Primary Succession on Slag Sites and Uncontaminated Soil: A Comparison

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Abstract

Slag, waste from the steel-making process, contains large amounts of calcium, magnesium, iron and other heavy metals. Because of its composition, low pH and water retention ability, slag is considered an inhospitable environment to plants. Nevertheless, the spontaneously generated plant communities on slag are surprisingly diverse, but the assembly and structure of such communities is poorly studied. Previous studies have identified slow process of succession due to low growth rate and slow accumulation of topsoil. Using two former industrial sites on the South Side of Chicago, IL, I investigated whether slag communities display similar patterns. I removed all vegetation from plots on both slag and non-slag soil to test whether primary succession differed over one growing season (4 months). To directly assess plant growth, selected species were planted on both sites and harvested. I show that primary succession differed on slag and non-slag soils. The recruitment process on slag, measured by percent cover and number of recolonized species, was overall slower; however, the difference was not significant until 6-8 weeks of experiment, suggesting potential stage-dependent effect of slag on plant growth. Functional trait analysis found that graminoid and early successional species preferentially colonized slag plots. Slag plots recovered slower from disturbance, suggesting a slow succession process that would hinder natural recovery. However, slag also has the potential of hosting flora of analogous habitats native to the area, serving as plant refuge. Restoration effort should be informed by the low possibility of natural recovery, and its potential as native plant refuge.

Introduction

Human activities have drastically modified natural landscapes, creating many unique artificial systems. One such example is the urban ecosystem, which encompasses the biological activities and ecological processes that results from a built environment. With high disturbance, largely patchy habitat and highly variable soil composition, urban ecosystems generally have unique community dynamics (Swan et al. 2011). Among many urban habitats, industrial dumps have gained attention from both ecologists and public because of its unique environmental and public health concerns (Bayless et al. 1998, Lundholm and Richardson 2010). Slag, one common type of industrial dump, is the byproduct of steel production that contains a large amount of heavy metals and alkaline earth metal compounds (Bayless and Schulz 2003). This

industrial waste is estimated to cover over 52 km^2 of land surface in northeastern Illinois and northwestern Indiana in the greater Calumet region (Bayless and Schulz 2003), with sites usually covered in a layer of slag material that varies in size from small granules to big chunks to large contiguous surfaces (Kay et al. 1997). The chemical composition, as well as thin topsoil, low water retention and low organic matter makes slag site a generally inhospitable place for plant life (Bayless and Schulz 2003, Reddy and Amaya-Santos 2017). Although variable in specific contents, steel slag is generally comprised of calcium and magnesium silicate, compounds of iron, manganese and other heavy metals (Bayless and Schulz 2003). Some slag contains organic pollutants such as polycyclic aromatic hydrocarbons (Reddy and Amaya-Santos 2017). Because of the high calcium and magnesium content, the pH of slag is generally basic. Depending on specific composition of slag, some contents may leach into nearby water bodies, causing significant pollution (Bayless et al. 1998); even more strikingly, the Big Marsh site in the Calumet region hosts a pond with pH higher than 12 (Reddy and Amaya-Santos 2017).

Due to its history as a center for steel production, the Calumet region has experiences extensive dumping of slag. Typically, slag and other waste was dumped on land adjacent to industrial plants, into pits, lakes or wetlands, or used as fill (Kay et al. 1997). Remediation of industrial dumps as sources of contamination has always been a major public health concern (Reddy and Amaya-Santos 2017); therefore, multiple slag sites in the region have undergone different degrees of restoration. Some, such as the Big Marsh Park, have been sites for active ecological restoration and have been transformed into vibrant urban parks (Chicago Park District 2014), while others such as U.S. Steel South Works have remained relatively untouched for decades. Common methods to prepare sites for ecosystem construction include phytoremediation or capping with topsoil. Nevertheless, it has been suggested that contaminants in slag might leach into the soil and affect plant growth even when capped with topsoil (Daniel Spencer and Lauren Umek, *personal communication*), and experiments with different plants in order for phytoremediation resulted in low survival (Reddy and Amaya-Santos 2017; Lauren Umek, *personal communication*). Furthermore, in some cases, other debris such as construction and demolition waste has also been dumped at slag sites, complicating the land use history and making restoration more difficult.

Slag sites, however, harbor diverse vegetation, and plant communities are comparable to those in urban disrupted land or vacant lots, with many non-native and weedy grasses, forbs and shrubs; native forbs and grasses are also common. The only trees successfully established on slag are cottonwood (*Populus deltoides*) and mulberry (*Morus alba*). Typical wetland species, such as sedges and common reed (*Phragmites australis*), are found near depressions on slag. Overall, given the harsh environment of slag sites, such spontaneous plant communities are amazingly diverse.

Naturally, such spontaneous vegetative communities raise the question of how they have assembled. According to classical successional theory, soil formation is enhanced by primary successional species that can facilitate succession by other species (Clements 1916). Those species are generally drought- and heat-resistant, fast growing and produce larger amount of litter that integrate into the soil, increasing organic material content (Cowles 1899, Clements 1916, Kazakou et al. 2006). Therefore, the ability of the primary successional community to provide organic material for soil is important for the survival of later successional species, and the recovery of an ecosystem from disturbance (facilitation model; see Connell and Slatyer 1977). Without soil formation, many natural habitats display extremely slow or arrested primary succession. For instance, alvar habitats, comprised of thin topsoil on limestone bedrock, similarly have high pH, shallow topsoil and low nutrient availability; due to the low growth rate of early successional plants, the community is "arrested" at a primary successional stage and does not succeed to later stages (Stark et al. 2004, Tomlinson et al. 2008).

A slag site can be considered as a habitat after disturbance: most plants were killed by dumping. As disturbance ends (no further dumping) and erosion breaks the contiguous slag surface, primary succession takes place and the present community assembles. Previous studies have estimated successional trajectories of other industrial sites, predicting recovery to the original climax community (Smith et al. 1997, Řehounková and Prach 2008). However, it is possible that slag site plant communities, though they have been relatively undisturbed for long periods of time, may have arrested primary succession. Because slag sites are usually characterized by a thin layer of topsoil with high pH, they might be comparable to alvars in displaying "arrested" soil and plant community development. The environmental stress on slag may lower growth rates of early successional plants, resulting in less accumulated plant litter and decreased soil formation (Chapin 1991). This hypothesis is supported by the fact that some

sites, including the Big Marsh and the Van Vlissingen parks, have been undergoing succession for decades but could not support survival of later-successional species such as most trees and shrubs without addition of compost (Reddy and Amaya-Santos 2017). Nevertheless, more concrete evidence is still necessary to determine whether such slag plant communities are arrested in primary successional stage by the lack of nutrients.

I tested whether primary succession was slowed down in slag soils, including whether 1) slag sites differed from non-slag sites by soil composition and vegetation, 2) the primary succession process differed for slag and non-slag sites, and 3) the plant growth rate were reduced in slag versus non-slag sites, 4) plants on slag differed in their functional traits. Over the course of one growing season (June to October 2018), a combination of plant surveys, soil testing and controlled experiments on two types of site, **Slag** and non-slag soils (hereafter **Reference**), were used to test the three hypotheses. I evaluated functional traits to assess if plant communities varied between Slag and Reference sites.

Glossary

Site Selection
BM: Big Marsh
VV: Van Vlissingen
Locale: CPD properties on which experiments were set up; BM or VV
Site: Slag or Reference (non-slag soil) at each locale
Methods
Plot: 1.2 × 1.2 m squares of land on which all vegetation was cleared; basic unit of experiments
Block: consist of either 3 (for 2 blocks at both Reference sites) or 5 (all else) plots of different purposes; basic unit of replication
Germination Experiment: measurement of germination and growth in plots after vegetation removal; two treatments (see below)
Treatment: in germination experiment, each plot was either untreated (removal only) or treated with topsoil
Focal Species Experiment: selection of 3 species to plant in plots for measurement of growth by biomass
Focal Species
AS: *Asclepias syriaca*, common milkweed
BC: *Bouteloua curtipendula*, side-oats grama
SS: *Solidago speciose*, showy goldenrod

Methods

Figure 1. Maps and photos showing experimental site. **A**: regional map showing location of site; **B**: zoom-in satellite image showing Big Marsh (BM) and Van Vlissingen (VV) parks; **C**: photo of slag site at BM; **D**: photo of slag site at VV.

I. Site Selection

The study was conducted at two locales: Big Marsh Natural Area (**BM**) and Van Vlissingen Natural Area (**VV**), both Chicago Park District properties located on Chicago's Southeast Side. The locales were chosen so that they both contain Slag (**S**) and non-slag Reference (**R**) sites (Mark Bouman and Lauren Umek, *personal communication*) and are in close proximity to each other (1.9 km), to minimize variance in climate. Slag at Big Marsh was deposited at BM (**BM-S**) more recently than **VV-S**, on which construction debris was also deposited (Lauren Umek, *personal communication*). The reference site at BM (**BM-R**) mainly consists of construction waste capped by thick top soil and has been seeded with a common tall-grass prairie seed mix by Chicago Park District (Lauren Umek, *personal communication*). **VV-R** is surrounded by sparsely spaced cottonwood (*Populus deltoides*). All sites are in full

sun, except for a few plots at VV-R which encounter partial shade for no more than 2 hours per day. Both slag sites contain slight depressions which allow standing water to accumulate after heavy rainfall. The experimental site at VV-S is surrounded by *Phragmites*-dominated shallow slag-bottomed wetlands.

The study spanned 4 months, from Jun 5, 2018 (planting focal species) to Oct 6, 2018 (final harvest). Weather data of study sites were drawn from National Weather Service (National Weather Service Forecast Office 2018). Highest temperature ranged from 17.8 (Jun 22) to 36.1 ºC (Aug 4), and low temperature ranged from 8.9 (Jun 6) to 24.4 ºC (Jul 1). Highest precipitation was 6.00 cm (Aug 7). Typically, weather at study sites was highly variable temporally and geographically; during the study period, the weather was characterized by consecutive hot and sunny days, continuous rainfall in prolonged period and occasional local thunderstorms.

II. Plant Survey and Soil Tests

Plant surveys were conducted on Jul 9, 2018 at VV-S and Jul 31, 2018 at BM-S using the censusing procedure from Northern Indiana Restoration Monitoring Inventory (NIRMI). At each site, a 50 m × 20 m survey plot was set up, and a comprehensive list of species and cover data was recorded according to NIRMI procedure (NIRMI, 2010). At BM-S, parts of the experimental plots were included in the survey. Species lists produced at BM-R and VV-R were in close proximity of experimental plots (<= 10m), providing a regional species list for identification of species germinating in experimental plots. Floristic Quality Assessment (FQA, Taft et al. 1997) was also performed on species lists using Universal FQA Calculator (Freyman et al. 2015) to evaluate conservation values of each site. The assessment estimates habitat quality based on the coefficient of conservation for each species present; the coefficient is determined by whether the species is disturbance-adapted or associated to undisturbed natural area (Taft et al. 1997).

Category	Variables Measured
Nutrient Level	Organic matter (percentage), N, P, K
Chemical Properties	Mg, Ca, pH, Cation Exchange Capacity (CEC)
Heavy Metal Content	Zn, Mn, Fe, Cu, As, Cr, Pb
Physical Properties	Sand, silt, clay (all percentages)

Table 1. Variables measured by soil test, in four major categories

Samples of soil at each site were drawn from 10 different spots < 0.5 m from experimental plots, with an approximate depth of 10 cm. Samples were also taken from the commercial topsoil used in germination plots (see *Experiment 1: Germination*). Two distinctive types of soil were observed at VV-S, therefore sampled separately and designated VV-S-1 (block A-D) and VV-S-2 (block E). Samples from each site were then combined, and ~ 400 mL soil was drawn from each mixture for laboratory analysis. A total of 6 samples (one per each site for BM-S, BM-R, VV-R; two for VV-S; one from commercial topsoil) were tested. Soil was analyzed for variables listed in **Table 1** by A&L Great Lakes Laboratories (Fort Wayne, IN).

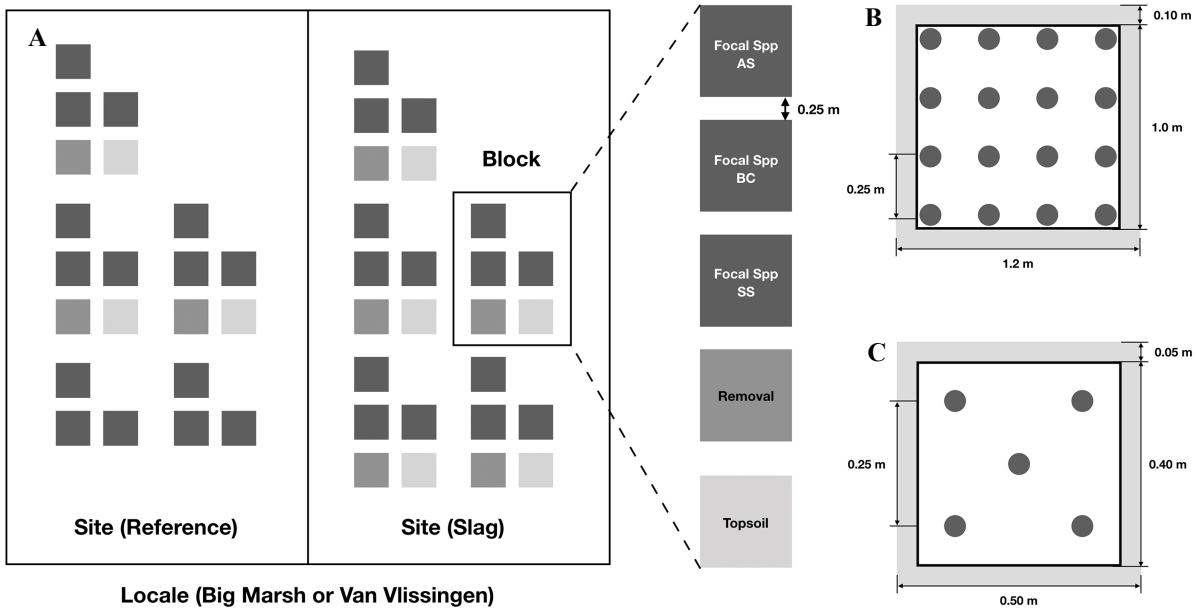

Figure 2. Experimental setup and plot maps. **A**: General map showing number of plots, and relationships between locale, site and block, with configuration within each block; each plot is randomly ordered and separated by at least 0.25 m. **B**: Configuration of 16 individuals within each BC and SS focal species plot. **C**: Configuration of 5 individuals within each AS focal species plot.

III. Experimental Setup

The basic unit of replication was the block, which consisted of five experimental plots: three focal species and two germination (**Figure 2A**). Each site hosted five blocks, named A-E. The order of plots within block was randomized. Two blocks on each Reference site contained only three focal species plots because of spatial constraint. Overall, each Slag and Reference site hosted 25 plots and 21 plots, respectively.

Experiment 1: Germination

To test what species initiate primary succession, all vegetation was removed from experimental plots (1.0 × 1.0 m, with additional buffer zone of 0.2 m on each edge), followed by either no further manipulation (hereafter **removal**) or cover by commercial topsoil (5 mm thick; hereafter **topsoil**). Removal plots simulated the recruitment of plants into newly disturbed habitat; topsoil plots characterized the "background dispersal rate" because the effect of growth from seed bank or vegetative structures remaining in the soil was eliminated.

To measure the generation/growth rate of plants in germination plots, monitoring photos were taken weekly during the course of 16 weeks. An aluminum quadrat (0.25 × 0.25 m) was placed at one corner of the 1.0 × 1.0 m plot area, and a photo was taken at approximately 1.5 m above each of the four corners in a horizontal position, yielding four photos. Each photo was then cropped to leave only the area inside quadrat and imported to ImageJ (Abràmoff et al. 2004). The percentage of green vegetation (hereafter **cover**) was extracted; cover of a plot was then determined by the arithmetic average of cover values from all four photos.

To identify the species diversity within germination plots, species presence/absence data for experimental plots was recorded from weekly monitoring photos selected from three dates, Aug 22, Sep 5 and Sep 26 (day 77, 91 and 112). A species was considered present if observed in any of the three records. To discover possible time trend in diversity patterns, the number of species present in each plot over the whole experimental period was also counted and recorded from monitoring photos.

Experiment 2: Focal Species

To directly measure the growth rate of plants on Slag and Reference sites, three native focal species with high conservation value were manually planted in experimental plots. For side-oats grama (*Bouteloua curtipendula*, **BC**) and showy goldenrod (*Solidago speciosa*, **SS**), each 1.0 × 1.0 m plot (with buffer zone of 0.2 m on each edge) hosted 16 plants in 4 × 4 pattern (**Figure 2B**); due to a sourcing constraint, only five common milkweed (*Asclepias syriaca*, **AS**) were planted in a 0.5 × 0.5 m plot (with buffer zone of 0.1 m on each edge; **Figure 2C**). Seedings less than a month old were sourced from Cardno Native Plant Nursery (Walkerton, IN). All plants were watered daily during the first two weeks and at least once a week

afterwards. No other manipulations (intensive weeding, application of fertilizer or pesticide) or physical protection were done.

IV. Biomass Harvest

The aboveground biomass was quantified twice in the Focal Species plots. An initial harvest was done at week 2 (Jun 20, 2018) where two plants (one for AS plots) were harvested for each focal species plot, and a final harvest was done at week 17 (Oct 6, 2018), with all plants in focal species plots were harvested. The central 0.25 × 0.25 m portion of each germination plot harvested to include an equal portion of each corner that contributed to the cover measurement. Individuals of focal species harvested at week 2 were omitted for the final harvest. Therefore, a maximum of 14 (4 for AS plots) plants were harvested from each plot, exact number depending on actual survivorship. All biomass was left at room condition for no more than 12 hours before drying at 80 ºC for at least 96 hours. The biomass in germination plots were measured as a mixture of species; each focal species plant was measured individually.

V. Data Analysis

All data analyses and plotting were done in R (R Core Team 2013) using packages *ade4* (Dray and Dufour 2007), *cluster* (Maechler et al. 2018), *dendextend* (Galili 2015), *factoextra* (Kassambara and Mundt 2017), *FD* (Laliberté and Legendre 2010), *ggvegan* (Simpson 2017), *lindia* (Lee and Ventura 2017), *nlme* (Pinheiro et al. 2018), *PCAmixdata* (Chavent et al. 2017), *tidyverse* (Wickham 2017) and *vegan* (Oksanen et al. 2018).

Soil Tests

Soil data were scaled, centered, and analyzed by Principal Component Analysis (PCA). Two methods of transformation were performed on the original data set. Because sand, silt and clay content were compositional measurements (in percentages that added up to 1), one measurement was sequentially excluded from the analysis while the remaining two were log-transformed. In the second transformation, all measurements were log-transformed. The resulting sets of vectors characterizing each soil sample were then extracted as independent variables in a linear regression of cover and biomass.

Cover and Biomass

I tested whether site, locale or treatment of germination plots affected cover and biomass. Cover and biomass data were analyzed using one-way ANOVA analyses to evaluate site (Slag or Reference) and locale (BM or VV) effects, respectively; cover data was further analyzed by treatment (removal or topsoil). To exclude the potential effect of low species number on low cover at slag sites, the relationship between cover and species number at each time in removal plots was analyzed by linear regression to detect potential effect of slag on recovery. Because the experiment involved a block design, Linear Mixed Effects (LME) models were also fitted for block effect as a source of random error. Biomass estimates were divided into three groups: initial harvest (focal species only; 2 individuals per plot for BC and SS, 1 for AS), final harvest of focal species and final harvest of germination plots. All groups were analyzed using Welch's t-test and Wilcoxon's test when applicable, with biomass on Slag lower than on Reference as alternative hypothesis. Data from final harvest of germination plots were further analyzed with two-way ANOVA of site and treatment effects.

To explore possible relationships between soil composition and plant growth, both cover and biomass measurements were analyzed using linear regression as dependent variables, with soil measurements extracted from PCA as independent variables. To compress the time series data of cover, the maximum was taken for each plot. Missing sample due to mortality was designated 0 in biomass analysis. Linear regression analyses used a Bonferroni correction; with 16 soil measurements, the adjusted p value was $0.05/16 = 0.003125$.

Species Diversity and Community Composition

Species presence-absence data from germination plots and on both Slag and Reference sites was compiled from three days of monitoring photos and NIRMI plant survey, respectively (see *Experiment 1: Germination*). For both datasets, species richness (α diversity), plot dissimilarity (Whittaker's β diversity; Koleff et al. 2003) and native status were analyzed. Non-metric multi-dimensional scaling (NMDS) analysis was performed on germination plots presence-absence data to further evaluate the dissimilarity among sites and locales using the R package *vegan* (Oksanen et al. 2010). Species number from each plot over the experimental

Trait Category	Trait Name
Lifestyle	Species type (herb, grass, vine, shrub, tree) Life history (annual, biennial, perennial) Growth form (grass, tall forb, short forb, shrub, tree) Functional group (graminoid, forb, legume, woody)
Physiology	Growth habit (erect, decumbent, procumbent, sprawling, vine) Shoot structure (leafy, semirosette, rosette) Canopy length (quantitative, in cm)
Regeneration	Regenerative strategy (widespread seed, vegetative spread, seasonal by seed) Seed number (quantitative) Seed dry mass (quantitative, in mg) Seedbank longevity (short: under 1 yr; medium: 1 to 5 yrs; long: >5 yrs) Lateral spread (<0.01 m, 0.01-0.25 m, >0.25 m) Phenology (early: before June; summer: June to July; late summer: after July)
Primary Production	Leaf dry matter content (LDMC; quantitative, in mg/mg) Specific leaf area (SLA; quantitative, in mm^2/mg)
Native status	Native or Nonnative

Table 2. Plant functional traits used for analysis

period was also compiled from monitoring photos and subsequently tested for site effect using ANOVA.

I tested whether functional traits differed in communities colonized removal plots on Slag and Reference, selecting traits that are important to plant strategies in diverse environments (Cornelissen et al. 2003, Kazakou et al. 2006, Wright et al. 2004, 2006, Pérez-Harguindeguy et al. 2016; **Table 2**). Functional traits and native status for species present in germination plots were obtained from Illinois Wild Flowers (Hilty 2017), the TRY database (Kattge et al. 2011) and Grime et al. (2014). To calculate a species distance matrix based on functional traits, which consisted of both quantitative and nominal data, two methods were used. First, a Gower's distance (Gower 1971) was calculated using the function *gowdis* from R package *FD* (Laliberté and Legendre 2010). Second, I computed the distance matrix by the function *dist.ktab* from R package *ade4* (Dray and Dufour 2007) following Pavoine et al. (2009); the method takes two separate matrices of quantitative and nominal data, computes Gower's distance matrices separately, then combine the two results. Species were clustered based on the distance matrix. Mixed-effect PCA using the R package *PCAmixdata* (Chavent et al. 2017) were performed to identify functional traits that best characterize species distances. Canonical (Constrained) correspondence analysis (CCA; Ter Braak 1986) was used to detect the potential correspondence between environmental variables and species distribution in germination plots. Model selection based on maximum likelihood method was then performed used the R function *ordistep* to obtain environmental variables that best explain species composition.

RLQ model was constructed to evaluate the association between functional traits and environmental variables (Dolédec et al. 1996) with the package *ade4* (Dray and Dufour 2007).

R, **L** and **Q** stand for matrices with environmental variables, species distribution (abundance or presence-absence) and functional trait information, respectively; the fourth-corner method was used to analytically detect correlations between those matrices (Dray and Legendre 2008). Specifically, the fourth corner method permutes variable elements of community matrices with a Monte-Carlo test and compares the statistics of random versus observed matrices, detecting potential effects of environmental variables (Dray and Legendre 2008). For the purpose of this study, I permuted both site and species vectors to generate 1000 randomized presence-absence matrices. First, the significance of associations between each environmental variable and functional trait was evaluated using the function *fourthcorner*; then, the $S_{\mathbf{RLQ}}$ statistic (multivariate statistic similar to correlation coefficient; see Dray and Legendre 2008) using the function *fourthcorner2* to determine the overall association between environmental variables and functional traits.

Results

I. Plant Survey

Slag sites at BM and VV hosted 66 and 44 species, respectively. Most species were herbaceous and relatively short in stature, forming a sparse cover. Dominant species included rough false pennyroal (*Hedeoma hispida*) and *Dicanthelium spp*. Rosette-forming forbs were also common, including fleabanes (*Erigeron spp*.), goldenrods (*Solidago spp.*) and plantains (*Plantago spp.*). Invasive European buckthorn (*Rhamnus cathartica*) and glossy buckthorn (*R. frangula*) are examples of shrubs on slag. Although seedlings of several trees, including Siberian elm (*Ulmus pumila*), red maple (*Acer rubrum*), green ash (*Fraxinus pennsylvanica*) and common juniper (*Juniperus communis*), have been spotted sporadically on slag, the only established trees are cottonwood (*Populus deltoides*), mulberry (*Morus alba*) and sumac (*Rhus spp.*; not in survey); individuals of both species display significantly slow growth and stunted stature compared to those growing on non-slag soil. Many invasive wetland species, such as common reed (*Phragmites australis*) and cattail (*Typha* × *glauca*), has either established monoculture or dominated the plant community in depressions on slag.

Some species with high conservational value, such as Crawe's sedge (*Carex crawei*), elliptic spikesedge (*Eleocharis elliptica*) and nodding lady's tresses (*Spiranthes cernua*) have also established on slag. According to FQA, VV-S is of higher quality by hosting more native

and high-conservation value species than BM-S. At BM-S, percentages of native and non-native species were 55.6% and 44.4%, respectively; the adjusted Floristic Quality Index (FQI) for the community was 19.4, with wrinkle-leaf goldenrod (*Solidago rugosa*) having the highest FQI of 6. At VV-S, percentages of native and non-native species were 79.4% and 20.6%, respectively; the adjusted FQI for the community was 40.1, with *Carex crawei* and *Elliocharis elliptica* both having the highest FQI of 10. According to FQI measures, VV-S falls into the range of natural Midwest prairie quality (30-40; Alison 2002, Bowles et al. 2006, Hanson and Gibson 2014). For a complete species list, see **Table S1A**.

II. Soil Test Results

PCA of soil measurements showed significant difference between Slag, Reference and commercial topsoil (CTRL; **Figure S1**). The first two principle components explained 42.99% and 29.57% of total variance. Specifically, Slag samples were characterized by high Ca, Mn, K, Zn, Cr, sand content and higher CEC (**Table 3**). Both transformations resulted in the same clustering pattern and same set of indicative variables; PCA result from second transformation was shown to display all soil variables.

Category	Variables
I. Slag	Ca, Mn, K, Zn, Cr, Sand, CEC, pH
II. Reference	Cu, Pb, Mg, Clay, Silt
III. Topsoil (CTRL)	Organic matter, P, N, As, Fe

Table 3. Characterization of soil samples by measurements, from PCA (**Figure S1**).

III. Growth Measurement: Percent Cover

Site Effect

	Slag Effect, BM		Slag Effect, VV		Locale Effect, Slag		Locale Effect, Reference	
	Removal	Topsoil	Removal	Topsoil	Removal	Topsoil	Removal	Topsoil
F	14.45***	42.64***	61.99***	57.37***	86.05***	33.19***	2.556	27.16***

Table 4. ANOVA results of slag (between slag and non-slag sites) and locale effects (between BM and VV) on cover in germination plots, with degree of freedom 1. Significance levels: $p < 0.05$ (*), $0.01 < p < 0.05$ (**), $p < 0.01$ (***).

Overall, Slag plots showed lower plant cover than Reference plots at both locales. ANOVA analysis on cover showed significant site effect between Slag and Reference with both treatments at both locales. Furthermore, site effect was not distinctive until later stage of the experiment, as shown by time series plots of cover between sites of the same locale and treatment (**Figure 3**, **A**-**D**), with ANOVA indicating a site difference starting at day 49 or 63 (**Table S4A**). Removal plots on both slag sites showed slower increase of cover with

increasing species number, indicated by smaller slope of regression lines (**Figure 4**, **Table S4C**). Both removal and topsoil plots on BM showed significant block effect, indicating a high heterogeneity within site (block effect σ/overall σ = 2.422/7.308 and 2.552/8.163, respectively). Block effect was not significant for both treatments on VV.

Locale Effect

In addition to a site effect, there was a significant locale effect on cover across sites and treatments, except between removal plots on Reference sites (**Table 4**). Although the exception is not statistically significant (ANOVA, $p = 0.113$), there appears to be a divergence toward the end of experiment, after day 84. Overall, the high divergence between same types of plots on different locales indicated that BM and VV could not be treated as replicates.

III. Growth Measurement: Biomass

Germination Plots

Slag biomass was lower than Reference biomass at both BM and VV for both removal and topsoil plots, using Welch's t-test on log-transformed data (**Figure 5D**, **5E**; **Table S5A**). Two-way ANOVA on site (Slag or Reference) and treatment (removal or topsoil) showed consistent effect of slag ($F = 21.335$ and 33.53, respectively; both $p < 0.001$) but not treatment (between removal and topsoil; see **Table S5B**). This result corresponds to observation during the experiment. Wilcoxon's test was not applicable due to low number of samples.

Focal Species Plots

Initial harvest of focal species showed little effect of site or locale. The biomass of BC on Slag was higher than on Reference (both Welch's and Wilcoxon's tests, $p < 0.05$); the significance was not robust using log-transformed biomass data (**Table S5A**).

At the end of the growing season, the biomass on Slag soils was significantly lower than on Reference soils for BC and SS (both Welch's and Wilcoxon's tests, $p < 0.01$; **Figure 5B** and **4C**). Notably, the high mortality (43/70, or 61.4%) of SS on BM Slag resulted in a medium biomass of 0. Strong effect of slag was still detectable using only survived SS biomass; however, if all zeroes caused by mortality were removed, no significant difference in SS

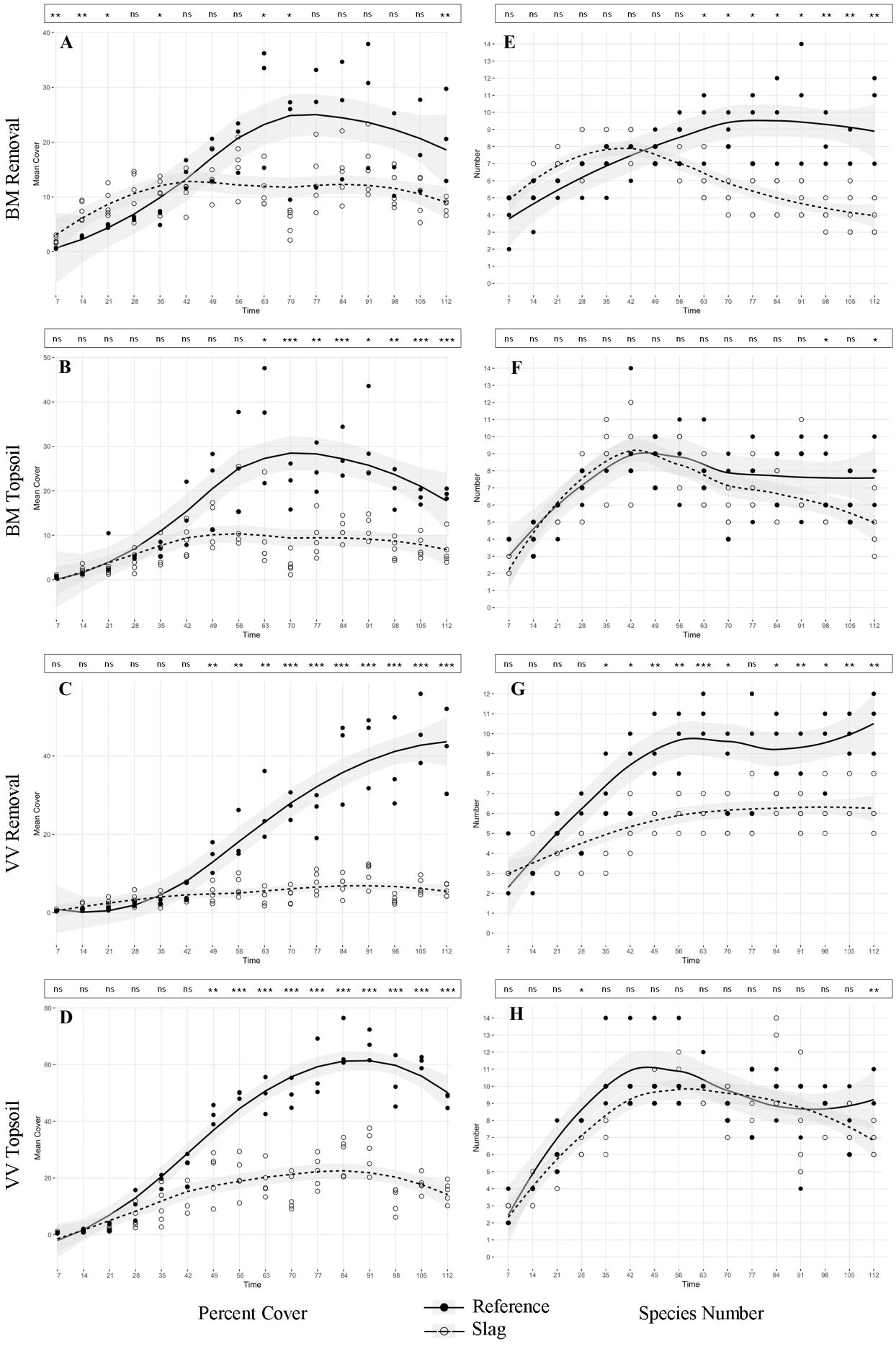

Figure 3. Cover and Species numbers of germination plots between sites at each locale, showing the effect of slag. A-D: cover (in percentage); E-H: species number. *x* axes show time from start of experiment in days. Shades along regression lines show ranges of standard deviation. Notations above each graph shows the site-effect ANOVA result using data from that specific day. Significance levels: $p > 0.05$ (ns), $p < 0.05$ (*), $0.01 < p < 0.05$ (**), $p < 0.01$ (***). See **Table S4A-B** for complete statistics.

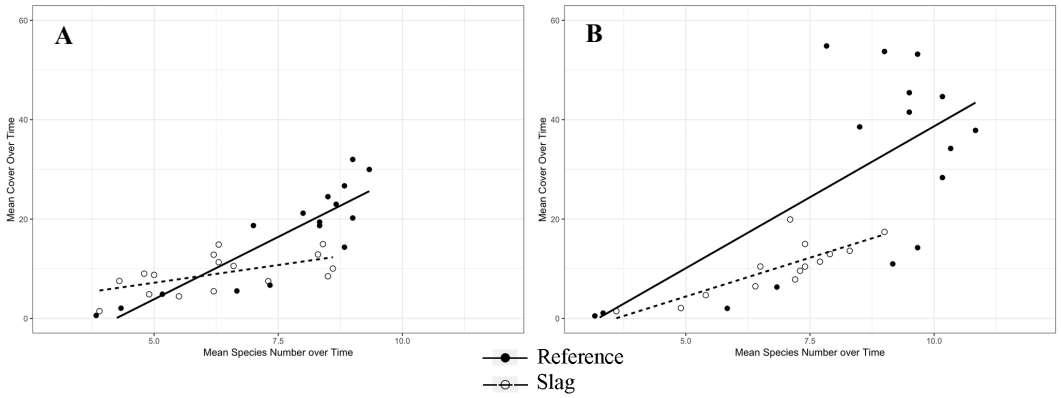

Figure 4. Linear regression of mean percentage cover versus mean species number at each time in removal plots, showing different slopes on Slag and Reference sites. **A**: BM; **B**: VV. See **Table S4C** for complete statistics.

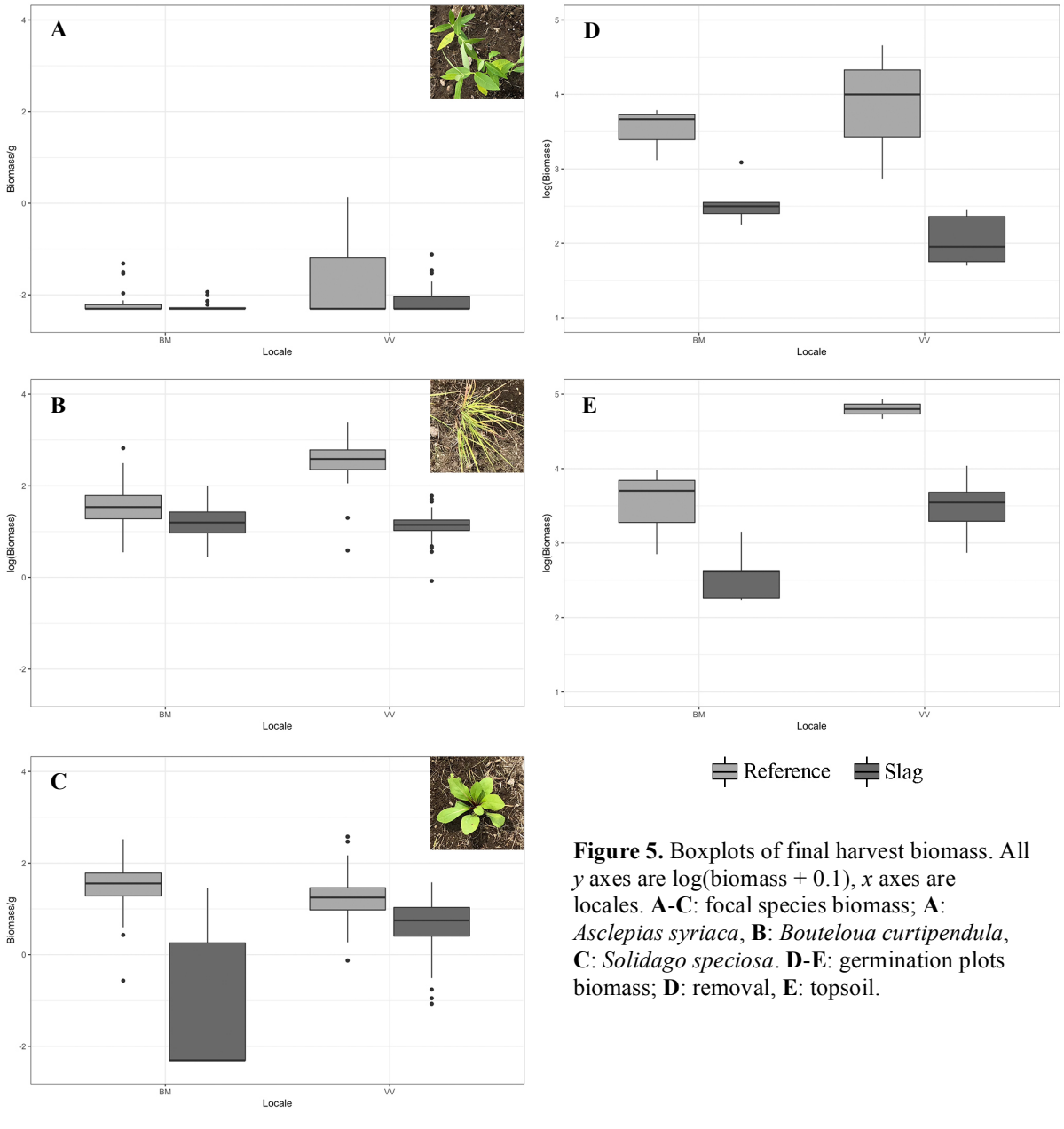

Figure 5. Boxplots of final harvest biomass. All *y* axes are log(biomass + 0.1), *x* axes are locales. **A**-**C**: focal species biomass; **A**: *Asclepias syriaca*, **B**: *Bouteloua curtipendula*, **C**: *Solidago speciosa*. **D**-**E**: germination plots biomass; **D**: removal, **E**: topsoil.

biomass was detected between BM-S and VV-S. The high mortality of AS across all sites and locales (54/80, or 67.5%) led to little difference in final biomass (**Figure 5A**); no difference could be detected even zeroes were excluded because most biomass collected were newly generated sprouts from remaining underground parts after herbivory or desiccation. Even though some tests were marginally significant, data were too few to be informative. No mortality was observed for BC at all sites.

Similar to cover data, block effect was analyzed by linear mixed-effect (LME) models on biomass of each species on each locale. BC at both BM and VV and SS at VV showed significant block effect (block effect σ/overall σ = 0.7642/1.929, 1.382/3.538 and 0.38/1.607, respectively).

IV. Effect of Soil on Growth

Slag soil variables mostly correlated negatively to plant growth, measured in both cover and biomass when cover and biomass were fitted to each soil variable with linear regression (LR) models using both untransformed and log-transformed data (**Table S6**). The transformation yielded no difference in slope and little difference in R^2 and p value. With only a few exceptions, most variables in category I (Slag; see **Table 3**) negatively correlated with plant growth, measured in either cover or biomass, of both experiments; most variables in category II (Reference) and III (Topsoil) positively correlated with plant growth. However, the R^2 and p values of most models were very low or not significant, likely due to limited soil data.

V. Species Diversity and Community Composition

In the original experimental design, topsoil plots were set up to exclude germination of local seed bank and characterize the background dispersal rate. However, the commercial topsoil was not sterile. 11 nonnative species, including purslane (*Portulaca oleracea*), smooth crabgrass (*Digitaria ischaemum*) and redshank (*Persicaria maculosa*), appeared in all topsoil plots but not removal plots. I therefore performed germination assays with topsoil only in indoor trays to avoid any possible propagules from outside. Six species germinated from topsoil trays, indicating that our topsoil was contaminated by predisposed seeds. Therefore, many results of species diversity and community composition analyses were significantly biased, and analyses on community structure (see below) either were done separately for

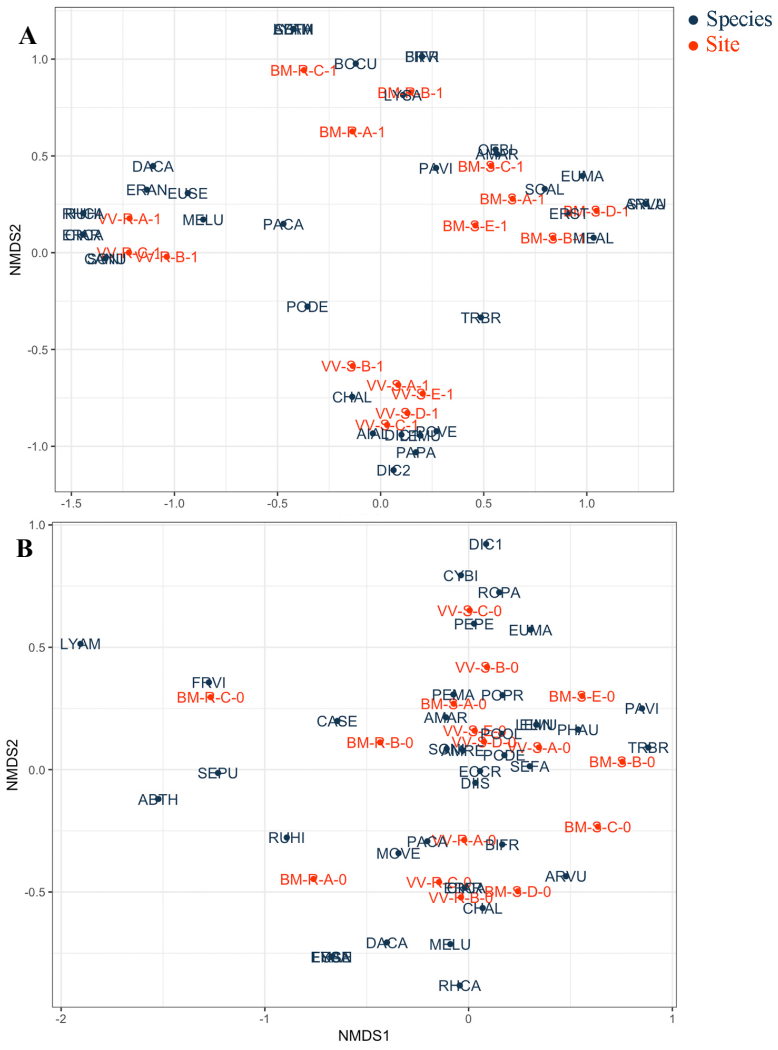

Figure 6. NMDS results based on species presence-absence of germination plots. **A**: removal; **B**: topsoil

removal and topsoil plots or excluded data from topsoil plots. For full species presence-absence data, see **Table S1B**.

α and β Diversity

Removal plots on slag hosted less species at both locales (ANOVA on site effect, $F = 26.18$ and 47.82 for BM and VV, respectively; both $p < 0.01$), although the difference between Slag and Reference did not diverge until day 63 at BM, a pattern similar to "time threshold" reported in site effect of cover (**Figure 3E**, **3G**). In contrast, a distinct site effect could not be observed for topsoil plots (ANOVA on site effect, $F = 2.374$ and 2.921 for BM and VV, respectively; both $p > 0.05$).

β diversity (as Whittaker's β) showed that removal plots on the same site were significantly more similar (**Figure S2**); Slag or Reference plots across locales did not show much similarity. Topsoil plots showed relatively weaker similarity both within and across sites, but a generally higher similarity across plots was observed due to contamination of seeds.

Removal and topsoil plots showed different patterns of species clustering (**Figure 6A**). Removal plots showed four distinctive clusters corresponding to four sites according to species composition in each plot with NMDS. Most species resided in those clusters, indicating their constrained distribution in one type of sites; cottonwood (*Populus deltoides*) and fluxweed (*Trichostema brachiatum*) were present in plots across multiple sites and therefore were not clustered.

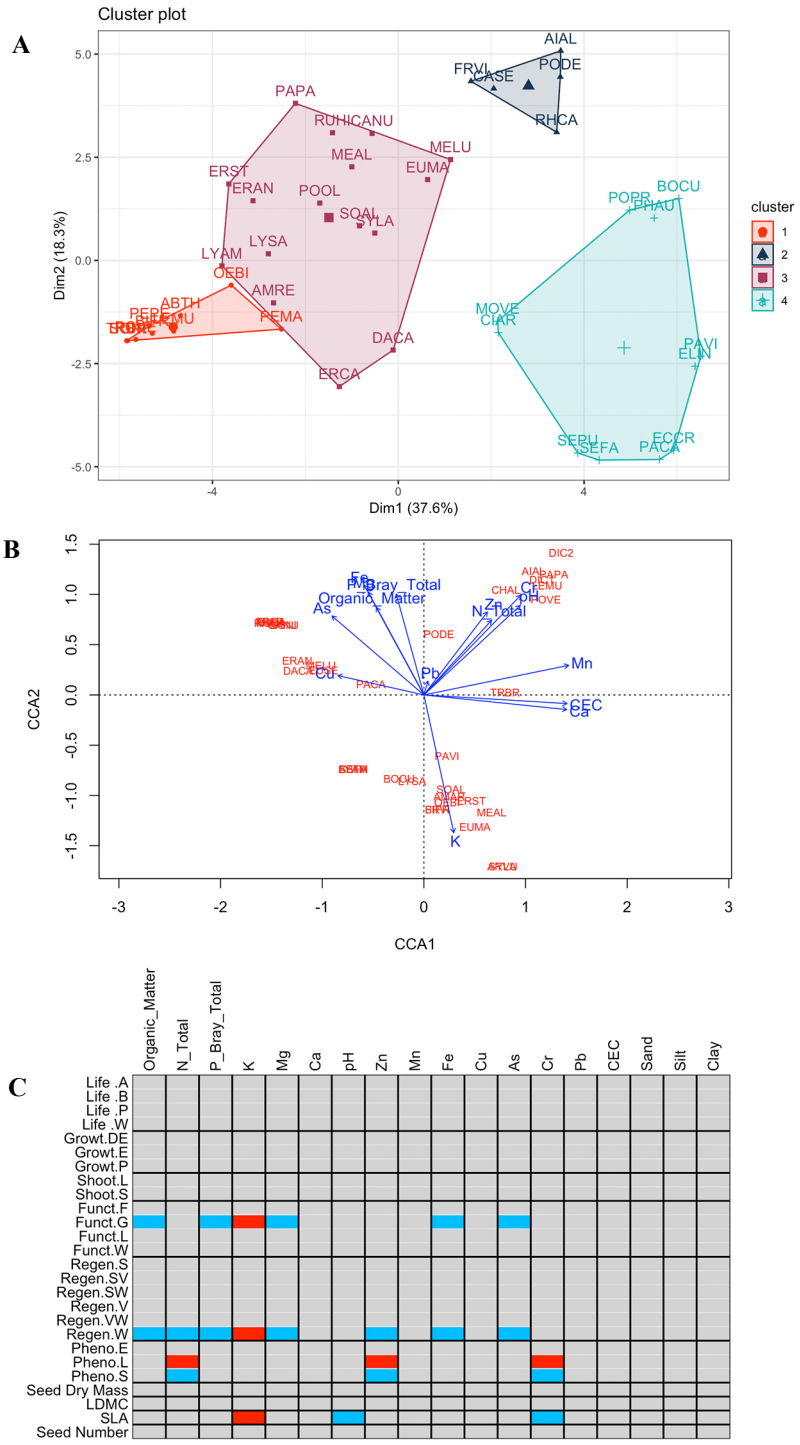

Figure 7. Structure of assembled communities in germination plots. **A**: clustering plot based on functional traits of all species appearing in germination plots. **B**: CCA result showing association between species and environmental variables. **C**: fourth-corner analysis result showing association between functional traits and environmental variables; significant associations are colored, with positive as red and negative as blue. See **Table S1C** for a complete list of species abbreviations; see **Table S3** for complete functional traits data with abbreviations.

Topsoil plots showed a more diffused pattern: plots were not aggregated by site, and species formed one central cluster and diffused in the NMDS space (**Figure 6B**). The diffused pattern remained even when species germinated in topsoil indoor or species only present in topsoil plots were removed (result not shown).

Native Status

Although Reference removal plots had lower species richness, Slag removal plots had significantly higher proportion of native species (Welch's t-test, $p = 0.0357$). This difference is apparent between VV-R and VV-S removal plots (**Figure S3A**). Topsoil plots generally had higher number of nonnative species due to seed contamination, and the difference between Slag and Reference is not significant (Welch's two-sided t-test, $p = 0.3449$; **Figure S3B**). Removal plots on non-slag sites have higher number of native species than topsoil plots on slag sites ($p < 0.001$); species germinated from commercial

topsoil in greenhouse, or species only present in topsoil plots were mostly nonnative (**Figure S3 C-D**, **Table S1B**), suggesting that topsoil was a source of nonnative species.

Species Distribution and Functional Traits

Four clusters, one graminoid, two forbs, and one miscellaneous with shrubs, trees and some forbs (**Figure 7A**), were derived from the distance matrix calculated by *dist.ktab* (see Methods-Species Diversity and Community Composition); clustering analyses based on the two distance matrices both showed robust patterns of clustering with very similar species composition. Mixed-effect PCA did not provide further insight to the specific functional trait composition of those clusters (result not shown): the analysis identified one tightly packed forb and two diffused tree and graminoid clusters. The Hopkins statistic ranged from 0.20 to 0.30, implying that the clustering was valid.

Species Distribution and Environmental Variables

Results from CCA again showed significant clustering for both species and sites (**Figure 7B**). BM plots on both sites were more similar, while VV-S and VV-R were segregated. Similar to the results from NMDS, each cluster of plots had a distinctive collection of species. A model selection with R function *ordistep* from R package *vegan* (Oksansen et al. 2018) using all environmental variables identified K, Mn and N as three most significant variables determining species distribution. Limiting environmental variables to category I (Slag) and category II + III (Reference; see **Table 3**) yielded different results: for Slag, a collection of Ca, pH and Mn explained most of the variations in species distribution; for Reference, the explanatory environmental variables changed to organic matter, N and Mg. Only presence-absence data of removal plots were used for this analysis; therefore, measurements from topsoil was excluded.

Functional Trait Distribution and Environmental Variables

Figure 7C shows the result of fourth-corner analysis; associations with significant *p* values are colored, with red denoting positive and blue, negative associations. Graminoid species (*Funct.G*) and species with widespread seeds (*Regen.W*) generally associated most strongly with the same set of environmental variables: organic matter, P, Mg, Fe, As (negative)

and K (positive). The result could be explained by the categorization of most graminoid species as widespread seed producers. Species flowering in late summer (*Pheno.L*) were positively associated with N, Zn and Cr; the reverse was detected for summer-flowering species (*Pheno.S*). The specific leaf area (SLA) was negatively associated with pH and Cr and positively associated with K. Although some individual associations were significant, the overall association was not significant as determined by the $S_{\mathbf{RLQ}}$ statistic, with $p > 0.50$. The analysis used data from removal plots only; because this analysis requires higher data integrity, seed bank longevity and lateral spread were excluded from this analysis because of low availability of data. See **Table S3** for complete functional traits data.

Discussion

Understanding plant communities on slag and other uniquely urban habitats is valuable to both ecological theory and conservation practice. Fragmented urban habitats with unique environmental conditions provide ecologists with great opportunities of studying environmental filtering, metacommunities, plant invasion and rapid local adaptation to urban environment (Swan et al. 2011); community composition and growth of self-assembled vegetation on contaminated sites would be informative to conservation methods such as phytoremediation (Lundholm and Richardson 2010, Reddy and Amaya-Santos 2017, Řehounková and Prach 2007). By manipulative experiments and functional trait analysis, I characterized spontaneous plant communities on slag from two perspectives: 1) how environmental variables affect plant growth and subsequently succession, and 2) how environmental variables affect plant community assembly. Results indicate that plant growth was significantly hindered by slag soil, and community composition on slag did not represent communities on non-slag soil of close proximity. However, the heterogeneity between slag sites and blocks within each slag site contributed significantly to the results. Block effect was significant for both cover and biomass, indicating strong effect of within-site heterogeneity on plant growth. Difference in community composition between BM-S and VV-S also suggested significant difference between Slag sites. Given these data and general info on land use histories, BM-S and VV-S should not be regarded as replicates, but rather two distinctive sites. Better characterization of heterogeneity within each site would require more extensive soil sampling.

Effect of Slag on Plant Growth

Plant growth, measured by both percent cover and biomass, was negatively affected by slag soil. Overall, site effect between Slag and Reference was significant for all measurements of plant growth, agreeing with observation and previous studies (Reddy and Amaya-Santos 2017). Specifically, the low cover in untreated germination plots directly indicated the slow recovery of plant communities on slag, even considering the effect of less species (**Figure 3**, 4). Combining these results with previous studies that discovered arrested primary succession on natural habitats with similar soil profile, it is tempting to infer that with such low growth rate, slag vegetation might also experience arrested, or at least delayed primary succession. Linear models showed that environmental variables characterizing slag (category I in **Table 2**) negatively correlated with plant growth (**Table S6**). Although the robustness of this result was significantly undermined by the small number of soil measurements, the effect was strikingly consistent among all measurements of growth. Clear evidence of Cr inhibiting plant growth in terms of root elongation has been identified in a lab setting (Peralta 2001), and high alkalinity and high calcium concentration also negatively affect water retention and absorption of essential nutrients such as N and P (Russell 1950). However, the linear regression results are not necessarily generalizable and causative. For instance, the association between As and plant growth was positive due to a high As content in commercial topsoil, which had higher growth due to higher organic matter, N and P contents. Other factors may also bias the result: a shallow soil layer might also cause bonsai effect (plants with limited root space are generally smaller), in addition to soil chemical properties (Passioura 2002). This part of analysis would benefit greatly from an increased number of soil samples.

Plant colonization (germination and early growth) was not affected by slag soil. Cover and species number did not show significant site effect until after early stage of experiment, at day 49, 56 or 63 (**Figure 3**, **Table S4A-B**). Generally, there was a "time threshold" before which no site effect could be observed. Monitoring photos confirmed this "time threshold": before day 49-63, plots on both slag and non-slag soil were occupied by similar amount of growth. These results indicate that the germination or recruitment of plants on unoccupied habitat was not influenced by soil composition. Previous literature has equivocal evidence on the inhibitory effect of heavy metal ions on seed germination and seedling growth (Xiong 1998, Peralta et al.

2001, Li et al. 2005, Di Salvatore et al. 2008, Sethy et al. 2013). Among heavy metals measured in soil tests, Cr, Cu and Pb have been identified as having negative effects on both germination and seedling growth (Xiong 1997, Peralta et al. 2001, Li et al. 2005), but Zn has not shown significant effect on either seed germination or both processes (Li et al. 2005, Peralta et al. 2001). Nevertheless, all those studies were conducted in a lab setting with one or a few species. Environmental conditions in the field could be more complex, and species on slag might be more tolerant to heavy metal contamination because of previous exposure.

A significant dip in BM-S cover was observed for both untreated and topsoil plots (**Figure 3A**, **3B**); SS individuals planted on BM-S also suffered a mortality of more than 50% (**Figure 5C**). This was likely caused by a heat wave between two monitoring sessions on Aug 4, with 36.1 ºC being the highest temperature during experiment. Since heat stress leads to increased evapotranspiration and subsequently elevated water loss, a speculation could be that the high sand content at BM-S has lower water retention rate, resulting in more severe desiccation (Cowles 1899). An alternative, non-exclusive explanation might be the lack of shading, since vegetation on BM-S was the sparsest among four sites. This would indicate a potential effect of positive density dependence, which has been described in many contexts as highly variable (Antonovics and Levin 1980, Goldberg et al. 2001). However, none of other sites experienced such dramatic mortality caused by the heat wave, suggesting other possible explanations, including the significantly different environmental conditions between Slag sites.

Slag Effect on Community Structure

Collectively, Slag and Reference sites from both locales fit under the metacommunity framework proposed by Leibold et al. (2004); each block could be approximated as heterogeneous patch, and species present in each patch are determined by specific environmental factors. This species sorting paradigm corresponds to the so-called environmental filtering effect: that the environmental conditions of habitats shape distribution of species (Kraft et al. 2015, Cadotte and Tucker 2017). Theories on environmental filtering predict that environmental factors should strongly shape community composition by selecting for species with traits that confer higher fitness, resulting in clustering of those traits (Weiher et al. 1998, Fukami et al. 2005, Kraft et al. 2008). Studies have shown that this clustering effect is more visible on functional traits than species (Fukami et al. 2005), and at larger than smaller

spatial scale (Kraft et al. 2008, Cornwell and Ackerly 2009, Bello et al. 2013). According to these predictions, either species composition or functional traits, or both, should be clustered due to the strong filtering effect of slag.

Although species compositions of untreated plots were more similar within each site, general similarity between Slag sites was not detected (**Figure 6A**, **S2A**). This observation is partly explained by CCA result (**Figure 7B**), which showed significantly different soil compositions between the two Slag sites. This effect due to locale difference potentially selected for different assemblages in untreated plots. The clustering was more obvious in functional traits (**Figure 7A**, **7C**): Graminoid species (grasses, sedges) aggregated with high K content, which characterizes BM-S in CCA; late-summer flowering species aggregated with high N, Zn and Cr content, all of which characterize VV-S. More strikingly, SLA corresponds positively with K and negatively with pH and Cr, meaning that species with high SLA clustered at BM-S. Graminoids are iconic early-successional species (Cowles 1899, Clements 1916), and a high SLA implies high investment in primary production, often a characteristic of fast growing, early successional species (Kazakou et al. 2006). Therefore, BM-S generally hosted more early-successional species than VV-S. Late flowering is generally associated with either competitive or ruderal plant species (Grime 1977) and has been shown to associate with higher relative reproductive success (Molau 1993); therefore, plants with high competitiveness and rapid spread via seed generally aggregated on VV-S. The reason that two slag sites display different aggregations of traits is unclear. Despite this difference, plants colonizing untreated Slag plots were generally early successional species with high growth rates, consistent with the prediction. Compared to Slag plots, Reference plot species are not characterized by early-successional traits. One possibility is that regenerations from the seed bank largely dominated the recolonized community, making the latter less representative of an early successional community.

However, it is worth noting that functional trait data analyzed were obtained from a database, not *in situ*. Therefore, failure of detecting strong evidence of environmental filtering might be due to changes of plant functional traits on slag. For instance, results from experiments have shown significantly slower growth of species on slag for both early-successional species in untreated germination plots and late-successional species planted in focal species plots. Therefore, traits associated with production, such as SLA and LDMC might

change due to low growth rate and would not be accurately captured by measurements from online database. Long term monitoring would also provide more information on community assembly, persistence and resilience.

An underlying assumption of the experimental design is that there is no dispersal barrier between the two locales or sites within each locale. Although this assumption is generally satisfied by the close proximity of sites, it has not been experimentally tested. Studies have argued that dispersal is important to environmental filtering (Kraft et al. 2015, Germain et al. 2017): The correspondence between effect would be greatly undermined if other factors, such as dispersal barriers, contribute to the different compositions of subcommunities. Therefore, to confirm the effect of environmental filtering, further experiments should be conducted to exclude possible species sorting by dispersal barriers.

Implications for Conservation

The succession status of slag sites could be important to conservation and management practices. Although some plants may successfully uptake contaminants, phytoremediation has not obtained satisfactory results due to low survival (Reddy and Amaya-Santos 2017). On the other hand, studies have shown that industrial ecosystems such as sand-gravel pits, alkaline waste and limestone quarry floor have been successfully colonized with species suitable for these habitats, displaying normal succession processes (Ash et al. 1994, Řehounková and Prach 2008, Tomlinson et al. 2008, Lundholm and Richardson 2010). One implication from such results is that these industrial sites are able to provide habitat for natural succession process without remediations such as seeding or topsoil capping: Smith et al. (1997) predicted that a slag dump from 1918 would have accumulated enough organic material to support pine forest in 75 years; Řehounková and Prach (2008) predicted that 25 years of natural succession would restore gravel-sand pits aged 1-75 years back to grassland, woodland or wetland. However, these conclusions are derived from specific conditions, that the site contains enough organic material (Smith et al. 1997), that recovery of community is defined by resemblance of species to the target climax community (Řehounková and Prach 2008), or that successful establishment of several species can start succession (Ash et al. 1994); none of them explicitly addressed the slow growth rate of recolonized species and its potential effect on soil formation, highlighted by Start et al. (2004).

Although many species successfully colonized slag, it is not guaranteed that the community would proceed further along a successional trajectory. Empirical evidence from both germination and focal species experiment suggests that species on slag developed much slower than on non-slag soil; slag plots were colonized by less species and accumulated less biomass. Given current evidence and conclusion by Start et al. (2004), the succession process on slag would be slow. Even if slag vegetation could reach its "climax" community in 25-75 years, outlined by previous studies, the time scale would be too large for urban conservation, especially the remediation of contaminated soil that would raise public health concerns. Furthermore, the goal of restoration for slag sites is hard to define. Previous studies compared industrial sites to the surrounding natural communities, such as pine forest (Smith et al. 1997), grassland, woodland or wetland (Řehounková and Prach 2008). Embedded in the urban matrix, the "original" natural community of slag sites is not readily identifiable, and rehabilitation of slag to its "original natural state" (climax community; e.g. tallgrass prairie) would be extremely difficult. Moreover, topsoil used to cap contaminated sites generally contain seeds of nonnative, weedy species. Slag plots capped by topsoil had more nonnative species than non-slag plots without topsoil (**Figure S3B-D**, **Table S1B**), indicating that topsoil could be a significant source of nonnative species, risking of introducing new invasive species by restoration practices.

Nevertheless, unique urban and industrial habitats might serve as plant refuges, providing an alternative concept of restoring slag habitats. Because of its similarities to naturally occurring habitats such as alvar or dolomite prairie, slag sites have the potential of hosting rare plants that could only inhabit such environments (Lundholm and Richardson 2010). For instance, Tomlinson et al. (2008) found significant overlaps between flora of alvar habitat and artificial quarry floor through natural colonization. Furthermore, species that are adapted to natural habitats with high metal content, such as serpentine soil or mining sites, could establish in industrial ecosystems (Cooke and Johnson 2002, Whiting et al. 2004, Lundholm and Richardson 2010). Inhabitable by many competitors, habitats such as slag could provide a refuge for those rare species. Relatively high proportion of native flora and many species of very high conservation index were identified at both BM-S and VV-S; more strikingly, the latter has high adjusted FQI, suggesting habitat quality comparable to natural habitats. Depressions on VV-S hold water during rainy season, supporting many native fen and marsh

species such as *Carex crawei* and *Eleocharis elliptica*; native plants with high conservation values planted on VV-S, *Bouteloua curtipendula* and *Solidago speciosa*, both displayed 100% survival. These results suggest that VV-S has the potential as an urban refuge of rare plants, especially for native species on similar habitats such as wetlands and dolomite prairie. The restoration would be more practical if the target habitat is natural analogs of slag sites, such as alvar or dolomite prairie (Tomlinson et al. 2008, Lundholm and Richardson 2010). Those habitats formed by limestone outcrops are native to Midwest, hosting many species that are particularly adapted to their environments (Baskin and Baskin 2000, Corbett and Anderson 2006). Given the similarity of environmental conditions and many overlapping species such as *Carex spp*., *Eupatorium spp*. and *Panicum virgatum*, VV-S could be restored as these natural habitats.

Conclusion

Although slag has been viewed as a contaminated wasteland, it is a unique urban-industrial ecosystem that has the potential of plant refuge. By plant surveys and manipulative experiments, I have shown that unfavorable environmental conditions significantly lowered the growth and recovery rates of slag communities in terms of percentage cover, biomass and number of recolonizing species. The composition of slag communities generally corresponds to early successional communities, but the low growth rate may significantly reduce the rate of succession. Unlike many other industrial systems, natural recovery of slag sites might not be feasible in short term, requiring active restoration effort. While topsoil capping might be the most effective method of increasing organic matter, it risks introducing nonnative species, further lowering habitat quality. Although challenging for restoration and remediation, slag could potentially host rare plants from natural, analogous habitats, such as flora found at dolomite prairie, alvar and limestone outcrop habitats, all of which native to Midwest. Therefore, in addition to "radical" remediation such as topsoil capping, burning and intensive weeding of undesirable species, further restoration should pay attention to building slag sites as refuges of native plants, conserving regional biodiversity.

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