

Phytostabilization of Mine Tailings Using Compost-Assisted Direct Planting: Translating Greenhouse Results to the Field

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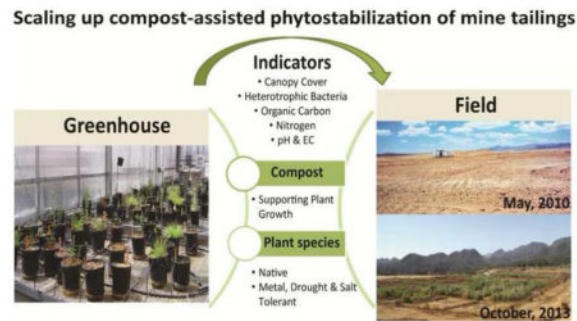
Abstract

Standard practice in reclamation of mine tailings is the emplacement of a 15 to 90 cm soil/gravel/rock cap which is then hydro-seeded. In this study we investigate compost-assisted direct planting phytostabilization technology as an alternative to standard cap and plant practices. In phytostabilization the goal is to establish a vegetative cap using native plants that stabilize metals in the root zone with little to no shoot accumulation. The study site is a barren 62-hectare tailings pile characterized by extremely acidic pH as well as lead, arsenic, and zinc each exceeding 2000 g kg⁻¹. The study objective is to evaluate whether successful greenhouse phytostabilization results are scalable to the field. In May 2010, a 0.27 hectare study area was established on the Iron King Mine and Humboldt Smelter Superfund (IKMHSS) site with six irrigated treatments; tailings amended with 10, 15, or 20% (w/w) compost seeded with a mix of native plants (*buffalo grass*, *arizona fescue*, *quailbush*, *mesquite*, and *catclaw acacia*) and controls including composted (15 and 20%) unseeded treatments and an uncomposted unseeded treatment. Canopy cover ranging from 21 to 61% developed after 41 months in the compost-amended planted treatments, a canopy cover similar to that found in the surrounding region. No plants grew on unamended tailings. Neutrophilic heterotrophic bacterial counts were 1.5 to 4 orders of magnitude higher after 41 months in planted versus unamended control plots. Shoot tissue accumulation of various metal(oids) was at or below Domestic Animal Toxicity Limits, with some plant specific exceptions in treatments receiving less compost. Parameters including % canopy cover, neutrophilic heterotrophic bacteria counts, and shoot uptake of metal(oids) are promising criteria to use in evaluating reclamation success. In summary, compost amendment and seeding, guided by preliminary greenhouse studies, allowed plant establishment and sustained growth over four years demonstrating feasibility for this phytostabilization technology.

Graphical Abstract

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Keywords

Mine tailings; phytoremediation; phytostabilization; direct planting; fertility islands

1.0 Introduction

Mine tailings are the main product remaining after ore beneficiation and, if left unreclaimed, can contribute to particulate dispersion into the surrounding environment (Csavina et al., 2011; Mendez and Maier, 2008; Root et al., 2015). In legacy sites, mine tailings particulates often have associated metal(loid) contaminants because extraction technologies 50 – 100 years ago were not as efficient as those used in modern mining operations. Human health risks arising from dispersion of metal(loid)-containing particulates from legacy sites can result from various routes of exposure including inhalation of particles transported by wind and ingestion of contaminated soil (particularly for children) or food due to the deposition of wind- or water-borne particles onto soil or garden vegetables (Csavina et al., 2011; Henry et al., 2013; Mendez and Maier, 2008; Ramirez-Andreotta et al., 2013).

The US Environmental Protection Agency (EPA) has estimated that remediation costs for National Priority List (NPL) hardrock mining sites will exceed US \$7.8 billion for 63 NPL sites inventoried in 2004 with an additional US \$16.5 billion needed for future sites using current technologies (Lovingood et al., 2004). The most commonly used technologies are based on constructing an inert or biological cap over the mine tailings (ITRC, 2009). The goal is to have germination and establishment of a vegetative cap followed by plant succession eventually leading to a stable vegetative community on the site. However, such capping strategies can be very expensive (Kempton et al., 2010).

An alternative technology to capping is phytostabilization, which is the use of a vegetation cover planted directly into the tailings that acts to immobilize metals in the rhizosphere and to reduce above ground wind and water erosion processes (Mendez and Maier, 2008; USAEC, 2014). However, direct planting alone is not feasible for many legacy sites because acidic conditions and high metal(loid) content prevent plant germination and growth. A further complication is the need for drought-tolerant plant species which are generally adapted to the alkaline conditions found in most arid environments (Saslis-Lagoudakis et al., 2015). Therefore the phytostabilization process often must be “assisted” through the addition of amendments that may include compost, biosolids, lime, and/or fertilizers (Brown

et al., 2004; Clemente et al., 2012; Conesa et al., 2007; Lee et al., 2014; Li and Huang, 2015; Madejón et al., 2010; Santibañez et al., 2012). The vegetative cap created by assisted phytostabilization should result in the phyto-catalyzed stabilization of inorganic contaminants in the root zone driven by organic matter, plant root exudates and the associated rhizosphere microbial community (Mendez and Maier, 2008; Santibañez et al., 2012). Further, there should be limited above ground biomass accumulation of metal(loid)s to prevent the movement of contaminants into the surrounding ecosystem and food chain through grazing or plant death and decay (Henry et al., 2013; Mendez and Maier, 2008; Pérez-de-Mora et al., 2011).

There are few reported field studies that have evaluated the feasibility of assisted phytostabilization of mine tailings in semi-arid environments (Brown et al., 2009; Clemente et al., 2012; Cordova et al., 2011; Pardo et al., 2014; Santibañez et al., 2012). The goal of this study was to determine whether assisted phytostabilization could be successfully implemented at the Iron King Mine and Humboldt Smelter Superfund (IKMHSS) site which has mine tailings that are characterized by extreme acidity and high levels of arsenic, lead, and zinc (Root et al., 2015). We specifically wanted to determine whether successful results from greenhouse trials (Solís-Dominguez et al., 2012) could be scaled to the field. The parameters evaluated in the field trial included percent canopy cover, plant shoot tissue metal(loid) uptake, neutrophilic heterotrophic bacterial counts (NHC), pH, total carbon (TC), total organic carbon (TOC), total nitrogen (TN), electrical conductivity (EC). These parameters were used to assess progress in transitioning the original mine tailings ecosystem to include soil properties more characteristic of a plant-sustaining matrix.

2. Materials and methods

2.1 Site description

The IKMHSS was active from the late 1800s until 1969 producing gold, silver, copper, lead, and zinc, leaving behind a mine tailings pile comprising approximately 62 hectares adjacent to the town of Dewey-Humboldt, Arizona (North 34°31'57", West 112°15'9") (USEPA, 2010)(Fig. 1). The top of the mine tailings pile is at an elevation of 1464 m and the surface of the site is an orange gossan zone that is vulnerable to erosion (Hayes et al., 2014; USEPA, 2010). The surficial tailings are characterized by low pH and nutrient content and elevated concentrations of a range of metal(loids) including arsenic, lead, copper, cadmium, chromium, and zinc as well as pyrite (Tables 1 – 3). In contrast, the surrounding area is a Chaparral biome influenced by three ephemeral waterways with *Balon gravelly sandy clay loam* (BgD) as the predominant soil type. Vegetation in the area is dominated by rubber rabbitbrush (*Ericameria nauseosa*), shrub live oak (*Quercus turbinella*), and broom snakeweed (*Gutierrezia sarothrae*) among other plants. White willow (*Salix spp*), Arizona walnut (*Juglans major*), and cottonwood (*Populus fremontii*) are present in riparian areas (USEPA, 2009).

2.2 Site preparation

In May 2010, a compost-assisted phytostabilization trial was established on a 0.27 hectare area on the IKMHSS mine tailings. The six treatments tested (with four replicates each)

were: (1) unamended control; (2) 10% compost - seeded with buffalo grass and mesquite; (3 & 4) 15% and 20% compost - unseeded; (5 & 6) 15% and 20% compost seeded with a mixture of six native plants. All treatments were laid out in a randomized block design with the exception of the controls which were located at the far corners of the study area (Fig. 1, controls are labeled with the number 1). This was done to prevent contamination of the control plots with compost during site preparation and tilling of compost into the subsurface.

A tractor was used to rip and till the site to a depth of about 38 cm and divided into 24 experimental plots (9.6 m × 15 m per plot) each bermed to about 50 cm to prevent cross contamination between treatments. A dairy manure-green waste compost from Arizona Dairy Compost LLC (Anthem, AZ) was weighed using a truck scale and added to each plot according to treatment: the 10% compost treatment received 228 t ha⁻¹; 15% compost treatments received 342 t ha⁻¹; and 20% compost treatments received 456 t ha⁻¹. The compost was tilled into each plot to a depth of 15 cm.

Plots were then seeded according to treatment based on preliminary greenhouse results (Solís-Dominguez et al., 2012). The six native desert plants used in this study and their seeding rates were: grasses, 90 kg ha⁻¹ buffalo grass (*Buchloe dactyloides*); 56 kg ha⁻¹ arizona fescue (*Festuca arizonica*); shrubs, 56 kg ha⁻¹ quail bush (*Atriplex lentiformis*), 11 kg ha⁻¹ mountain mahogany (*Cercocarpus montanus*); trees, 0.15 kg ha⁻¹ mesquite (*Prosopis juliflora*), and 1 kg ha⁻¹ catclaw acacia (*Acacia greggi*) (seed source: Desert Nursery, Phoenix, AZ). The 10% compost treatment, considered a suboptimal rate (Solís-Dominguez et al., 2012), received only seeds from the two plants that grew most successfully in the greenhouse as measured by biomass production (buffalo grass and mesquite). The 15 and 20% compost treatments received a mixture of the six seeds, all of which grew in the greenhouse, to represent a range of plant canopy covers and rooting patterns (e.g. grasses, shrubs, and trees). Buffalo grass, arizona fescue, quail bush, and mountain mahogany were broadcast by hand and raked into the tailings. Straw was scattered over the plot surface at a rate of 6 t ha⁻¹ and crimped 10 cm deep into the tailings surface to decreased compaction by irrigation, seeds predation by birds, dispersion by wind, and water evaporation from the substrate. Large seed species (mesquite and acacia) were soaked in water for 24 h and then planted by hand at a depth of 2.5 cm along seed lines that were 30.5 cm apart alternating the two species. Seeds and straw were applied at night to avoid losses due to high daytime winds.

A sprinkler irrigation system was installed to supplement rainfall throughout the growing season (Table 4). Plots were irrigated every 7 to 10 d depending on the observed plant status and monitored weather conditions. Due to limited availability and reliability of the water source irrigation applications were limited to between 0.5 and 1.0 inches of water. During periods of appreciable rainfall irrigation was postponed. Irrigation was suspended when visual signs of winter senescence were observed. Irrigation was reinitiated the following spring once temperature below freezing had ceased and signs of spring growth were observed. A fence was built around the study area to avoid additional stress of wildlife grazing. In July 2010, an on-site wireless Vantage Pro™ 2 Plus weather station (Davis Instruments Corp, Hayward, CA) was placed in the center of the study area to monitor major

weather variables including air temperature, relative humidity, wind speed and direction, rainfall, and soil temperature.

2.3 Sampling and analysis

To evaluate the immediate effect of compost in tailings, triplicate 100 g surface samples were troweled into plastic soil bags from each plot at a depth of 0–8 cm at the initiation of the study (May 2010). Samples for physical and chemical characterization were subsequently air-dried and sieved to a 2 mm mesh and stored at room temperature. The triplicate samples from each plot were combined to create a composite plot sample for each time point. Samples were analyzed for pH and EC from aqueous solutions of a 1:2 mass ratio of tailings in 18.2 M Ω deionized (Milli-Q, Barnstead) water reacted for 1 h. Twenty grams of the composite samples were finely ground according to McGee et al., (1999) and analyzed for TC, total inorganic carbon (TIC), TOC, and TN (Shimadzu TOC-VCSH analyzer with solid state module SSM-5000A, Columbia, MD). Detection limits were determined separately for each batch of samples collected in May and October. The range of detection limits for TC analysis was from 0.03 to 0.135 g kg⁻¹ dry tailings, from 0.45 to 0.1 g kg⁻¹ for TIC, and from 0.0020 to 0.016 g kg⁻¹ for TN. Total elemental analysis of compost and tailings was by ICP-MS (ELAN DRC-II, Perkin Elmer, Shelton, CT) and detailed in Hayes et al. (2014). Analyses were performed at the University of Arizona Laboratory for Emerging Contaminants (ALEC, <http://www.alec.arizona.edu/>). Plant available metal(loid)s in the tailings is operationally defined here as the sum of a two-step sequential extraction: 1) 18.2 M Ω lab pure water (Milli-Q) and 2) 1 M NaH₂PO₄ at 1:100 solid to solution ratio (n=4) with detection after filtration (0.45 μ m GHP, Acrodisc) as above by ICP-MS.

2.4 Canopy cover and changes in edaphic factors by assisted direct planting

Canopy cover and species composition were estimated on a yearly basis beginning in October 2010 using transect and quadrat sampling (Lutes et al., 2006; Swanson, 2006). Observations were made within a 1 m² quadrat frame placed at 3 m increments along two 15 m diagonal transects across each plot (Coulloudon, 1999; Elzinga et al., 1998). Four additional observations were made at random locations within the plot. At the same time, a second annual core was randomly collected from each plot using a 2" soil probe (AMS Inc., American falls, ID) to a depth of 22 cm, the auger was carefully cleaned between samples. Sub-samples were collected from the auger to characterize depths of 8, 15, and 22 cm. These samples were treated as describe previously (e.g. sieved, dried, stored at 4°C) and analyzed for TC, TIC, TOC, and TN, pH and EC to evaluate changes in edaphic factors by assisted direct planting.

2.5 Neutrophilic heterotrophic bacteria

Triplicate 1 m cores were collected from each treatment plot on an annual basis during the spring/summer months (May–June) using a JMC ESP plus soil core sampling kit (Clements Associates Inc., Newton IA) with butyrate plastic liners (91.4 cm \times 3.18 cm). Each liner was capped then sealed with vinyl tape at both ends to minimize post collection oxidation and immediately placed on ice for transport back to the laboratory. Cores were processed upon arrival at the lab by cutting and removing the top 0–20 cm of each core, with the remaining

71 cm stored at -4°C for subsequent analyses. Tailings from 0–20 cm were removed from the core liner sleeve and homogenized. A composite of each treatment plot was generated by combining 10 g of homogenized 0–20 cm tailings from each of the triplicates and analyzed immediately for Neutrophilic heterotrophic bacteria (NHC). The remaining sample was split and archived at -80°C and -20°C .

One gram of the composited core samples from the top 0–20 cm was placed in a tube containing 9.5 ml of sterile distilled water and vortexed for 2 min to determine NHC. Serial dilutions were performed and 0.1 ml from each was plated in triplicate on R2A agar (Bacton Dickenson and Company) with 200 mg L^{-1} of cyclohexamide (to suppress fungal growth). All plates were incubated for 5 days at 23°C and then enumerated. All NHC are reported as colony forming units (CFU) per gram dry weight of sample (Solís-Dominguez et al., 2012).

2.6 Metal(loid) uptake into plant tissue

Plant shoot tissue from the dominant plant species in each plot was collected in October of each year to assess uptake of metal(loid)s. Shoot tissue samples were washed with a 0.1% HCl solution and dried on a Blue M force air oven (Thermal Product Solutions, New Columbia, PA) at 65°C . Samples were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ), passed through a 40-mesh (0.42 mm) screen, and microwave digested (MARS6, CEM Corp., Matthews, NC) using USEPA method 3052 for total element concentrations of As, Pb, Zn, Cd, Cu, and Ni (USEPA, 1996). Quality controls for the digestion included: sample duplication; digestion blank controls with: distilled water, HNO_3 , and hydrogen peroxide; and digestion of a Standard Reference Material NIST 1573a (tomato leaves) as an external quality control (Ramirez-Andreotta et al., 2013). Samples were analyzed at ALEC by ELAN DRC-II ICP-MS (Perkin Elmer, Shelton, CT) using at least one quality control solution from a second source, e.g., NIST 1643e Trace Metals in water.

2.7 Acid potential

The acid potential (AP) of tailings samples was determined based on the pyritic fraction of the tailings (Sobek, 1978). The pyrite fraction of the surface tailings was determined by Rietveld refinement of Synchrotron transmission powder x-ray diffraction (ST-XRD) as described previously (Hayes et al., 2014). While Rietveld XRD may not always detect minor phases ($<5\text{ wt } \%$), pyrite has strong and unique reflections that could be fit to about 0.3 wt %. The pyrite fraction (% pyt) in the tailings was used to calculate the AP, where $\text{AP} = \% \text{pyt} \times 16.7$; as kg acid equivalents per metric ton tailings expressed in mass equivalents of CaCO_3 neutralizing capacity of 2 moles CaCO_3 ($\text{MW} = 2 \times 100.087$) to 1 mole of FeS_2 ($\text{MW} = 119.975$) to kg CaCO_3 equivalent per ton of material (Parker and Robertson, 1999). The range of AP for the tailings samples were 0 to 154 kg acid equivalents per ton tailings with an average of 48.0 kg acid equivalents per ton tailings for the non-control samples.

2.8 Statistical Analysis

Normality of the data were analyzed using goodness of fit test, and verified by using residual/predicted plots. When data were highly differing from normal distribution, they were log transformed. Significant differences over time for canopy cover, NHC, TOC, TN, pH and EC were detected by employing one-way ANOVA ($p < 0.05$) by treatment and

significant differences between means were determined by Tukey's test ($p < 0.05$). Metal(loid) accumulation in shoot tissue data were square-root transformed to reduce the influence of outliers before performing a one-tail Paired t-Test ($p < 0.05$) to evaluate the increase of metal(loid) concentration in plant shoot tissue with time. In order to evaluate the effect of rate of compost with time in the accumulation of metal(loid)s in leaves, a 2-way Anova ($p < 0.05$) was performed. Principal Component Analysis (PCA) was used to compare changes in the treatments based on geochemical parameters evaluated in this study. In order to reduce influences from outliers and different scales data was square-root transformed before performing the PCA. All analyses were conducted using JMP®, Version 11.0. (SAS Institute Inc., Cary, NC, 1989–2007).

3.0 Results

3.1 Tailings characteristics before and after compost amendment

The IKMHSS surface tailings have a loam texture, 34.7% sand, 44.8% silt, and 20.4% clay, and are comprised dominantly of quartz, albite, pyrite, gypsum, jarosite, plumbojarosite, and goethite or ferrihydrite (Solís-Dominguez et al., 2012). Selected properties including pH, EC, TC, TOC, TN, NHC, metal(loid), and pyrite content of the compost, the tailings (both unamended and compost-amended), and an off-site soil sample are provided in Tables 1–3.

3.2 Plant germination, growth, and canopy cover

The control treatment which was irrigated but did not receive either compost amendment or seeds remained barren of plants for the duration of this study. In contrast, all treatments receiving compost amendment and seeds showed germination and plant growth achieving a 30 to 39% canopy cover within the first five months (Fig. 2). This was equivalent to the canopy cover measured in the off-site surrounding area (dashed line on Fig. 2). Canopy cover was subsequently measured yearly for three years and showed a trending but not significant decline in the 10% compost treatment, no significant change in the 15% compost treatment, and a significant increase to 61% canopy cover in the 20% compost treatment.

Treatments that received compost but no seeds showed less than 6% canopy cover after 5 months but the canopy cover increased significantly over the subsequent three years reaching 21 and 36% in the 15 and 20% compost treatments, respectively. This was likely a result of seed deposition following blooming and seeding of quailbush from the neighboring seeded plots as well as from volunteer species from off-site. Quailbush plants in these plots were visually much larger and more spatially separated than those in the composted seeded plots (Fig. 3).

Germination and growth of the six plant types tested varied considerably in this field trial. Despite showing success in greenhouse studies, three of the six seed types applied (mountain mahogany, mesquite, and catclaw acacia) did not establish at the site. A fourth plant, Arizona fescue showed a small amount of growth in 2010 (5.8% of the plant composition for the 15% compost + seeds treatment and 2.2% for the 20% compost + seeds treatment). However, the percentage declined substantially by 2011 and the plant was not observed in 2012 or beyond.

For the 10% compost-amended treatment plots that received only buffalo grass and mesquite seeds, species composition was dominated by buffalo grass, ranging from 97.2% (2010) to 84.2% (2013) with the remainder represented by annual weeds that were not in the original seed mix.

Quailbush dominated in the 15 and 20% compost-amended seeded treatments. In 2010, the average quailbush composition in the 15% and 20% seeded treatments was 78.5%, buffalo grass composition averaged 17.4% and the remainder was annual weeds. Between 2010 and 2013, quailbush declined to an average of 47.8% while buffalo grass was maintained at 25.2%, and there was an increase in annual weeds. Blooming and seeding of quailbush and buffalo grass first occurred in 2011 and was observed on a yearly basis thereafter.

3.3 Accumulation of metal(loid)s into plant shoot tissue

Plant shoot tissues collected from the dominant plants, quailbush and buffalo grass, in the 10, 15, and 20% compost-amended and seeded treatments were analyzed for uptake of metal(loid)s. Results from the 2010 and 2013 samplings show that foliar accumulation generally did not exceed the Domestic Animal Toxicity Limits (DATL NRC, 2005) with some plant specific exceptions (Table 2). The exception for quailbush was that shoot tissue Zn levels were similar to or slightly higher than the DATL in the 15 and 20% compost treatments. For buffalo grass, shoot tissue As exceeded the DATL by 2-fold in the 10% compost-amended treatment after three years and Cu levels for all treatments were similar to the DATL.

The amount of compost added had an impact on whether or not there was an increase in shoot tissue metal(loid) concentrations between 2010 and 2013. There was no increase in metal(loid) concentration for the elements examined in 20% compost-amended treatments, there was an increase in two metals, Zn and Ni, in the 15% compost-amended treatments, and there was an increase in four metal(loid)s, As, Pb, Zn, and Ni, in the 10% compost-amended treatments.

3.4 Neutrophilic heterotrophic bacteria

Initial NHC in the unamended tailings were low, 1.43×10^2 CFU g^{-1} dry tailings, and did not change over the duration of the study (Fig. 4). Compost amendment caused an immediate 1 to 3 log increase in NHC to 2.08×10^3 , 7.33×10^4 , and 1.02×10^5 CFU g^{-1} dry tailings for the 10, 15, and 20% compost treatments, respectively. A further increase of 1 to 2 logs occurred at 12 months across all composted treatments. Thereafter, NHC did not change significantly in any treatment except for the 20% compost unseeded treatment which showed a significant 1.5 log decline from 2011 to 2013. For comparison, NHC in two off-site samples that were collected in May 2015 averaged 2.18×10^6 g^{-1} dry tailings, a value similar to the 15 and 20% compost amended treatments and slightly higher than the 10% compost amended treatment (dashed line, Fig. 4).

3.5. Changes in edaphic factors

Pyrite content was measured in 8 cm depth tailings immediately following compost addition in all treatments and controls to estimate acid potential in the tailings. Values for pyrite

content ranged from 1.0 to 4.5 wt% in samples with tailings, with the variability likely due to heterogeneous mixing of upper and lower layers of the tailings during the ripping and tilling process and dilution by compost addition (Table 3). Samples collected at 41 months showed a notable loss of pyrite in all treatments and the control, which was attributed to oxidation (depleted 33% to 89%, average 61.4%). The off-site sample and compost did not contain pyrite.

Due to potential acid production by pyrite oxidation, pH was measured in all treatments. The unamended control samples had an average pH of 2.5 ± 0.1 with no significant difference over time (0 to 41 months). Compost amendment immediately increased the tailings pH by 3–5 log units (Table 1). A regression analysis comparing the increase in pH to the amount of compost added showed a significant positive relationship ($R^2 = 0.959$, $P < 0.205$). Subsequent yearly measurements were made at three depths, 8, 15, and 22 cm corresponding to the well-mixed tailings-compost zone (8 cm), the interface between the well-mixed zone and unamended tailings (15 cm), and unamended deeper tailings (22 cm), respectively. In the well-mixed zone, the pH was maintained for the first 17 months in all treatments (Fig. 5A). Thereafter, from 17 to 41 months, there was a trend of declining pH among all compost-amended treatments. This decline was significant in the 10 and 15% seeded treatments.

There was no time zero measurement of pH at the 15 cm interface between the well-mixed zone (15 cm) and unamended tailings (22 cm). However, there was a trending decline in pH between 17 and 41 months similar to that observed at 8 cm (Fig. 5B). The decline in pH was significant for the 15% seeded and unseeded treatments.

Likewise, there was no time zero measurement of pH at 22 cm which is below the layer of compost amendment. The average pH in this zone among all compost-amended treatments was 2.8 ± 0.4 with no significant difference among treatments for the 41 month duration of the study. This average is significantly higher than the unamended control (2.5 ± 0.1) likely because some organic matter leached down to 22 cm.

Total organic carbon was below detection limits in the unamended tailings (Table 1). At 8 cm, compost addition immediately increased TOC levels to between 54 and 124 g kg^{-1} dry tailings (Fig. 6A). A linear regression analysis comparing TOC to the amount of compost added showed a significant positive relationship ($R^2 = 0.869$, $P < 0.004$). Subsequent measurements of TOC in 2011, 2012, and 2013 showed a downward trend in most cases but values were not significantly different from the initial measurement (Fig. 6A). Despite this downward trend, the TOC values in all of the composted treatments were significantly higher than TOC values in off-site soils (Table 1, Fig. 6A, dashed line).

Total organic carbon at 15 cm (interface between well-mixed zone and unamended tailings) averaged $28.6 \pm 7.6 \text{ g kg}^{-1}$ dry tailings across all time points (17, 29, and 41 months) with no significant differences among treatments (data not shown). TOC at the 22 cm was below detection limits for all compost-amended treatments.

Total nitrogen was below detection limits in unamended tailings (Table 1). At 8 cm depth, compost addition immediately increased TN to 5.0 to 8.6 g kg^{-1} dry tailings with a

significant positive relationship between TN and the amount of compost added ($R^2 = 0.95$, $P = 0.001$). In 2011, TN increased by 10 to 66% in the compost-amended treatments although this increase was only significant for the 15% compost + seeds treatment (Fig 6B). In the two following years, 2012 and 2013, TN levels returned to levels similar to those measured in 2010. Total nitrogen values in all of the composted treatments were significantly higher than TOC values in off-site soils (Table 1, Fig. 6B, dashed line).

Total nitrogen at the 15 cm interface between the well-mixed zone and the unamended zone averaged 3.2 ± 0.9 among all the composted treatments from 2011 to 2013 with little variation. Total nitrogen was below detection limits at the 22 cm for all samples tested.

Unamended tailings had an EC of 8.3 ± 0.7 ms cm^{-1} (Table 1). Compost addition immediately increased the tailings EC to 17 to 27 ms cm^{-1} and there was a significant positive relationship between the amount of compost added and EC ($R^2 = 0.87$, $P = 0.0067$) (Fig. 6C). However, this increase was transient and measurements in subsequent years (2011, 2012, and 2013) showed a decline to an average of 4.2 ± 0.8 among the composted treatments which was significantly less than the EC in the unamended control treatments. The EC at the 15 cm interface between the well-mixed zone and the unamended tailings averaged 5.9 ± 1.5 ms cm^{-1} and at the 22 cm averaged 5.3 ± 1.4 . The EC values in all treatments were significantly higher than those found in off-site soils (Table 1, Fig. 6C, dashed line)

3.6. Principal Component Analysis

A PCA bi-plot was generated to compare the measured soil quality parameters (pH, NHC, TOC, TN, EC) and % canopy cover to the six treatments tested over time (Fig. 7). The first two axes of the PCA together explained 81.0% of the total variation. The first axis explained 60.7% of the total variation and was positively correlated with pH, NHC (as CFU), TOC, and TN and % canopy cover. The second axis, which explained 20.3% of the total variation, was positively correlated with EC. The position of a treatment along the length of a bi-plot arrow indicates the relationship between the treatment and the parameter measured.

The unamended control treatment samples were tightly clustered for all years and were negatively correlated to all parameters measured, except EC. The 2010 samples from the remaining five treatments, which were collected immediately after compost addition, clustered together and were positively correlated with EC, pH, TOC, and TN. In subsequent years (2011, 2012, and 2013), these five treatments remained clustered together with a positive correlation to pH, NHC, TOC, TN, and % canopy cover. But the samples from 2011, 2012, and 2013 formed a separate cluster from the 2010 samples suggesting an influence of plants on these treatments. The composted treatments (all years sampled) were not tightly clustered in comparison to the unamended control treatment samples indicating that there was great heterogeneity in the field after the compost was mixed into the tailings.

4.0 DISCUSSION

The IKMHSS mine tailings studied are considered a “worst case scenario”, being highly acidic, and saline with elevated levels of metal(loid)s. The native tailings have not supported

plant growth for decades. In this 41-month field trial a single application of compost with supplemental irrigation has supported the establishment of a stable vegetative cover that is similar in density to the region surrounding the IKMHSS site. Key to the success of this technology was the combined impact of compost as a conditioner to improve soil quality parameters and to provide a source of organic matter and a microbial inoculum that acted to disrupt the established tailings ecosystem (Mendez et al., 2007; Solís-Dominguez et al., 2012). Two previous greenhouse studies on IKMHSS tailings supported the design of this field trial. The first was a 60-day pot study which showed that NHC and pH increased in composted and planted treatments while bioavailability of metal(oids) decreased (Solís-Dominguez et al., 2012). The second was a 1 year mesocosm study where metal solubilization and acidification was reduced in the presence of healthy plants in composted treatments (Valentín-Vargas et al., 2014).. Both studies showed that prior to the addition of compost, the IKMHSS tailings are dominated by a chemolithoautotrophic sulfur- and iron-oxidizing community that supports an ecosystem characterized by extreme acidity. Compost addition disrupts the established IKMHSS ecosystem by providing an inoculum of nutrient cycling and plant growth promoting bacteria (Pérez-de-Mora et al., 2011; Shi et al., 2011) which, in the present study, increased initial NHC by 1 to 3 orders of magnitude depending on the amount of compost added. The NHC showed a further 10-fold increase after 12 months, a “vegetation effect” (Berg and Steinberger, 2010), which was then maintained for the next two years (Fig. 4). Accompanying the increase in NHC, these previous studies also showed an associated decrease in iron oxidizers (1.6×10^4 MPN g^{-1}) of up to 1.5 orders of magnitude (Solís-Dominguez et al., 2012). It has been suggested that the addition of organic carbon and the concomitant increase in pH from compost addition diminishes chemoautotrophic sulfur oxidizer activity (Johnson and Hallberg, 2008). Additionally, redox micro-environments can develop where decreased fO_2 drives reducing conditions, which can alter the stable mineral phases in the rhizosphere. Reduced micro-environments effect the activity and partitioning of iron and sulfur between aqueous and solid phases, and can limit chemoautotrophic microbial activity, e.g. ferrous iron oxidation (Johnson and Hallberg, 2008). This shift allows for development of carbon and nitrogen cycling activities associated with “healthy” soil processes and diversity in the soil microbial community (Epelde et al., 2010; Mendez et al., 2007; Zornoza et al., 2015).

4.1 Translation of greenhouse results to the field

The IKMHSS field trial design was based on successful results from previous 60-day greenhouse pot studies (Solís-Dominguez et al., 2012). The greenhouse results scaled effectively to the field for several key parameters including the amount of compost required and the amount of metal(loid) accumulation into shoot tissues. The fact that important greenhouse results scaled effectively to the field suggests that greenhouse trials are a useful preliminary step for this technology. Mine tailings characteristics vary quite widely from site to site in terms of mineralogy, pH, and metal content. As such, preliminary greenhouse trials that can precisely determine the minimum amount of compost or other amendments necessary, as well as plants that can be successfully established, would likely enhance the successful implementation of this technology.

We note, however, that individual plant success was one aspect that did not translate exactly from the greenhouse to the field. Some plants that showed promise in the greenhouse trials did not successfully grow in the field. Examining greenhouse results more carefully shows that four of the six plants tested (buffalo grass, mesquite, quailbush, and cataclaw acacia) produced higher amounts of biomass (up to 14 g pot⁻¹) in greenhouse studies while mountain mahogany and arizona fescue produced much lower amounts of biomass (up to 1 g pot⁻¹) (Solís-Dominguez et al., 2012). In contrast, in the field, only buffalo grass and quailbush produced biomass that was sustained over the 3.5 years studied. The difference between greenhouse and field results could be due to competition among the plant species occurring in the field. Competition was not tested in the greenhouse studies, rather plants were tested individually in separate pots. The difference could also be due to a need to optimize seeding rates in the field, something that should be tested in future studies. A final difference to consider is that short term climate controlled greenhouse trials do not account for the effect of on-site year round seasonal climatic effects on plant germination and growth (e.g., large daily temperature variations, freezing autumn and winter temperatures, wind, daily and seasonal humidity changes, storms).

Aside from considerations of competition, the plants with better performance in the field (quailbush and buffalo grass) have two notable attributes that may have helped them survive. Buffalo grass is a C4 photosynthetic plant and quailbush is known for the ability to change from the C3 to C4 pathway in response to salinity and temperature (Srivastava et al., 2012; Zhu and Meinzer, 1999). Plants with C4 photosynthesis are common in hot and arid environments, with more efficient photosynthesis wherein they close their stomata to reduce evapotranspiration and water loss during the day (Zhu and Meinzer, 1999). Second, previous results have shown that buffalo grass and quailbush seedlings have the ability to alkalinize their environment and prevent acidification (Solís-Dominguez et al., 2012). The ability of quailbush to prevent acidification was also demonstrated in a 12-month mesocosm study (Valentín-Vargas et al., 2014). This ability may provide a competitive advantage which can be assessed with an easy screening test available (Solís-Dominguez et al., 2012).

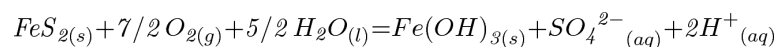
4.2 Self-propagation and fertility island effects

Propagation of quailbush through blooming-seeding cycles and buffalo grass through stolon propagation were observed on a yearly basis in the composted and seeded plots beginning in 2011. The ability to self-propagate resulted in the appearance of new quailbush and buffalo grass plants each year, often under the canopy of an established plant. In addition, the compost amendment combined with a robust vegetative cover maintained levels of TOC and TN over the period of this 41-month study that were substantially higher than in off-site soils. This in turn helped support of a variety of annual plants that were observed each year and that contributed to leaf litter development as the annuals went through senescence, death, and decay. One long-term goal of this project will be to determine whether plants can survive in the long-term, and if so, what type of ecological succession takes place. Escarré et al. (2011) and LeFebvre and Jacobs (2014) report that seeds from pioneer vegetation were better able to tolerate high concentrations of metal(loid)s and acidic pH. This is an interesting observation and seeds are being collected to determine whether pH or metal tolerance changes in subsequent generations.

One surprising result from this study was the development of a robust plant cover in the composted but unseeded treatments over the 41 months of this study. The composted unseeded plots were established to separate the effect of compost alone and compost plus plants in the development of soil quality parameters during this field trial. However, even though care was taken to exclude seeds in unseeded plots, seeds from the planted plots either blew or were carried by birds or animals into the neighboring unplanted plots almost immediately following the initial seeding. In subsequent years seeds were further supplied to the unseeded plots by the yearly blooming and seeding cycles in the seeded plots. What is most interesting, particularly about the quailbush plants in the composted unseeded plots is that single plants developed a much more robust biomass than the plants in the composted seeded treatments (Fig. 3). These plants created fertility islands in the composted unseeded plots that resulted in the gradual increase in plant canopy cover over time (Alday et al., 2014; Berg and Steinberger, 2010). This is an intriguing result that needs to be explored further. It suggests that in an extremely acidic site like the IKMHSS, while it may be necessary to provide compost over the entire area, it may not be necessary to seed the entire area which would result in a cost-savings. Further, in tailings that are not acidic, these results suggests that it would be worth exploring whether the use of spatially separated fertility islands (treated with compost and seeds) within a site would allow only a fraction of the site to be reclaimed with the expectation that the fertility islands would gradually spread out to encompass reclamation of the entire site (Alday et al., 2014; Santini and Fey, 2013).

4.3 The relationship between pyrite content and pH

Implementation of the field study at IKMHSS imposed an immediate disequilibrium in the tailings, which had been relatively undisturbed for ca. 50 years, in two ways: 1) through the addition of organic matter, seeds and irrigation, and 2) through the mixing of the top ca. 25 cm layer which varied in pyrite content due to the propagation of the oxidation front. Earlier work has quantified this variation showing that the upper 15 cm layer of undisturbed tailings have a lower pyrite content (1.4 to 2 wt%) than tailings below 15 cm (> 10 wt%) (Hayes et al., 2014). Following these perturbations, all treatments as well as the unamended control showed a decrease in pyrite after 41 months suggesting that acid generation was occurring according to the equation:



Results indicate that the compost, and possibly plant root exudates, buffered acid generated by pyrite oxidation to a large extent. The 20% compost treatments buffered the pH most effectively with no significant decrease during the 41 months examined. The 20% compost seeded plot showed a pyrite oxidation from 3.5% to 0.4% from 2010 to 2013, 89% decrease in pyrite. While the 20% compost without seeds showed just 33% decrease in pyrite. This suggests a plant effect that needs to be further examined, but the effect of compost and plants could not be separated in this experiment. However, we have shown previously that the plants used in this study are capable of stabilizing pH through alkalization of the rhizosphere (Solís-Dominguez et al., 2012). The lower compost applications (10% and 15%) showed significant decrease in pH as the proton consumption capacity of the compost was

consumed by acid generation from pyrite oxidation, with final pH of about 3 and 4, respectively. These results further suggest that a combination of lime and compost in tailings could prevent acidification of the tailings, and future experiments should include lime amendments (Chaney et al., 2014).

5.0 Conclusions

This field trial demonstrates that direct planting using compost-assisted phytostabilization technology can be used to establish plants in a highly acidic metalliferous mine tailings and that greenhouse results can guide successful translation of this technology to the field. Direct planting as a remediation strategy for mine tailings is of interest because the more commonly used soil cap and plant technology requires larger amounts of resources to create the cap. Such resources can be difficult and costly to obtain, and in some instances, result in removal of vegetation and top soil from adjacent undisturbed areas. Finally, results suggest that easily measured soil quality parameters, such as pH, NHC, TOC, TN, and EC can provide information on the progression of the phytostabilization process and the transition of tailings materials into a substrate that can successfully support plant growth.

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Highlights

- Cap and plant is the current costly standard for mine tailings reclamation
- We assessed direct planting for remediation of acidic metalliferous mine tailings
- 60-day greenhouse pot studies translated successfully to this 41-month field trial
- A single compost application supported plant establishment and soil development
- Direct planting with compost addition is a viable alternative technology for treatment of mine tailings



Figure 1.

(A) An aerial view of the IKMHSS tailings. The black square delineates the study area. The inset shows the location of the IKMHSS site (black diamond) in Yavapai County (highlighted in red), AZ. (B) a map of the 24 plots (9.6 m × 15 m per plot) showing the location of all treatments and replicates. Numbers indicate treatment: (1) unamended control; (2) 10% compost – seeded with buffalo grass (BG) and mesquite (MQ); (3) 15% compost – unseeded; (4) 20% compost – unseeded; (5) 15% compost – seeded; (6) 20% compost –seeded.

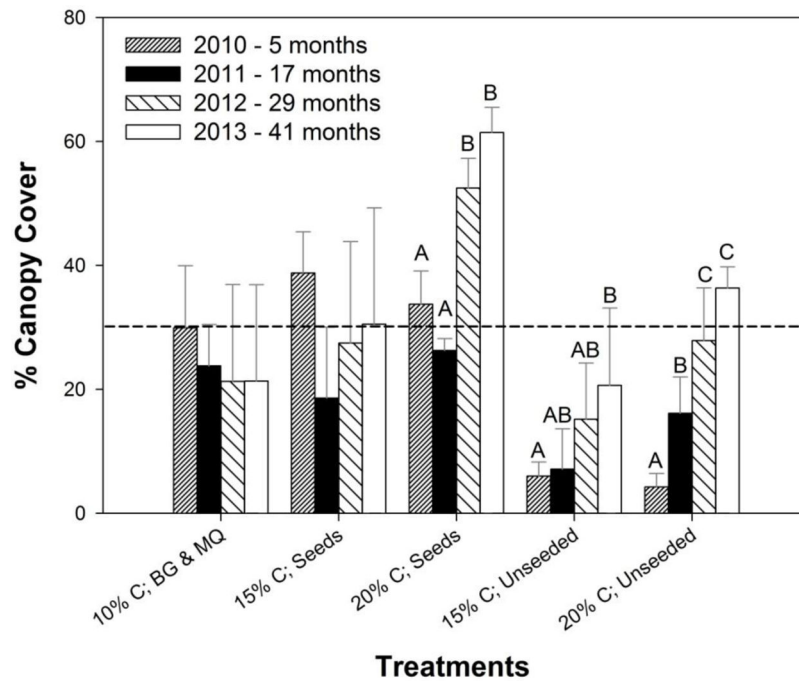


Figure 2. Effect of 10, 15, and 20% compost amendment on canopy cover (average + 1 standard deviation, n = 4) at 5, 17, 29, and 41 months. No plants grew in the unamended tailings control. The dashed line denotes the average canopy cover in the immediate surrounding area (34°29'54.90"N; 112°15'15.18"W). A one-way ANOVA was performed for each treatment. Means identified with different letters are significantly different by year ($p < 0.05$; Post-Hoc Tukey-Kramer test, n = 4). C = compost, BG = buffalo grass, and MQ = Mesquite.

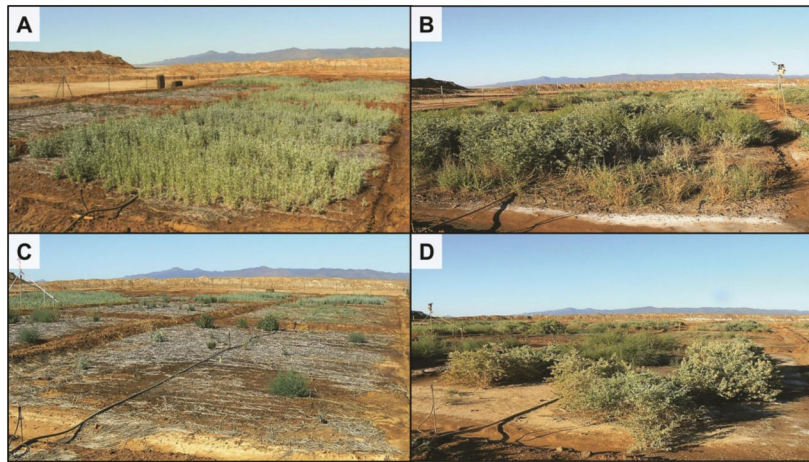


Figure 3.
A comparison of quailbush plants in the 20% compost seeded treatment (A) 2010; (B) 2012 and the 20% compost unseeded treatment in (C) 2010; (D) 2012.

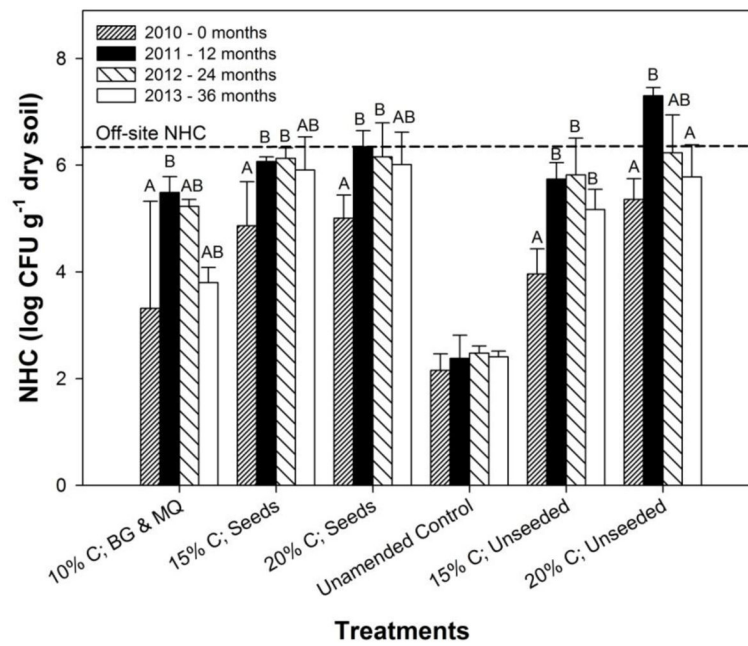


Figure 4. Effect of treatment on NHC expressed as log CFU g⁻¹ dry tailings (average ± 1 standard deviation in parentheses, n = 4). The dashed line denotes 6.84±0.2 as the average NHC in soil samples collected from two off-site areas (34°30'44.90"N; 112°15'38.37"W and 34°30'49.48"N; 112°15'34.04"W). A one-way ANOVA was performed for each treatment. Means identified with different letters are significantly different by year (p < 0.05; Post-Hoc Tukey-Kramer test, n = 4). C = compost, BG = buffalo grass, and MQ = Mesquite.

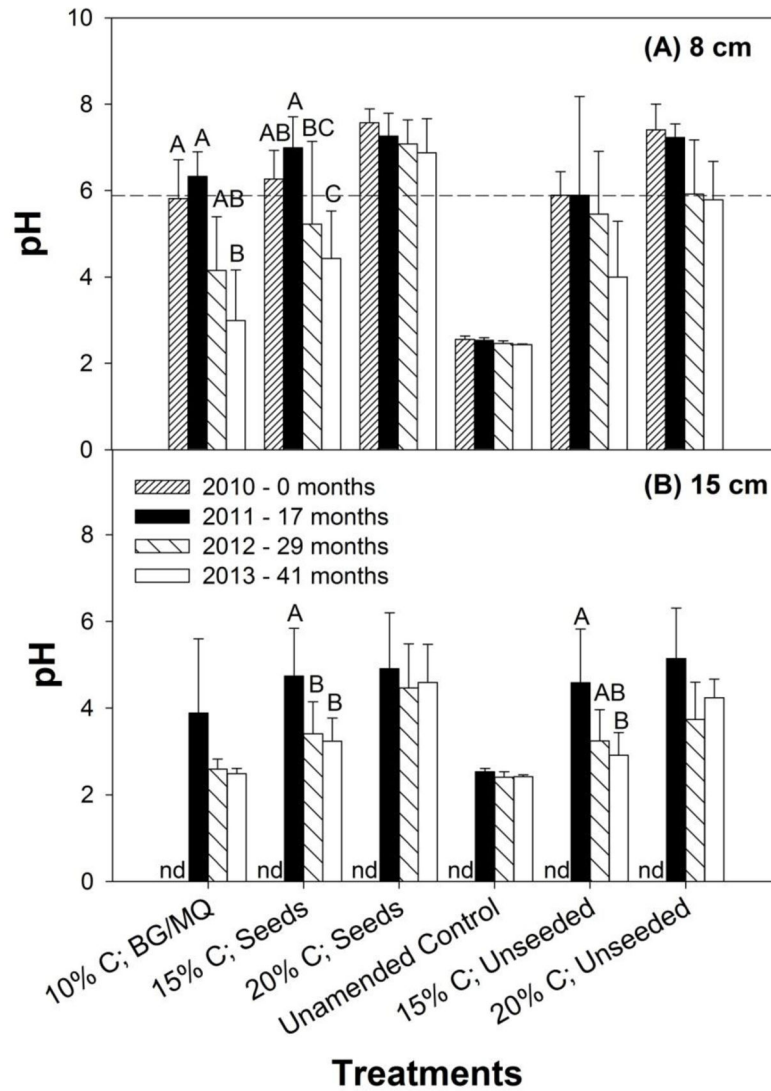


Figure 5. Effect of treatment on pH at (A) 8 cm (well-mixed phase) and (B) 15 cm (interface between the well-mixed phase and the unamended tailings). Values presented are the average \pm 1 standard deviation in parentheses, $n = 4$). The dashed line denotes the average pH in soil samples collected from two off-site areas ($34^{\circ}30'44.90''N$; $112^{\circ}15'38.37''W$ and $34^{\circ}30'49.48''N$; $112^{\circ}15'34.04''W$). A one-way ANOVA was performed for each treatment. Means identified with different letters are significantly different by year ($p < 0.05$; Post-Hoc Tukey-Kramer test, $n = 4$). C = compost, BG = buffalo grass, and MQ = Mesquite.

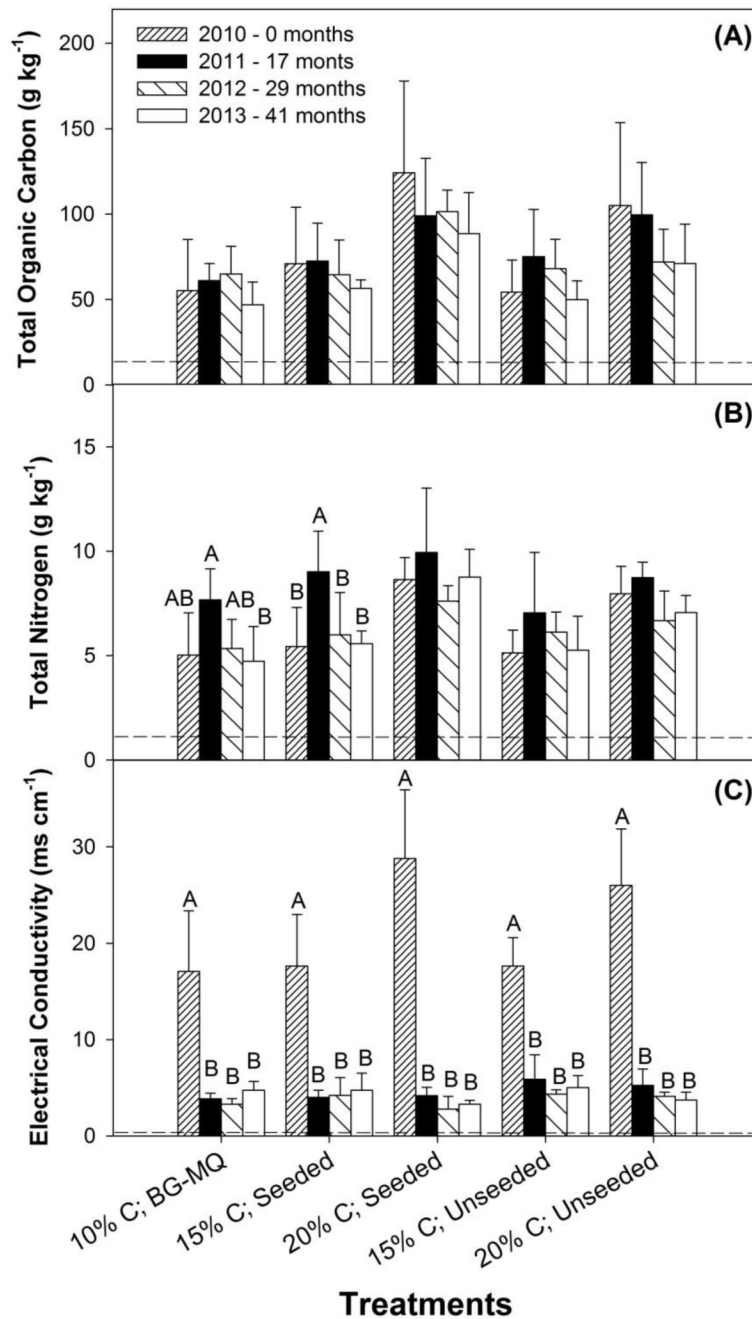


Figure 6. Effect of treatment on (A) TOC; (B) TN; and (C) EC at 8 cm (average \pm 1 standard deviation in parentheses, $n = 4$). The dashed line denotes the average (A) TOC; (B) TN; and (C) EC in soil samples collected from two off-site areas ($34^{\circ}30'44.90''N$; $112^{\circ}15'38.37''W$ and $34^{\circ}30'49.48''N$; $112^{\circ}15'34.04''W$). A one-way ANOVA was performed for each treatment. Means identified with different letters are significantly different by year ($p < 0.05$; Post-Hoc Tukey-Kramer test, $n = 4$). C = compost, BG = buffalo grass, and MQ = Mesquite.

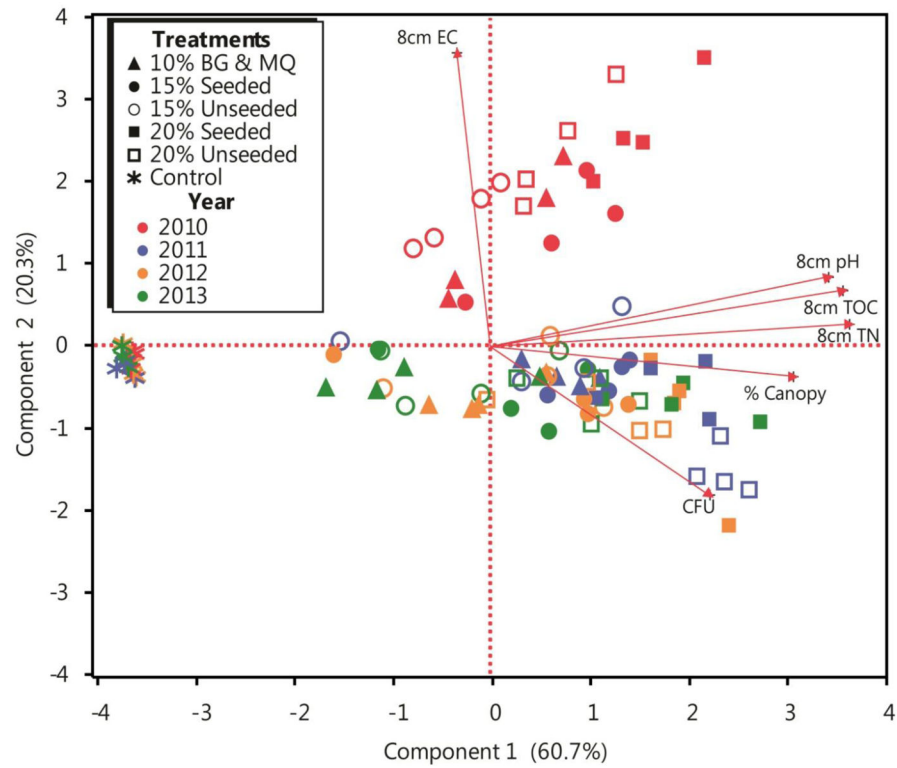


Figure 7. PCA comparing measured parameters (pH, NHC, TOC, TN, EC, and % canopy cover) in the six treatments over time. Arrows represent the relationship (direction and strength) of each measured parameter with the treatments. Please refer to the electronic version for clarification of colors.

Table 1

Characteristics of IKMHSS tailings before after compost amendment

Treatments	pH	EC (ms cm ⁻¹)	ANC ^a g kg ⁻¹	TC g kg ⁻¹	TOC ^b g kg ⁻¹	TN g kg ⁻¹	NHC Log CFU g ⁻¹ dry soil
Unamended Tailings ^c	2.5 (0.1)	8.3 (0.7)	na	bdl	bdl	bdl	2.16 (0.31)
Tailings + 10% compost	5.8 (0.9)	17.1 (6.3)	14.8(1.9) ^b	56.3 (30.7)	55.2 (29.8)	5.0 (2.02)	2.98 (2.58)
Tailings + 15% compost	6.1 (0.6)	17.6 (4.0)	22.8 (2.9)	63.3 (26.7)	62.7 (23.3)	5.3 (1.4)	4.6 (0.8)
Tailings + 20% compost	7.5 (0.4)	27.0 (6.2)	29.7 (3.9)	118 (49.9)	114.6 (48.4)	8.3 (1.4)	5.8 (1.0)
Compost	9.3 (0.2)	34.4 (1.2)	148 (19)	260 (21.2)	249 (21.2)	11.3 (3.6)	7.8 (0.5)
Off-site	5.9 (0.2)	0.07 (0.03)	na	11.0 (2.62)	11.0 (2.6) ^c	1.1 (0.2)	6.84 (0.2)

EC = electrical conductivity, ANC = Acid neutralizing capacity; TC = total carbon by IR, TOC = total organic carbon, TN = total nitrogen, NHC = neutrophilic heterotrophic bacterial counts. Values are average and (standard deviation) for n = 4 for unamended tailings and tailings + 10% compost and n=8 for tailings + 15% and tailings + 20% compost;

^aANC measured as standardized 0.1 N H₂SO₄ consumed on titration of a compost suspension to pH 4.0 following kg⁻¹ tailings equivalent;

^bTOC was calculated from the (Wong et al. 1998) expressed as g CaCO₃ difference between measured TC and non-purgeable total inorganic carbon;

^ctailings + compost ANC values calculated from wt% compost.

Table 2
Accumulation of metal(loids) in plants shoot tissues in IKMHSS mine tailings based on Toxicity Limits (DATL).

Element	PA ^a	Total ^b mg kg ⁻¹	DATL ^c	Plant species ^d	Accumulation of metal(loids) in the shoot (mg kg ⁻¹)					
					10% Compost		15% Compost		20% Compost	
					2010 ^e	2013 ^e	2010	2013	2010	2013
As	610(120)	2590	30	BG	14.3(2.6)	77.1(24.7)*g	24.8(18.4)	27.8(17.6)	14.8(1.4)	11.7(5.2)
				QB	np ^f	np	19.7(5.6)	8.0(5.8)	11.8(3.3)	4.9(2.4)
Pb	4.2(2.8)	2200	100	BG	8.7(1.5)	34.2(5.8)*	11.9(8.6)	14.3(4.7)	8.1(1.8)	6.5(2.2)
				QB	np	np	12.3(5.0)	5.4(4.6)	6.4(2.2)	3.1(1.0)
Zn	18001900	2000	500	BG	236(173)	461(14.5)*	207(156)	225(43.7)	147(78)	167(69)
				QB	np	np	655(229)	936(305)*	506(253)	675(211)
Cd	na ^h	7.1	10	BG	0.6(0.5)	0.5(0.2)	0.4(0.3)	0.3(0.1)	0.39(0.37)	0.2(0.1)
				QB	np	np	2.1(1.1)	1.4(0.9)	1.4(0.9)	1.4(0.7)
Cu	96(25)	127	40	BG	38.5(18.6)	42.6(14.5)	44.7(11.8)	49.2(10.7)	45.8(17.9)	51.2(20.1)
				QB	np	np	10.4(3.1)	9.4(0.8)	11.9(3.3)	8.9(2.6)
Ni	3.7(1.9)	9.67	100	BG	0.6(0.2)	3.1(0.7)*	0.5(0.4)	2.3(1.0)*	7.3(9.9)	2.5(0.8)
				QB	np	np	4.1(4.5)	0.9(0.03)	3.9(4.2)	1.0(0.2)

^aPA is plant available, defined here as metal(loid) solubilized by a sequential extraction using DI H₂O and 1 M NaH₂PO₄ at 1:40 solid to solution.

^bTotal metal(loid) concentration in the unamended IKMHSS tailings. Values are expressed as the average (standard deviation) of n = 4 except for the 2013 15% compost values are based on n= 3.

^cDATL= Domestic Animal Toxicity Limit. Values are the maximum tolerable levels for cattle (National Research Council, 2005).

^dBG = buffalo grass, QB = quailbush.

^ePlants leaves were collected 5 months after seeding in 2010 and at 41 months in 2013.

^fnp = not planted, not reported because quailbush was not planted in this treatment.

^gA one-tail paired t-test was performed to compare 2010 and 2013 samples.

^h2013 values with an asterisk are significantly higher than the 2010 values (α > 0.05).

Freeze-dried homogenized compost (0.2 g) was added to Teflon microwave vessels, reacted with the aqua regia and hydrogen peroxide overnight, then placed in a CEM MDS-2100 microwave digestion system until the compost was completely digested. Averaged TC and TN (n=3) were analyzed on separate splits of the compost with a Shimadzu TOC-VCSH with TN module. Loss on ignition (LOI) is a mass difference of freeze dried samples and samples heated to 900 °C for 15 minutes. See Hayes et al., 2014 and Root et al., 2015 for further detailed geochemical characterization of the tailings prior to phytostabilization. Initial phosphate concentrations were 11.1 g kg⁻¹ in the compost and 2.73 g kg⁻¹ in the tailings.

Table 3

Pyrite and acid potential of controls and samples taken preferentially after compost addition and at 41 months

Sample	2010				2013 (41 months)				
	Pyrite (%)	AP ^a	χ^2 ^b	Pyrite (%)	AP ^a	χ^2 ^b	Pyrite (%)	AP ^a	χ^2 ^b
20% Seeded	3.5	58.4	4.1	0.4	6.67	5.5			
15% Seeded	2.1	35.0	5.5	1.4	23.4	15.7			
10% BG & MQ	4.5	75.1	23.7	0.8	13.4	22.3			
20% Unseeded	1.2	20.0	4.4	0.8	13.4	7.0			
15% Unseeded	1.7	28.4	22.7	0.7	11.7	15.5			
Unamended Control	1.0	16.7	41.8	0.3	5.01	48.5			
Off-site 0–3"	0	0	na						
Off-site 3–5"	0	0	na						
Compost ^c	0	0	na						

^aThe acid potential (AP) is expressed as kg of CaCO₃ equivalent per metric ton of mine waste as described in Materials and Methods.

^b χ^2 represents the goodness of fit (3,5° to 77° 2θ, Cu Kα). $\chi^2 = \sum [(I_{obs} - I_{calc})^2 / \sigma^2(I_{obs})] / (n - p)$; where I = the intensity, $\sigma(I_{obs})$ = the estimated error of the measure (fixed to 10% of the counts), n = the number of points used for the refinement simulation and p = the number of parameters estimated.

^c composition of the compost: C:N = 12.0, loss on ignition (LOI) = 41.3%(6.1), reported on a dry-weight basis from homogenized and sieved to 100 mesh sample of compost. *na* indicates no Rietveld refinement was performed because pyrite reflections were not present.

Table 4

Irrigation and rainfall at the IKMHSS field site from 2010 to 2013.

Time Period (months)	Dates	Irrigation applied (mm)	Precipitation (mm)	Total (mm)
5	5/2010 – 10/2010	378	238	626
12	11/2010 – 10/2011	365	261	626
12	11/2011 – 10/2012	322	401	724
12	11/2012 – 10/2013	387	83	470

Table 5

Plant composition based at the IKMHSS field site from 2010 to 2013.

Treatments	Plant type	Plant Composition (%)			
		2010	2011	2012	2013
20% C; Seeded	Grasses	13.8(4.1)	13.6(3.7)	23.6(11.9)	24.8(16)
	Shrubs	86.1(4.0)	83.4(3.2)	67.9(14.6)	56.4(12)
	Trees	0.0	0.0	0.0	0.0
	Weeds	0.0	2.9(4.4)	8.5(8.9)	18.8(14)
15% C; Seeded	Grasses	0.0	2.5(3.3)	1(0.6)	2.6(4)
	Shrubs	27.3(12.5)	58.9(41.2)	45.9(34.0)	40.2(28)
	Trees	0.0	0.0	0.0	0.0
	Weeds	72.7(12.5)	38.7(41)	53.1(33.3)	57.2(28)
10% C; BG & MQ	Grasses	28.9(8.2)	47.3(24.1)	26.6(10.3)	25.4(11)
	Shrubs	70.9(8.1)	50.5(24.2)	41.9(22.5)	39.2(20)
	Trees	0.0	0.0	0.0	0.0
	Weeds	0.2(0.2)	2.1(3.4)	31.4(32.6)	35.3(30)
20% C; Unseeded	Grasses	0.0	0.0	0.0	0.0
	Shrubs	14.7(26.4)	26.5(49)	18.0(22.5)	19.4(16)
	Trees	0.0	0.0	0.0	0.0
	Weeds	85.3(26.4)	73.5(49)	82.0(25.4)	80.6(16)
15% C; Unseeded	Grasses	97.2(3.4)	97.5(2.1)	79.5(13.7)	84.2(10)
	Shrubs	0.0	1.6(2.3)	7.1(8.7)	0.8(2)
	Trees	0.0	0.0	0.0	0.2(0)
	Weeds	2.8(3.4)	0.9(0.5)	13.3(6.5)	14.8(9)
Control	Grasses	0.00	0.00	0.00	0.0
	Shrubs	0.00	0.00	0.00	0.0
	Trees	0.00	0.00	0.00	0.0
	Weeds	0.00	0.00	0.00	0.0

Values are expressed as the average and (standard deviation) n=4 from randomized vegetative cover survey.