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Mycorrhizal Infection Can Ameliorate Abiotic Factors in Urban Soils

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Abstract
Once abandoned, urban and post-industrial lands can undergo a re-greening, the natural regeneration and succession that leads to surprisingly healthy plant communities, but this process is dependent upon microbial activity and the health of the parent soil. This study aimed to evaluate the effects of arbuscular mycorrhizal fungi (AMF) in facilitating plant production in post-industrial soils. In so doing, we helped to resolve the mechanism through which AMF ameliorate environmental stress in terrestrial plants. An experiment was established in which rye grass (*Lolium perenne*) was grown in two heavy metal-contaminated soils from an urban brownfield in New Jersey, USA, and one non-contaminated control soil. One set of the treatments received an AMF inoculum (four species in a commercial mix: *Glomus intraradices, G. mosseae, G. etunicatum and G. aggregatum*) and the other did not. Upon harvest, dried plant biomass, root/shoot ratio, AMF colonization, and extracellular soil phosphatase activity, a proxy for soil microbial functioning, were all measured. Plant biomass increased across all treatments inoculated with AMF, with a significantly higher average shoot and root mass compared to non-inoculated treatments. AMF colonization of the roots in contaminated soil was significantly higher than colonization in control soil, and the root/shoot ratio of plants in contaminated soils was also higher when colonized by AMF. Mycorrhizal infection may help plants to overcome the production limits of post-industrial soils as is seen here with increased infection and growth. The application of this mechanistic understanding to remediation and restoration strategies will improve soil health and plant production in urban environments.

Keywords Soil · Contamination · Mycorrhizae · Plant growth

Introduction

The soils of urban and post-industrial landscapes support distinct ecological communities and can present valuable opportunities to increase green space as human populations move increasingly into cities. Likewise, within these post-industrial landscapes, contaminated soils offer an extreme condition to study the complex interactions between soil fungi, plants, and industrial contaminants [1]. The stress of high concentrations of heavy metals affects plant-soil dynamics. Although trace amounts of some heavy metals are beneficial to plant functioning, higher amounts can be toxic to growth [2–4]. Additionally, contaminants can limit the diversity [5] and functionality [6] of soil microbial communities [7–9]. This can result in lowered soil enzymatic activity [10–12] and, occasionally, curiously high enzymatic activity [6]. Despite the effects of metal contamination, it is possible that these communities may adapt and flourish over time [5].

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Some plants [13] and microbial communities [14] have mechanisms adapted for acclimation to heavy metals. Within an urban brownfield, Hagmann et al. [6] identified one contaminated site with high metal load and high enzymatic activities, suggesting a functioning and well-adapted soil microbiota. Further investigation of this same brownfield site found a distinct mycorrhizal community composition that was reflective of the enzymatic responses found by Hagmann et al. [6] and indicated adaptation to soil metal contamination [15].

It is possible that the distinct mycorrhizal community identified by Evans et al. [15] also increased the plants' abilities to withstand environmental stresses. An increase in anthropogenic soil contamination and understanding of the role of mycorrhiza has led many to investigate the importance of mycorrhiza and particularly arbuscular mycorrhiza (AMF) in toxic ecosystems. When exposed to high levels of heavy metals, an increase in stress-acclimating genes was seen in *Glomus intraradices* [16]. Additionally, some AMF species have the ability to bind and absorb soil metals [17]. However, there is a wide range of responses to heavy metals across different species from sensitive to potentially well adapted [18, 19]. Due to these different responses, moderately contaminated soils see slight increases in AMF diversity, while highly contaminated conditions result in sharp decreases in diversity [20]. With new technologies and phylogenetic studies uncovering high diversity levels, it is likely that the understanding of AMF responses to contamination and adapted species has the potential to grow [21]. A thorough sequencing of heavy metal contaminated soils from across one post-industrial site showed that the soils support surprisingly high diversity with respect to fungi and bacteria [5], but the composition of those communities, especially the fungi, varies with metal load [15].

AMF colonization has been seen to facilitate the survival and growth of plants in heavy metal conditions [16], and numerous studies have shown that AMF can increase plant growth in heavy metal-contaminated soils [22–26]. Likewise, increased colonization rates of tolerant AMF species have been found under experimentally high heavy metal conditions [27], and plants grown in lead contaminated soils show increased growth with AMF present, particularly for plant species more dependent on the symbiosis [23, 26]. AMF facilitation of plant growth in contaminated soils is not always seen and varies depending on fungal and plant species [28]. Further, facilitation can be affected by abiotic soil properties that override the beneficial effects of symbiotic fungi [29]. In this experiment, we expected plants grown in contaminated soils to depend upon AMF more than those in non-contaminated soils because AMF has been shown to help alleviate stress [16].

Based on the known contributions of AMF in alleviating stress in contaminated soil conditions, we sought to resolve the mechanism of AMF-facilitated primary production in contaminated soils. Given the trade-offs of mycorrhizal infection in restrictive environments, it is logical that AMF would be more beneficial to plants in contaminated soils than in otherwise clean control soils [1]. A growth chamber experiment tested this idea with both control and field-collected contaminated soils inoculated with a commercial AMF suspension and subsequent monitoring of plant growth and soil metrics of AMF presence. AMF are the best mycorrhizal inocula because they are known to infect rye grass (*Lolium perenne*), the experimental plant, that species is also found commonly within the contaminated site from which soils were collected. This research informs a deeper understanding of plant-microbe interactions in urban soils with important implications for the restoration of degraded lands [30].

Materials and Methods

Liberty State Park

The soils of Liberty State Park (LSP), Jersey City, New Jersey, USA, served as the study system for this experiment. Once an estuary on the Hudson River, this land has seen substantial human impact as a railyard with development and industrial use occurring from the mid-nineteenth to mid-twentieth centuries, followed by abandonment in the late 1960s and subsequent natural forestation. LSP supports a surprisingly robust, biodiverse, and naturally colonizing temperate deciduous forest [31, 32]. The mechanisms that support this forest are not clear, but their resolution sheds valuable light on remediation of contamination and restoration of post-industrial and reclaimed lands. Currently an urban brownfield, the portion of this park with restricted access, offers a unique case study of contaminated soils. The 100-hectare region of non-remediated soils of LSP has been well mapped and shown to have varying levels of heavy metal contamination between sites, including arsenic, chromium, lead, zinc, and vanadium [33] in addition to organic pollutants [34]. Specifically, this experiment utilizes soils collected from LSP sites 146 and 25R. The soils of these two sites are heavily contaminated with metal loads above the surrounding threshold [33] and above the Soil Clean Up Criteria of the NJ Department of Environmental Protection (https://www.nj.gov/dep/rules/rules/njac7_26d.pdf). Site 146 is densely vegetated and has high measured enzyme activities, whereas 25R is barren of vegetation and has enzyme activities below detection limits.

Experimental Design

A potted growth chamber experiment was conducted to compare the role of AMF in plant growth in contaminated soils. Specifically, the experiment established a factorial design with three soil contamination levels and the presence of AMF inocula or not. The AMF inocula was a commercial mixture of four taxa (*Glomus intraradices, G. mosseae, G. etunicatum and G. aggregatum*) (Root Naturally, LLC, Denver, CO, USA). The design included two factors. The first is three soil types: a control, commercial potting soil (PS) (Scott's Miracle Grow Potting Mix); a less contaminated soil shown to have high function and dense vegetation (146) (soil characterized in [6]); and a more contaminated soil with low function and no vegetation (25R) (soil characterized in [5]). Nutrient analysis and characterization of the contaminants can be found in Hagmann et al. [6] and Singh et al. [5]. The second is the inoculation treatment with AMF added to half the treatments and sterile water to the other half. Experimental units are labeled as the soil type and either +/- representing AMF+ or AMF-. For example, PS+ indicates potting soil with AMF inocula. Each experimental treatment combination was replicated six times for a total of 36 pots (two units were lost to low germination in PS+ and 146+ leaving 5 replicates in those treatment combinations).

Each soil was first coarse sieved through 2-mm mesh and then sterilized by autoclaving through two wet cycles with the assumption that the native microbial community would be significantly reduced. Sterilized soils were potted into 700-mL pots, and half of the pots inoculated with 5 g (approximately 600–700 fungal spores) of the commercial AMF suspension. Ten winter rye grass seeds (*Lolium perenne,* non-sterilized) were sewn into each experimental pot. Approximately six seeds were planted, but in 3 weeks post-germination, plants were culled to leave the largest remaining plant per pot. Over the course of the experiment, each pot was watered with equal tap water twice a week (20–40 mL per pot), and the experiment was maintained in a growth chamber with diurnal settings of 12 h day at 24 °C and 65% moisture and 12 h nights at 16 °C and 55% moisture. To evaluate the effects of AMF under different soil metal conditions, the following responses were measured: plant biomass (root and shoot dry weights including root/shoot ratio), AMF colonization of roots (as percent colonization), and soil extracellular enzyme activity (phosphatase).

Plant Biomass

After 105 days of growth, plants were harvested and separated into plant roots and shoot then oven-dried at 70 °C over 2 days before weighing.

AMF Colonization

To confirm inoculation treatments and quantify infection, staining of experimental roots using the classical AMF root staining methodology including trypan blue stain followed by the gridline interest method to quantify percent AMF colonization [35] was used. Specifically, roots were cleared with 10% KOH at 60 °C for 90 min until translucent, followed by three rinses with tap water. Clearing of roots grown in PS followed the same procedure with time extended to 2 h to account for greater thickness. Roots were then acidified with cold 2 N HCl for 2 min and stained for 20 min at 60 °C with stain that follows: 0.05% trypan blue, 50% glycerol, 48% water, and 2% 2 N HCl. Destaining followed the staining procedure with 50% glycerol, 48% water, and 2% 2 N HCl. After completion of staining, roots were viewed with an optical zoom stereo microscope (Nikon SMZ1000) and then evaluated for positive or negative AMF colonization of 200 grids per root squash to determine percent of root length colonized. This procedure was carried out on five individual pseudoreplicates that were averaged for each experimental unit (where n=5 or 6).

Enzymatic Activity

Extracellular phosphatase activity of the experimental soils was measured for all treatments and replicates at the completion of the experiment. Phosphatase activity is known to correlate with AMF colonization and activity in the soil [36]. The procedures of Hagmann et al. [6] and the fluorometric assay protocol developed by Marx et al. [37] were both modified as needed to measure the amount of 4-methylumbelliferone product formed by the phosphatase enzymes present in each soil sample. Moisture was analyzed with 2.0 g of soil in a drying oven at 70 °C for 24 h. Phosphatase activity was expressed as moles of reaction product produced per hour per g of dry soil.

Statistical Analysis

A two-way factorial ANOVA was used (JMP®, Version 13.2 PRO. SAS Institute Inc., Cary, NC, 1989-2007) in which soil type (PS, 146 and 25R) and AMF inoculation (AMF+ or AMF-) were the fixed factors to determine effects on the following response variables: plant growth (root, and shoot dry biomass and root/shoot ratio), as well as percent AMF colonization and soil phosphatase activity. The ANOVA was followed by a Tukey HSD analysis for pair-wise comparisons. In the case of the analyses for AMF colonization and enzymatic activity of the soil, the roots were too fragile and small to stain and phosphatase levels were below detectable levels for 25R plants and soils. Therefore, they were excluded from statistical analysis. For that reason, a t-test

was used to analyze AMF infection in the roots of 146 and PS soils. All data will be made available upon request.

Results

Plant Biomass

To evaluate the effects of AMF on plant growth in soils of different contamination levels and vegetation history, we compared the growth of ryegrass plants among the six treatment combinations. Plants grown in potting soil and 146 grew noticeably bigger than those in 25R (Supplementary Figure). Average root biomass was always greater in treatments inoculated with AMF (Fig. 1, $F_{1,34} = 6.76$, $p < 0.05$) across all soil types. Among soil types, the greatest average root biomass was seen in PS, and the lowest was in 25R, with significant differences in root mass between pair-wise comparisons of all soil types (Fig. 1, $F_{2,34} = 225$, $p < .0001$).

The analysis revealed a significant interaction in shoot plant biomass by two-way ANOVA depending upon soil type and AMF presence (Fig. 2, $F_{2,34} = 23.7$, $p < .0001$). As in the main effects for roots, treatments with AMF had greater average shoot masses (Fig. 2, $F_{1,34} = 36.5$, $p < .0001$) across all soil types. Likewise, we found significant difference in shoot plant biomass by two-way ANOVA depending upon soil type (Fig. 2, $F_{2,34} = 404$, $p < .0001$); again, among soil types, the average shoot mass was seen to be greater in PS compared to both LSP soils, 146 and 25R (Tukey HSD, $p < .0001$) and greater in 146 compared to 25R (Tukey HSD, $p < 0.001$).

Root/shoot ratio was calculated and found to be significantly different depending upon soil type (Fig. 3, $F_{2,34} = 27.5$, $p < .0001$). The root/shoot ratio was found to be significantly higher in 146 compared to both PS and 25R (Tukey HSD, $p < .0001$). However, though non-significant, the root/shoot ratios of plants growing in PS versus those grown in 25R soil varied depending upon inoculum and soil type. The root/shoot ratio for inoculated plants in PS was approximately 50% lower than the uninoculated plants, and the ratio for inoculated plants in 25R soil was approximately 50% higher than uninoculated plants in the same soil (Fig. 3).

AMF Colonization

Quantification of AMF root colonization using the stained roots found that roots from 146+ had significantly greater average colonization than those from PS+ (Fig. 4, t-test, $t_8 = -2.19$, $p < 0.05$). Roots from 25R were not stained because they were too fragile and there was insufficient root tissue. Stained roots from PS- and 146- had no evidence of AMF colonization.

Enzymatic Activity

Soil phosphatase activities were found to be significantly greater in PS soils than 146 soils (Fig. 5, $F_{1,34} = 8.68$, $p < 0.01$), with no significant impact of AMF inoculum ($F_{1,34} = 0.124$, $p > .05$). Phosphatase levels in both 25R treatments, whether they were inoculated with AMF or not, were below detection limit.

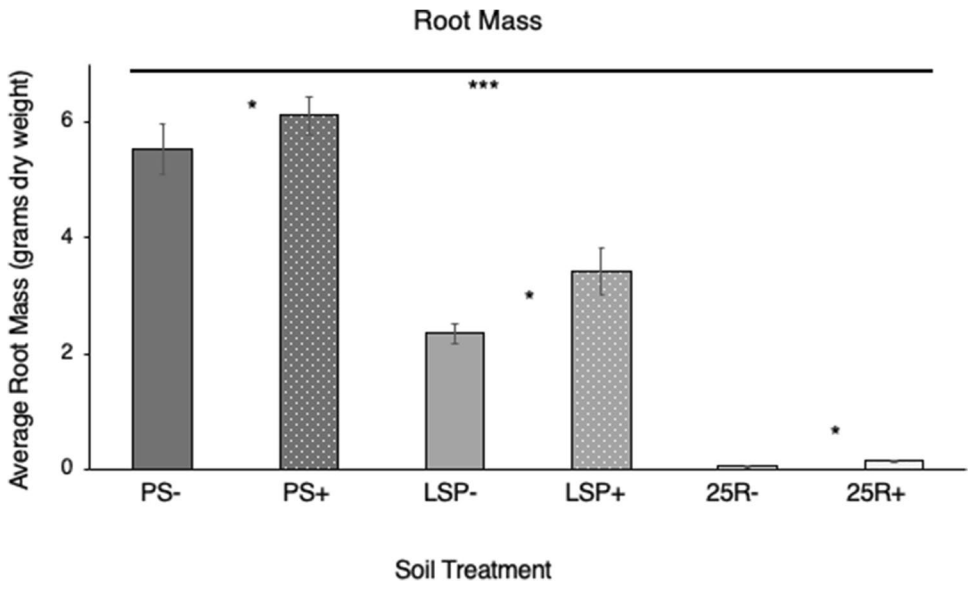

Fig. 1 Root mass (grams dry weight) of experimental plants across treatments. Significant difference found in total plant biomass by two-way ANOVA depending upon soil type ($F_{2,34} = 225$, $p < .0001$). Significant difference found in total plant biomass by two-way ANOVA between AMF treatments ($F_{1,34} = 6.76$, $p < 0.05$). (*** indicates $p < 0.001$ and * indicates $p < 0.05$)

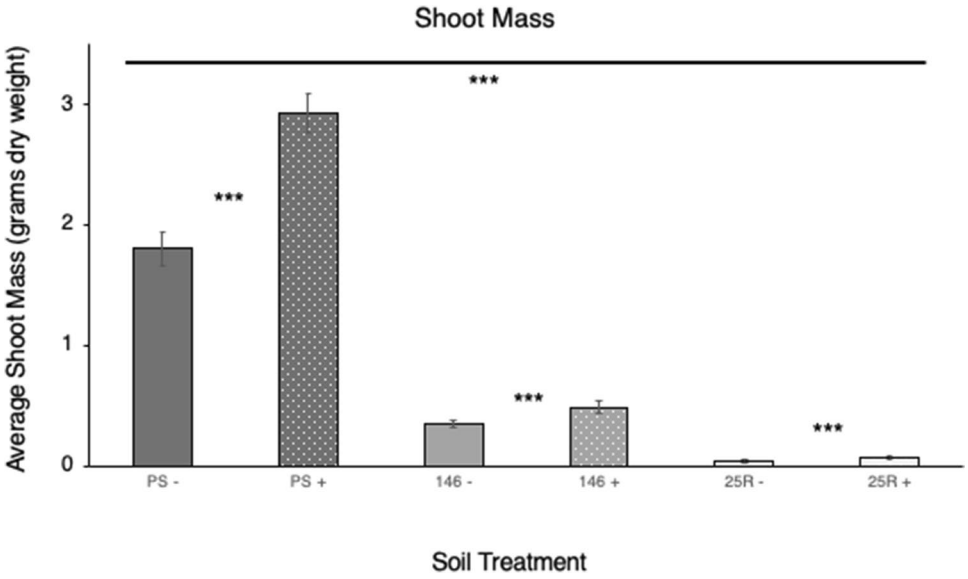

Fig. 2 Shoot mass (grams dry weight) of experimental plants across treatments. Significant interaction found in shoot plant biomass by two-way ANOVA depending upon soil type and AMF presence ($F_{2,34} = 23.7$, $p < .0001$). Significant difference found in shoot biomass by two-way ANOVA depending upon soil type ($F_{2,34} = 404$, $p < .0001$). Significant difference found in total plant biomass by two-way ANOVA between AMF- and AMF+ treatments ($F_{1,34} = 36.5$, $p < .0001$). (*** indicates $p<0.001$)

Discussion

The brownfield soils of LSP are characterized by known and measured heavy metal contamination [33]. High levels of heavy metals can limit plant growth [4]. Because of the stress heavy metals pose on plant growth, and facilitation theory [38], we hypothesized and then demonstrated that plants would have lower mass in LSP soils compared to non-contaminated potting soil controls. We also expected the plants in metal contaminated soil to capitalize on mycorrhizae to a greater degree than those in PS when mycorrhizal spores were available [1]. This was realized when we measured AMF colonization rates and found them to be higher in plants of contaminated soils (Fig. 4), and in addition, we also found an increased allocation of biomass to roots in contaminated soils when AMF were present as opposed to uncontaminated controls (Fig. 3).

Soil type, and therefore contamination level and nutrient availability, was a significant factor across all measured responses including root mass, shoot mass, root/shoot ratio, AMF root colonization, and soil phosphatase levels. The root/shoot ratio was highest in the plants potted in site 146 soil (Fig. 3). This trend supports the notion that AMF can facilitate plant growth in contaminated soils, and plants may capitalize on AMF colonization more than they would in hospitable soils. This result has been seen when AMF

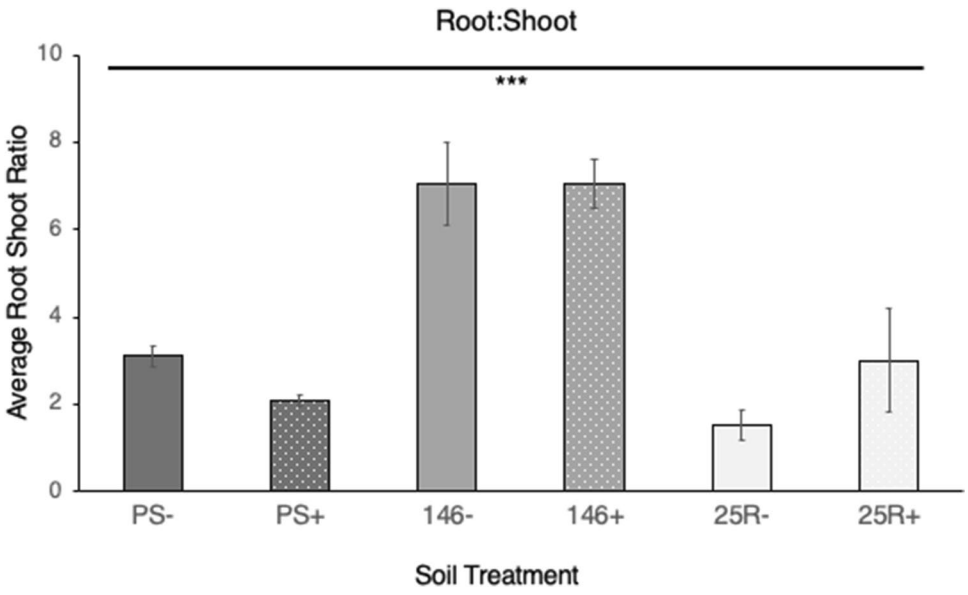

Fig. 3 Average root/shoot ratio of experimental plants across soil treatments. Significantly greater root/shoot of plants grown in LSP 146 compared to both PS and LSP 25R was found by two-way ANOVA with Tukey post hoc test ($p < .0001$). The main effect of soil was found to be a significant factor affecting root/shoot ratio ($F_{2,34} = 27.5$, $p < .0001$). (*** indicates $p<0.001$)

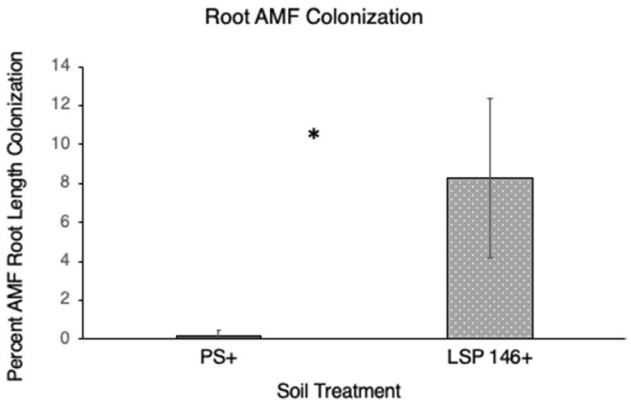

Fig. 4 Percent root AMF colonization of experimental roots inoculated with AMF. Roots from LSP 146+ were found to have significantly higher percent AMF colonization compared to PS+ (t-Test, $t_8 = -2.19$, $p < .05$). Five samples were counted from each replicate for PS+ and LSP 146+. (* indicates $p<0.05$)

inoculation improved plant success in cadmium-contaminated soils [12] and when the addition of earthworms together with AMF increased microbial activity and soil health [39]. The benefits of AMF colonization can only be realized when plants can germinate and produce roots. In the highly restrictive soils of site 25R, plants could not overcome the abiotic limitations of the soil to build roots and mycorrhizal relationships. Notably, root biomass was also relatively low in the potting soil (Fig. 2), though the mechanism explaining this is different from those in the contaminated soils. Root mass in potting soil is likely relatively lower as those plants are free from nutrient limitation and allocate more biomass to shoots as seen in the differences in the root/shoot ratios [40, 41] (Fig. 3).

The effect of AMF on plant growth may have been influenced by heavy metal toxicity on the fungi themselves. Although some AMF species have been found to be heavy metal resistant, there is a wide response to heavy metal stress across AMF species [18]. In addition to limitations on root growth itself, as we likely observed in plants grown in 25R soils, it is possible that the strains of AMF present in the commercial inoculum we used were sensitive to heavy metals, as well as other uncharacterized contaminants in LSP soils. Prior work at this site has proven the presence of diverse AMF in each of these soils [5] suggesting a tolerance to metals at the site. However, as a baseline, recall that all soils were sterilized prior to starting this experiment and inoculation with the AMF. Therefore the legacy of naturally occurring and metal-resistant fungi from the site would not have persisted in this experiment. In addition, the degree of root colonization can vary depending upon form of inoculum used and AMF species present [42]. Overall, our root colonization was low compared to field study findings [43].

Measurement of soil phosphatase levels found that AMF was not a significant factor. This suggests that the presence of AMF did not affect nutrient cycling in these experimental soils. Therefore, the increased allocation to roots and colonization by AMF in is not likely associated with nutrient enrichment. Past studies have shown that AMF can increase soil enzyme activities, including phosphatase [44, 45]. This increase is positively related to fungal density [44], suggesting that the AMF present in these experimental soils may not have developed to high enough densities to significantly affect soil enzymatic activities or that abiotic soil factors not associated with fungal community composition limited soil enzymatic activity [46]. In this study, soil type significantly impacted phosphatase activities, with 146 being significantly

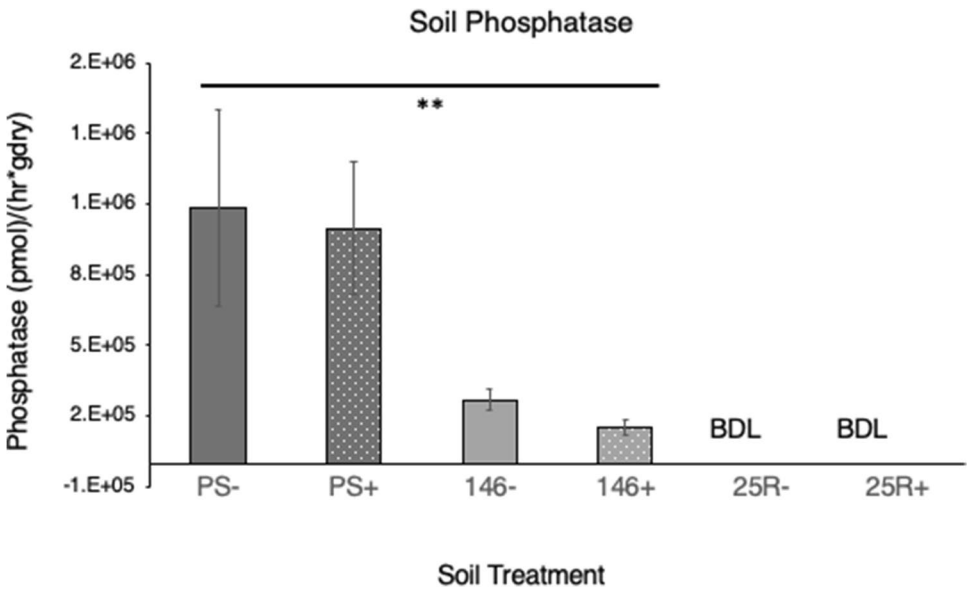

Fig. 5. Soil phosphatase in experimental pots at termination of experiment. Significant difference in phosphatase was found in experimental treatments depending on soil type, with greater phosphatase in PS (Two-way ANOVA, $F_{1,34} = 8.68$, $p < 0.01$). AMF inoculum found not found to significantly affect phosphatase ($F_{1,34} = 0.124$, $p > .05$). Phosphatase levels for LSP 25R were below detection limit (BDL) and excluded from statistical analysis

lower than PS, and 25R below detectable levels. These consistent results may be accounted for by the presence of heavy metals in LSP soils, which can suppress the activity of soil microbial communities and phosphatase levels [9].

The goal of this study was to explore the role of AMF in the facilitation of plant growth in contaminated soils. This study used soils of different heavy metal contamination levels from LSP and a non-contaminated commercial potting soil to compare plant growth with and without AMF present. We tested the hypothesis that plants would benefit from AMF colonization to a greater degree in contaminated soil rather than in nutrient-rich and hospitable potting soil. These results support the common finding that AMF can facilitate plant growth in contaminated soils, but also we show something intriguing. Plants allocated more biomass to the roots when AMF are present in contaminated soils, and this is affirmed by the fact that they support significantly more mycorrhizal colonization. The results we present here demonstrate the beneficial presence of mycorrhizal infection within the ongoing balance between soil microorganisms and the abiotic factors in moderating feedbacks between plants and soils [47].

Conclusions

The soils of LSP present a unique case study in urban soil ecology; these results can inform a deeper understanding of the mechanisms of facilitation in restrictive soils. AMF inoculation increased plant growth across all soils types. However, additional findings provide insights into the mechanisms behind the increased plant growth. We found no change in soil phosphatase activity when AMF was added to the soils, suggesting that general increased microbially mediated nutrient cycling was not the mechanism by which AMF facilitated plant growth. One possible mechanism may be increased metal tolerance such that plant growth is greater. Interestingly, a greater density of AMF was seen in the plant roots in contaminated soil. In the presence of AMF, plants also allocated a greater fraction of their biomass to roots in contaminated soils compared to non-contaminated soil. Plants therefore had both larger root surface areas *and* higher densities of AMF infections in contaminated soils. These changes may have maximized the presence and thus the protective effects, including metal tolerance, of AMF in the heavy metal laden soil. The results of this study increase the understanding of the role of AMF in soil health and urban plant and soil interactions [48, 49]. Resolving mechanisms of microbial and plant interactions in contaminated soils will support environmental movements to re-green post-industrial lands and improve urban quality of life [50].

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Data availability Data will be made publicly available upon request.

Code availability Not Applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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