Effect of metal contamination on microbial enzymatic activity in soil

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Abstract

Anthropogenic metal contamination is a pervasive problem in many urban or industrial areas. The interaction of metals with native soil communities is an important area of research as scientists strive to understand effects of long-term metal contamination on soil properties. Measurements of free soil enzyme activities can serve as useful indicators of microbial metabolic potential. The goals of this study are to determine extracellular soil enzymatic activities with respect to corresponding metal concentrations within a site of long-term contamination. These data are examined to understand relationships between extracellular soil enzyme activities and persistent metal loads in situ. Here we present such results from a rare research opportunity at an un-remediated, urban brownfield in Jersey City, NJ, USA. The soils of the site developed over the last 150 years through the dumping of urban fill from New York City as well as industrial rail use. The site was abandoned and fenced in the late 1960s, and within it, there is a mapped gradient of metal concentration in the soils, including As, Pb, Cr, Cu, Zn, and V. We measured soil enzymatic potential (alkaline phosphatase, cellobiohydrolase, and L-leucine-aminopeptidase) across four plots within the site and at an uncontaminated reference site that is of the same successional age and geographic influence. We found the highest enzymatic activities for all three activities measured at the site with the greatest soil metal loads and a particularly strong relationship among enzyme activity and the metals V and Cr. Our results differ from many experimental studies that show decreased soil enzyme activity in soils experimentally treated with metals. The results may indicate the effects of long-term adaptation of soil communities within these metal contaminated soils.

1. Introduction

The global increase in human development is rapidly changing our world (DeFries et al., 2004), and has generated unique soils that vary in contaminant nature and concentration, moisture, and nutrient levels (Pouyat et al., 2007). The process of understanding how soil communities are affected by human impacts includes resolving differences in the structural and functional character of the biotic community. Within the soil, the presence and concentration of contaminants will modulate interactions between soil organisms (Krumins et al., 2015). Research on soil function, specifically enzymatic activity, in these systems is an increasingly important approach to a more holistic understanding of soils in contaminated environments.

In contaminated soils, the activity of the microbial community is intertwined with soil abiotic properties (Schimel et al., 2007). Functions like cellulose degradation or nitrogen cycling can be measured by using soil enzymatic activities as indicators (Burns et al., 2013). When microbial communities adjacent to industrialized sites were analyzed, they showed lower enzymatic function with higher soil metal loads (Wang et al., 2007) as well as lower community diversity (He et al., 2010). Lower enzymatic activities have also been observed when soil was experimentally contaminated with heavy metal loads. For example, Kandeler et al. (2000) showed lower urease, alkaline phosphatase, and xylanase activities for soils experimentally contaminated with Zn, Cu, Ni, V, and Cd compared to soils that were not experimentally contaminated.

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However, in other studies, high soil metal loads were associated with high enzymatic activities (Kızılkaya, 2004; Pascual et al., 2004), and in other studies, shifts in the microbial community composition and functioning were found with metal load (Zhang et al., 2007). These results highlight the complexity of the effect of heavy metal contamination on soil enzyme activities and the need to consider the experimental details of each study.

The goal of this research was to determine the relationship between elevated heavy metal concentrations in soil and extracellular enzyme activities that are proxy measures for nutrient cycling. To better understand relationships between persistent metal contamination and extracellular enzymatic activities, we have measured soil metal loads and determined the extracellular enzymatic potential of the soil across a gradient of heavy metal contamination within an unremediated urban brownfield. Further, we compare these results to those of a relatively uncontaminated reference site of similar successional age and geography, but very different historical usage. The results of this study will help answer an important question relevant in restoration ecology: What are the effects of long-term metal contamination on microbial nutrient cycling in soils?

2. Materials and methods

2.1. Site description

Our primary research site is located within Liberty State Park (LSP), Jersey City, NJ (40°42′16″N, 74°03′06″W), in the piedmont physiographic region of New Jersey (Fig. 1). The un-remediated soils of LSP have been given their own series designation by the USDA Natural Resource Conservation Service, the Ladyliberty Series (National Cooperative Soil Survey, 2012). Historically, intertidal mudflats and salt marsh characterized the area. Between 1860 and 1919 the area was filled with construction debris and municipal waste from New York City to build and develop the Central Railroad of New Jersey. Rail service through the terminal ran until 1967. It was abandoned until 1970 after which much of the area was remediated and established as a state park, now dominated by landscaped recreational areas. The most contaminated portion of the park, our study site, was fenced off from human use and access since 1969 and has not been remediated, restored, or managed.

The plant community is characterized by mid-successional temperate, deciduous forest growing in existing contamination. The forest is dominated by Betula populifolia (gray birch), Populus deltoids (cottonwood) and Populus tremuloides (quaking Aspen). Other significant forest species encountered sporadically include Prunus serotina (black cherry) and Acer rubrum (red maple) (Gallagher et al., 2008). Plant community composition varies very little among the four sites with a slightly higher density of graminoids and herbaceous plants in the understory of some sites that are both of low and high metal load (notably sites 48 and 146, see Section 2.2). Those sites tend to have higher soil moisture (Table 1). Contamination caused by rail and industrial use at the site is primarily composed of heavy metals (As, Cr, Cu, Hg, Pb, Zn, V) (Gallagher et al., 2008) and, in isolated areas, organic pollutants. The organic pollutants have not yet been characterized. However, the exact source or origin of particular contaminants across the site will likely never be known. The research plots lie along a well-characterized soil metal gradient within the fenced and inactive portion of the park. Based on measurements in 2006 (Gallagher et al., 2008), the total soil metal loads at sites 48 and 43 are below the critical threshold level whereas levels at sites 14 and

Fig. 1. Aerial view of primary research site, Liberty State Park (LSP) (A) with respect to the Statue of Liberty (B) and Ellis Island (C) in New York City Harbor. The four research sites are situated within the inactive portion of the park, and they are numbered 48, 43, 14 and 146 in increasing order of relative, overall metal contamination. As such, the sites lie along the southern boundary of the well-characterized brownfield (park inactive). Photo credit: Robison Aerial Inc.
146 exceed the threshold. The critical threshold level is defined on a scale that total soil metal load (a scale from zero to five) that is the rank order summation of the log transformed concentration of the individual metal species (Jiang et al., 2001). Previous work established the value of three as a critical threshold, above which, both productivity and diversity were negatively impacted (Gallagher et al., 2008).

The reference site is located within the Rutgers University Hutcheson Memorial Forest (HMF) in Franklin Township, New Jersey (40°30′N, 74°34′W). Similar to LSP, HMF is located within the piedmont physiographic region of New Jersey. Soils are characterized by well drained silty loams developed from the Brunswick formation of Triassic red shale (Ugolini, 1964). The site was primarily agricultural for centuries, but it also contains a 26 ha area of old-growth forest that is over 300 years old. HMF was established as a nature preserve and research station in 1955. As our reference to the forest at LSP, we sampled soils from an area within the Buell-Small Succession Study, a chronosequence of plots that was allowed to undergo succession beginning in 1968 (Cadenasso et al., 2008). This HMF plot is dominated by Juniperus virginiana (eastern red cedar) and A. rubrum. Both LSP and HMF have been subject to influences as they are only 63 km apart, and they have undergone succession for similar amounts of time.

2.2. Soil collection

In July of 2013, soils were collected from four sites within LSP designated 48, 43, 14, and 146, with low to high total metal loads, respectively. In September 2013, soils were collected from the reference site at HMF. These soil samples were used for the alkaline phosphatase, cellobiohydrolase, and L-leucine-amino-peptidase enzyme assays. The site labels are arbitrary and do not correspond to any measurements but the labels have been in place for decades, and they can be used to relate historic sampling in the park (Gallagher et al., 2008).

At each collection, five soil mini-cores (2 cm diameter) 4 m apart at a depth of 1–5 cm were collected along a linear transect at each of the five sites (one site at HMF and four sites at LSP (48, 43, 14 and 146)). Therefore, we sampled five times across a gradient in total metal load at LSP and once within HMF. Only large roots were avoided when soil samples were collected. All soils were separately sieved (2 mm) and stored at 4 °C. Consolidated soil samples from each site (48, 43, 14, 146 and HMF) were prepared as follows: Soil (5 g) from each subsample (collected 4 m apart from each other at one site) were combined so that, for each site, there was a consolidated sample containing equal amounts of each of the 5 individual subsamples.

The extracellular enzyme activity measurements were conducted in 96-well plates over the time period lasting from six to 29 days after soil collection. The change in fluorescence intensity was recorded over 6 h for the cellobiohydrolase assay and the phosphatase assay, and over 3 h for the L-leucine-amino-peptidase assay to ensure that the linear increase in product fluorescence was analyzed and any effects from pre-equilibration were omitted. Each assay was conducted on ten samples in one 96-well plate at a time. Eight wells were used for each sample, three wells for samples (each sample reading was an average reading from three wells) and five wells to generate the standard curve. A separate standard curve was generated for each soil sample to account for any effects of a particular soil sample on MUB fluorescence intensity (see Section 2.4 for more information on the enzyme assay). Further, each sample was analyzed a minimum of three independent times to ensure that the analytical error also accounted for any error from weighing different analytical aliquots from a soil sample. We did not see significant changes, neither increases nor decreases, in enzymatic activities over the time period we conducted the assays, indicating that the fact that the samples were analyzed over a period of 23 days did not affect the accuracy of the data. We have also measured these enzyme activities for a different study and the relationships between the activities of soils from different sites remained the same (unpublished data).

2.3. Metal and nutrient concentration determination

The total metal concentrations (As, Cr, Cu, Pb, V, Zn) of the four LSP soils and one HMF soil were determined by metal digestion followed by analysis by ICP–MS using EPA method 3050B (Acid Digestion of Sediments, Sludges, and Soils) (Reeder et al. 2006). Briefly, 1 g of dry soil was weighed and 10 mL of 30% HNO3 was added to the soil and the mixture was refluxed at 95 ± 5 °C for 15 min. After cooling, 5 mL of concentrated HNO3 was added and samples refluxed at 95 ± 5 °C for 30 min; this step was repeated until no brown fumes formed. The samples were then reduced to 5 mL by heating at 95 ± 5 °C. After cooling, 2 mL of deionized water and, slowly, 3 mL of 30% H2O2 were added. Aliquots (1 mL) of 30%
H$_2$O$_2$ were added and heated at 95 ± 5 °C until effervescence had stopped or was minimal. The samples were reduced to 5 mL, cooled and filtered using Fisherbrand P5 filter paper, and then diluted to 50 mL with deionized water. The samples were further diluted by a factor of 20 in 1% nitric acid and analyzed for metal concentrations (Cu, Zn, Cr, V, Pb, and As) using an Inductively Coupled Plasma Mass Spectrometer (ICP–MS) (Thermo Fisher X Series) and an ASX-510 Autosampler CETAC (Analysis was done on three analytical replicates and is presented in Table 1). The dilution factors for ICP–MS for the metal extractions were ≥1000 and therefore the matrix effect was minimized. A method blank was carried through each sample preparation and analytical process to ensure that samples were not contaminated from the metal extraction protocols. A new standard curve was obtained after every 15–18 samples and used to analyze the metal concentrations in the samples preceding it; the following SPEX CertiPrep reference materials were used: #PLZN2-2Y (Zn), #PLCR2-2Y (Cr), #PLCU2-2Y (Cu), #PLAS2-2Y (As), #PLAS2-2Y (Pb) and # PLV2-2Y (V).

Cornell Nutrient Analysis Laboratory measured soil nutrient concentrations (C, N and P) and pH for soil collected from HMF, and the Rutgers University Soil Testing Laboratory carried out the same measurements for LSP (Table 1). Phosphorus was measured using the Kjeldahl method. Rutgers measured nitrogen using the H$_2$O$_2$ method (for reference see EPA Method No. 351.1) while Cornell measured nitrogen using the Kjeldahl method. Phosphorus was measured using inductively coupled plasma–atomic emission spectrometry (ICP–AES) following EPA Method No. 6010B, and the pH was measured in water. Soil carbon was measured as Loss on Ignition (LOI) (at approx. 400 °C at Rutgers and 500 °C at Cornell). We suggest a limited interpretation of our nitrogen concentration measurements, as both laboratories did not follow the same procedure. Rutgers measured nitrogen using the kjeldahl method (for reference see EPA Method No. 351.1) while Cornell measured nitrogen after combustion at 950 °C in a carbon/nitrogen analyzer (NC 2100, Analytik Jena, Jena, Germany). Both laboratories use known standards for calibration and quality control before and after measurements for nitrogen and phosphorus concentrations.

2.4. Enzymatic activity assays

Three different enzyme activities (phosphatase, cellobiohydrolase, and l-leucine-amino-peptidase) were measured for the consolidated samples from each site. The assay was done in triplicate (three different wells for technical replication) in each 96-well plate, and the entire assay was repeated a minimum of three times for each soil sample. Wet soil (0.1 g for phosphatase assay and 0.5 g for cellobiohydrolase and l-leucine-amino-peptidase assays) was suspended into 100 mL 0.1 M MES buffer, pH = 6.0 and the solution was homogenized using a sonicator at an output of about 25 W for 3 min. The resulting slurries were stirred continuously as samples (160 mL) were transferred to wells in a 96-well black plate.

Triplicate wells containing soil slurry and substrate analogs were prepared for each soil sample. The fluorescent substrate analogs used were 4-MUB-phosphate (Sigma–Aldrich #M8883, 350 μM in well), MUB-β-d-cellobioside (Sigma–Aldrich #M6018, 650 μM in well), and l-leucine-7-amino-MUC (Sigma–Aldrich #L2145, 400 μM in well) for the phosphatase, cellobiohydrolase, and l-leucine-amino-peptidase assays, respectively. A fluorescent plate reader (Molecular Devices M3) was used at 30 °C to measure the fluorescence intensity (320 nm ex./450 nm em. for the phosphatase and cellobiohydrolase assays, and 350 nm ex./440 nm em. for the l-leucine-amino-peptidase assays). Time points were collected every 15 min over 6 h for the cellobiohydrolase assay, every 20 min over 6 h for the phosphatase assay, and every 15 min over 3 h for the l-leucine-amino-peptidase assay.

To generate a standard curve for the fluorescent products, 4-methylumbelliferone (MUB) (phosphatase and cellobiohydrolase assays) or 4-methylcoumarin (MUC) (l-leucine-amino-peptidase assay), 160 μL of soil slurry and 40 μL of fluorescent product were combined to yield concentrations of 0, 500, 1000, 1500, and 2500 pmols of product in separate wells. These wells were analyzed simultaneously with the soil-substrate wells. The emission intensities from the wells containing soil slurry mixed with different amounts of fluorescent product were used to generate a standard curve (fluorescence emission intensity versus concentration) at each time point. These standard curves were used to determine the amount of product in the wells containing substrate and soil slurry. The time dependence of the amount of product generated was analyzed to determine the enzymatic activity the amount of product generated in 1 h by 1 g of oven-dry soil in units of nmols/g oven-dry weight/h. All enzyme activity measurements were calculated on the basis of gravimetrically determined oven-dry weights (70 °C for 24 h) of soils.

2.5. Testing metals as inhibitors or activators of extracellular enzyme activities

To determine whether metals in solution act as an inhibitor or activator of extracellular phosphatase activities, standard stock solutions of V, Cr, Cu, Zn, Pb were added to the assay and the effect of each metal on the enzymatic activity was analyzed. Specifically, increasing concentrations of each metal in the study were added to soil from the LSP site with the lowest metal load (site 48). The final concentration in this assay varied from no added metals to approximately the highest concentration of each metal found across the sites in the study (see Table 1). Soils for these experiments were collected from LSP in May 2014 as described above in Section 2.2. A solution (140 μL) of soil slurry (0.1 g soil + 100 mL 0.1 M MES, pH = 6.0) was added to each well in a 96-well plate. Next, 20 μL of a solution of a metal (V, Cr, Cu, Zn, As, or Pb) was added to each well to give the following metal concentrations in individual wells: V(V) (0.0075, 0.03, 0.12 ppm), Cr(III) (0.005, 0.019, 0.077 ppm), Cu(II) (0.005, 0.018, 0.073 ppm), Zn(II) (0.008, 0.033, 0.133 ppm), As(III) (0.002, 0.008, 0.031 ppm), and Pb(II) (0.039, 0.158, 0.630 ppm). After the metal solutions were added to the soil slurry, 40 μL MUB at varying concentrations was added as described above (Section 2.4). The enzymatic reaction was monitored for 6 h at 15-min intervals at 320 nm excitation and 450 nm emission.

2.6. Testing the soil as an inhibitor/activator of extracellular enzyme activities

To determine whether the metal contaminated soil acts as an inhibitor or activator of extracellular enzymatic activities, soil from the highest metal load site (site 146) was first rendered enzymatically inactive by autoclaving a 1 g sample (30 min at 121 °C, 15 psi). After autoclaving, phosphatase activities of the soils were measured as described above (Section 2.4) and the autoclaved soils were found enzymatically inactive. Soils for these experiments were collected from LSP in October 2013. To assess the ability of the enzymatically-inactive soil to inhibit/activate phosphatase activities, a slurry of the autoclaved soil was prepared in 0.1 M MES buffer (pH 6.0). The phosphatase enzyme assays were conducted as described (Section 2.4), with the modifications that the soil was enzymatically inactive and a commercially available phosphatase enzyme was used to represent soil phosphatases. Briefly, 150 μL of soil slurry was added to the wells of six columns in a black 96-well plate. MES buffer (0.1 M, pH = 6.0) was added to the remaining six columns. Then 40 μL substrate, 4-MUB-phosphate, was added to the three rows of the black 96-well plate to obtain 3 replicates, for a final concentration of
350 μM 4-MUB-phosphate in the well (total volume 200 μL). To determine whether the inactive, autoclaved soil from site 146 could inhibit or activate the phosphatase activity in the assay, 10 μL of a solution of alkaline phosphatase from *Escherichia coli* (Alkaline Phosphatase from *E. coli*, Sigma Aldrich #P5931 diluted to 1 × 10^{-3} units/μL) was added to all wells. The reaction progress was analyzed by measuring the time dependence of product concentration using a microplate reader (320 nm em./450 nm ex.) for 1 h at 30 °C.

### 3. Results

Across the four study sites within LSP and the reference site of HMF, soil enzymatic activity was distinctly higher in site 146 for L-leucine-amino-peptidase (Fig. 2A), cellobiohydrolase (Fig. 2B) and phosphatase (Fig. 2C). Therefore we carried out Pearson correlations between pairs of each of the five data points (with a correction for multiple comparisons (Benjamini and Hochberg, 1995; Verhoeven et al., 2005)) to resolve the effects of abiotic soil factors including individual metal loads on enzymatic activity (carried out in SAS Version 9.1 (SAS Institute, Cary, NC)). The correlations showed a strong relationship between V and Cr and the highest metal site (primarily driven by site 146). There was no significant correlation between enzyme activity and the other nutrients or heavy metals (Table 2). These correlations are qualitatively similar when extracellular enzyme activities are compared to exchangeable metals rather than total metals (unpublished data). The result that there is no correlation between nutrients and enzyme activities must be viewed with some caution because the nutrient analysis for the samples from LSP and analysis for the soil from HMF were conducted in two different, commercial laboratories. With that said, we expect the two laboratories to yield comparable data because solutions of known concentrations were used as standards in both laboratories.

One possible explanation for the high enzymatic activities found at the site with the highest metal concentrations is that the enzyme activities are elevated because the metals in the soil serve as catalytically important cofactors or activators. To test this possibility, a control experiment was designed where additional metals were added to the soil slurry used in enzymatic assays (Section 2.5). Varying concentrations of each metal in the study were added to soil from site 48. Soil from site 48 was used because the metal concentrations and the enzyme activities are relatively low. Using soil from site 48 allowed us to determine whether additional metals in the enzymatic assay result in higher enzymatic activities that are more similar to those found for soils from site 146. The final concentration in this assay ranged from no added metals to approximately the highest concentration of each metal found across the sites (Table 1). If the enzymatic activity is higher with higher concentrations of added metals, then metals are likely serving as cofactors or activators. On the other hand, if the added metals have no effect on activity, then the high enzymatic activities found in the high metal site are not simply due to the metals in the soil serving as cofactors or activators but rather the long-term interaction between metals and soil must have resulted in altered soil properties through another mechanism. The results showed that there was no change in the phosphatase activity when additional metals were added to the soil from site 48 (see Supplement Figure S1). All phosphatase activities for soil samples with added metals, regardless of metal, were within one standard deviation of control soil from site 48 with no metals added. In this control experiment, we confirmed that simple addition of metals to the assay without incubation of the soil with the metals does not affect activity. This means that the short amount of time

![Fig. 2.](image-url)
(about 5 min) for which the metals interacted with the soil enzymes in this control experiment, was not enough to result in activation of the phosphatases in the soil via metal binding. A different process must be taking place to explain this correlation, which could involve the microbial community.

Another possible reason for the high enzymatic activities found at the site with the highest metal concentrations is that the metal containing soil itself (or some component of it) serves as an activator of enzymatic activities. In the soils from LSP, the metals may be bound to the organic and inorganic matter in the soil and metals in complexes may influence the enzymatic reaction differently from metals in solution. To test the ability of site 146 soil to serve as an activator of phosphatase activity was explored in another control experiment (Section 2.6). Upon autoclaving the soil to eliminate enzyme activity, the phosphatase activity was measured and compared to non-autoclaved soil. The results showed that autoclaving the soil eliminated 100% of the phosphatase activity; no detectable increase in fluorescence indicating product formation was observed (the rate of substrate consumption) for which the metals interacted with the soil enzymes in this control experiment, was not enough to result in activation of the phosphatases in the soil via metal binding. A different process must be taking place to explain this correlation, which could involve the microbial community.

Phosphatase, cellulohydrolase, and l-leucine-amino-peptidase enzymatic activities, representative of C, N, and P cycling, respectively, were determined at four LSP sites lying on an increasing total metal concentration gradient and compared to a site of similar successional age, HMF. Alkaline phosphatase hydrolyzes phosphomonoesters and releases phosphate, cellulohydrolase is involved in the hydrolysis of complex carbon components, and l-leucine-amino-peptidase releases leucine and other hydrophobic amino acids from the N-terminus of a polypeptide. The data showed that all three enzyme activities were highest at the site with the highest metal concentrations, 146 (Fig. 2). One might suspect that metals should result in lower rather than higher enzyme activities (Kuperman and Carreiro, 1997) by causing protein denaturation, forming a complex with the substrate, interacting with the residues involved in enzyme function, or by reacting with the enzyme–substrate complex (Hemida et al., 1997). However, after having conducted the control experiments where metals were added to the enzymatic assays and the ability of the soil itself to act as an inhibitor or activator was assessed, it is clear that the metals or the soil themselves are not simply activating the enzymes studied and that something else must be responsible for the high enzymatic activities observed at site 146.

The distinctly high enzymatic activities at site 146 are significantly correlated with Cr and V concentrations in the soil. A possible explanation is that the presence of elevated Cr and V in the soil over 40 and more years has resulted in an altered soil microbial community resulting in soil with high extracellular enzyme function and nutrient turnover. The correlative data does not imply a causative relationship between Cr and V concentrations; it is possible that Cr and V are co-contaminants with another unmapped pollutant/s, which in fact is responsible for the elevated extracellular enzyme activities at site 146. We did not find significant correlations between the enzyme activities and nutrient levels in the soil. The fact that all three enzyme activities were elevated similarly to each other (Fig. 2) further suggests that elevated enzyme activity at 146 is not due to nutrient limitation.

Other researchers have shown declines in enzymatic activity associated with soil metal load. Kandel et al. (2000) added metals in the laboratory setting to the soil to relatively high concentrations, Zn (300 ppm), Cu (50 ppm) and Cd (3 ppm). They found that metals resulted in decreased enzyme activities. It is difficult to compare these results to our own because their study was conducted in the laboratory, and the metals interacted with the soil for a shorter time than in this study. In a field-experiment conducted at a military base in Maryland in the USA, lower extracellular soil enzyme activities were also found associated with heavy metal contamination. At this site, the metal concentrations were higher (Zn (1353 ppm), Cu (569 ppm), and Pb (1340 ppm)) than at our study site LSP 146 (Zn (140 ppm), Cu (76 ppm), and Pb (414 ppm)), making it difficult to make direct comparisons between the sites and findings (Kuperman and Carreiro, 1997). The metal concentrations from a mining effluent studied in Spain had concentrations of Zn (113 ppm), Cu (31 ppm), and Pb (15 ppm), and their research showed lower enzyme activities at the contaminated site compared to the non-contaminated counter part (Hinojosa et al., 2004). Conversely at the most contaminated site in our study, LSP site 146, we saw the highest enzyme activities for all three enzymes studied. These differences highlight the importance of studying metal concentrations in the context of the rest of the soil environment and carefully considering the details of the study site and its history.

The metal concentrations at LSP are likely typical of many urban brownfields. The history of LSP makes our study sites...
an excellent opportunity to deeply understand the long-term feedbacks between metals and soils within a naturally assembling deciduous forest. The study sites at LSP were contaminated over time (more than 100 years) and left undisturbed since 1969 and remain so today allowing possible effects of these metals on microbial communities and enzymatic function to play out over time, in the absence of human intervention. Metals interacted with the soil for decades, rather than for a shorter time period of days to months (Hinojosa et al., 2004). Effect of metals on enzyme function may in part depend on the length of time the soil-metal system has been left to “adjust and adapt”, and the study site at LSP allows for study of extracellular enzyme activities in a system that has been interacting for over 40 years. This study is different from many other related studies in a second important way: In this work, enzyme activities are investigated in soil that interacted with metals in nature rather than in the laboratory — or green house setting. Experimental data obtained in a laboratory or green house setting may not fully reflect what happens in nature for diverse reasons. Specifically, the ‘parent’ ecological community in the field will serve as the pool from which diverse microorganisms may seed this system. Conversely, a laboratory or even a green house will provide a much more limited pool of microorganisms to seed that system.

5. Conclusion

Here we report the finding that, at the brownfield LSP, extracellular soil enzyme activities are notably high at a site with the highest heavy metal loads, and there is a strong relationship between these enzyme activities and Cr and V in particular. These findings are surprising because heavy metal contamination is often thought to result in lower rather than higher enzymatic activities. The control experiments discussed above suggest that added metals or the metal laden soil did not directly activate nor inhibit the enzymes in our assays and that other explanations for the high enzymatic activities at LSP site 146 must be explored. To understand these results, the context must be considered. When we measured the extracellular enzyme activities, the metals had been present in the soil and the field had been unmanaged for over 40 years, left alone to naturally succeed. This is in critical contrast with studies in which the contamination has been only recently introduced or a study that is conducted in a manipulative laboratory setting. This case study demonstrates the capacity of some heavy metal contaminated soils to enzymatically function well under seemingly restrictive conditions.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.soilbio.2015.09.012.

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