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Barren to green in a single application: Revitalizing brownfield soil with simulated root exudates

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ABSTRACT

Barren, metal-contaminated soils lack plants and root exudate inputs, exhibit low microbial abundance and functioning, and often require soil revitalization to revegetate. While the effects of simulated root exudates (SRE) have been investigated in uncontaminated, vegetated soils, their potential for remediating post-industrial barren, contaminated soils has not been examined or leveraged. We asked whether priming brownfield soils with a laboratory-prepared SRE solution stimulates native soil microbial metabolism and functioning and how long the effects last. Moreover, we compared a cost-effective single SRE addition to repeated SRE additions. We collected soils from a metal-contaminated, abandoned industrial rail yard (barren and vegetated sites) and a vegetated agricultural reference site, established microcosms, and treated the soils with either a single or repeated SRE addition/s. By day 30, SRE-enriched barren, brownfield soils showed significantly higher soil respiration rates than the untreated control soils. Phosphatase activities were significantly higher even 210 days after a single SRE addition. Plants were introduced 282 days after the single SRE addition. The average shoot height (16 ± 0.3 cm) and total plant biomass (0.5 ± 0.02 g) of plants grown in single addition SRE enriched barren soil were significantly higher than the controls (9 ± 0.9 cm and 0.3 ± 0.02 g, respectively). The increased soil microbial functioning and productivity indicate that a single SRE application holds promise as a field-ready technology to revitalize barren, poorly functioning brownfield soils. SRE application may also be a pragmatic and innovative approach to enable successful phytoremediation and re-greening of industrial barrens.

1. Introduction

Brownfield soils with persistent contaminants are ubiquitous and there is an urgent need to develop innovative and pragmatic technologies to remediate and manage these sites. These soils often display oxidative stress, poor plant productivity, limited nutrient bioavailability, and low indigenous microbial abundance (Afegbua, 2014; Bardgett et al., 2014; Patra et al., 2020; Reeder et al., 2006). When they become barren, brownfield soils no longer optimally maintain soil structure, immobilize pollutants, or provide ecosystem

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services (Gallagher et al., 2018; Salisbury et al., 2020). Barren soils also lack root exudate inputs that are necessary to sustain microbial communities (Grobelak et al., 2017; Ibraheem, 2007; Sun et al., 2010). Conversely, in vegetated soils, roots can anchor contaminants in place and shoots can sequester contaminants from the soil. Moreover, plant roots secrete a variety of chemical compounds, root exudates, into the soil (Haichar et al., 2014; Kunc and Macura, 1966; Shukla et al., 2011; Wang and Lambers, 2019). These exudates, comprising simple metabolites (sugars, organic acids, amino acids), nourish soil microbes (Hale et al., 1978; Sasse et al., 2018), promote microbial functioning, facilitate nutrient cycling, and improve soil quality (Han et al., 2022; Kuzyakov, 2010; Liu et al., 2022; Wang et al., 2022; Zhu et al., 2014).

The need to develop simple, sustainable, soil remediation technologies that target post-industrial sites is pressing because there are an estimated 450,000 brownfield sites in the United States alone (United States Environmental Protection Agency, 2022). One eco-friendly restoration technique that is aesthetically pleasing and requires low maintenance is phytoremediation (Awasthi et al., 2020; Balkrishna et al., 2022; Kafle et al., 2022; Madhav et al., 2024; Montreemuk et al., 2023). Successful implementation of phytoremediation requires that soil quality is conducive to plant productivity, (Aghili and Golzary, 2023; Balkrishna et al., 2022; Kafle et al., 2022; Kumar, 2019) and approaches that revitalize barren and poorly functioning post-industrial soils are needed to support plant productivity and meet urban land management demands. Contaminated soils house microbial strains that survive in and exhibit resistance against the specific contaminants in that soil, (Ahmed et al., 2022; Ceci et al., 2019; Emenike et al., 2018; Liao et al., 2021) and root exudates can support these contaminant-resistant microbial communities with benefits for plants (Badri et al., 2009; Dundek et al., 2011; Wang et al., 2020).

Even if there are no plans for phytoremediation, regreening a barren, contaminated soil has significant value. For example, planted soil ties down contaminants and prevents their migration to groundwater. Moreover, urban green spaces have been shown to provide mental health benefits for humans and increase urban habitats for wildlife (Aghili and Golzary, 2023; Ayala-Azcárraga et al., 2019; Bertram and Rehdanz, 2015; Besha and Esmaeilian, 2020; Plunz et al., 2019). Moreover, before adding contaminant-sequestering plants at a barren site, the nutrient cycling capability and microbial functioning of the barren soil must be addressed. After all, to successfully implement phytoremediation, the soil must be able to support plant growth.

Researchers have investigated priming agricultural and contaminant-spiked soils with natural and simulated root exudates (SRE) (Bali et al., 2020; Baudoin et al., 2003; Dakora and Phillips, 2002; Vonk et al., 2022; Xie et al., 2012). Studies have shown that the addition of soil amendments can improve soil nutrient cycling and plant productivity (Beesley et al., 2011; Ghosh and Maiti, 2021; Hartley et al., 2009; Rinklebe and Shaheen, 2015). In contrast, the potential of SREs to restore soils from industrial barrens has not been investigated. Industrial barrens are post-industrial sites where persistent contaminants have created unvegetated, inactive soils (Kozlov and Zvereva, 2007). We hypothesized that priming barren, poorly functioning, contaminated soil with a chemical solution that mimics natural root exudates is a promising approach to revitalizing and revegetating barren brownfield soils.

The effects of priming soils with amendments can recede over time, highlighting the need to further investigate the longevity of the effects of an SRE addition to industrial barrens (Bastida et al., 2019; Bernard et al., 2022; Kuzyakov et al., 2000; Wiszniewska et al., 2016). To address the longevity of the SRE addition technology, we monitored our microcosms for an extended period of more than 320 days after SRE additions. Many post-industrial sites are difficult to access and repeated SRE additions may not be practical or feasible from an environmental management perspective. To efficiently implement SRE technology, the dosing strategy must be optimized. Previous work has not addressed optimal dosing strategies, specifically whether a single SRE addition is sufficient in reviving microbial functioning or if repeated interventions are necessary. We compared the effectiveness of a single SRE addition to a multiple SRE addition strategy.

In this microcosm experiment, we enriched metal-contaminated soils from a post-industrial site located within the Liberty State Park (LSP) and an uncontaminated, vegetated reference site, Hutcheson Memorial Forest (HMF), with a solution of SREs containing simple metabolites, in order to stimulate native soil microbes. Each soil type was subjected to three treatments: a single addition treatment where SRE was added once on the first day of the experiment (single SRE addition), a repeated addition treatment, where SRE was added 14 times in total over a period of 30 days (repeated SRE addition), and a control (sterile tap water only). We compared the effects of the three treatments on the soil respiration rate over 150 days, soil extracellular phosphatase activity (PA) over 270 days, and plant biomass at day 320. The results indicated that SRE application technology may be beneficial in restoring industrial barrens into green spaces for public recreation and in preparing barren brownfield soils for remediation through phytoremediation.

2. Materials and methods

2.1. Study site description and soil collection

We collected soil for the microcosms from two contaminated study sites located next to each other at LSP, Jersey City, NJ (40̊42’16 N, 74̊ 03’ 06 W); vegetated site 25 F and barren site 25 R This area 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 25 F) has shown high microbial function, while the barren area (LSP site 25 R) has shown low microbial function (Hagmann et al., 2015, 2019; Singh et al., 2019; Vaidya et al., 2020). Abundant vegetation has grown at site 25 F despite the absence of human intervention and the presence of high contaminant levels (Gallagher et al., 2018, 2015, 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 25 R site has opposite characteristics; little vegetation, high contaminant concentrations, and low PA (Hagmann et al., 2019; Singh et al., 2019; Vaidya et al., 2020).

The soil collected from Hutcheson Memorial Forest (HMF), Somerset County, owned by Rutgers University, NJ was used as the

reference soil. The reference site HMF was a historical farmland and was selected because it has remained undisturbed since 1969, similar to LSP. Previous research on HMF soil has shown little contamination, substantial vegetation, and healthy soil (Hagmann et al., 2015, 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 the experimental microcosm set-up on day 0.

2.2. Bulk soil characteristics

The bulk soil characteristics of experimental soils vegetated HMF, vegetated 25 F, and barren 25 R are listed in Table S1 in the supplementary information. The PA was highest in vegetated 25 F soil, high in vegetated HMF, and low in barren 25 R soil. The pH was acidic in all soils (Table S1). Moisture content was lower in barren 25 R soil than in vegetated 25 F and HMF soil (Vaidya et al., 2020) (Fig. S3). The heavy metal concentrations in 25 F and 25 R soils were higher than in HMF and above the threshold limits specified by the NJDEP guidelines (New Jersey Department of Environmental Protection, 2020). Pb, As, and Cu concentrations at the barren 25 R site were two times higher than in vegetated 25 F soil (Table S1) (Singh et al., 2019).

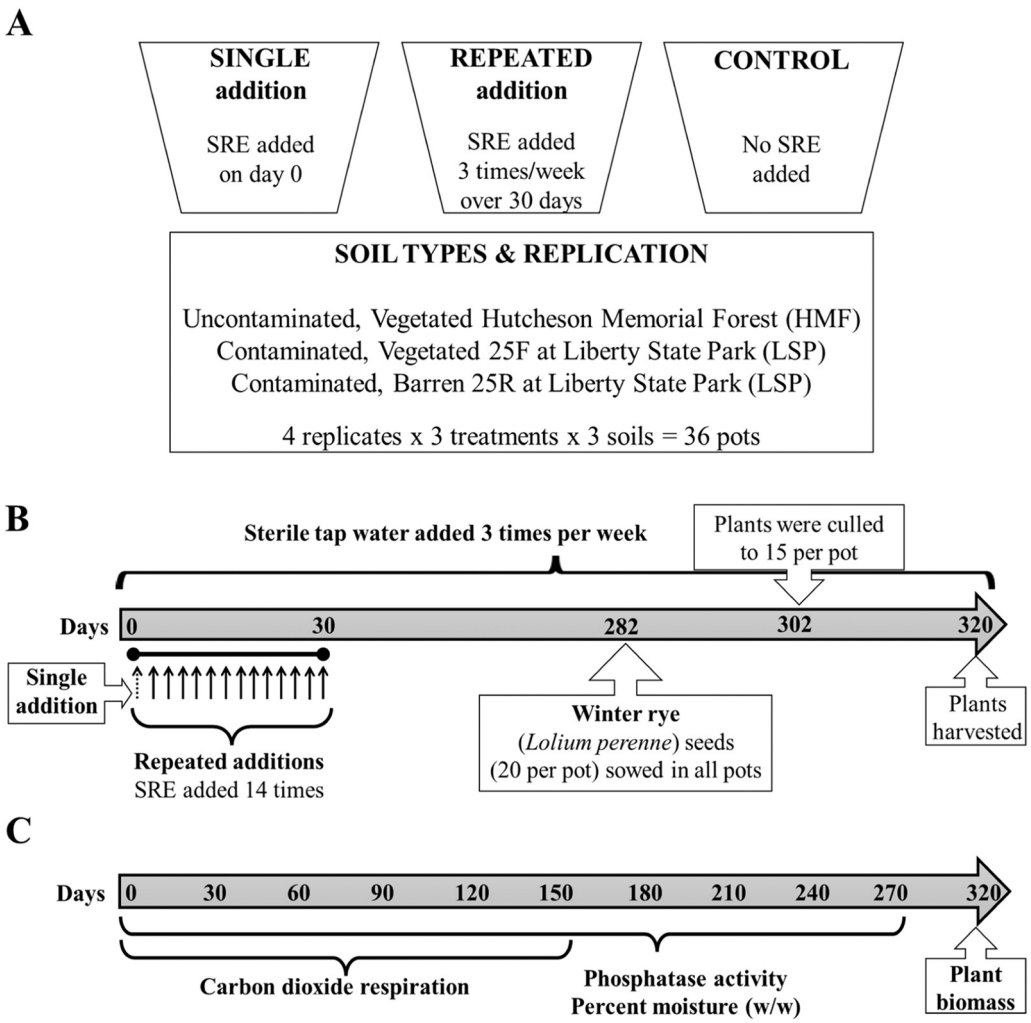

Fig. 1. Experimental design (A) describes SRE additions scheme, soil types, replicates and experimental set-up. Timeline of treatments added (B) shows the addition of simulated root exudate (SRE) solution and planting of winter rye seeds. The dotted arrow represents single SRE addition on day 0 (single addition), while the filled black arrow represents repeated SRE additions in which SRE was added three times per week for 30 days (14 additions in total). Timeline of analytical measurements (C) shows the sampling time points and response variables. The response variables measured were soil CO_2 respiration rate (soil CO_2 emission), PA phosphatase activity, percent moisture, and plant biomass. Sterile tap water was added by soil weight three times per week over 320 days to each pot in a climate-controlled incubator.

2.3. Experimental design

We established a factorial design (Fig. 1) and tested two independent variables, treatment and time. We used three base soils with different properties (vegetated HMF, vegetated 25 F, and barren 25 R) as case studies. We subjected the soils to two experimental treatments (single and repeated SRE additions) and a control. We set up four replicate pots for each treatment (n=4), therefore there were 12 pots per soil type and 36 pots in total (4 replicates x 3 treatments x 3 base soils). For the single SRE addition treatment, we treated the replicate pots from each soil type 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 (14 additions). By the end of the 30 days, the single and repeated SRE addition pots received the amounts of exudates as shown in Fig. 1B. The control pots received sterile tap water only by weight three times weekly. After the 30-day exudate addition regimen, we watered all pots by soil weight three times weekly. We measured soil respiration rate daily for 90 days and then weekly until day 150 and PA and soil moisture content every 30 days for 270 days. We measured plant biomass and shoot height after harvest on day 320.

The mass of soil added to each pot differed by soil type, as it was based on the volume occupied by the soil in the pot (6 in height x 4 in bottom diameter). Pots with soil from HMF consisted of 500 g of soil. For LSP soils, pots with soil from site 25 F contained 341 g, and site 25 R occupied 495 g. 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 simple metabolites such as sugars, organic acids, and amino acids (Fig. 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

2.5.1. Soil respiration rate

Soil CO_2 respiration rate (soil CO_2 emission) 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 vol 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.

2.5.2. Phosphatase activity

The extracellular 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. PA was expressed as micromoles of reaction product per hour per gram of dry soil.

2.5.3. 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 soil).

2.5.4. Plant biomass and shoot height

Winter rye (*Lolium perenne*) seeds (20 per pot), which are native to the site (Gallagher et al., 2011; 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. We culled these to 15 after two weeks of plant growth, to maintain overall capacity in SRE treated and untreated soils. 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 investigated the effects of single and repeated SRE additions on soils from three sites: HMF, 25 F, and 25 R. Each soil type was subjected to three treatments: 1) a single SRE addition, 2) 14 repeated SRE additions; and 3) sterile tap water only control. To identify any statistically significant differences among treatments and time points, we used a two-way repeated measures analysis of

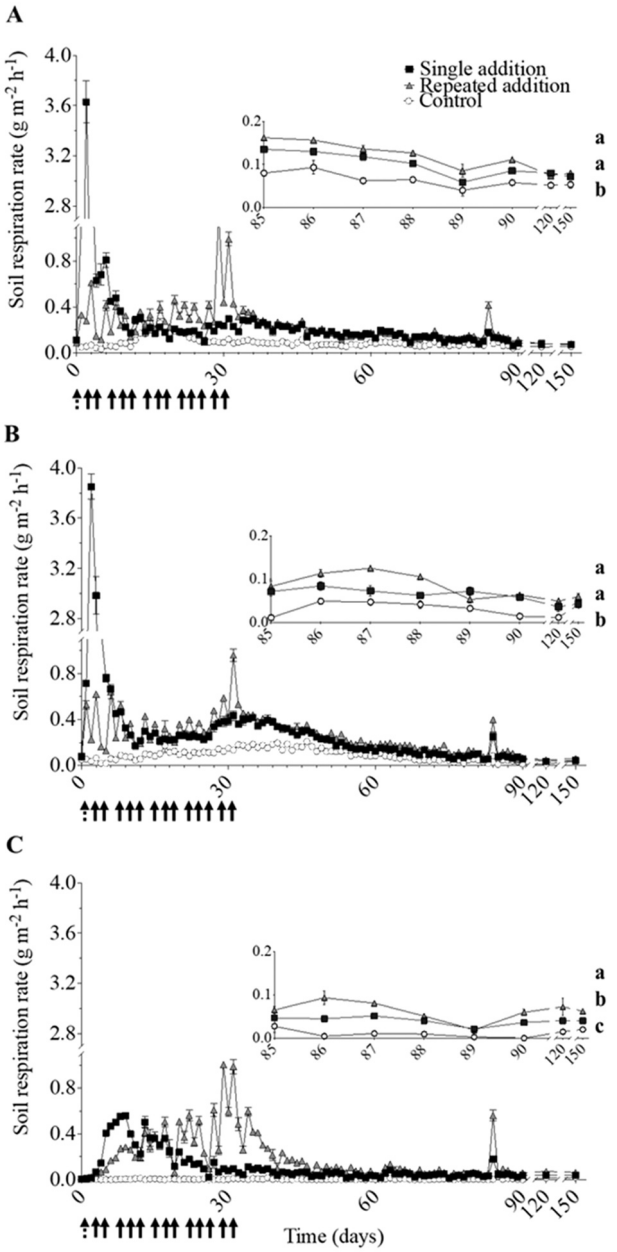

Fig. 2. Soil respiration rates measured as soil CO_2 emission (g m^{-2} h^{-1}) over 150 days for three treatments: a single SRE addition (black squares), repeated SRE additions (gray triangles), and sterile tap water only (control; white circles). Data are shown for soils collected from three sites: vegetated, uncontaminated HMF (A), vegetated, contaminated 25 F (B), and barren, contaminated 25 R (C). 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 additions in total). The insets show the data from day 85–150 to emphasize differences in soil respiration among treatments. Within each inset, lower-case letters indicate statistically significant differences among the treatment means (day 0–150) at $p < 0.05$. Error bars show the standard error (SE) of the mean (n = 4, ± 1 SE).

variance (ANOVA). The response variables were soil respiration rate (104 measurements over 150 days), PA (10 measurements over 270 days), and moisture (10 measurements over 270 days). A square root transformation was done on the soil respiration rate and PA values to make them more normally distributed. The independent variables were treatment (single, repeated, and control) and time. Treatment effects were analyzed separately for each soil type and each response variable. We first examined the main effects of treatment and time and the interaction effect between them. Then, we analyzed individual contrasts using Tukey's Honestly Significant Differences (HSD) post hoc test at $p < 0.05$.

We analyzed the differences in plant shoot height and biomass means separately for the three different soil types using a one-way

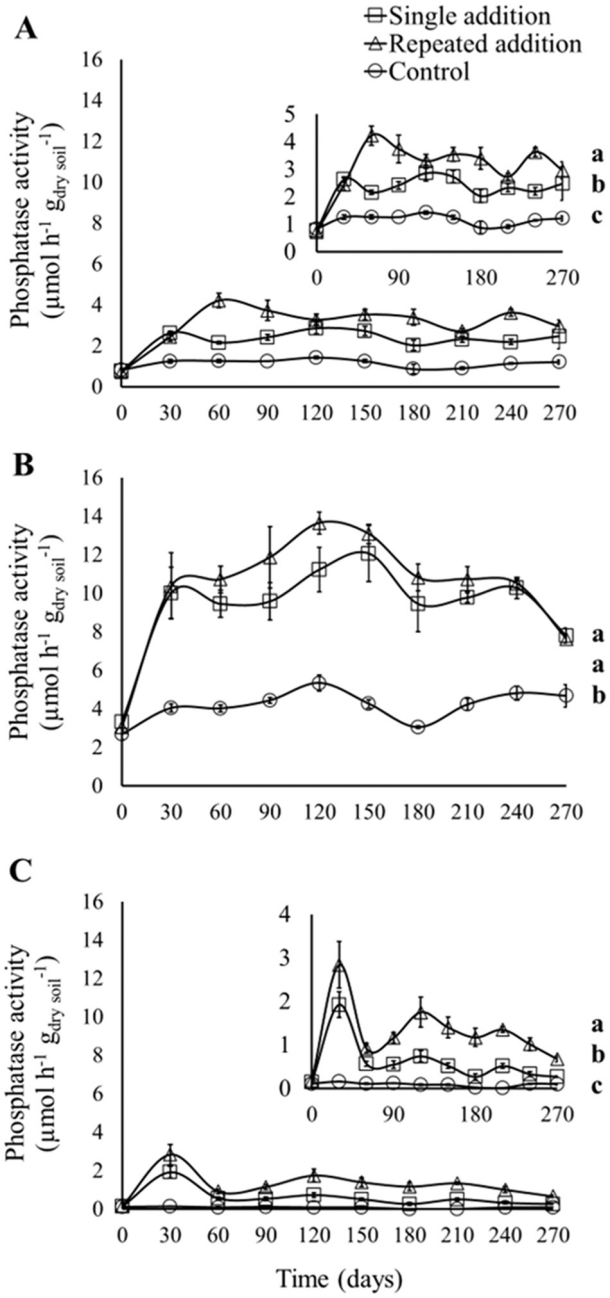

Fig. 3. Extracellular phosphatase activities (µmol h^{-1} $g^{-1}_{dry\ soil}$) are shown over 270 days for a single SRE addition (squares), repeated SRE additions (triangles), and sterile tap water only (circles). Data are shown for soils from vegetated, uncontaminated HMF (A), vegetated, contaminated 25 F (B), and barren, contaminated 25 R (C). The insets in Figs. 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 obtained from repeated measures two-way ANOVA from day 0 to day 270 (ten time points). Standard errors (SE) of the mean are shown ($n = 4$, $\pm$ 1 SE).

ANOVA, where treatment was the independent factor. This analysis was followed by Tukey's HSD post hoc test to identify significant mean differences among the treatments. All statistical analyses were carried out in RStudio using R version 4.1.1 (RCoreTeam, 2021).

3. Results and discussion

3.1. Effect of treatments on soil respiration rate

Before treatments, the vegetated soils respired CO_2 more rapidly (HMF: 0.1 ± 0.02 and 25 F: 0.08 ± 0.01 g m^{-2} h^{-1}) than the barren soil (0.003 ± 0.002 g m^{-2} h^{-1}; Fig. 2). The single SRE addition to vegetated soils resulted in an accelerated soil respiration rate. For example, from day 0 to day 2, the soil respiration rates increased 69-fold in HMF (0.1 ± 0.02–3.6 ± 0.2 g m^{-2} h^{-1}) and 48-fold in 25 F (0.08 ± 0.01–3.9 ± 0.1 g m^{-2} h^{-1}; Fig. 2A and B). The single SRE addition to barren 25 R also resulted in an acceleration but the increase was more gradual (Fig. 2C). It took nine days for the 25 R soil respiration rate to reach a maximum value, increasing 3,000-fold from 0.003 ± 0.002 on day 0–0.6 ± 0.02 g m^{-2} h^{-1} on day 9. The highest fold acceleration was much larger in barren, contaminated 25 R (3,000-fold) compared to the vegetated soils HMF (69-fold) and 25 F (48-fold) soils. We used a repeated measures two-way ANOVA separately for each soil type to determine whether there were statistically significant differences among the treatments or time points. The analysis showed that a single SRE addition was effective in yielding higher respiration rates in all three soils. In vegetated HMF soil, the single SRE addition yielded a statistically significantly higher mean respiration rate (0.24 ± 0.01 g m^{-2} h^{-1}) compared to its control (0.088 ± 0.002 g m^{-2} h^{-1}) ($p < 0.0001$, Tukey HSD). Similarly, the single SRE addition resulted in statistically significant increases ($p < 0.0001$, Tukey HSD) in the mean respiration rates from 0.086 ± 0.009–0.28 ± 0.02 g m^{-2} h^{-1} in vegetated 25 F and from 0.005 ± 0.001–0.11 ± 0.01 g m^{-2} h^{-1} in barren 25 R.

The repeated SRE additions (14 SRE additions; Table S2) to barren 25 R soil resulted in a large increase in soil respiration rate (6,000-fold; day 0 to day 30). In comparison, repeated SRE additions to HMF and 25 F resulted in only 13- and 7-fold increases in soil respiration rates (day 0–30), respectively. A repeated measures ANOVA showed that repeated SRE additions resulted in significantly higher mean soil respiration rates compared to the control in each soil type (Fig. 2, $p < 0.0001$). Together the data indicate that both single and repeated SRE additions resulted in increased soil respiration rates in all three soils but the fold increases were much larger in the barren, poorly functioning 25 R soil. Contaminated soils that are unable to support vegetation lack consistent nutrient inputs in the form of root exudates, which are required to sustain microbial communities (Badri and Vivanco, 2009; Haichar et al., 2014; Kuzyakov and Blagodatskaya, 2015). Priming the poorly functioning, contaminated 25 R soil with SREs may have invigorated contaminant-stressed and nutrient deficient microbial communities.

After ceasing SRE additions on day 0 (single SRE addition) and day 30 (repeated SRE addition) (Fig. 1), we observed decreased soil respiration rates and expected respiration rates to quickly drop to the values observed for the control. We continued to observe statistically significantly higher respiration rates in SRE enriched vegetated HMF and 25 F soils than their controls, even on day 60 (Fig. S1). Repeated measures ANOVA showed that mean respiration rates for barren, contaminated 25 R soil were 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}, $p < 0.0001$, Tukey HSD, Fig. 2C inset).

The results indicate that SRE enrichments created a legacy within the soil that persisted for at least a month (see Supplement and Fig. S1). The legacy effects of SRE additions were inferred from increased respiration rates in all three soils, especially in barren 25 R soil. SRE intervention increased microbial metabolism in barren 25 R soil; both treatment types, single and repeated SRE additions, were effective. A single SRE addition significantly increased soil respiration and that the effects lasted longer than we had expected (≥ 30 days). A single SRE addition may therefore be sufficient to increase microbial metabolism and perhaps revitalize dormant microbial communities in some barren, contaminated soils for the duration of a biologically relevant period (Blagodatskaya and Kuzyakov, 2008; Hamer and Marschner, 2005).

3.2. Effect of treatments on phosphatase activity

Before treatments, the extracellular PAs in vegetated soils (HMF: 0.77 ± 0.03; 25 F: 3.0 ± 0.03 μmol h^{-1} $g^{-1}_{dry\ soil}$) were higher than the PA in barren 25 R soil (0.15 ± 0.03 μmol h^{-1} $g^{-1}_{dry\ soil}$). These values are comparable to previously published PA values for soils collected at the same sites (Balacco et al., 2022; Hagmann et al., 2015, 2019; Singh et al., 2019; Vaidya et al., 2020, 2022). SRE additions resulted in increased PAs in all three soils. For example, the single SRE addition increased PAs by 3.5-fold, 3.0-fold, and 13-fold in HMF, 25 F, and 25 R, respectively, from day 0 to day 30 (Fig. 3). Correspondingly, with repeated SRE additions, PAs increased by 3.4-fold in both HMF and 25 F, and 15-fold in 25 R soils from day 0 to day 30 (Fig. 3). In comparison, PA increased by only 1.5-fold from day 0 to day 30 in the untreated (control) HMF, 25 F, and 25 R soils.

To determine whether the PA increases were statistically significant, we used a repeated measures two-way ANOVA. We found that both treatments resulted in significantly increased PAs compared to the controls ($p < 0.001$, Tukey HSD) (Fig. 3). When averaged over time including day 0, mean PAs for SRE treated 25 R soils were 0.6 ± 0.2 μmol h^{-1} $g^{-1}_{dry\ soil}$ (single SRE addition) and 1.3 ± 0.2 μmol h^{-1} $g^{-1}_{dry\ soil}$ (repeated SRE addition) compared to only 0.1 ± 0.01 μmol h^{-1} $g^{-1}_{dry\ soil}$ for the untreated 25 R control.

The phosphorus cycle is a vital nutrient cycle in soil ecosystems that involves the mineralization of phosphate when microbial biomass multiplies (Frossard et al., 2000; Garg and Bahl, 2008; Glæsner et al., 2019; Nannipieri et al., 2011). Phosphatases are enzymes exuded by multiplying microbes that release phosphorus from organic matter, making it bioavailable (Burns, 1982; Dick, 1994; Martinez, 1968; Nannipieri et al., 2011; Narendrula-Kotha and Nkongolo, 2017). High phosphatase activity typically reflects active microbial communities and high rates of organic matter decomposition in soil (Chander et al., 1997; Nannipieri, 1994; Zhang et al.,

2015). Hence, phosphatase activity is used as a proxy for soil functioning (Adetunji et al., 2017; Dumontet et al., 1992; Eivazi and Tabatabai, 1977; Tyler, 1976; Yurong et al., 2017).

In vegetated soils, "root exudate induced priming effects" from established vegetation have been shown to accelerate microbially mediated enzyme activities despite environmental stresses such as Cd and As concentrations (Bali et al., 2020; Vithanage et al., 2012). When soils are vegetated, a continuous supply of metabolites becomes available for soil microbiota to produce enzymes for cycling of metabolites in the soil. In contrast, barren soils lack this supply of metabolites due to minimal leaf litter and debris, resulting in poor enzymatic function. Adding exudate mimics (SREs) and leaf litter additives have been shown to stimulate microbially mediated enzymatic reactions since these amendments nourish soil microbiota (Kuzyakov, 2010, 2018; Tian et al., 2019; Vaidya et al., 2022; Vamerali et al., 2009). In the experiments reported here, the addition of simple metabolites (sugars, amino acids, organic acids) may have stimulated the contaminant-exposed microbes in 25 R soil, perhaps explaining the increased PAs observed for SRE treated soils. Similar results were previously observed after repeated SRE additions to poorly functioning soil (Vaidya et al., 2022).

Even after 210 days from the single SRE dose, PA of the treated 25 R soil (0.5 ± 0.06 μmol h^{-1} $g^{-1}_{dry\ soil}$) was significantly higher compared to the untreated 25 R control (0.01 ± 0.005 μmol h^{-1} $g^{-1}_{dry\ soil}$) ($p < 0.0001$, Tukey HSD) (see supplement, Fig. S2). Our data

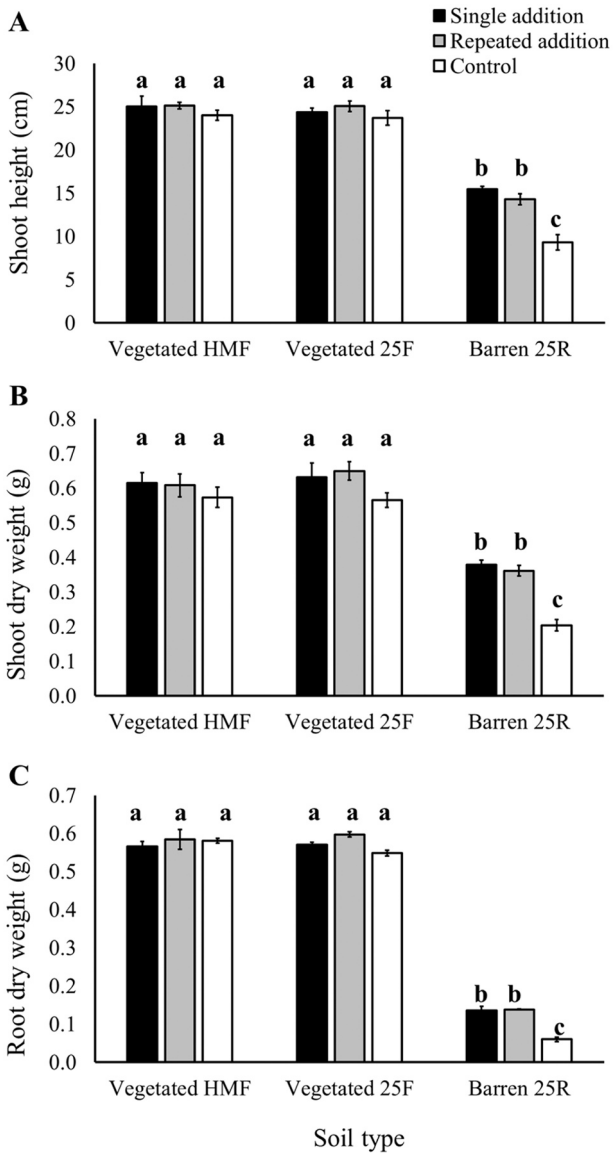

Fig. 4. The shoot height of grass blades (cm) (A), shoot mass ($g_{dry\ weight}$), and root mass ($g_{dry\ weight}$) (C) are shown for all treatments and soil types. The treatments are single SRE addition (black bars), repeated SRE additions (gray bars), and control (sterile tap water only; white bars. Lowercase letters indicate statistically significant differences (shoot height $p < 0.001$; shoot mass $p < 0.0001$) among treatments within a soil type. Bars represent the mean of four replicates and the standard error (SE) of the mean ($n = 4$, $\pm$ 1 SE).

indicate that a single SRE intervention on day 0 left a legacy of increased P mineralization in the barren 25 R soil that lasted for at least 210 days after the single dose was added. Previously, Wang et al. (2022) demonstrated that adding sugar (sucrose, fructose, glucose) solutions twice a week for four weeks to uncontaminated agricultural soil increased alkaline PA. To our knowledge, the effects of a single SRE addition on PA have not previously been demonstrated on contaminated, barren soils.

There were no statistically significant differences in moisture levels among treatments within each soil type during the 270 days of the experiment (see supplement, Fig. S3). We expected this because we watered the pots regularly based on the soil mass measured on day 0. The percent moisture content means among the three soils were statistically significantly different (Fig. S3, $p < 0.0001$). Percent moisture levels were higher in vegetated soils. This may be due to the presence of root hairs that bind soil aggregates and water molecules.

3.3. Effect of treatments on plant shoot height and biomass

We compared the shoot heights of winter ryegrass grown in soils treated with single SRE addition, repeated SRE additions (Table S2), and control in three soil types, HMF, 25 F, and 25 R. The shoot heights did not vary significantly with treatment in vegetated HMF or 25 F (Fig. 4A and B). In contrast, plants were significantly taller when grown in barren 25 R soils treated with single or repeated SRE additions compared to control soils (single SRE addition: 15.5 ± 0.3 cm, repeated SRE additions: 14.3 ± 0.6 cm, and control: 9.3 ± 0.9 cm) (one-way ANOVA, $p < 0.0003$, $p < 0.001$, respectively, Tukey HSD) (Fig. 4C). These results indicate that SRE addition had the biggest effect on plant height in barren and contaminated 25 R soil. Contaminated soils can lack nutrients and/or microbial abundance and diversity, thus exhibiting reduced germination and plant biomass (Gerhardt et al., 2009; Smith et al., 2006; Xie et al., 2012). The results here show that either a single or multiple SRE additions can increase shoot height of ryegrass grown in a contaminated, barren soil.

We compared dry shoot and root masses of winter ryegrass grown in soils treated with single SRE addition, repeated SRE additions, and control. The findings are qualitatively similar to those described above for shoot height. When we grew the rye grass in vegetated HMF or 25 F soils, the shoot and root masses did not vary among the three treatments. In contrast, plants grown in the barren 25 R soil had significantly larger shoot and root masses when treated with either a single SRE addition (shoot mass 0.4 ± 0.01 g, root mass 0.2 ± 0.1 g) or repeated SRE additions (shoot mass 0.4 ± 0.02 g, root mass 0.1 ± 0.002 g) compared to the controls (shoot mass $= 0.2 \pm 0.02$ g, root mass $= 0.06 \pm 0.006$ g) ($p < 0.0001$, Tukey HSD) (Fig. 4C).

In the barren 25 R soil, a single SRE addition resulted in significantly larger plants than the control, indicating that a single SRE addition to a barren and poorly functioning soil might serve as a cost-effective and easy-to-implement treatment to restore soil quality and increase plant productivity in industrial barrens. A combination technology that begins by conditioning the soil with SREs and then deploying phytoremediation would facilitate urban regreening efforts (Gavrilescu, 2022; Zheng et al., 2022). Adding SREs to barren, contaminated soils can supply the essential nutritional stimuli to revive the native microbial metabolic processes, which can, in turn, support germination and plant growth.

4. Conclusions

Enriching a barren, metal contaminated, inactive soil from a post-industrial site (25 R) with a single SRE addition significantly increased microbial metabolism, soil enzymatic function, and plant biomass. The addition of SREs to this extreme soil environment may have provided the native microbial community with essential metabolites that supported microbial metabolism and functioning, as indicated by increased soil respiration rates and PAs. The increased microbially mediated PA persisted for over 210 days after the single SRE addition, suggesting that the soil system reached a new stable functional state. This altered functional state coincided with greater mean biomass and shoot height of winter ryegrass in SRE enriched barren, contaminated 25 R soils compared to the untreated controls. Both single and repeated SRE treatment schedules increased soil function.

From a practical perspective, full-scale remediation requires the reduction of metal concentrations in the soil. Phytoremediation is an appealing approach to remove metals from contaminated soil (Balkrishna et al., 2022; Madhav et al., 2024), yet it is only possible when the soil can grow plants. Here we showed that applying SREs to a barren soil can reinvigorate microbial function and enable plants to grow. As an initial step, SRE addition can condition barren soil, nourish contaminant-tolerant native soil microbiota, restore microbial abundance, and improve soil quality before planting. As such, a combination strategy of SREs and phytoremediation can be useful for remediating barren soils.

One important consideration for the SRE addition technology is its potential impact on metal bioavailability (Gray et al., 2006; Li et al., 2019; Luo et al., 2024; Song et al., 2022; Tyler, 1974; Wuana and Okieimen, 2011). SREs contain organic acids, which can decrease soil pH and therefore change metal speciation soil (Bali et al., 2020; Kim et al., 2010; Luo et al., 2024; Reeder et al., 2006). Moreover, citric acid has been shown to complex trivalent metals, depending on the soil pH, and alter the mobilization of metals in soils (Jones, 1998; Jones and Kochian, 1996; Montiel-Rozas et al., 2016). Future work will provide additional insights into the effects of SRE addition technology on metal bioavailability in barren soils.

In rootless, contaminated soils that are deficient in organic metabolites, revitalizing soil microbes is critical for effective soil management. Traditional soil fertilization efforts often focus predominantly on inducing plant growth through plant nutrients, without targeting the soil microbial communities. In soils with poorly functioning or dormant microbial communities, revitalization of microbial communities can enhance microbial metabolism and functioning to support vegetation. A single SRE addition left a long-lasting legacy of restored PA in a barren, contaminated, inactive soil, perhaps reflecting the revival of contaminant-resistant microbial communities. Promoting plant growth in previously barren soils through a pragmatic and effective single SRE intervention can lay the

groundwork for phytoremediation and offer a promising strategy to remediate and re-green extreme, highly degraded soil environments.

CRediT authorship contribution statement

Jennifer Adams Krumins: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sarah E. Krisak:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Bhagyashree P. Vaidya:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Nina M. Goodey:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nina M. Goodey reports financial support was provided by National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103735.

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