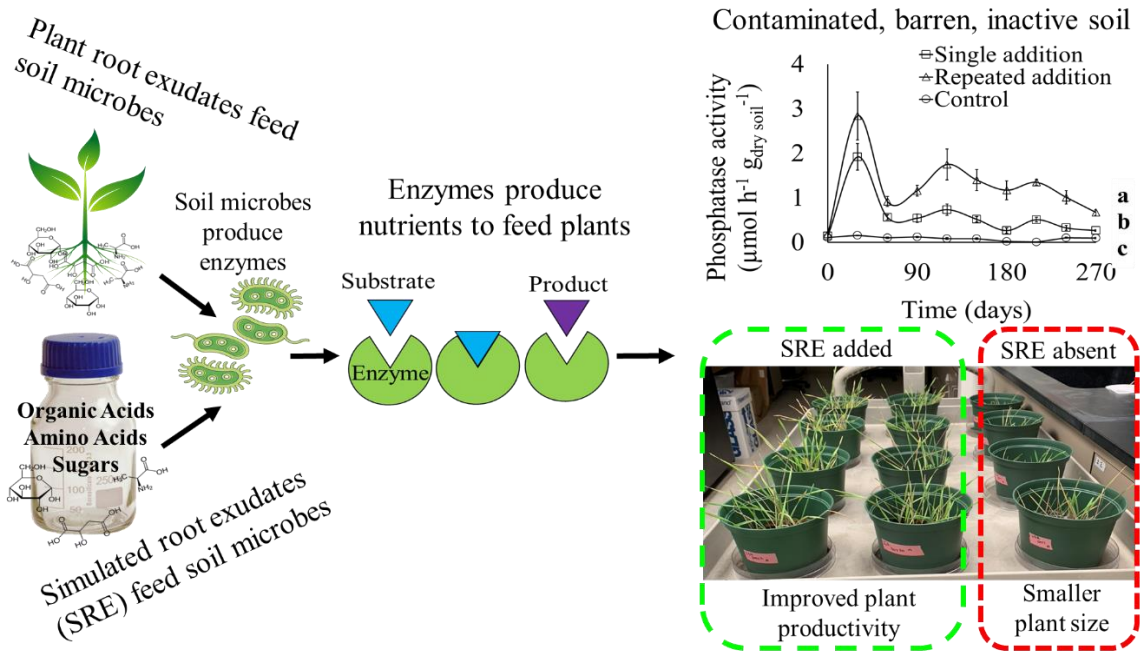
Figure S1. Soil phosphatase activities in vegetated 146 (A) and barren 25R (B) are shown for pots treated with different concentrations of artificial root exudate solutions. The phosphatase activities shown are an average of measurements at two time points (day 45 and 90). The five concentrations of artificial root exudate solution added to both soils were 0.1x, 0.5x, 1.0x, 2.0x, and 2.5x (see methods for details). Each treatment consisted of four replicates for vegetated and barren soils. A control (0.0x) for both soils replicated four times received only sterile tap water and no exudates. Adding 0.5x and higher concentrations of artificial root exudate solutions yielded significantly ($P < 0.0001$, Tukey HSD) increased phosphatase activities in barren 25R soil. From these results, 2.5x exudate solution was used in the main experiment to ensure maximum microbial activity occurred to influence nutrient cycling processes for a lasting effect towards revitalizing barren 25R soil. Lower case letters indicate statistically significant differences at $P < 0.05$ and ns denotes no significance, $P > 0.05$.

In vegetated 146 soil, phosphatase activity did not vary significantly with the concentration of the exudate solution that was added (Fig. S1A). In contrast, phosphatase activities were found higher when exudate solution with higher concentrations were added to barren soil 25R ($P < 0.001$, Tukey HSD) (Fig. S1B). In barren 25R soil, phosphatase activity between untreated (0.0x) and treated (0.1x) and between treatments 2.0x, and 2.5x exudate solutions, showed little change ($P > 0.05$, Tukey HSD) (Fig. S1B). In a previous study, Vaidya et al. (2020) reported that plants increased the activity of barren 25R soil. The findings from that study suggest that root exudates resulted in an increased soil function and were thus likely one mechanism by which plants increased soil 25R's activity. Based on the results from dose response experiment, we selected the highest concentration (2.5x) of the artificial root exudate solution concentration for the main experiment reported in section 3.1.

CHAPTER 4

Project 3. *A single addition of simulated root exudates increased microbial function and plant biomass in a barren, metal contaminated, inactive soil*

Graphical abstract



Article 3. *A single addition of simulated root exudates increased microbial function and plant biomass in a barren, metal contaminated, inactive soil*

Abstract

Brownfield sites are areas that were contaminated by anthropogenic activities. Our study site, an abandoned rail yard, is located within Liberty State Park in Jersey City, New Jersey. The contaminated section of the park has been closed off and left undisturbed since 1969. Previous studies have reported polycyclic aromatic hydrocarbons (PAHs) and heavy metals (As, Pb, Cu) above threshold levels at the site. While some areas of the park yield natural plant growth despite high contaminant levels, others remain barren. The roots of the plants in vegetated areas naturally secrete root exudates into the soil, which include a variety of sugars, amino acids, and organic acids. These exudates nourish microbes within the soil, thus promoting microbial metabolic function and improving soil quality. To restore microbial function in barren, contaminated, inactive brownfield soil from site 25R, we used a simulated root exudate solution (SRE) prepared in the lab. By measuring carbon dioxide emission rates, also known as soil respiration and phosphatase activity, considered markers of microbial function and soil health, over 150 and 270 days, respectively, we examined whether the effects of single and repeated SRE additions differed and if the effects depended on soil type. We also evaluated if the enriched soils supported plant productivity. By day 30, SRE enriched 25R soil showed a significantly higher soil respiration rate than the control, which was treated with sterile tap water. Until day 210, phosphatase activities were significantly higher in SRE enriched 25R soils than in the control. The legacy effect of a single SRE addition lasted over 270 days. The shoot height (16 ± 0.3 cm) and total plant biomass (0.5 ± 0.02 g) of plants grown in enriched 25R soil (single SRE addition) were significantly higher than the control (9 ± 0.9 cm, 0.3 ± 0.02 g, respectively).

Studying plant-soil-microbial interactions exclusive to this site may be relevant for developing "green" remediation strategies to clean up barren brownfield sites.

Keywords: root exudates, soil enzymes, heavy metals, brownfield, phosphatase activity, soil respiration

1. Introduction

Persistent organic and inorganic pollutants render post-industrial derelict soils unvegetated and inactive, referred to as industrial barrens (Kozlov & Zvereva, 2007). Without vegetation, such sites cannot maintain soil structure to immobilize the pollutants and provide ecosystem services (Gallagher et al., 2018; Six et al., 2000). To support plant productivity, avail ecosystem services, and meet urban land-use demands, restoring the soil quality of barren, inactive, contaminated sites has become necessary. Among the prevalent bioremediation techniques, phytoremediation - growing contaminant-tolerant plant species that trap and sequester soil pollutants – is considered a preferred "gentle-remediation option" (Cundy et al., 2013). For the successful implementation of phytoremediation, where specific plant species are grown to reduce contaminant toxicity, the soil quality has to support plant productivity (Cundy et al., 2016; Lopes et al., 2016; Masarovičová & Kráľová, 2019). Soils from contaminated sites typically display oxidative stress from the inorganic pollutants (Pb, As, Hg, Cu), limited nutrient bioavailability, and low abundance of indigenous microbes resulting in poor vegetation (Afegbua, 2014; Bardgett & Van Der Putten, 2014; Fayiga & Saha, 2016; Reeder et al., 2006). Inorganic pollutants have been reported to hinder microbial metabolic processes, growth, and survival (Kandeler et al., 2000; Kavamura & Esposito, 2010; Kozdrój & Van Elsas, 2000;

Markiewicz-Patkowska et al., 2005). That said, these challenges have promoted contaminant-resistant strains in soil microbial communities to prevail over the hindrance and establish ecological diversity (Ceci et al., 2019; Wang et al., 2007; Xie et al., 2016). While a small number of contaminant-resistant microbial communities may remain in the soil, industrial barrens lack nutrient inputs to sustain these microbial communities (Grobelak et al., 2017; Ibraheem, 2007; Sun et al., 2010). To overcome these limitations, various approaches have been reported (Liao et al., 2021; Lynch & Moffat, 2005; Megharaj & Naidu, 2017; Wang et al., 2021). A few of these approaches include growing contaminant-tolerant plant species that trap and sequester soil pollutants, adding genetically modified microbial communities that promote plant growth (phytostimulation), and adding nutrients to stimulate native microbial consortia (biostimulation) (Andre et al., 2010; Beesley et al., 2011; Grobelak et al., 2017; Rinklebe & Shaheen, 2015; Siyar et al., 2019).

In vegetated areas, the plant roots naturally secrete a variety of chemical compounds called “root exudates” (Kunc & Macura, 1966; Rovira, 1969). These root exudates are typically simple metabolites (sugars, organic acids, amino acids) that provide nourishment to the soil microbes (Hale et al., 1978; Sasse et al., 2018). Studies have shown that plant species in natural environments also exude specific compounds through their roots to signal pest attacks (Sasse et al., 2018; Tiwari & Lata, 2018). Before introducing plants to contaminated soils, soil quality restoration can provide a better chance for the vegetation to establish, given the limited enzymatic function at such sites. A promising approach is to prime contaminated soils with root exudate compounds, either collected directly from plants roots or as a solution of root exudate compounds (SRE) prepared in a laboratory that mimic the natural root exudate constituents

(Dundek et al., 2014; Hamer & Marschner, 2005; Kuzyakov, 2010; Vaidya et al., 2022; Wang et al., 2020).

Priming agricultural soils and contaminant-spiked soils with natural and simulated root exudates were researched intensively (Bali et al., 2020; Baudoin et al., 2003; Dakora & Phillips, 2002; Xie et al., 2012). To our knowledge, the efficacy of simulated root exudates in restoring an aged, barren, contaminated, inactive soil has been recently demonstrated by Vaidya et al. (2022) for the first time. However, the most optimal dosing strategies have not been investigated. For example, it is not known whether a single SRE addition is sufficient for soil revitalization in derelict soils, or if regular intervention through repeated additions is needed to restore soil function. Studies have shown that regular addition of soil amendments (biochar, compost) improve soil nutrient cycling and plant productivity (Beesley et al., 2011; Ghosh & Maiti, 2021; Hartley et al., 2009; Rinklebe & Shaheen, 2015). Soil function is determined by measuring phosphatase activity, a proxy for phosphorus cycling in soils.

Based on a previous experiment (Vaidya et al., 2022), we know that repeated SRE additions significantly improved phosphatase activity in a barren, contaminated, inactive soil. Brownfield sites are difficult to access and repeated SRE additions may not be practically feasible from an environmental management perspective. So, we asked if a single SRE addition was sufficient to stimulate soil function in a post-industrial barren soil, and for how long. We examined the influence of a single SRE addition on the soil and whether the addition was impactful enough to leave a legacy.

To answer this question, we studied three soil types, two from the LSP brownfield site, one vegetated and enzymatically active (25F) and the other barren and poorly functioning (25R). The third is from an uncontaminated, vegetated reference site Hutcheson Memorial Forest

(HMF) owned by Rutgers university in Somerset County, NJ. The reference site HMF was a historical farmland and selected because it has remained undisturbed since 1969 similar to our study site. Our study site is located within Liberty State Park (LSP), New Jersey, USA, which was an industrial rail yard, filled with construction refuse from New York. The site was left undisturbed since 1969 and today, a thick forest covers most of the site except for a few barren areas. Despite high contaminant levels, an area with vegetation (LSP site 25F) has shown high microbial function, while the barren area (LSP site 25R) has shown low microbial function (Hagmann et al., 2015; Hagmann et al., 2019; Singh et al., 2019; Vaidya et al., 2020). Hagmann et al. (2019) have found polycyclic aromatic hydrocarbons and high heavy metal concentrations in LSP soils.

Soils from LSP and HMF were enriched with a solution of simulated root exudates (SRE) containing sugars, amino acids, and organic acids. Each soil type was enriched with three treatments: a single addition treatment where SRE was added once (single SRE addition), a repeated additions treatment, where SRE was added 14 times in total over 30 days (repeated SRE addition), and a control that was treated with only sterile tap water (control). We compared the effects of the three treatments on the soil respiration rate over 150 days, soil extracellular phosphatase activity, percent soil moisture ($w/w_{(dry\ soil)}$) over 270 days, and plant biomass at day 320. We investigated the longevity of the improved soil function following a single SRE addition and whether this treatment resulted in altered plant productivity. In this study, we stimulated native brownfield microbes with a single SRE addition and cultivated plants in enriched soils. This strategy may be beneficial in restoring industrial barrens into green spaces for public recreation.

2. Materials and methods

2.1 Study site description and soil collection

We collected soil from two contaminated study sites located next to each other in Liberty State Park (LSP), Jersey City, NJ (40˚42’16N, 74˚03' 06W); vegetated site 25F and barren site 25R. This area of LSP has been fenced off from human access and left un-remediated since 1969. Thick vegetation has grown at site 25F despite the absence of human intervention in this area of LSP (Gallagher et al., 2018; Gallagher et al., 2015; Gallagher et al., 2011). Previous studies at LSP have mapped and determined soil contaminant concentrations (Gallagher et al., 2008; Hagmann et al., 2019; Salisbury et al., 2017). The adjacent 25R site has opposite characteristics; little vegetation, high contamination concentrations, and low phosphatase activity (Hagmann et al., 2019; Singh et al., 2019; Vaidya et al., 2020). The reference soil was collected from Hutcheson Memorial Forest (HMF) in Somerset County, owned by Rutgers University, NJ. Previous research on HMF soil has shown little contamination, substantial vegetation, and healthy soil (Hagmann et al., 2015; Hagmann et al., 2019). This area has a similar chronosequence to Liberty State Park. Fresh soil samples were collected from five markers, 5 m apart, along three parallel transects 10 m apart at each site, with a field grid of 16 by 20 m. In total, fifteen samples from each site were combined to form a composite sample of that site. The composite fresh soil samples were sieved (2 mm) and stored at 4 °C until experimental set-up on day 0 when treatments were added.

2.2 Bulk soil characteristics

The bulk soil characteristics of experimental soils vegetated HMF, vegetated 25F, and barren 25R are listed in Table S1 in the supplementary information. The phosphatase activity

was highest in vegetated 25F soil, high in vegetated HMF, and low in barren 25R soil. The pH was low in all soils. Moisture content was lower in barren 25R soil than in vegetated 25F and HMF soil (Vaidya et al., 2020). The heavy metal concentrations in 25F and 25R soils were higher than in HMF and above the threshold limits specified by the New Jersey Department of Environmental Protection guidelines (NJDEP, 2020). Cu, As, and Pb concentrations at the barren 25R site were two times higher than in vegetated 25F soil (Table S1) (Singh et al., 2019).

2.3 Experimental design

We established a factorial design (Fig. 1) and tested three independent variables: soil type, treatment, and time. We used three soils with different properties for this study (vegetated HMF, vegetated 25F, and barren 25R). Each soil was subjected to two experimental treatments: single and repeated SRE additions and control with four replicate pots for each treatment. For the single SRE addition treatment, the replicate pots from each soil type were treated with a single dose of SRE solution on day one of the experiment. For the repeated SRE additions treatment, each pot received a dose of SRE solution three times weekly over 30 days (a total of 14 additions). By the end of the 30 days, the single and repeated SRE addition pots will have received the amounts of exudates as shown in (Figure 1B). The control pots received sterile tap water only by weight three times weekly. After the 30-day exudate addition regimen, all pots were watered by soil weight three times weekly. We measured phosphatase activity and soil moisture content at a 30-day interval for over 270 days. Soil respiration rate was measured daily for 90 days and then weekly until day 150. Plant biomass concentrations in the soils were measured after harvest on day 320.

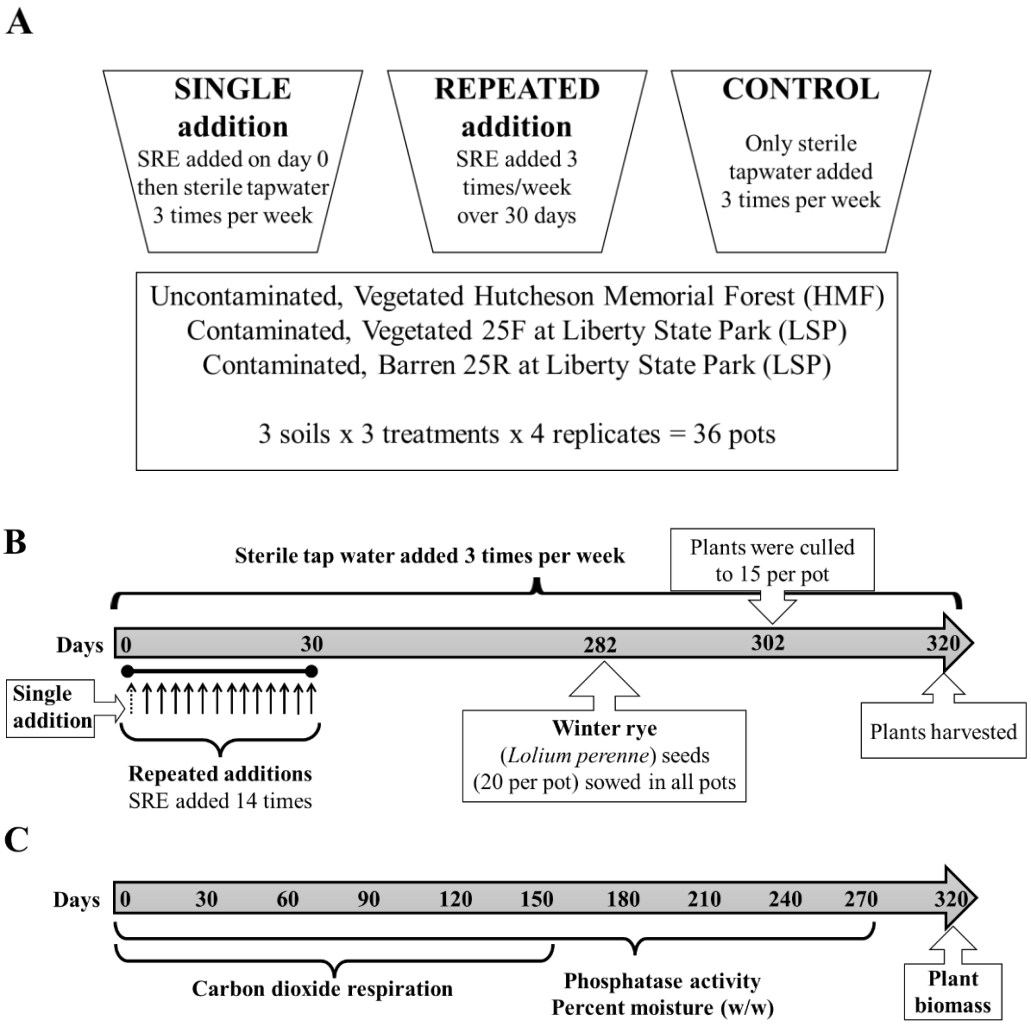


Figure 1. Experimental design (A), timeline (B) of events showing the addition of simulated root exudate solution (SRE), planting of winter rye seeds, and timeline (C) of the sampling time points when the soil was sampled from each pot to measure the response variables during the 320 days of the experiment. The response variables measured were carbon dioxide respiration, phosphatase activity, percent moisture, and plant biomass. The dotted arrow represents single SRE addition on day 0 added to single SRE addition pots, while the filled black arrow represents repeated SRE additions in which SRE was added three times per week for 30 days (14 times total). Sterile tap water was added over 320 days to each pot in a climate-controlled incubator.

Each soil type was equally distributed in 12 pots. The mass of soil added to each pot was based on the volume occupied by the soil in the pot (6 in height x 4 in bottom diameter). Each pot with soil from HMF consisted of 500 g of the soil. For LSP soils, pots with soil from site 25F contained 341 g, and site 25R occupied 495 g. The experiment consisted of a total of 36 pots.

After each soil respiration measurement and watering, all pots were randomly distributed within an automatic growth chamber (Percival Scientific Inc, E36L) for storage. The relative humidity was set to 65%, the photo-period of the day/night cycle was 10.5/13.5 h, and the day/night temperatures were 24 °C and 16 °C, respectively.

2.4 Simulated root exudate (SRE) solution preparation

The SRE solution was prepared by combining selected sugars, organic acids, and amino acids (Figure 1B, supplementary material Table S2) modified from the recipe developed by Steinauer et al. (2016). Our chosen concentration of SRE solution was 2.5 times higher than that reported by Steinauer et al. (2016). The SRE solution was stored at 4 °C. The total amount (mmol) of SRE added to enrich soils through a single SRE addition and 14 repeated SRE additions per pot are shown in the supplementary material (Table S2). For the single SRE addition, one addition (60 mL) per pot resulted in adding 3.0 mmol of each sugar, 1.2 mmol of each organic acid, and 0.43 mmol of each amino acid. For repeated additions, one addition (10 mL) per pot resulted in adding 0.3 mmol of each sugar, 0.1 mmol of each organic acid, and 0.03 mmol of each amino acid.

2.5 Measurement of soil quality indicators

Soil respiration rate

Soil CO_2 respiration rate was measured using an infra-red CO_2 Gas Analyzer (PP Systems EGM-5 CO_2 Gas Analyzer) with a soil respiration chamber attachment (SRC-2). The dimensions of the SRC-2 chamber were: 150 mm height x 100 mm diameter; 1171 mL volume, 78 cm^2 area, 15.0 volume to area ratio; termination settings 180 s maximum measurement duration (dT) and

5000 ppm maximum CO_2 respiration change (dC). The electric and pneumatic connections between the SRC-2 chamber and the EGM-5 system were fastened before powering on the device for its warmup period. To begin the SRC measuring process, a plot number corresponding to each pot was entered into the EGM-5 system to identify each measurement. The chamber was then held in the air for flushing for 24 s between two measurements. After flushing, the chamber was placed on the soil surface within 9 s. The respiration chamber was placed within an experimental pot such that the open circular rim of the chamber was fixed directly on the soil surface within the pot. Then, the system was allowed to calibrate for 12 s on top of the soil surface before recording the linear soil respiration rate ($g\ m^{-2}\ h^{-1}$) for each pot over a 180 s period. The chamber was wiped with 70% ethanol between each measurement.

Phosphatase activity

The extracellular phosphatase activity (PA) in soil was measured using the fluorometric assay protocol developed by Marx et al. (2001) and adapted to analyze LSP soils in 2015 by Hagmann et al. (2015). The fluorescence emitted by the 4-methylumbelliferone product formed by the reaction of extracellular phosphatase enzymes and the substrate methylumbelliferyl phosphate in each soil sample was measured. Composite soil samples combined from locations around the soil surface within the pots were collected and analyzed at ten-time points over the 270 days of the experiment. Phosphatase activity was expressed as micromoles of reaction product per hour per gram of dry soil.

Moisture content

Soil moisture content was measured by drying approximately 2.0 g of soil scooped from each pot in an aluminum crucible at 100 °C in a conventional oven for 24 hours (Schmugge et al., 1980) and expressed as percent dry soil weight ($w/w_{(dry\ soi)l}$).

Plant biomass

Winter rye (*Lolium perenne*) seeds (20 per pot), which are native to the site (Balacco et al., 2022; Salisbury et al., 2020) were sowed in all pots on day 282 after adding the treatments. On day 285, 15 – 20 rye grass seeds had germinated in all pots. To maintain similar overall capacity in all pots, on day 302, plants in each pot were culled to 15. On day 320, after 33 days from seed germination, plants were harvested. Plants from each pot were separated into shoots and roots. Roots were gently tapped and washed with tap water to remove soil particles. Shoots and roots were stored in brown paper bags and kept for drying in a conventional oven at 70 °C for 13 days. Shoot and root mass of plants from each pot was measured, and then total plant biomass was calculated. The shoot height of plants in each pot was measured using a ruler at the time of harvest.

2.6 Data analysis

This study aimed to determine the effects of single and repeated SRE additions on the soil function of soils from three sites. Among the three study sites, two were from the LSP brownfield site, contaminated 25F (vegetated) and 25R (barren). The third reference soil was from an uncontaminated, historically agricultural HMF site. The LSP soils displayed similar abiotic character in relation to contamination but were different in their biotic character with

respect to vegetation history. The soils from these sites were subjected to three treatments: single SRE addition and 14 repeated SRE additions, compared with untreated controls over 270 days. The effects of SRE solution on the study soils were examined by measuring response variables: phosphatase activity, soil respiration rates, moisture, and plant biomass. The independent variables were soil type (vegetated HMF, vegetated 25F, barren 25R), treatments (single, repeated, and control), and time (270 days). All statistical analyses were carried out in RStudio using R version 4.1.1 (RCoreTeam, 2021). Due to characteristic differences among the soil types, treatment effects were analyzed separately for each soil type. To analyze differences in the response variable and trends over time, we used a two-way repeated measures analysis of variance (ANOVA). The response variables include soil respiration rates over 150 days, phosphatase activities, and percent moisture over 270 days after priming the soil with SRE once, repeatedly, and with no treatment. With this analysis, we analyzed the effect of the treatments over time, then compared the two treatments with respect to the controls and the interaction effect between the treatments and time within each soil type, followed by a subsequent analysis of individual contrasts using Tukey's Honestly Significant Differences (HSD) as the post hoc test at $P < 0.05$. Appropriate transformations to the data were made to adhere to the normality distribution and assumptions during data analyses. A square root transformation was made on the phosphatase activity and soil respiration rate (response variables) to rescale the data and reduce positive skewness. Differences in plant biomass were analyzed using a one-way ANOVA where the soil types and treatments were the fixed factors. Tukey's HSD post hoc test was used to identify the mean differences among the soil treatments and the soil types.

3. Results and discussion

3.1 Effect of treatments on soil respiration rate

Before adding SRE, the soil respiration rates for the vegetated soils (HMF: 0.1 ± 0.02 and 25F: 0.08 ± 0.01 g m^{-2} h^{-1}) were higher than the barren 25R soil (0.003 ± 0.002 g m^{-2} h^{-1}) (Fig. 2A, B, C). SRE enrichment significantly increased soil respiration rates compared to the control, which received only sterile tap water. The single SRE treatment on vegetated soils resulted in a sharp spike in soil respiration rates within two days (Fig. 2A, B). The rise in soil respiration rate for the barren 25R soil was more gradual and took nine days to reach a maximum value. For example, after the single SRE addition, the soil respiration rates in vegetated HMF increased from 0.1 ± 0.02 on day zero to 3.6 ± 0.2 g m^{-2} h^{-1} on day 2, and from 0.08 ± 0.01 on day zero to 3.9 ± 0.1 g m^{-2} h^{-1} on day 2 in vegetated, contaminated 25F (Fig. 2A, B respectively). In barren, contaminated 25R, the single SRE addition resulted in an increase in soil respiration rate from 0.003 ± 0.002 g m^{-2} h^{-1} on day zero, reaching a maximum value of 0.6 ± 0.02 g m^{-2} h^{-1} on day 9 (Fig. 2C).

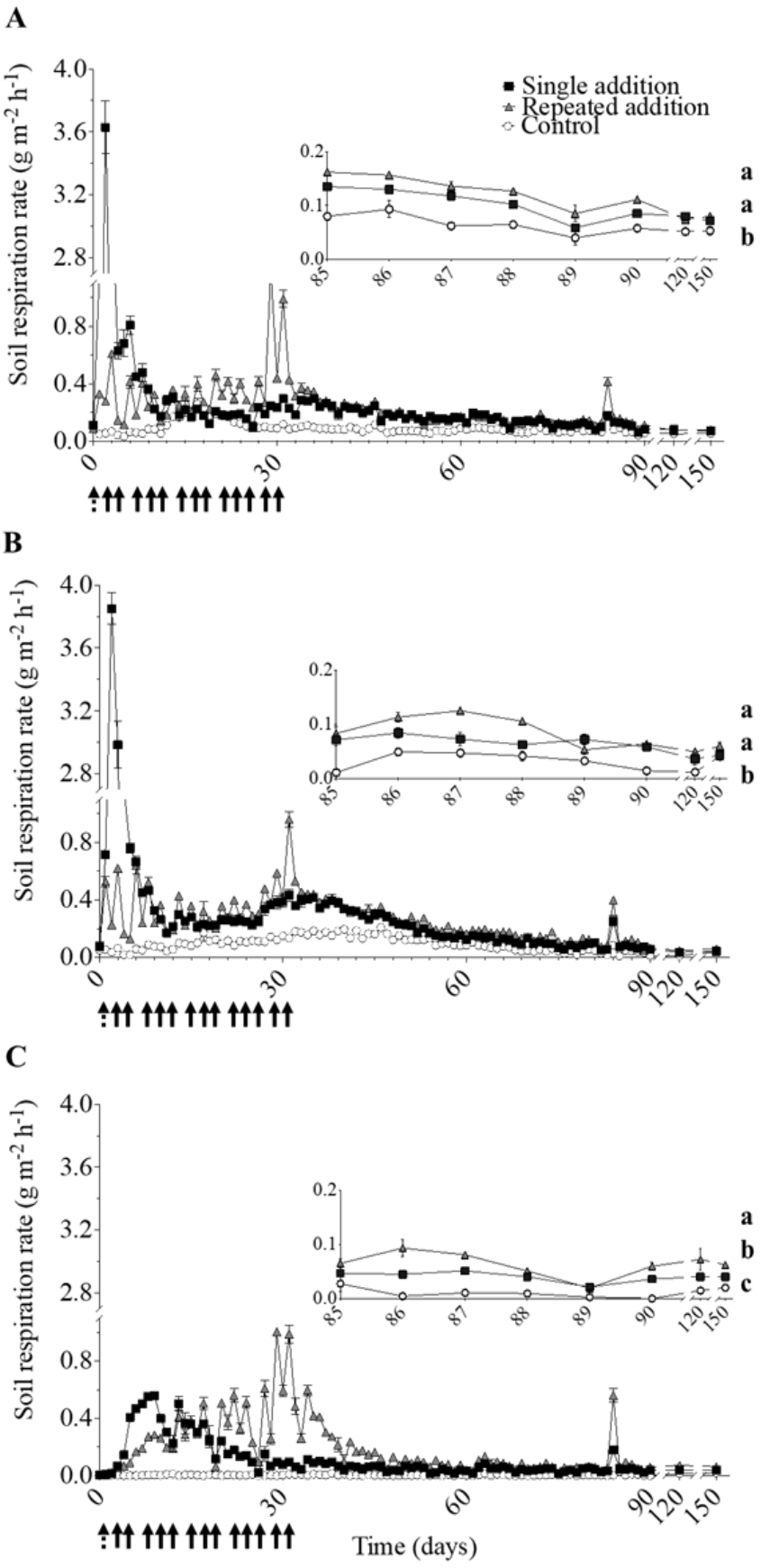

A
B
C
Soil respiration rate (g m-2 h-1)
Single addition
Repeated addition
Control
a
a
b
a
a
b
a
b
c
Time (days)

Figure 2. Soil respiration rates measured as soil carbon dioxide emission (g m^{-2} h^{-1}) over 150 days for three treatments: single SRE addition (black squares), repeated SRE additions (gray triangles), and sterile tap water only (control; white circles). The dotted arrows indicate the timing of the single SRE addition on day 0. The black arrows indicate repeated SRE additions that began on day 0, and continued three times per week for 30 days (14 times total). The insets show the data from day 85 to day 150 to emphasize differences in soil respiration among treatments, which persist for over four months after SRE enrichment. Data are shown for soils collected from three sites: vegetated, uncontaminated HMF (A), vegetated, contaminated 25F (B), and barren, contaminated 25R (C). Soil respiration spiked within three days after a single SRE addition in the vegetated soils while the barren soil respiration spiked within ten days. Error bars show the standard error (SE) of the mean (n = 4, ± 1 SE).

Each soil type exhibited a distinct response to the type of SRE treatment (single or repeated SRE additions). SRE treatments resulted in larger percent increases in soil respiration rate in barren 25R soil compared to the vegetated soils. In barren, contaminated 25R soil, the percent increase in soil respiration rate after single SRE addition was 19,304 % from day zero to day 9. In vegetated HMF, the largest percent increases in respiration rate were 3,128 % on day 2 for the single SRE addition. We observed a similar trend in the vegetated, contaminated 25F soil where the largest percent increases in respiration rate were 4,661 % on day 2 for single SRE addition. In contrast, after 14 repeated SRE additions to the barren 25R soil, the largest percent increase in soil respiration rate (39,138 %) was observed on day 29. In vegetated HMF, the largest percent increase was 1,268 %, and 704 % in vegetated 25F on day 29 after 14 repeated SRE additions.

A repeated measures ANOVA showed that the repeated SRE additions resulted in significantly higher soil respiration rates compared to the control in all soils. The single SRE treatment resulted in higher mean respiration rates (0.24 ± 0.01 g m^{-2} h^{-1}) compared to its control in vegetated HMF (0.088 ± 0.002 g m^{-2} h^{-1}) ($P < 0.0001$, Tukey HSD). We observed a similar trend in barren 25R soil that received single SRE treatment (0.10 ± 0.001 g m^{-2} h^{-1}) compared to its control (0.005 ± 0.001 g m^{-2} h^{-1}) ($P < 0.0001$, Tukey HSD). After ceasing SRE additions on

day 0 (single SRE addition) and day 30 (repeated SRE addition) of the experiment (Fig. 1), we observed decreased soil respiration rates and expected respiration rates to quickly drop to the values observed for the control.

Surprisingly, we observed significantly higher respiration rates in SRE enriched vegetated HMF and 25F soils than their controls, even on day 150 (Fig. 2A, B inset). Respiration rates for barren, contaminated 25R soil remained significantly higher for both the single (0.10 ± 0.001 g m^{-2} h^{-1}) and repeated (0.18 ± 0.002 g m^{-2} h^{-1}) SRE additions than the control (0.005 ± 0.001 g m^{-2} h^{-1}) on day 150 ($P < 0.0001$, Tukey HSD) (Fig. 2C inset). The results indicate that SRE enrichment created a legacy effect within the soil that persisted for at least 150 days. The legacy effects of SRE treatments (single and repeated SRE additions) on the native soil microbial communities resulted in higher soil respiration rates than the control in barren 25R soil (Fig. 2C, inset). SRE intervention was essential in barren 25R soil; both the treatment types – single and repeated SRE additions, were effective. Therefore, a single SRE addition may be adequate for some barren, contaminated soils from a practical management perspective. We found only one study where simulated root exudates were added to a barren soil: Adamczyk et al. (2021) studied uncontaminated, barren, alpine soils that have shown positive effects of SRE addition on soil respiration rates similar to what we observed.

3.2 Effect of treatments on phosphatase activity

During the SRE intervention experiment, we discovered that a single SRE addition effectively increased phosphatase activity in treated study soils. In cellular metabolic processes, phosphatase catalyzes the cycling of phosphorus from soil organic matter, hence used as a proxy to evaluate soil functioning (Martinez, 1968; Nannipieri et al., 2011; Tyler, 1976). To determine

the change in phosphatase activity in SRE enriched and control soils, we measured phosphatase activities at ten time points over 270 days (Fig. 3). Before SRE treatment, the average phosphatase activities were 0.77 ± 0.03 μmol h^{-1} $g_{dry\ soil}^{-1}$ in vegetated HMF soil (Fig. 3A), 3.0 ± 0.03 μmol h^{-1} $g_{dry\ soil}^{-1}$ in vegetated, contaminated 25F soil (Fig. 3B), and 0.15 ± 0.03 μmol h^{-1} $g_{dry\ soil}^{-1}$ in barren, contaminated 25R soil (Fig. 3C). The activities are comparable to values reported in previous publications for these soils (Balacco et al., 2022; Hagmann et al., 2015; Hagmann et al., 2019; Singh et al., 2019; Vaidya et al., 2020; Vaidya et al., 2022).

The addition of SRE to vegetated HMF and 25F, and barren 25R soils resulted in significantly increased phosphatase activities compared to the controls when averaged over time ($P < 0.001$, Tukey HSD) (Fig. 3A, B, C). For example, the mean phosphatase activities for 25R treated with SRE were 0.6 ± 0.2 μmol h^{-1} $g_{dry\ soil}^{-1}$ (single SRE addition), 1.3 ± 0.2 μmol h^{-1} $g_{dry\ soil}^{-1}$ (repeated SRE addition) and only 0.1 ± 0.01 μmol h^{-1} $g_{dry\ soil}^{-1}$ for the untreated 25R control. Phosphatase activity increased 3.5-fold from day 0 to day 30 in SRE enriched vegetated HMF and 3.2-fold in 25F soils and only 1.5-fold in their respective controls (Fig. 3A, B). The largest increase from day 0 to day 30 (12.8-fold for single SRE addition and 14.9-fold for repeated SRE additions) in phosphatase activities occurred in barren 25R soil enriched with SRE compared to its control (1.5-fold) (Fig. 3C). There were no significant differences in percent moisture content in any of the soil types among the treatments and controls (Fig. S1).

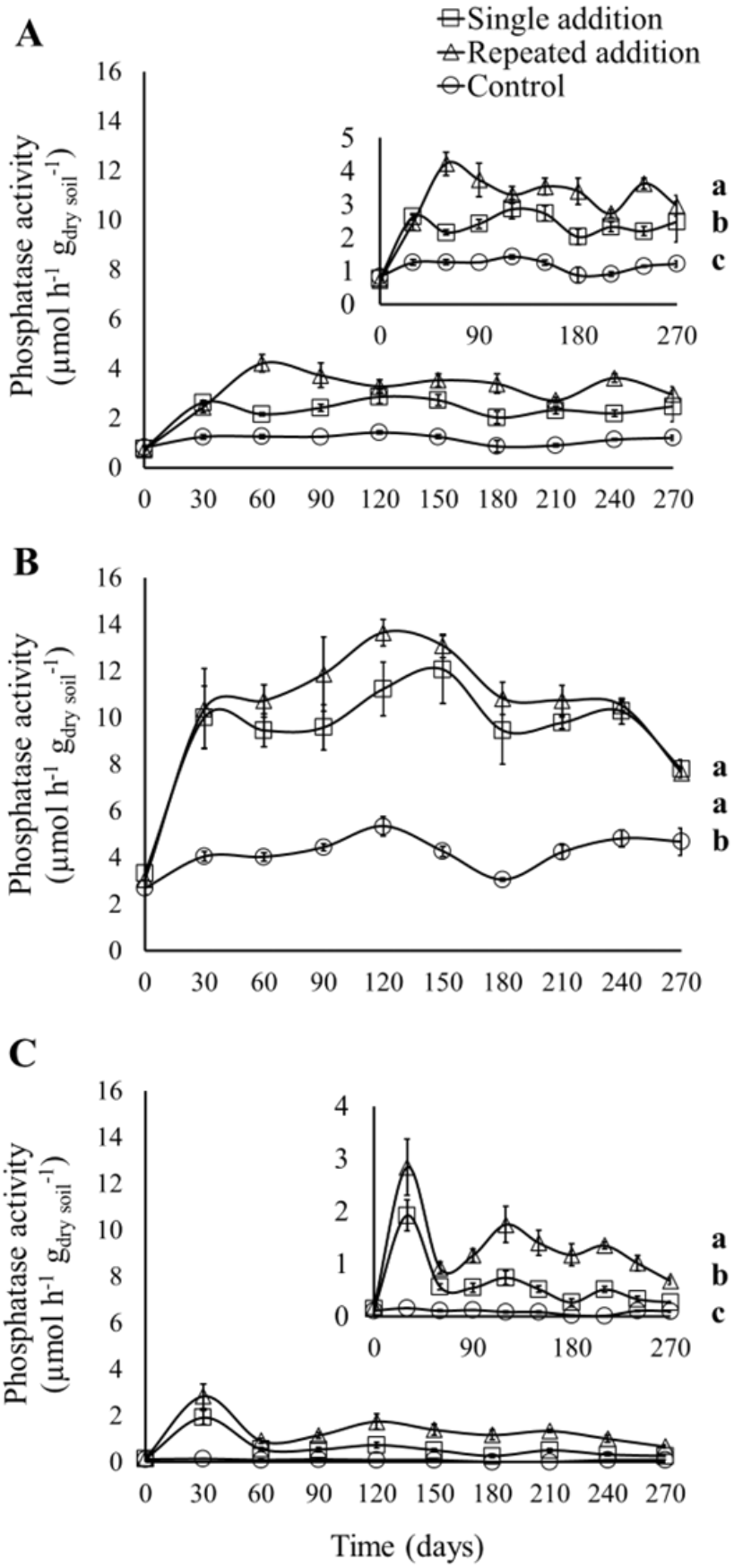

A
Single addition
Repeated addition
Control
Phosphatase activity (µmol h-1 gdry soil-1)
a
b
c
B
Phosphatase activity (µmol h-1 gdry soil-1)
a
a
b
C
Phosphatase activity (µmol h-1 gdry soil-1)
a
b
c
Time (days)

Figure 3. Extracellular phosphatase activity (μmol h^{-1} $g_{dry\ soil}^{-1}$) increased significantly in soils enriched with SRE over 270 days. Data are shown for a single SRE addition (squares), repeated SRE additions (triangles), and sterile tap water only (circles). Data are shown for soils from vegetated, uncontaminated HMF (A), vegetated, contaminated 25F (B), and barren, contaminated 25R (C). The insets in figures A and C highlight the differences among the treatments and the control. Lowercase letters indicate statistically significant differences (P < 0.0001) among treatments within a soil type for phosphatase activity. Symbols represent the mean soil phosphatase activity, and the standard errors (SE) of the mean are shown (n = 4, ± 1 SE).

Our results indicate that a single SRE addition to barren 25R soil produced significantly higher phosphatase activities (0.6 ± 0.2 μmol h^{-1} $g_{dry\ soil}^{-1}$ than the control (0.1 ± 0.01 μmol h^{-1} $g_{dry\ soil}^{-1}$) when averaged over time (P < 0.0001, Tukey HSD) (Fig. 3C, inset). This indicates that a single SRE treatment was adequate to restore microbially-mediated phosphatase activity in barren 25R soil. The contaminant-exposed microbes in 25R soil responded favorably to the readily available simple metabolites (sugars, amino acids, organic acids) received from a single SRE enrichment, as was shown by increased phosphatase activities observed for treated soils. Similar results were previously observed after repeated SRE additions to poorly functioning soil (Vaidya et al., 2022).

This is the first time we have implemented a single SRE addition to LSP soils. We observed even after 210 days from the addition of the single SRE dose, phosphatase activity of the treated 25R soil (0.5 ± 0.06 μmol h^{-1} $g_{dry\ soil}^{-1}$) was significantly higher compared to the untreated 25R control (0.01 ± 0.005 μmol h^{-1} $g_{dry\ soil}^{-1}$) (P < 0.0001, Tukey HSD) (Figure S2). Our data indicate that a single SRE intervention on day zero left a legacy of increased P cycling in the barren 25R soil that lasted for at least 210 days after the single dose was added. Wang et al. (2022) demonstrated that adding sugar (sucrose, fructose, glucose) solutions to uncontaminated agricultural soil increased alkaline phosphatase activity similar to our results that have shown higher acidic phosphatase activity.

3.3 Effect of treatments on plant shoot height and biomass

We examined whether SRE enrichment increased plant productivity in the treated soils. We compared the shoot heights of winter ryegrass in SRE enriched vegetated HMF and 25F, and barren 25R soils and their controls, which only received sterile tap water. We sowed 20 seeds and culled them to 15 after two weeks of plant growth, to maintain overall capacity in SRE treated and untreated soils. Shoot heights of plants in SRE enriched HMF and 25F soils did not vary among the three treatments (single SRE addition, repeated SRE additions, and control) (Fig. 4A, B). Interestingly, the shoot heights of plants in SRE enriched barren 25R soil were significantly higher than the shoot heights of plants in the control pots (single SRE addition: 15.5 ± 0.3 cm, repeated SRE additions: 14.3 ± 0.6 cm, control = 9.3 ± 0.9 cm) ($P < 0.0001$, Tukey HSD) (Fig. 4C). These results indicate that SRE addition had the biggest effect on plant heights in barren 25R soil. There were no significant differences in shoot height between the single SRE treated soils compared to soils that received repeated SRE additions. Soils from contaminated sites often lack nutrients and/or microbial abundance and diversity, factors that can reduce germination and plant biomass (Gerhardt et al., 2009; Smith et al., 2006; Xie et al., 2012). The results here show that a single SRE treatment can reverse the effects of soil contamination on shoot height.

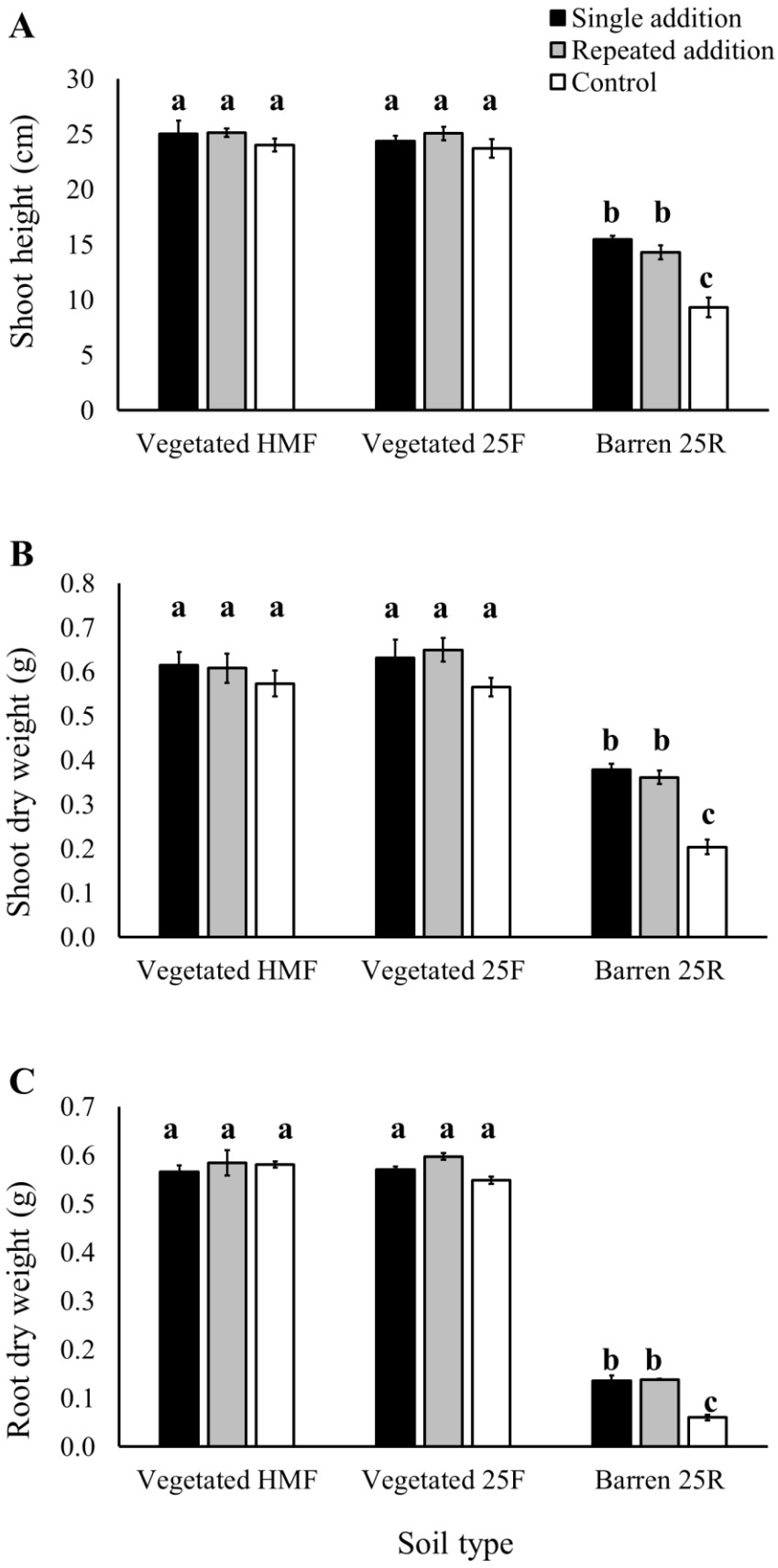

A
Single addition
Repeated addition
Control
Shoot height (cm)
30
25
20
15
10
5
0
a a a a a a
b b
c
Vegetated HMF
Vegetated 25F
Barren 25R
B
Shoot dry weight (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a a a a a a
b b
c
Vegetated HMF
Vegetated 25F
Barren 25R
C
Root dry weight (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a a a a a a
b b
c
Vegetated HMF
Vegetated 25F
Barren 25R
Soil type

Figure 4. The mean shoot heights (cm) of grass blades in each pot are shown for all treatments and soil types (A). Total plant biomass ($g_{dry\ weight}$) distributed as mean shoot mass (B) and root mass (C) are shown for treated and untreated control soils. Winter rye seeds (20) were added to all pots on day 282, and plants were harvested on day 320 of the experiment. The shoot mass of plants grown in enriched barren 25R soils was significantly higher compared to 25R control soil. Data are shown for treatments single SRE addition (black bars) and repeated SRE additions (gray bars), and the control (white bars), which received sterile tap water only. Lowercase letters indicate statistically significant differences ($P < 0.0001$) among treatments within a soil type for the shoot height (A) and plant biomass (B). Bars represent the mean shoot height, shoot mass, and root mass of four replicates and the standard error (SE) of the mean (n = 4, ± 1 SE).

Next, we examined the biomass of the 15 plants in each pot. We measured dry shoot and root mass and compared these masses between the SRE enriched and control soils. We observed that the shoot and root masses of the winter ryegrass plants in HMF and 25F soils did not vary among the three treatments (single SRE addition, repeated SRE addition, and control). In contrast, shoot and root masses of the plants in SRE enriched 25R soils (single SRE addition: shoot mass 0.4 ± 0.01 g, root mass 0.2 ± 0.1 g, repeated SRE additions: shoot mass 0.4 ± 0.02 g, root mass 0.1 ± 0.002 g) were statistically significantly greater than the masses of the plants from the control pots (control: shoot mass = 0.2 ± 0.02 g, root mass = 0.06 ± 0.006 g) ($P < 0.0001$, Tukey HSD) (Fig. 4C). Notably, in the barren 25R soil, a single SRE addition resulted in significantly increased plant biomasses. This suggests that it might be possible to implement a single SRE addition to a barren and poorly functioning soil to increase plant productivity in a brownfield or industrial barren.

4. Conclusions

Enriching a barren, metal contaminated, inactive soil from a post-industrial site with SRE once increased microbial function and plant biomass. Our results showed that the SRE enrichment to barren soil 25R enhanced soil respiration. Moreover, microbially-mediated phosphatase activity persisted for over 270 days and increased plant life even after ceasing SRE addition. The biomass of winter rye plants was greater and shoot heights were longer in SRE enriched 25R soils than in the untreated control. In barren 25R soil, SRE treatment was beneficial regardless of the treatment type, whether single or repeated additions. Further research into determining the effect of SRE on soil microbial abundance at selected time points of the experiment would provide deeper insights into the impact of each treatment type. Another aspect to explore would be replacing the SRE compounds with waste materials with similar chemical compositions to make it cost-effective. A single SRE intervention restored microbially-mediated phosphatase activity in a contaminated, barren, inactive soil. The strategy to stimulate native microbial communities with a single intervention followed by phytoremediation may be a promising approach to remediate extreme environments.

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Appendix: Supplementary material

Table S1. Bulk soil characteristics (µg/$g_{dry\ soil}$) in the soils from LSP sites 25F and 25R and reference site HMF. Soil cleanup criteria (µg/g) in non-residential direct contact soil as per the New Jersey Department of Environmental Protection guidelines (NJDEP, 2020).

Soil properties	Vegetated HMF	Vegetated 25F	Barren 25R[a]
Vanadium (µg/$g_{dry\ soil}$)	38.3 ± 0.5[b]	114 ± 13[b]	106 ± 17
Chromium (µg/$g_{dry\ soil}$)	26.7 ± 0.5[b]	120 ± 3[b]	216 ± 32
Copper (µg/$g_{dry\ soil}$)	20.4 ± 2.5[b]	2,714 ± 470[b]	7,460 ± 1,659
Zinc (µg/$g_{dry\ soil}$)	42.5 ± 1.7[b]	8,451 ± 1971[b]	20,736 ± 4,690
Arsenic (µg/$g_{dry\ soil}$)	4.8 ± 0.1[b]	850 ± 265[b]	1325 ± 269
Lead (µg/$g_{dry\ soil}$)	30.0 ± 0.4[b]	8,824 ± 1817[b]	21,965 ± 4486
pH	4.85[e]	5.06	4.95
Moisture (% $_{w/w\ dry\ soil}$)	25.5 ± 0.2	47.0 ± 0.3[c]	5.9 ± 0.1
Organic matter (%)	7.4 ± 0.6	15 ± 0.3[c]	13 ± 0.4
Water holding capacity (%)	58 ± 1.1	49 ± 2.8[c]	29 ± 0.8
Phosphatase activity (µmol h^{-1} $g_{dry\ soil}^{-1}$)	0.6 ± 0.04	2.1 ± 0.1[d]	0.11 ± 0.01

[a] Soil properties of site barren 25R compared with data reported by Vaidya et al. (2020).

[b] Heavy metal concentrations (µg/$g_{dry\ soil}$) in the soils from the study sites with standard error shown (n = 3). (Singh et al., 2019)

[c] Soil percent moisture, organic matter content and water holding capacity in the soils from the study sites with standard error shown (n = 4).

[d] Phosphatase activity in the soils from study sites with standard error shown (n = 4).

[e] Soil pH of reference site Hutcheson Memorial Forest (HMF), New Jersey, USA, compared with data reported by Hagmann et al. (2015).

Table S2. Simulated root exudate (SRE) solution composition adapted from the work reported by Steinauer et al. (2016). The amounts of exudate compounds added to each pot in a single SRE addition are shown in the third column. The amounts of exudate compounds added to each pot over 14 repeated SRE additions are shown in the fourth column.

SRE composition	Exudate compounds	Amount (mmol) added to each pot on day 0 in a single addition	Amount (mmol) added to each pot after 14 repeated additions
Sugars	Glucose, Fructose, Sucrose, Maltose	3.0 mmol per sugar	3.5 mmol per sugar
Organic acids	Acetic acid, Citric acid, Lactic acid, Malic acid, Succinic acid	1.2 mmol per organic acid	1.4 mmol per organic acid
Amino acids	Alanine, Arginine, Glycine, Histidine, Leucine, Asparagine, Glutamic acid	0.43 mmol per amino acid	0.44 mmol per amino acid

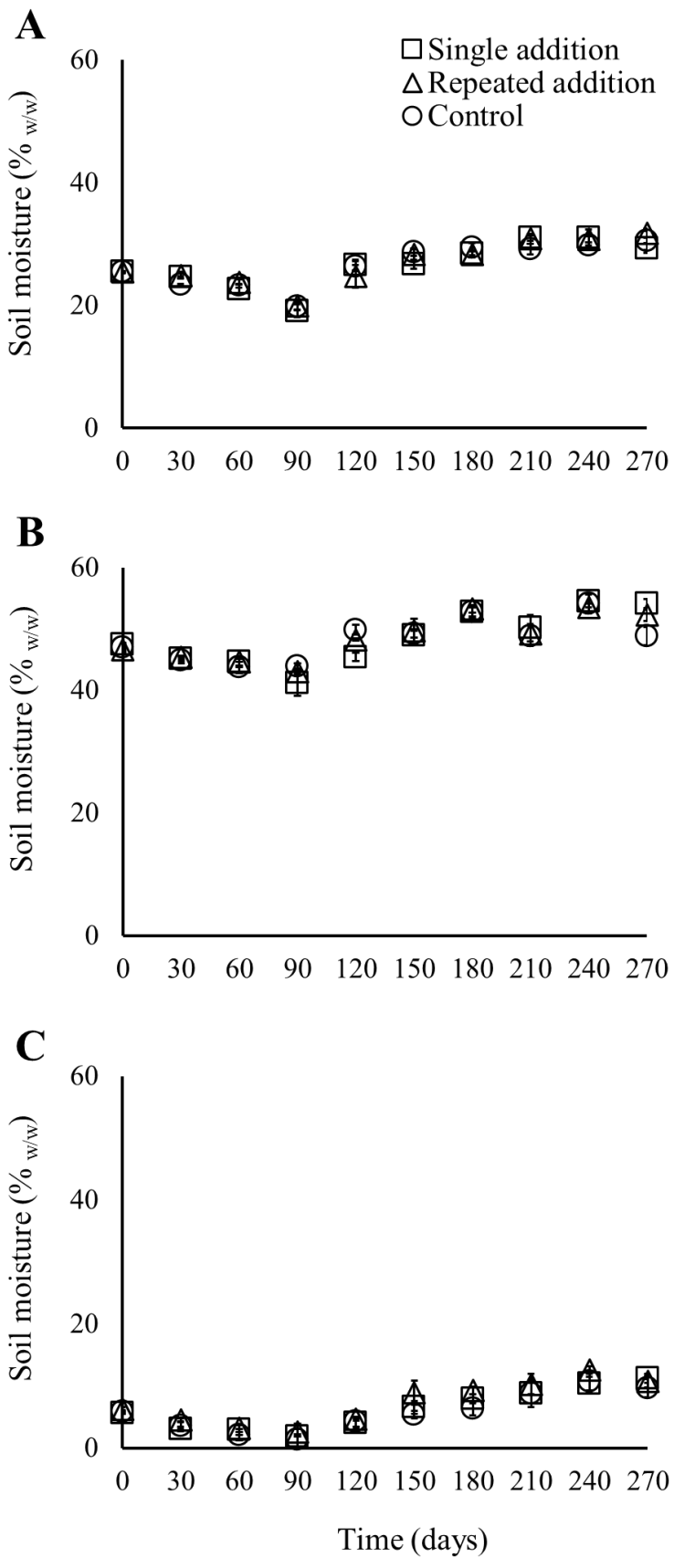
A
Single addition
Repeated addition
Control
Soil moisture (% w/w)
60
40
20
0
0 30 60 90 120 150 180 210 240 270
B
Soil moisture (% w/w)
60
40
20
0
0 30 60 90 120 150 180 210 240 270
C
Soil moisture (% w/w)
60
40
20
0
0 30 60 90 120 150 180 210 240 270
Time (days)

Figure S1. Soil percent moisture (% $_{w/w(dry\ soil)}$) did not vary among treatments over 270 days. Data are shown for single SRE addition (squares), repeated SRE additions (triangles), and control, which received sterile tap water only (circles). Data represent percent moisture in soils from vegetated, uncontaminated HMF (A), vegetated, contaminated 25F (B), and barren, contaminated 25R (C). The single and repeated SRE additions did not vary among the soil moisture content means analyzed by repeated measures two-way ANOVA over 270 days ($P > 0.05$, Tukey HSD). Symbols represent the mean soil moisture of four experimental replicates and the standard error (SE) of the mean (n = 4, ± 1 SE).

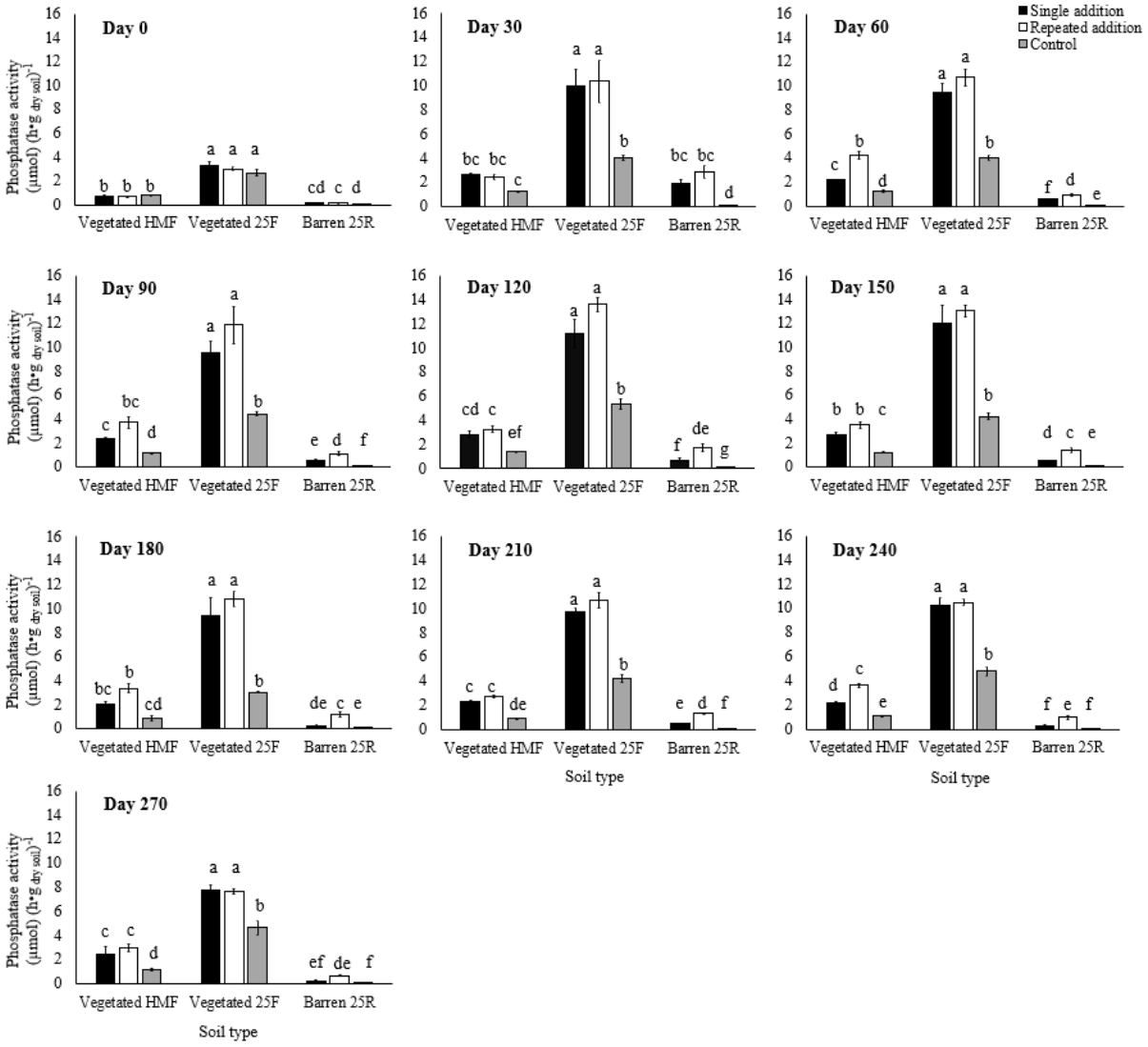

Figure S2. Extracellular phosphatase activity (µmol h^{-1} $g_{dry\ soil}^{-1}$) increased significantly in soils enriched with SRE solution at each time point over 270 days. Data are shown for a single SRE addition (black bars), repeated SRE additions (white bars), and sterile tap water only (gray bars). The single and repeated SRE additions varied among the phosphatase activity means analyzed by two-way ANOVA at each time point ($P < 0.05$, Tukey HSD). Lowercase letters indicate statistically significant differences ($P < 0.05$, Tukey HSD) among treatments within a soil type for phosphatase activity. Bars represent the mean soil phosphatase activity, and the standard errors (SE) of the mean are shown (n = 4, ± 1 SE).

CHAPTER 5

Conclusions

Post-industrial landscapes are often located near residences. Many post-industrial sites are abandoned and remain barren because the soils there contain persistent organic and inorganic pollutants that impact soil organisms (Kozlov & Zvereva, 2007; Sun et al., 2010). For example, soils containing high concentrations of heavy metals such as Pb, As, and Cu have been reported as harmful to human and environmental health (Alengebawy et al., 2021; Li et al., 2018; Maddela et al., 2022). In vegetated soils, plant-soil-micro- and macro-organisms, through their interactions cycle nutrients across trophic levels (Soliman et al., 2022). Contrarily, in barren, contaminated, inactive soils, high contaminant concentrations and limited soil nutrients suppress soil microbial abundance, inhibiting vital feedback loops and ecosystem services (Gianfreda et al., 2006; Krumins et al., 2015). Industrial barrens are undesirable because they do not support agriculture, provide ecosystem services to wildlife, or capture toxic compounds to protect groundwater (Kozlov & Zvereva, 2007; Sun et al., 2010).

Our study site at Liberty State Park (LSP) has high contamination levels and forest cover, a rare combination (Evans et al., 2015; Gallagher et al., 2008). Though natural attenuation has improved most parts of the study site, some sections remain barren and display enzymatically inactive soils (Hagmann et al., 2019; Singh et al., 2019; Vaidya et al., 2020). The research presented through this dissertation evaluated a remediation pathway to restore soil functioning of a post-industrial, barren, rail yard, left undisturbed for over five decades. The main focus of my research was finding out whether plants (phytoremediation) or native microbial stimulation (biostimulation) might increase soil function. In this study, I added natural and simulated root

exudate compounds to a barren, contaminated, inactive soil from a post-industrial site and studied its effects on soil functioning.

The results from the first project (chapter 2) indicated that switchgrass and winter ryegrass species native to the study site germinated in the barren, contaminated, inactive 25R soil in the climate-controlled growth chamber. Phosphatase activity in the barren 25R soil significantly increased when plants were present. The results also indicated that mixing two contaminated soils, a high functioning with a low functioning one was not as effective as growing plants. An important point to note here is that soils collected from the site were from the top 10 cm, which corresponded with the biologically active zone and maximum root concentration (Gallagher et al., 2008).

From these outcomes, we can derive that mixing the abiotic and biotic components of the soil may not yield desired soil function. The toxic abiotic components can adversely impact the biotic components from both active (site 146) and inactive (site 25R) soils, which may reduce microbial functioning. Another aspect to consider is microbial competition for soil nutrients among the active and inactive soils can impact soil function. Apparently, plants have proven to support soil organisms and aid microbial functioning.

In the second phase of this project, switchgrass and winter ryegrass seeds were added to the soil mix experiment. Soil phosphatase activity in barren 25R soil increased substantially in the presence of plants over the course of 65 days. Possibly, the plant roots might have supplied nutrients (organic acids, sugars, amino acids) to stimulate the contaminant-stressed microbes in the barren 25R soil. These results guided the design of my next project where I thought of preparing a solution of root exudate constituents in the laboratory to mimic what plant roots naturally exude in soils.

A few limitations of these lab-scale experiments were: 1) laboratory-scale experimental results could not be the true representatives of those observed in field conditions due to seasonal and weather disparities, 2) the time-scale of laboratory experiments (within a year) was smaller than those carried out at the field-scale (decades), and 3) the limited amount of soil that could be transported from the field site to the laboratory for setting the experimental pots.

In the second project (chapter 3), I periodically added a laboratory-prepared simulated root exudates solution (SRE) to the barren 25R soil and found that the exudate compounds significantly enhanced phosphatase activity and plant biomass in the enriched soil. The results showed that adding the exudates solution increased phosphatase activity substantially in only 40 days. The plants grown in pots enriched with SRE showed greater shoot mass than those grown in unenriched soils. During 205 days of this experiment, phosphatase activity remained significantly higher in soils enriched with exudates solution. After 200 days, the plants in unenriched and enriched soils began senescing, which was why this experiment was terminated at day 205. Another issue that limits phytoremediation success is that the plant species involved are selected based on fast growth and are site-specific. Besides that, the competition for available nutrients between plants and soil microbes modulates their growth (Krumins, 2014).

In the third project (chapter 4), I studied whether a single SRE addition was adequate to stimulate native microbial function and restore phosphorus (P) cycling in barren soil, and assessed the practical application of this strategy. In soils treated with a single addition of SRE, the carbon dioxide respiration rates and phosphatase response were significantly greater than in the control soils treated with sterile tap water. Plant biomass and shoot heights of plants (winter rye grass) in the SRE enriched soils were significantly larger than the plants grown in the control soils. To summarize, a single addition of SRE left a legacy effect that allowed plant biomass

accumulation and restored P cycling in the barren 25R soil. Realistically, a single instead of repeated interventions may save time and manual effort making its implementation more practical. However, having established that a single addition of SRE was effective in a laboratory-scale experiment, this strategy needs further investigation for a field-scale implementation.

As seen in the results from project 2 (chapter 3), soil function in enriched soil was maintained for 205 days. The results from the third project (chapter 4) showed that soils enriched with single and repeated additions of exudate solution maintained significantly higher phosphatase activities than untreated soils, lasting over 270 days. An interesting point to note was that after day 240, soil function declined in both contaminated soils. The fall in soil enzymatic function may indicate a reduction in microbial activity due to nutrient limitations. That means a follow-up second dose of "single addition" might be necessary while deploying this remediation strategy. Alternatively, as shown in the results from project 3, by planting seeds on day 282, a natural input of root exudates was provided eight months after ceasing the SRE addition. One of the limitations of planting seeds was that plants began senescing, hence were harvested at day 320. The limitation of plant growth indicated that a second dose of SRE would be beneficial and sustainable to maintain soil function. As seen from the 30-day plant growth phase followed by senescence, SRE injection was long lasting and effective.

Using the results from the laboratory-scale experiments conducted as a part of this dissertation and reflecting on the outcomes from each project, in my opinion a single remediation strategy such as phytoremediation may not be adequately effective in increasing soil function in contaminated, inactive, barren post-industrial soil. For instance, I found that mixing barren, inactive 25R soil with active 146 soil was not as effective as adding plants, which supported

nutrient cycling and microbial functioning. Therefore, supporting soil microorganisms with nutrients that aid their metabolic functioning was beneficial for restoring soil enzymatic activities. At the same time, adding any abiotic component to contaminated soils needs careful assessment to ensure soil's physico-chemical characteristics remain minimally altered. Moreover, for robust soil functioning, often a delicate balance between abiotic and biotic factors is necessary. Hence, to plan a remediation pathway for a barren, inactive soils, combining two strategies, such as biostimulation of native microorganisms followed by phytoremediation or another dose of nutrient stimulation, might enhance the remediation efforts. Based on the above findings and to reach more sound conclusions, I propose the following future directions:

1) Application of SRE may be repeated at other barren, contaminated sites.
2) Regulating the diversity of the compounds in the SRE composition to make it cost-effective (Strickland et al., 2015; G. Wang et al., 2022; J. Wang et al., 2021; L. Wang et al., 2021).
3) Substituting the exudate compounds with low-cost waste materials that have similar chemical composition (Beesley et al., 2011; Ghosh & Maiti, 2021; Hartley et al., 2009; Kuppusamy et al., 2016).
4) Analyzing the degradation of polycyclic aromatic hydrocarbons and sequestration of heavy metals in enriched soils.
5) Examining the microbial community structure before and after SRE treatment.
6) Designing a carefully planned field experiment to understand the practical issues while implementing this methodology.

Some key points while designing a field-experiment are:

1) the duration of SRE interventions

2) the time of the year when these interventions can be successfully applied to the field site, for example, the spring season
3) fluctuations in moisture levels and weather conditions
4) site-specific, fast-growing plant species
5) cost and time efficient implementation and use of resources

A preliminary field experiment could provide valuable information on these aspects. A tentative design could be as follows: four plots of 1 m x 1 m size, four treatments - plants, single addition of exudate solution, repeated addition of exudate solution, control (no plants or exudate solution), begin springtime, and monitor until late fall. Some variables that could be measured include soil respiration rate, phosphatase activity, microbial cell count, and plant biomass.

In an experiment conducted by a colleague, Eshariah Dyson, contamination levels and phosphatase activity along soil depth (top 10 cm) were analyzed. She found that the barren, inactive 25R soil from the LSP site has a one-inch surface layer with highest concentrations of heavy metals (Pb, Cu, As) (unpublished data) compared to the deeper layers. In a follow up experiment, she found that if the top 10 cm layer remained intact, the gemination rate was low, while in mixed soil the germination rate improved (unpublished data). Therefore, the barren, inactive region (25R) of our study site might be restored by removing the surface layer, followed by tilling, organic matter addition, and planting.

Recent advances in soil remediation recommend combination of physical, chemical and biological technologies (Awasthi et al., 2022; Song et al., 2022; X. Wang et al., 2022; Zheng et al., 2022). Researchers also raise caution concerning the secondary pollution resulting from using one strategy or an additive to the contaminated soil (Balkrishna et al., 2022; Kafle et al., 2022; Oladoye et al., 2022; Rahman, 2022).

Through the experiments presented in this dissertation, I investigated the effects of simulated root exudates on soil quality indicators (phosphatase activity, soil respiration rate, glomalin content, plant biomass) of a post-industrial, barren, contaminated, inactive soil. The results indicated beneficial effects of the simulated exudates on soil properties, microbial function, and plant growth. These outcomes are important and further contribute to the understanding of beneficial effects of simulated root exudates in restoring the soil functioning of barren, contaminated, inactive soil systems.

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