A combined compost and endophyte addition improves phytostabilization by a native perennial grass in metal contaminated mine tailings

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Abstract

Background and Aims:

Re-vegetation of contaminated and disturbed landscapes can reduce the transport of toxic elements while improving soil fertility. This study evaluated whether the planting of a perennial grass with diazotrophic microbial endophytes and municipal waste compost—alone and in combination—improved phytostabilization of potentially toxic trace elements in dolomite-amended tailings from a historically mined polymetallic mineral deposit.

Methods

We grew *Bouteloua curtipendula* seedlings in tailings with hazardous concentrations of As, Cd, Pb, Mn, and Zn. We evaluated how plant growth, organic matter accumulation, and minor and trace element mobilization responded to microbial endophyte and organic amendments.

Results

Although most of the added endophytes were not uniquely identified, the best plant growth and fertility outcomes were achieved with a combination of amendments: dolomite to reduce acidity, a compost topdressing to accumulate nitrogen in the tailings, and a mixed consortium endophyte seed coating to synergistically increase organic carbon and grass biomass yields. Combining amendments also improved phytostabilization: compost reduced the shoot forage toxicity of *B. curtipendula* seedlings without reducing phytostabilized contaminant yields in the rhizosphere, while endophyte inoculated grass marginally reduced total and water-extractable concentrations of toxic trace elements through enhanced mobilization.

Conclusion

The most effective means of reclaiming these acidic, polymetallic tailings is with a simultaneous dolomite, compost, and endophyte seed treatment.

1. Introduction

Abandoned mines have created a legacy of environmental damage. Mines have a small footprint, covering less than 0.5% of the Earth's ice-free land surface (European Commission, Joint Research Center 2018; Maus et al. 2020). However, the landscape-level disturbances caused by the extraction and processing of metal and mineral resources can be dispersed across a much larger area (Tost et al. 2020; Meyfroidt et al. 2022). Environmental hazards can be particularly pronounced when toxic mine waste is released in the environment, directly impacting human and ecosystem health (Dudka and Adriano 1997; Bridge 2004; Sonter et al. 2017). In the United States, liability to mitigate the environmental hazards from mining follows a "polluter pays" principle, but for historic and abandoned mines the burden can fall to the federal government. There are an estimated 100,000–500,000 abandoned hardrock mines on state,

federal, and private land in the western United States (U.S. Environmental Protection Agency 2004). On federal lands alone, about 15% of these abandoned mines are confirmed or suspected environmental hazards (Government Accountability Office 2020). Many of these mines are small, remote, or in semi-arid or arid climates. Traditional methods of environmental remediation, like offsite removal, are difficult at remote mines in arid climates where biological or in-situ approaches like phytostabilization may be more appropriate (Mendez and Maier 2008a).

Phytostabilization is the accumulation of contaminants in plant roots without substantial transfer to above ground tissues, resulting in less mobilization of soluble contaminants through soil and into surface or groundwater. Because phytostabilization involves re-vegetation, it also comes with the indirect benefits of increasing belowground carbon, improving slope stability, and improving the visual appearance of mine waste piles (Ranjan et al. 2015). Native plants can grow well in landscapes disturbed by mining (Yoon et al. 2006) because they are typically adapted to the soils and climates of the area and are less likely to aggressively spread (Mendez and Maier 2008a). Even so, re-vegetation of mine tailings is difficult. Tailings often have poor physical structure prone to erosion and compaction, low water holding capacity, low fertility (few to no organics and soil microorganisms), high salinity, and extreme pH values in addition to high concentrations of phytotoxic trace elements like Pb, Zn, and Cu (Tordoff et al. 2000). Even plants tolerant of the local climatic and environmental stressors can have trouble establishing and growing if seeded into mine waste in the absence of organic, microbial, and/or inorganic amendments (Mendez and Maier 2008b; Macdonald et al. 2017; Xie and van Zyl 2020). Successful growth after directly seeding plants into mine waste requires a careful selection of soil amendments that can alleviate some environmental stressors to plants (Gil-Loaiza et al. 2016; Yan et al. 2020).

For sulfidic mine tailings, successful phytostabilization typically involves adding lime to increase the pH along with a source of nutrients and microorganisms (e.g., compost) (Córdova et al. 2011; Yang et al. 2016; You et al. 2018). Not only do these amendments directly improve plant growth by adding potentially limiting essential macro, micro, and trace elements, they can reduce the bioavailability of phytotoxic metals to plants and microorganisms if metals are immobilized onto the amendments (McGrath et al. 1995; Wong 2003; Walker et al. 2004). For example, although compost is a potential source of both organic and inorganic contaminants (Farrell et al. 2010), it can reduce phytotoxicity by immobilizing toxic metals and metalloids through complexation or sorption (Ruttens et al. 2006; Smith 2009; Forján et al. 2016). In greenhouse and field experiments, direct planting with compost reduces the bioavailability of toxic elements, improves re-vegetation success of acidic, polymetallic mine tailings, and re-establishes soil and root-associated microbial communities (Solís-Dominguez et al. 2012; Gil-Loaiza et al. 2016). Soil and root-associated microorganisms are critical for plant growth and nutrient acquisition (Richardson et al. 2009) and influence the bioavailability of metals and metalloids (Jing et al. 2007; Wang et al. 2017; Domka et al. 2019). For example, metals and metalloids can sorb onto mycorrhizal fungal hyphae associated with plant roots, stabilizing metals and metalloids in the rhizosphere (i.e., the soil on and near plant roots), potentially reducing their bioavailability and toxicity (Meier et al. 2012; Riaz et al. 2021). Microbial endophytes—which are bacteria, archaea, fungi, and/or protists inhabiting the inner plant tissues for at least some portion of their life (Hardoim et al. 2015)—can produce organic acids and

siderophores that solubilize minerals, releasing mineral associated nutrients (e.g., phosphorus) and contaminants (e.g., Pb, Zn) (Ma et al. 2016; Manoj et al. 2020; Papik et al. 2020). Conversely, they can secrete compounds like extracellular polysaccharides that stabilize solubilized contaminants (Gadd et al., 2000). Therefore, whether plant-associated microorganisms can increase (Ma et al. 2011b), decrease (Madhaiyan et al. 2007), or have no impact (Zhang et al. 2013) on the solubilization or plant uptake of contaminants is ecosystem-specific and contingent on the plants, microorganisms, and amendments selected for phytoremediation (Mendez and Maier 2008a; Kohler et al. 2015).

The purpose of our experiment was to assess whether adding a compost topdressing or seeds coated with microbial endophytes—alone or in combination—improved the germination, growth, and stabilization of potentially toxic elements by a native perennial grass seeded in tailings from a polymetallic mine in southeastern Arizona, USA. The perennial grass *Bouteloua curtipendula* is tolerant to metals found in local mine waste (e.g., Cu, Pb, Mn, Zn), and stabilizes trace elements in the rhizosphere rather than in foliage (Eddleman, 2012; Leewis 2022). The endophytes coating the seeds are a mix of bacteria that fix nitrogen and solubilize phosphorus (Table 1). We hypothesized that *B. curtipendula* germination and organic matter accumulation would improve with a compost topdressing, but that the best growth would be observed when a seed coating of diazotrophic endophytes was added along with the compost due to increased N and P availability. We also hypothesized that root stabilization of potentially toxic trace elements would be greatest for plants with both a compost topdressing and endophyte-coated seeds, due to synergistic benefits to plant growth, organic matter accumulation, and trace element stabilization. Finally, we hypothesized that *B. curtipendula* growth and phytostabilization would reduce the environmental risk posed by the mine tailings due to immobilization in the rhizosphere.

2. Materials and Methods

2.1. Polymetallic mine geology and tailings characteristics

The Blue Nose mining complex (31.44782, -110.73293, 1600 m elevation) was a series of historical polymetallic mines in the Harshaw mining district of SE Arizona. The small surficial and near-surface ore deposits were mined for silver, lead, and gold with copper, zinc, and manganese as secondary commodities. Waste tailings, or the material remaining after value elements have been removed from ore, were produced during historical operation (1884–1956) of the mine and were deposited in piles. The tailings are finely textured, with 70% of the particles ≤ 125 µm, and have a relatively low specific surface area of 5.97 m^2 g^{-1}. The tailings are acidic (pH of 3.5 in a 1:5 m/v ratio) and infertile (i.e., low levels of nutrients (0.01% nitrogen; 0.7% phosphorus), organic carbon (0.21%), soluble organic carbon pools (10 µg C g^{-1}), and microbial biomass carbon (4 µg C g^{-1}; Creamer et al. 2022a)). The tailings pose a potential environmental hazard: concentrations of As (100 µg g^{-1}) and Pb (1.6%) are 10–20 times higher than the soil remediation regulatory limits (Arizona Department of Environmental Quality 2009). Water-soluble concentrations of Cd, Pb, Mn, and Zn also exceed surface water regulatory limits (Arizona Department of Environmental Quality 2019). Moreover, at least 60% of the Cd and Mn—and 90% of the Pb—in the tailings

are soluble, exchangeable, or associated with amorphous Fe and Al phases, indicating these elements are potentially mobile and bioavailable to plants (Creamer et al. 2022b).

2.2. Soil amendments

Dolomite was added to the tailings because *B. curtipendula* does not germinate well at pH values < 5.0 (Creamer et al., 2022a). Dolomite (52% $CaCO_3$ and 35% $MgCO_3$; Organic Garden Lime, Espoma, Millville, NJ) was ground to < 63 µm in a tungsten Shatterbox and mixed into the tailings (100X vertical rotation end over end). The addition of dolomite (35.34 mg per gram of tailings) increased the $pH_{1:5}$ from 3.5 to 5.1, increased the concentrations of total Ca (0.7 to 1.4%) and Mg (0.4 to 0.8%), and added 0.4% inorganic carbon. It also increased water-extractable calcium by 200 mg L^{-1} and halved the water extractability of Ag, Al, As, Cu, and Fe (Creamer et al. 2022b). Municipal waste compost (predominantly green waste) was collected prior to the experiment from the Shoreway Environmental Center (San Carlos, CA). We quantified the total carbon and total nitrogen content of the compost with high temperature combustion on a CN analyzer (Carlo-Erba NA 1500, CE Elantech, Lakewood, NJ, USA). Prior to the analysis, the compost was dried (40°C) and ground in an agate mortar and pestle to < 105 µm. The compost added no appreciable amounts of toxic elements (Creamer et al., 2023).

Seeds of native perennial grass, *B. curtipendula*, were collected within 20 km of the Blue Nose mine by Borderlands Nursery & Seed. The *B. curtipendula* seeds were coated in the laboratory with a mix of ten potentially beneficial microbial endophytes (Table 1). Endophyte identification, putative N fixation, and P solubilization activities have been characterized elsewhere (Doty et al. 2005; Doty et al. 2009; Xin et al. 2009; Knoth et al. 2013; Knoth et al. 2014; Firrincieli et al. 2015; Khan et al. 2015; Doty et al. 2016; Kandel et al. 2017a; Varga et al. 2020). Coating the seeds required: 1) growing endophytes to an optical density of 0.5 at 600 nm in a N-limited media (Rennie, 1981), 2) spraying the endophytes onto a monolayer of *B. curtipendula* seeds (50 mL per 30.5 g of seed for 1 min), and 3) drying the endophyte-coated seeds for 3 d at 25°C. Control seeds were processed similarly and coated with sterile media. A portion of seeds remained uncoated (free of sterile media or exogenous endophytes) for DNA analyses (see section 2.4). Control and endophyte-coated seeds were stored separately at 4°C until planting.

Table 1
Tree endophytes used to coat *B. curtipendula* seeds. Endophytes were isolated from the stems of wild willows, wild cottonwoods, or cottonwood hybrids from the Pacific Northwest (USA)

Closest 16S match	Putative activities		Genbank ID
	N fixation	P solubilization	
Herbaspirillium sp.[2]	yes	unknown	KU495919
Sphingomonas sp.[2]	yes	yes	KT984987
Pseudomonas sp.[2]	yes	yes	KU557506
Curtobacterium sp[2]	yes	yes	KU523564
Rhodotorula sp.[3]	yes	yes	EU563924
Rahnella sp.[2]	yes	yes	KU497675
Burkholderia sp[2]	yes	unknown	KU523562
Acinetobacter sp[2]	yes	yes	KU523563
Burkholderia sp.[4]	yes	yes	KF597275
Rhizobium sp.[1]	yes	yes	KT962907

[1]Doty et al., (2005); [2]Doty et al., (2009); [3]Firrincieli et al., (2015); [4]Kandel et al., (2017a); [5]Khan et al., (2015); [6]Knoth et al., (2013); [7]Knoth et al., (2014); [8]Xin et al., (2009)

2.3. Experimental Setup

2.3.1. Growth Chamber

The experiment was conducted in a 1.82 x 1.22 m indoor growing complex with six isolated chambers. Each 0.36 m^2 chamber contained a single experimental treatment. Each chamber was equipped with a small fan for continuous air circulation and a LED grow light (HIGROW 600W full spectrum light; 400–760 nm) hung from the center of the chamber and set on a 12 h diurnal cycle (Online Resource 1). Centered 40 cm below the lights was half a Deepot rack (D50T; Stuewe and Sons, Tangent OR) holding 8–16 cylindrical plant pots (D16H; 5 x 18 cm) in the perimeter pot positions of the rack. The average photosynthetically active radiation reaching the top of the plant pots at these edge positions, measured using a line quantum sensor (MQ-306; Apogee Instruments, Logan UT), was 1100 ± 219 μmol m^{-2} s^{-1} (ranked ANOVA: $P = 0.575$ between treatment chambers). Average air temperature was 34.2 ± 1°C with the lights on and 22.4 ± 0.2°C with the lights off. Average relative humidity was 29 ± 1.7% with the lights on and 51 ± 1.9% with the lights off (logged every 15 min with a HOBO U23 Pro v2 Temperature/Relative Humidity Data Logger; Onset Computer Corporation, Bourne, MA). We built a gravity-fed drip irrigation

system to automatically deliver water to each pot. Deionized water was held in a 20 L carboy 2.5 m above the ground. We connected the water carboy to low-flow button drippers (1 per pot; Ceta® Pressure Compensating Dripper with turbulent flow; Antelco, Florida, USA) with commercial polyethylene irrigation tubing (Rain Bird®). Each pot was watered with about 55 mL per day. The irrigating rate was 9.1 mL min^{-1} for 1–3 minutes, 2 to 6 times per day. Although we irrigated the pots similarly, the water content was higher without compost ($P < 0.001$; 52 ± 2% with compost versus 57 ± 2% without). The DI water contributed negligible quantities of organic carbon (< 0.5 ppm).

2.3.2. Growth Experiment

Bouteloua curtipendula seedlings were grown in dolomite-amended tailings for 56 days, with or without a 1.25 cm compost topdressing and with or without an endophyte seed coat in an unbalanced fully factorial design. We planted fifteen *B. curtipendula* seeds into the dolomite-amended tailings across the following four experimental treatments: (1) control seeds (i.e., no endophyte coating) and no compost topdressing (n = 16); (2) control seeds with a compost topdressing (n = 9); (3) endophyte-coated seeds without compost (n = 16); and (4) endophyte-coated seeds and compost (n = 9). We also had two experimental controls with unplanted (i.e., plant-free) pots with just dolomite-amended tailings (n = 8), or dolomite-amended tailings with compost (n = 8). Because *B. curtipendula* has low germination rate (10%), high mortality rate (50%), and slow growth in polymetallic mine tailings without soil amendments (Creamer et al., 2022a), we expected that for some of the pots, no plants would germinate or be alive by the end of the experiment. We therefore planted twice as many pots in the compost free-treatments (n = 16) and planted one extra pot in the compost treatments (n = 9) compared to the controls (n = 8), resulting in an unequal design.

Each plant pot was lined with 50 μm nylon mesh, filled with 240 g of dolomite-amended tailings. Pots were wet to 54 ± 3.4% of saturation by injected deionized water at 10 spots through the pot profile with a 15 cm long stainless-steel needle. After 24 h, fifteen *B. curtipendula* seeds, with or without endophytes (depending on the treatment), were inserted vertically into the tailings. Wet compost at 55% saturation was added as a 1.25 cm topdressing (i.e., 12 g dry weight or 15.3 mg compost C g^{-1} tailing) to the appropriate treatments. Pots were placed in the growing complex. Every 3 days we measured gravimetric water content and rotated pots counterclockwise in the rack. The height, width, and number of living grass blades was measured every 6 days. We calculated grass shoot biomass using the allometric relationship between grass blade area and shoot biomass for *B. curtipendula* grown in mine tailings (i.e., biomass in mg = 0.0305 * shoot area in mm^2; Creamer et al., 2022a). We thinned the pots of seedlings after 6, 9, and 12 days until one or two seedlings remained. After 56 days, or at the end of the experiment, all compost treatments had one seedling per pot, and 13 out of 16 pots without compost had 1–2 seedlings per pot (the remaining 3 pots did not germinate or all seedlings died).

2.3.3. Destructive Harvest

The pots were destructively harvested over three days to separate the *B. curtipendula* roots and shoots from the tailings, to separate the compost from the tailings, and to subsample the plants, compost, and

tailings for analyses. Pots were harvested on different days to minimize potential cross-contamination of endophyte DNA across treatments. Pots were laid horizontally on bleach sterilized aluminum foil and plastic-coated lab benches. The nylon mesh containing the tailings, compost, and plants was pulled from the pots and opened (Online Resource 1). The intact compost topdressing (if present) was separated and removed from around the plants and from the top of the tailings, and visible compost fragments were picked with forceps from the tailings. The compost was air-dried for 14 days. *B. curtipendula* roots and shoots were removed from the tailings by gently loosening the tailings around the roots, and then pulling the plant free. Some broken fine roots remained, and we recovered visible broken root fragments with forceps and included them with intact roots. The plant roots and shoots were rinsed with DI water on a 63 µm sieve to remove adhering fragments of tailings and compost. Shoots were cut from the roots with bleached scissors. Root and shoot biomass yields were determined gravimetrically while surface area and length was quantified with WinRHIZO™ Regular software after image acquisition with the LA2400 Scanner (Regent Instruments, Canada). Roots and shoots were stored at 4°C between paper towels for 2 days until grinding for DNA and trace element analyses (described below). The compost-free tailings from each pot were homogenized and subsampled with bleach sterilized plastic tools for DNA extraction and stored at -20°C until analysis. The remaining tailings were dried at 40°C for 7 days.

2.4. Analytical methods

We quantified the elemental composition of the compost, tailings, and extracts of the tailings. We ground the compost to < 105 µm under liquid N_2 in a ceramic mortar and pestle. Tailings were ground to < 105 µm in a Frisch pulverisette corundum planetary ball mill. We ground baked (450°C for 4 hr) sand to < 105 µm using both methods to use as process blanks. Organic matter concentrations were quantified using high temperature combustion with a CN analyzer (Carlo-Erba NA 1500, CE Elantech, Lakewood, NJ, USA). Samples were fumigated with 12 *M* HCl prior to organic carbon analyses (Harris et al., 2001). Total major, minor, and trace elements (60 total elements) in the tailings were oxidized and solubilized using Na_2O_2 fusion and HNO_3 dissolution prior to analysis by inductively coupled plasma mass spectrometry (ICP-MS) or optical emission spectrometry (ICP-OES) by AGAT labs (Canada), under a contract with the U.S. Geological Survey Mineral Resources Program (Taggart, 2002). AGAT laboratories also quantified fifty sodium pyrophosphate extractable elements from the compost to target organo-metal complexes (Zimmerman and Weindorf, 2010). Samples were analyzed after 2 hours shaking in 1:40 m/v of 0.1 *M* $Na_4P_2O_7$. In addition, fifty-one water-extractable elements were similarly quantified after shaking the tailings for 2 hr in 1:30 m/v of deionized water. The pH values of unground compost (1:10 w/v) and tailings (1:5 w/v) were measured after shaking with deionized water. Additional ancillary elemental data can be found in Creamer et al. (2023).

Plant roots and shoots were ground using methods allowing detection of added microbial endophytes (using 16S rRNA gene-based amplicon sequencing) and quantification of major, minor, and trace elements in the plants. The roots and shoots were surface sterilized by vigorous shaking in 70% ethanol for 3 min and then in 2.5% (v/v) sodium hypochlorite (bleach) for 5 min (Barra et al., 2016). Immediately after surface sterilization, plant samples were rinsed with sterile deionized water (3 times for 5 min).

Microbial growth was assayed after spreading 100 µl of the final wash solutions on Luria Bertrani (LB) agar plates and incubating at 28°C for 5 days. Surface-sterilized roots and shoots were stored at -80°C and aseptically ground in a ceramic mortar and pestle with liquid N_2. Plant samples from 2–5 pots were composited during grinding to allow for DNA and trace element extraction from the same root or shoot sample, while still allowing for within-treatment replication (n = 3 per treatment after compositing). An average of 25 mg of each plant sample was aseptically removed during grinding. This subsample was dried at 40°C, digested in concentrated HNO_3, and then 18 major, minor, and trace elements were quantified by ICP-OES by the U.S. Geological Survey in Menlo Park, California (Hornberger et al. 2009). The remaining ground plant material was transferred into a 2 ml Eppendorf tube and stored at -80°C prior to DNA extraction.

2.4.1. Total DNA extraction, quantification, and amplicon generation

We extracted total genomic DNA from the tailings (0.70 g), compost (0.50 g), and *B. curtipendula* roots (0.20 g) to assess added microbial endophyte colonization. Total genomic DNA was also extracted from surface sterilized, uncoated *B. curtipendula* seeds to identify native endophytes. There was insufficient material (< 0.20 g) for reliable extraction of genomic DNA from *B. curtipendula* roots grown without compost, and for *B. curtipendula* shoots. Total genomic DNA was extracted using the FastDNA SPIN kit for soil (MP Biomedicals, Ohio, USA) following the manufacturer's protocol with several modifications to enhance DNA yield (Valentín-Vargas et al., 2014). Resultant DNA was purified and concentrated using the DNA Clean and Concentrator-10 kit (Zymo Research Irving, CA, USA). DNA was quantified using a PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific Technologies, Wilmington, DE).

Bacterial 16S rRNA gene amplicons were generated using DNA extracted from tailings, compost, and roots to assess the colonization of added bacterial endophytes. The V4-V5 hypervariable region of the bacterial 16S rRNA gene was amplified using the 515f/926r barcoded primers as described previously (Kracmarova-Farren et al., submitted). Mock community DNA standards (ZymoBIOMICS Microbial Community DNA Standard, Zymo Research, Irvine, CA) were sequenced and subjected to bioinformatic processing as well. All further downstream steps, including library preparation and amplicon sequencing using Illumina MiSeq platform, were performed at the Core Facility for Nucleic Acid at the University of Alaska Fairbanks as follows: purified amplicons were pooled in equimolar concentrations using Sequal Prep Kit (Thermo Fisher Scientific Technologies, Wilmington, DE), and the final quality and concentration of the library was determined via NEBNext Library (New England BioLabs, Ipswich, MA). Libraries were spiked with PhiX (15%) and followed standard Illumina denature and dilute protocols. Amplicon libraries (10 pM) were loaded and sequenced using the Illumina MiSeq V3 2 by 300 chemistry.

Sequenced amplicons were processed using the DADA2 R-package (Callahan et al. 2016) with a few modifications. For the 16S rRNA gene, sequences were filtered and trimmed using the following parameters: trimLeft = c(0, 0), maxN = 0, maxEE = 2, truncQ = 2 after primer sequences removal. The

truncLen parameters were automatically calculated based on the quality scores conducted on the subset of samples to determine the most accurate numbers for truncation. To further reduce errors introduced during sequencing, amplicon sequence variants (ASVs) of 16S rRNA sequences were merged if they differed by only a single base. The most abundant sequence was used as a representative for that ASV. Taxonomy was assigned using the silva_nr_v132_train_set.fa.gz database for 16S rRNA gene ASVs (Callahan 2018). Sequences of plant origin were discarded from the 16S rRNA dataset. The dataset was rarefied to the smallest sample size: 4,400 sequences. We aimed to identify added endophytes in plant roots, tailings, and compost by comparing the curated sequence data with representative sequences of each added endophyte using a local BLAST + search with ≥ 99% sequence identity (Camacho et al. 2009). The same procedure was performed on sequences found in non-coated and surface-sterilized seeds which served as a control. Sequence data are available in NCBI Short Read Archive under the accession number PRJNA697926.

2.5. Calculations and Statistics

Our experiment is an unequal fully factorial design with two experimental factors: compost addition (yes or no) and seed type (no seeds planted, *B. curtipenula* seeds planted, or endophyte coated *B. curtipendula* seeds planted). We used a generalized linear model with Tukey's post hoc comparisons ($\alpha \leq 0.05$) on Box-Cox or log-transformed data (if non-normally distributed) to test for main and interactive effects of univariate ending data (e.g., organic carbon, root length). Data that were still non-normal (Shapiro Wilk) or had unequal variance (Levine's Test) after transformations were analyzed with a Kruskal-Wallis test followed by Tukey's post-hoc comparisons. Seedling growth rates were estimated for each pot from the slope of linear regressions of calculated shoot biomass relative to the days since planting. Coefficients of variation (R^2 values) for fitted linear regressions to calculate growth rates ranged from 0.74–0.98. Data for pots where all the plants died or where none germinated (9 out of 50 planted pots) were not included in the analyses. We used an analysis of covariance (ANCOVA), with days since planting as a covariate, to test for significant differences (testing for equal slopes) in the linear growth rates of aboveground biomass between experimental factors (unplanted pots were omitted). Differences in the normalized abundance of endophyte sequences identified via local BLAST + search were analyzed with a two-way ANOVA with plant type (no plant, plant, or plant and endophytes) and compost (present or not) as fixed effects. Data which did not meet assumptions of normality (Shapiro Wilk) or equal variance (Levine's Test) were analyzed with a Kruskal-Wallis test followed by Tukey's post-hoc comparisons. All analyses were conducted in R and R studio (version 4.2.2; R Core Team, 2014). Any errors shown in text, tables, and figures are standard deviations unless otherwise indicated.

We used multivariate approaches in PRIMER (version 7.0.21) to test for potential treatment effects of compost and endophyte addition on the concentration of major, minor, and trace elements in the tailings, compost, water extracts of the tailings, and in *B. curtipendula* shoots and roots. We included major element and minor or major trace elements that were commodities or were a potential hazard in these tailings: Ag, Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, P, Pb, S, Sb, Si, Zn. All other quantified minor or trace elements were omitted. We used unconstrained ordinations of the data (i.e., principal components

analysis, PCA) followed by permutational multivariate analysis of variance (PERMANOVA) on Euclidean distance matrices of mean centered element concentrations, using compost addition and seed type as the between subject factors. Permutational *P*-values in text are provided for 9999 permutations and reported as *P[perm]*. Homogeneity of dispersion was confirmed prior to PERMANOVA. Treatment effects on individual element concentrations were analyzed with a Kruskal-Wallis test only if they were strongly correlated (Pearson's $r \geq 0.6$) with PC1 or PC2. Element concentrations were mean centered prior to any correlations.

3. Results

3.1. Seedling establishment and survival was highest with combined amendments

In the absence of a compost topdressing, *B. curtipendula* seedlings had similar germination and growth in the dolomite-amended tailings, regardless of whether the seeds were coated with endophytes. Instead, root growth was marginally decreased (*P* values ranged from 0.06–0.1) while shoot growth was unaffected (Fig. 1). In contrast, *B. curtipendula* had higher germination success and growth when planted with compost than when planted without. About 50% of seeds germinated when planted with compost, but only 30% of the seeds germinated without compost ($P < 0.01$). Although the endophyte seed coating added without compost had minor negative impacts on root growth, the best above ($P < 0.001$) and belowground ($P < 0.01$) *B. curtipendula* growth was observed with a combined dolomite, endophyte, and compost treatment (Fig. 1). Shoot growth rates were also the highest when *B. curtipendula* seeds were coated with endophytes and planted with compost ($P < 0.001$; figure in Online Resource 1).

3.2. Organic carbon was highest with combined amendments

Without considering contributions from the compost topdressing, pots grown with compost had significantly higher organic carbon and nitrogen concentrations in the tailings layer than pots without compost ($P < 0.001$; Fig. 2**).** Like plant growth patterns (Fig. 1), organic carbon in the tailings progressively increased as more amendments were added. The greatest organic carbon concentrations were achieved with a combined compost and endophyte addition. Nitrogen primarily responded to compost addition, i.e., the highest nitrogen concentrations in the tailings were found when a compost topdressing was present, regardless of whether there were plants or whether the seeds were coated with endophytes. Unlike the tailings, concentrations of organic carbon and nitrogen in the compost layer were independent of *B. curtipendula* planting or endophyte addition ($P \geq 0.10$). On average, organic carbon and nitrogen concentrations in the compost were 271 ± 9.9 and 21.6 ± 0.90 mg g^{-1}, respectively, about 100–200 times higher than the concentrations of organic matter in the tailings. The resulting C:N ratio (12.5 ± 0.40) indicates a mass loss of carbon and gain in nitrogen during the experiment that is consistent with microbial oxidative decomposition of compost and downward mobilization of organic carbon (Creamer

et al. 2013). If we include contributions from both the compost and tailings layer, a total of roughly 3700 g m^{-2} of organic carbon and 300 g m^{-2} of nitrogen were present in the mine tailings with compost additions in our 18 cm long pots, regardless of whether the seeds were coated with endophytes. For comparison, this is roughly 2–3 times the organic carbon and nitrogen stocks to 20 cm measured in grassland ecosystems near our field site (Wheeler et al. 2007).

3.3. Toxic minor and trace elements were mobilized during the experiment

We quantified major, minor, and trace elements in the compost, tailings, water extracts, and *B. curtipendula* seedlings to determine if they were solubilized or immobilized during the experiment. Outside of the losses of some abundant elements—like the decrease of Mn from c.a. 800 to 500 ± 30 mg kg^{-1}, of Pb from c.a. 1.6 to 1.3 ± 0.04%, and of S from c.a. 1.4 to 1.1 ± 0.04%—changes in the solid phase elemental composition of the tailings during the experiment were minor (i.e., < 25% difference between starting to ending concentrations; Fig. 3a**)**. The changes in solid phase As and Pb concentrations in the tailings during the experiment were not large enough to lower their concentrations below soil remediation limits and thus the environmental risk of the tailings was not reduced. Although overall changes in solid phase concentrations of potentially toxic minor and trace elements in the tailings resulting from compost and/or endophyte additions were relatively modest (i.e., changed by less than 10% by the treatments), solid phase concentrations of Pb in the tailings—along with Cu, Fe, and Zn—were lower in pots with endophyte-coated *B. curtipendula* seeds ($0.001 \leq P \leq 0.01$; figure in Online Resource 1). The concentrations of these elements are highly correlated in the tailings (Pearson's $r \geq 0.75$) because Cu, Pb, and Zn bearing mineral phases are associated with Fe and/or S in these tailings that contained plumbojarosite, chalcopyrite, and sphalerite (Creamer et al., 2022b). A compost topdressing did not impact the solid phase concentrations of potentially toxic trace elements in the tailings ($P[perm] \leq 0.82$), but there was mobilization of Cd (1 to 3 mg kg^{-1}), Cu (35 to 50 mg kg^{-1}), Mn (200 to 800 mg kg^{-1}), and Pb (20 to 200 mg kg^{-1}) into the compost layer during the experiment (Online Resource 1). The $pH_{1:5}$ of the tailings after the growth experiment was slightly higher in pots with compost than in those without (6.65 ± 0.06 vs 6.71 ± 0.05, respectively; $P = 0.02$), but neither tailings pH values nor organic matter concentrations were related to changes in the distribution of solid phase elements (Pearsons's r ranged from 0.1–0.25).

Concentrations of major, minor, and trace elements in water extracts of the tailings were generally unrelated to solid phase concentrations; only water extractable Mn was highly correlated between the two pools (Pearsons's $r = 0.83$). After the experiment, all measured major, minor, and trace elements, except As, had lower water-extractable concentrations (Fig. 3b). If a plant was grown from uncoated seed (i.e., no added endophytes), Cd, Mn, S, and Zn extractability was higher ($0.003 \leq P \leq 0.07$). In contrast, if a plant was grown with compost or from endophyte-coated seeds, the extractability of Cu and Sb was higher ($P < 0.001$; PCA shown in Online Resource 1). As a result, water-extractable Cu only fell below surface regulatory limits for grazing livestock (ADEQ, 2019) in pots without endophytes or compost (Fig. 3b**).** The

extractability of Cu was correlated with organic carbon (Pearsons's $r = 0.60$) but only marginally with tailings pH ($r = 0.30$) due to higher pH and organic carbon in compost treated pots.

3.4. Phytostabilization of potentially toxic trace elements impacted by compost and endophytes

B. curtipendula roots had higher concentrations of all potentially toxic elements than the shoots ($P_{[perm]} = 0.0001$; Fig. 3c,d). Only Mg and Ca were higher in the shoots than in the roots with, on average, about 1300 ± 130 mg kg^{-1} Mg and 3900 ± 900 mg kg^{-1} Ca in shoots but only 670 ± 200 mg kg^{-1} Mg and 2800 ± 1900 mg kg^{-1} Ca in the roots. As a result, only Mg and Ca were efficiently translocated from *B. curtipendula* roots into the shoots, as indicated by shoot: root concentration ratios > 1 (i.e., the translocation factor; Thakur et al., 2016). Although foliar translocation was similar between treatments, compost lowered the concentration of all potentially toxic trace elements (except Cu and Zn) in plant shoots ($P_{[perm]} < 0.001$; Fig. 3d). As a result, growing *B. curtipendula* with compost lowered the shoot concentrations of Cd, Mn, and Pb below the forage limits for sheep and cows, although Cu and Zn were still above forage limits regardless of treatment (National Research Council, 2005). The per-plant yields of potentially toxic trace elements in *B. curtipendula* shoots, calculated by multiplying biomass yields by trace element concentrations, were also lower if planted with compost ($P \leq 0.01$). An endophyte coating on the seeds had a measurable, but less consistent, effect on the concentrations of major, minor, and trace elements in the shoots ($P_{[perm]} < 0.0015$; PCA in Online Resource 1), and marginally increased the shoot concentrations of Co from 0.84 to 2.5 mg kg^{-1} ($P = 0.052$). Although the concentrations of other potentially toxic trace elements were unaffected by the endophyte addition, foliar yields of Cd ($P = 0.023$), Mn ($P = 0.026$), and Zn ($P = 0.034$)—in addition to Co ($P = 0.022$)—were higher with endophytes due to great aboveground biomass yields (Online Resource 1).

There were no changes in root concentrations of potentially toxic trace elements for *B. curtipendula* plants with endophyte-coated seeds ($P_{[perm]} = 0.86$). However, root concentrations of all potentially toxic trace elements, except Cu, were decreased by growing *B. curtipendula* with compost ($P_{[perm]} < 0.0001$; Fig. 3c). As a result, the accumulation of potentially toxic trace elements in the roots, measured as the ratio of concentrations in roots relative to the tailings (e.g., the bioconcentration factor; Yoon et al., 2006), was decreased by compost additions ($P < 0.001$) for Cd (8 ± 2 to 2.5 ± 1.5), Co (19 ± 5 to 5 ± 2), Mn (29 ± 8 to 6 ± 3), and Pb (0.8 ± 0.2 to 0.2 ± 0.1). Total yields of potentially toxic trace elements in the plant roots were unaffected by compost addition ($P_{[perm]} = 0.11$), because although concentrations were lower, the roots were bigger if grown with compost.

3.5. The abundance of inoculants across treatments

Using our curated sequences, we identified whether the bacterial endophytes coated on the seeds were potentially present in tailings, compost, or *B. curtipendula* roots. Endophytes were not assayed in shoots due to low biomass yields. We used the same method to identify whether populations related to the added bacterial endophytes could be identified in uncoated *B. curtipendula* seeds to assess whether *B.*

curtipendula has indigenous taxa with highly (≥ 99%) similar 16S rRNA gene amplicons as the added endophytes. We detected 16S rRNA gene sequences of a total of seven out of the 10 added bacterial endophytes in the tailings, compost, seeds, or *B. curtipendula* roots. However, not all the identified sequences were consistently found in the same materials or treatments. Sequences highly similar to several of the added endophytes were also detected in materials from pots that were not planted with endophyte-coated *B. curtipendula* seeds as well as in the pre-treatment, non-coated seeds (Table 2). For example, 16S rRNA genes with ≥ 99% similarity to the added *Pseudomonas sp.* was identified in the pre-treatment compost and seeds. The same sequence was also the most abundant in *B. curtipendula* roots grown from both uncoated and endophyte-coated seeds. The sequences of the added *Rahnella sp.*, *Burkholderia sp.* (Genbank ID KF597276), and *Rhizobium sp.* were uniquely identified in materials from pots with endophyte-coated seeds, but the *Rhizobium sp.* was the only inoculated endophyte whose sequence was absent in un-inoculated, pre-treatment control seeds. As expected for bacteria that live within plants, the highest abundance and the greatest number of genera were identified in plant materials (i.e., roots and seeds).

Table 2

The 16S rRNA gene sequences potentially affiliated with bacterial endophytes (and relative abundances) in the compost, tailings, *B. curtipendula* roots and native endophytes in surface sterilized seeds. Filled gray cells were not sequenced because the material was not present. *B. curtipendula* shoots were not analyzed. None indicates no inoculants were detected. 16S rRNA gene amplicons with ≥ 99% similarity to the added *Sphingomonas*, *Rhodotorula*, and *Burkholderia sp.* (Genbank ID KU523562) were not identified in any material. *Rhizobium* was the only added endophyte uniquely identified in plants with endophyte-coated seeds. Only endophyte genera are provided; see Table 1 for the GenBank IDs associated with the added endophytes

Treatment	**Compost layer**	**Tailings layer**	***B. curtipendula* roots**	**Surface sterilized seeds**
Pre-treatment	*Sphingomonas* (0.02%)	None		*Pseudomonas* (0.81%) *Curtobacterium* (0.16%) *Rahnella* (0.28% *Acinetobacter* (0.06%) *Burkholderia* (0.02%) *Herbaspirillium* (0.005%)
Tailings		None		
Plant		None		
Endophytes		*Acinetobacter* (0.02%) *Burkholderia* (0.004%)		
Compost	*Herbaspirillium* (0.04%)	*Herbaspirillium* (0.02%)		
Plant and Compost	None	None	*Pseudomonas* (0.16%) *Curtobacterium* (0.06%) *Acinetobacter* (0.05%)	

Treatment	Compost layer	Tailings layer	*B. curtipendula* roots	Surface sterilized seeds
Endophytes and compost	None	*Rhizobium* (0.002%)	*Pseudomonas* (0.2%) *Curtobacterium* (0.06%) *Rahnella* (0.05%) *Acinetobacter* (0.03%)	

4. Discussion

4.1. Endophytes and compost synergistically improved *B. curtipendula* growth

Both compost and endophytes have established positive impacts on plant growth and re-vegetation of mine waste (Larney and Angers 2012; Santoyo et al. 2016; Rho et al. 2018). Organic amendments like compost are often required for successful plant growth in acidic, polymetallic tailings (Yang et al. 2016; Xie and van Zyl 2020). As observed in other studies, the municipal waste compost added during planting of *B. curtipendula* seedlings was an important source of nutrients and organics that facilitated plant germination and growth (Fig. 1). These improvements were realized regardless of whether the seeds were coated with microbial endophytes, and in some instances (e.g., for nitrogen) were independent of whether a seed was planted (Fig. 2). Our roughly 5% (w/w) compost additions are lower than those used for field scale re-vegetation of similar acidic metalliferous mine tailings in semi-arid environments (e.g., 15% w/w compost addition; Gil-Loaiza et al. 2016). We expected that the added diazotrophic endophytes coated on the seeds would have a similar function as compost, providing additional nutrients and soil microorganisms to improve plant growth (Wood et al. 2016) while reducing the bioavailability of phytotoxic elements (e.g., Cd, Pb) (Barzanti et al. 2007; Ma et al. 2016). However, per plant biomass yields for *B. curtipendula* grown with a combined compost and endophyte addition were only about 25% of those observed for buffalo grass grown in acidic polymetallic tailings (albeit in tailings with nearly 10 times less lead; Solís-Dominguez et al. 2012). This suggests further growth improvements in these tailings may be realized with higher compost addition rates. Lead toxicity may also be reduced with more compost. Lead (and other phytotoxic elements) sorbs onto compost (Geebelen et al. 2002; Smith 2009; Forján et al. 2016) and in our experiment Pb was mobilized into and stabilized in the compost layer.

Coating *B. curtipendula* seeds with endophytes improved plant growth and germination with compost, but there was no independent benefit of the endophyte-coated seeds on plant growth in the absence of compost (Fig. 1**).** In fact, we observed marginal decreases in root growth with the endophyte coating alone. Although endophytes can be mutualistic, there can be metabolic costs to the plant-endophyte

relationship. The ultimate impact on plant growth is thus dependent on the biotic and abiotic environment (Hoeksema et al. 2010; Partida-Martinez and Heil 2011). For instance, under an environmental stress like nutrient limitation, endophytes would only improve plant growth when that environmental stress is alleviated (Cheplick et al. 1989). The high metal concentrations of the tailings may have killed endophytes during seed germination (McGrath et al., 1995) when they were planted directly into the tailings rather than between the compost-tailings interface. It is also possible that the endophytes—which are derived from poplar and willow species (Doty et al. 2005; Doty et al. 2009; Xin et al. 2009; Firrincieli et al. 2015)—were unable to colonize *B. curtipendula* since endophyte communities can be species-dependent (Wang et al. 2012; Papik et al. 2020). However, endophyte species specificity can be plastic (Cobian et al. 2019), and the endophytes we used can colonize and improve the growth of both herbaceous (e.g., rice, corn) and woody plants in non-contaminated environments (Khan et al. 2015; Kandel et al. 2017b). Therefore, we suspect that the harsh environmental conditions of the tailings without a compost topdressing (e.g., nutrient limitations or metal toxicity) may have inhibited the growth benefits of the added endophytes.

But even when compost was present, and where we observed improvements in growth with an endophyte-coated seed, the sequence of only one of the added endophytes (*Rhizobium sp.*) was uniquely identified. The finding of an additional six sequences of the ten added endophytes in uncoated seeds indicates *B. curtipendula* has indigenous endophytes with ≥ 99% sequence identity with the inoculants. Identifying endophytes in plants grown from field-harvested seeds is not unexpected. Endophytes are ubiquitous in plants and finding an endophyte-free plant would be the exception rather than the rule (Partida-Martinez and Heil 2011). Although the endophytes selected were functionally redundant, finding the sequences of endophytes in both the endophyte coated and uncoated seeds weaken the causal link between endophyte presence and improved growth. However, it is possible that *Rhizobium sp.* was responsible for the growth benefits observed through direct (e.g., N_2 fixation) or indirect mechanisms (e.g., localized pH changes) (Kuiper et al. 2004; Valentín-Vargas et al. 2014).

4.2. Greatest improvements in soil fertility with a combined compost and endophyte addition

The establishment of a functional belowground ecosystem is critical to short- and long-term phytostabilization success (Marques et al. 2009). This includes not only restoration of the microbial community (Valentín-Vargas et al. 2014; Zhou et al. 2020) but also increases in soil organic matter and improvements in pH and soil structure (Huang et al. 2012). The rhizosphere is a hotspot of biological and geochemical activity relative to the surrounding bulk soil (Kuzyakov and Blagodatskaya 2015; Kuzyakov and Razavi 2019). Thus, developing an expansive root network is a target for areas undergoing remediation (Karthikeyan and Kulakow 2003; Saravanan et al. 2020) as it effectively increases the volume of soil affected by root processes (Ma et al. 2018). Plant roots cause local changes in pH and exude organic acids—both of which can cause the desorption and mobilization of metal(loid) contaminants (Yang et al. 2006; Schwab et al. 2008) as well as soil carbon (Keiluweit et al. 2015). Carbon formation is typically faster and more efficient in the rhizosphere (Sokol and Bradford 2018), and the

influence of an expanding rhizosphere on soil carbon storage is an active area of research (Fontaine et al. 2007; Guenet et al. 2018; Zhao et al. 2022). In addition to its importance to plant growth and soil fertility (Tiessen et al. 1994; Oldfield et al. 2019), restoration of organic carbon formation processes during phytostabilization can provide an indirect monetary benefit if we consider the social cost of carbon emissions ($10–90 USD per metric ton of CO_2; Stern et al. 2007; Nordhaus 2007).

In our study, compost was critical to forming organic matter (carbon and nitrogen) and allowing for microbial growth in the tailings. Although there was an additional increase in soil carbon with endophyte-coated seeds—likely due to the larger developing root network—it did not measurably increase nitrogen concentrations. Independent of the impacts on organic matter and microorganisms, a larger root network is beneficial to the physical and hydrologic properties of tailings (Huang et al., 2012). The poor hydraulic conductivity and compacted structure of mine tailings can be improved after growth of pioneer plants like grasses, allowing for other plant species to establish (Guittonny-Larchevêque et al. 2016). Reclamation is more successful long-term when multiple plants are revegetating the mine waste, either by diverse initial plantings or from ecological succession (Holl 2002; Juge et al. 2021). Thus, cost-effective amendments that provide the best improvements to soil structure, hydrology, organic matter, and microbial biomass for native plants—like our combined lime, endophyte, and municipal waste compost additions—should be expected to provide the best long-term outcomes for restoring belowground ecosystems (Cooke and Johnson 2002).

4.3. Optimal phytostabilization with a combined compost and endophyte addition

We quantified concentrations of potentially toxic major, minor, and trace elements in multiple pools (plants, compost, tailings, water) to provide a complete picture of contaminant solubilization and stabilization during the experiment. Overall, the concentrations of potentially toxic elements between the different pools were not strongly correlated, so that each pool provided unique information on the dynamics of elemental transformations during our experiment. A seed coating of endophytes had two primary effects: increasing *B. curtipendula* foliar yields of Cd, Co, Mn, and Zn and marginally lowering solid phase concentrations of Cu, S, Fe, Pb, and Zn (figures in Online Resource 1). The endophytes added in the seed coating produce siderophores and solubilizes calcium phosphate (Doty et al. 2009; Khan et al. 2015; Kandel et al. 2017a). Both these functions can enhance the mobilization of metal(loids) in mine waste by chelating iron (siderophores) or solubilizing minerals by phosphate solubilization (Ma et al. 2016). Copper, Pb, and Zn are held in Fe-bearing phases in these tailings (Creamer et al. 2022b). Potentially, the production of siderophores by endophytes in the seed coating may have mobilized these elements, resulting in their preferential loss from pots with added endophytes. Production of siderophores by endophytes has been associated with higher plant metal uptake (Ma et al. 2011b), although in our study only Zn had higher foliar yields (out of the Fe-associated elements Cu, Pb, and Zn). In the water extracts, Cd, Co, Mn, and Zn—which are all mobile elements in soils at pH 5—were moderately correlated with each other (Spearman's $r > 0.5$) and were less extractable from tailings with endophyte coated seeds. These elements (Cd, Co, Mn, Zn) were also the elements that had higher foliar yields with endophytes,

suggesting a potential higher mobility (and thus plant uptake) of these elements with endophytes. Most of the observed benefits of endophytes for phytostabilization come from their improvements to plant growth rather than metal(loid) solubilization (Ma et al. 2011a; Wood et al. 2016). In fact, higher solubilization of potentially phytotoxic elements (e.g., Pb, Cd) may have caused the marginally lower rhizosphere development observed for plants grown from endophyte-coated seeds in the absence of compost. Although our study indirectly suggests greater solubilization of Fe- and mineral-associated elements, the higher phytostabilization with the combined endophyte and compost addition predominantly resulted from the enhancement of foliar biomass rather than enhanced accumulation. For mine waste with appreciable amounts of Cd, Co, Mn, or Zn (e.g., Ni-Co laterites), applying endophytes with similar Fe-chelating and mineral solubilizing functions, or that allow for rhizosphere expansion, may improve foliar yields from native plants to aid with extraction focused remediation (i.e., phytoextraction) provided phytotoxic effects of mobilized elements are mitigated (e.g., through compost addition).

For successful phytostabilization, potentially toxic trace elements should not be translocated into foliar plant tissues (reducing potential grazing animal exposure) and should instead be immobilized on plant roots or in the soil (Mendez and Maier 2008a; Xie and van Zyl 2020). *Bouteloua curtipendula* is thus suitable for phytostabilization because foliar translocation was low ($<<1$) under our experimental conditions, but bioconcentration of potential contaminants in the roots was high, particularly for Cd, Co, and Mn (root: soil ratios $>>1$). The root concentrations of potentially toxic elements (i.e., the elements we would target for phytostabilization) were decreased in tailings with a compost topdressing. Lowered bioavailability and sorption of potentially toxic elements like Cd, Cu, Pb, Mn, and Zn after compost addition has been observed elsewhere (Geebelen et al. 2002; Smith 2009; Forján et al. 2016). Based on the observed mobilization of Cd, Mn, and Pb into the compost layer during our experiment and the lower water extractability of Pb, our results are consistent with lower bioavailability of Cd, Mn, and Pb with compost addition. Although compost lowered the concentrations of potentially toxic trace elements in both *B. curtipendula* roots and shoots, this translated to lower yields only in the shoots. Lowered foliar yields is beneficial for phytostabilization of tailings with high concentrations of potentially toxic trace elements (Mendez and Maier 2008a). In our study, this translated to a lower environmental risk to grazing animals.

4.4. Implications for mine site reclamation

The primary elements of concern in these tailings were As, Cd, Mn, Pb, and Zn. During the experiment only Mn and Pb (and to a lesser extent Zn) showed decreasing solid phase concentrations. If we consider the compost, water, and plants to be potential sinks for these elements, we recovered about 50% of the Zn and about 30% of the Mn, split equally between the water extract and the compost. However, less than 5% of the decrease in solid phase Pb was found in the plants, water, or compost, indicating that a substantial portion of the Pb was mobilized out of the bottom of the pot during the experiment. Despite high concentrations of these elements in *B. curtipendula* roots, because of their small size, less than 1% of these elements were recovered in *B. curtipendula* roots or shoots, suggesting higher stabilization potential for bigger plants (Sessitsch et al. 2013; Wood et al. 2016). Although suitable for enhanced Mn and Zn

stabilization in *B. curtipendula* roots or shoots, simply growing larger plants would likely stabilize only a portion of this mobilized Pb as the bioconcentration factor (root: soil ratio) of Pb for our experiment ranged roughly between 0.2 and 1. Instead, this Pb could be immobilized by greater compost additions, by selecting organic amendments targeted for high Pb sorption (e.g., biochar; Park et al. 2013), by phosphate amendments to form lead phosphate (Miretzky and Fernandez-Cirelli 2008; Zeng et al. 2017), or by placing sorbents in areas of water outflow (Delkash et al. 2015).

Remediation of sulfidic polymetallic mine tailings is challenging, particularly when concentrations of phytotoxic metals and metalloids are exceedingly high. Here, we establish that applying municipal waste compost and lime, along with a seed coat of diazotrophic endophytes, improves the growth of a widespread perennial grass, *Bouteloua curtipendula*, and provides the greatest increases in soil organic matter and trace element stabilization. A similar approach employed in the field, focused on increasing pH and providing a source of microorganisms and nutrients, would likely improve remediation resultd (e.g., Gil-Loaiza et al. 2016). This combination is particularly suitable for improved phytostabilization of Cd, Mn, or Zn into *B. curtipendula* roots while lowering foliar concentrations, or for targeted foliar concentration of Co. Compared to other studies, the exceeding high Pb concentration in tailings—roughly 16,000 mg/kg compared to a typical range of 10–4500 mg/kg for sulfidic tailings and established plant toxicity levels of 100–500 mg/kg (Mendez and Maier 2008a; Xie and van Zyl 2020)—are a barrier to effective re-vegetation of these barren tailings piles. A combination of compost and endophytes apparently reduced the toxicity of Pb in the tailings by enhancing stabilization (compost) and mobilization (endophytes). Direct planting of *B. curtipendula* seedlings into tailings with similarly elevated Pb concentrations may require additional amendments to immobilize Pb both to prevent offsite transport and improve plant growth, while our presented approach is appropriate for sulfidic tailings with substantial Cd, Mn, and Zn contamination.

Abbreviations

ANCOVA: analysis of covariance; **ANOVA:** analysis of variance; **ICP-OES:** inductively coupled plasma optical emission spectrometry; **ICP-MS:** inductively coupled plasma mass spectrometry; **PCA:** principal components analysis; **PERMANOVA:** permutational multivariate analysis of variance

Declarations

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Competing Interests

Authors C.C., A.F., M-C. L., J.P., M.F-K, O.U. and S.K have no relevant financial or non-financial interests to disclose. Author J.F. was supported by Intrinsyx Environmental to perform his work.

Author Contributions

All authors contributed to the study conception, design, and materials preparation. Data collection and analysis were performed by Courtney Creamer, Mary-Cathrine Leewis, Sean Kacur, Martina Kracmarova, Jakub Papík. Endophyte inoculum preparation, endophyte seed coat treatments, dried seed endophyte survivability enumeration were performed by John Freeman. The first draft of the manuscript was written by Courtney Creamer and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during the study are available in Creamer et al. (2023) at doi.org/10.5066/P9M2JW70. The sequence data associated with the study are available in NCBI Short Read Archive under the accession number PRJNA697926.

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Figures

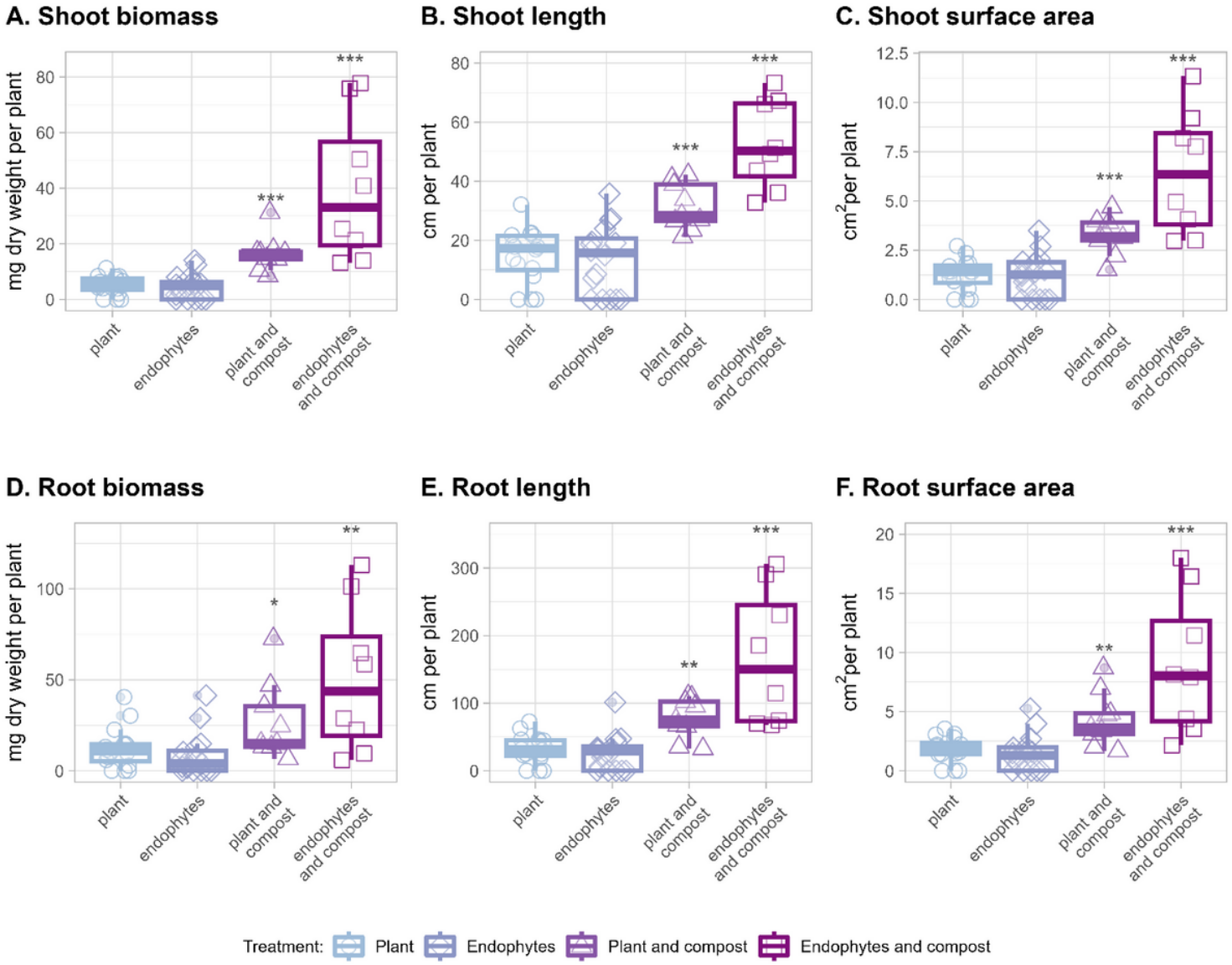

Figure 1

Improved plant shoot and root biomass with soil amendments. (a,d) Biomass yields (mg dry weight per plant) and (b, c, e, f) growth characteristics (length, surface area) for each plant are shown as open symbols overlaid by a boxplot. Significant increases in plant growth with endophyte and/or compost treatments relative to the plant-only controls are shown by asterisks (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$). The marginal ($P < 0.1$) decreases in root biomass, length, and surface area for between plants grown from endophyte coated vs uncoated seeds without compost are not shown.

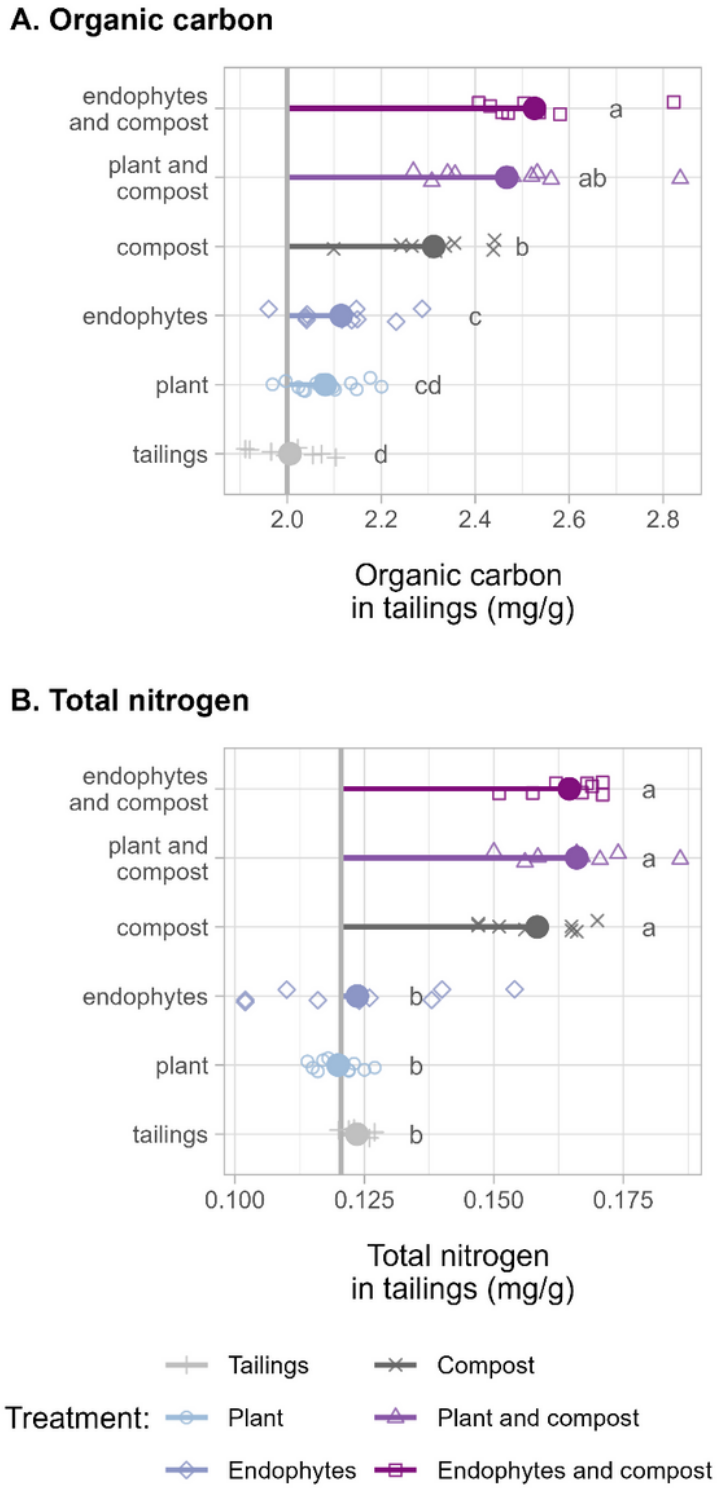

Figure 2

(a) Organic carbon concentrations and (b) total nitrogen concentrations in tailings. The average value for each treatment is shown as a larger dot relative to pre-treatment carbon and nitrogen concentrations in the tailings (gray vertical line). Open symbols show values for each pot within a treatment and are distinguished by color and shape. Significant differences ($P < 0.05$) between treatments are shown as lowercase letters

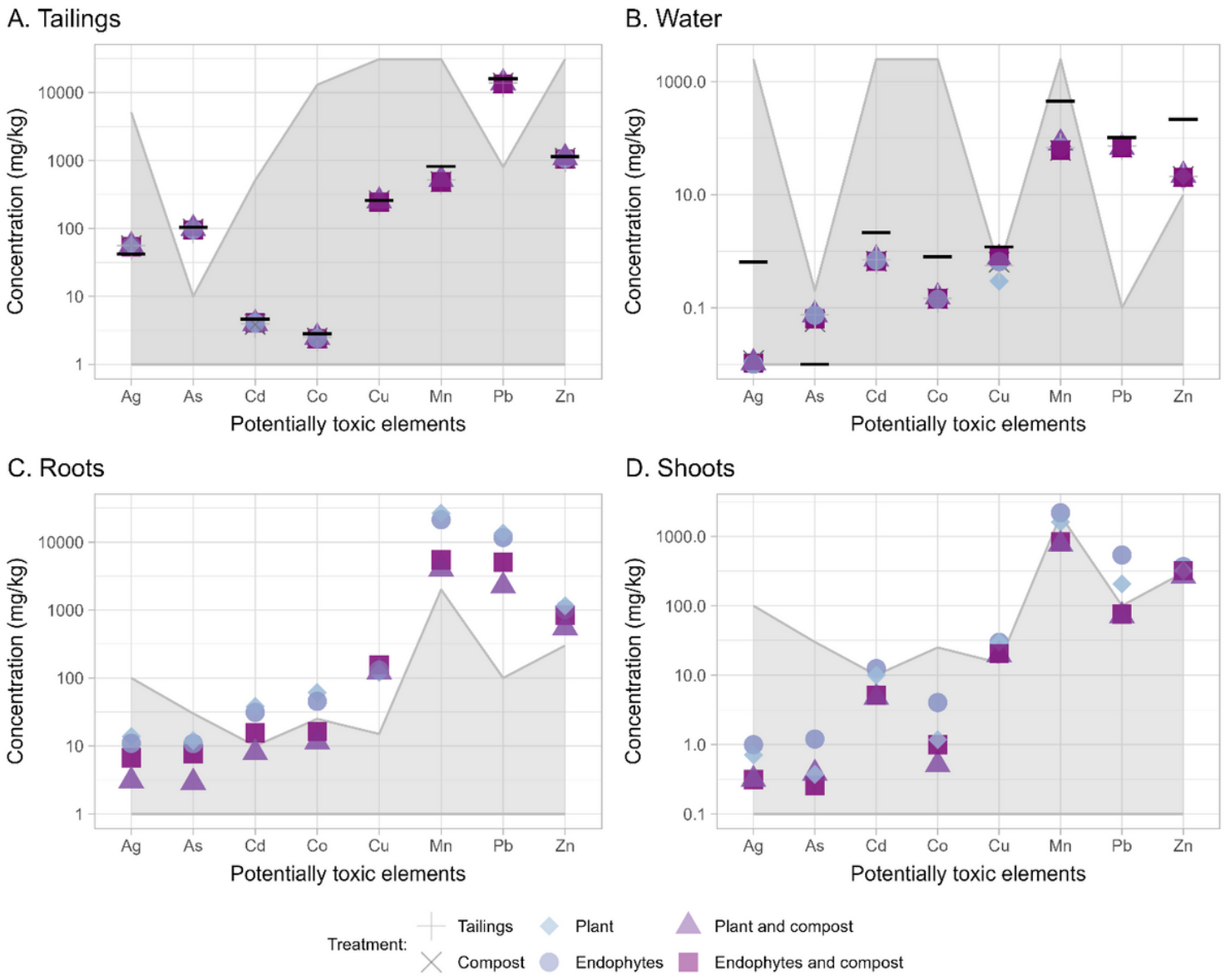

Figure 3

Minor and trace elements (Ag, As, Cd, Co, Cu, Mn, Pb, and Zn) measured in (a) tailings, (b) water extracts of the tailings, (c) plant roots, and (d) plant shoots. Concentrations are shown as averages (large circles) and treatments are distinguished by color and shape. Pre-treatment concentrations in the tailings and water extracts each element are shown by a black horizontal line. Regulatory limits for (a) soils (ADEQ, 2009), (b) drinking water for livestock (ADEQ, 2019), and (c,d) forage limits for grazing livestock (NRC, 2005) are shown as a gray fill: values that exceed regulatory limits are above the gray fill. The y-axis values, shown as a log scale, are similar between the tailings and roots, and are 10 times higher than y-axis values for the water extracts and shoots.

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