



A combined compost, dolomite, and endophyte addition is more effective than single amendments for improving phytoremediation of metal contaminated mine tailings

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Abstract

Background and aims Re-vegetation of mining-impacted landscapes reduces transport of toxic elements while improving soil fertility. This study evaluated whether the planting of a native perennial grass with a consortium of diazotrophic microbial endophytes and municipal waste compost—alone and in combination—enhanced plant growth while stabilizing metal(loids) 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 major, minor, and trace element mobilization and phytostabilization responded to microbial endophyte and/or compost amendments after the 45-day growth experiment.

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, compost to increase nitrogen, and a mixed consortium endophyte seed coating to synergistically increase organic carbon and grass biomass yields. Compost reduced shoot and root concentrations—but not yields—of contaminant metals. Endophytes increased foliar Cd, Co, Mn, and Pb yields but mobilized Pb and Zn from the tailings. Root stabilization of Cd, Co, Mn did not require amendments.

Conclusion The most effective means of revegetating these acidic, polymetallic tailings with the native *B. curtipendula* is with a simultaneous dolomite, compost, and endophyte seed treatment. Due to potential phosphate solubilization and siderophore production by this consortium of endophytes, strategies to capture solubilized metal(loids) may be needed for sulfidic tailings with metal(loids) associated with mobile mineral phases.

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Abbreviations

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

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. In these areas, biological or in-situ approaches like phytoremediation may be more appropriate (Mendez and Maier 2008a).

Phytoremediation uses plants to remove or immobilize contaminants in the environment, and it can be achieved through a variety of approaches (Tonelli

et al. 2022). Phytostabilization is the accumulation and immobilization of contaminants in plant roots without substantial foliar translocation, while phytoextraction is the foliar accumulation and then removal of contaminants (Salt et al. 1995). Because phytoremediation 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 establish in landscapes disturbed by mining if adapted to local soils and climate (Yoon et al. 2006), they are less likely to aggressively spread (Mendez and Maier 2008a), and they may be more effective at ecosystem restoration (i.e., restoring carbon cycling, recruiting other native plants; Thomas et al. 2022). 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 (e.g., Pb, Zn, and Cu; Tordoff et al. 2000). Even locally adapted plants can have trouble establishing if seeded into mine waste without 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 therefore requires a careful selection of soil amendments to alleviate environmental stressors (Gil-Loaiza et al. 2016; Yan et al. 2020).

For sulfidic mine tailings, successful phytoremediation 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 nutrients, they can also 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) 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 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 metal(loid)s in the rhizosphere (i.e., the soil on and near plant roots) and 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 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. A plant survey of the mining district identified *Bouteloua curtipendula* as the most widespread grass species growing in surface and mining-impacted soils with elevated metal concentrations (e.g., Pb, Zn; Lewis 2022). Therefore, *B. curtipendula* is a good native herbaceous candidate for phytoremediation in the area. Although non-native hyperaccumulators may be more tolerant to contaminant metals than the native *B. curtipendula* (e.g., *Brassica* sp. tolerance to Pb, Collin et al. 2022), non-native plants introduced for phytoremediation can become invasive and negatively impact native plant diversity (Neilson and Rajakaruna 2012). Our aim was therefore to increase the metal stabilization and growth potential of *B. curtipendula* with the optimal combination of amendments. The compost was sourced from a waste stream (i.e., municipal waste) and the consortium of bacterial endophytes coating the *B. curtipendula* seeds are commercially available (Intrinsyx Technologies, Sunnyvale, CA, USA) and have the potential to fix nitrogen, solubilize phosphorus, and produce siderophores (Table 1).

Table 1 Endophytes used to coat *B. curtipendula* seeds

Closest 16S match	Putative activities			Endophyte source	Genbank ID
	Nitrogen fixation	Phosphate solubilization	Siderophore production		
<i>Herbaspirillum</i> sp. ^{2,7}	yes	unknown	unknown	WW	KU495919
<i>Sphingomonas</i> sp. ^{2,5,6}	yes	no	yes	WW	KT984987
<i>Pseudomonas</i> sp. ^{2,5,7}	yes	yes	yes	WW	KU557506
<i>Curtobacterium</i> sp. ^{2,4-7}	yes	yes	yes	WW	KU523564
<i>Rhodotorula</i> sp. ^{3,5,8}	yes	no	yes	WP	EU563924
<i>Rahnella</i> sp. ^{2,5-7}	yes	yes	yes	WP	KU497675
<i>Burkholderia</i> sp. ^{2,5-6}	yes	yes	yes	WP	KU523562
<i>Acinetobacter</i> sp. ^{2,5,7}	yes	yes	yes	WP	KU523563
<i>Burkholderia</i> sp. ⁴	yes	yes	yes	WP	KF597275
<i>Rhizobium</i> sp. ^{1,5,7}	yes	yes	unknown	PTD	KT962907

Endophytes were isolated from the stems of wild willows (*Salix sitchensis*, WW), wild cottonwoods (*Populus trichocarpa*, WP), or cottonwood hybrids (*Populus trichocarpa* x *deltoides*: PTD) from the Pacific Northwest (USA). Listed activities were established in pure culture or through gene sequencing and are therefore putative

¹Doty et al. (2005); ²Doty et al. (2009); ³Firincieli et al. (2015); ⁴Kandel et al. (2017a); ⁵Khan et al. (2015); ⁶Khan et al. (2016); ⁷Knoth et al. (2013); ⁸Xin et al. (2009)

Because both compost and bacterial endophytes can increase plant growth and impact metal(loid) mobility, we hypothesized that combining a compost top-dressing with an endophyte seed coating would synergistically improve *B. curtispindula* growth, organic matter accumulation, and rhizosphere stabilization of contaminants.

Materials and methods

Polymetallic mine geology and tailings characteristics

The Blue Nose mining complex (31°26'52.2"N 110°43'58.6"W, 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 were not naturally re-vegetated following deposition. The tailings are finely textured, with 70% of the particles $\leq 125 \mu\text{m}$, and have a relatively low specific surface area of $5.97 \text{ m}^2 \text{ g}^{-1}$. The tailings are acidic (pH of 3.5 in a 1:5 m/v ratio) and infertile, with only 0.01% nitrogen, 0.7% phosphorus, 0.21% organic carbon, $10 \mu\text{g C g}^{-1}$ of soluble organic carbon, and $4 \mu\text{g C g}^{-1}$ of microbial biomass carbon (Creamer et al. 2022a). The tailings pose a potential environmental hazard: concentrations of As ($100 \mu\text{g g}^{-1}$) and Pb (1.6%) are 10–20 times higher than the soil remediation regulatory limits in Arizona (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 2022). 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 bio-available to plants (Creamer et al. 2022b).

Soil amendments

Dolomite was added to the tailings because *B. curtispindula* 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 $< 63 \mu\text{m}$ 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 $\text{pH}_{1:5}$ 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 ($40 \text{ }^\circ\text{C}$) and ground in an agate mortar and pestle to $< 105 \mu\text{m}$. The compost added no appreciable amounts of toxic elements (Creamer et al. 2023).

Seeds of native perennial grass, *B. curtispindula*, were collected within 20 km of the Blue Nose mine by Borderlands Nursery & Seed. Seeds were collected locally, which would allow for vertical transmission (i.e., parent to offspring) of native endophytes or other bacterial symbionts adapted to the local environment (Frank et al. 2017). We coated the collected *B. curtispindula* seeds with a mix of ten potentially beneficial microbial endophytes (Table 1) that are commercially available (Intrinsyx Technologies, Sunnyvale, CA, USA). The endophyte strains in the consortium were isolated from native North American poplar and willow trees and have established benefits on plant growth, nutrient acquisition, and mineral solubilization (putative N_2 -fixation, P-solubilization, and siderophore production activities; Doty et al. 2005, 2009, 2016; Xin et al. 2009; Knoth et al. 2013, 2014; Firrincieli et al. 2015; Khan et al. 2015; Kandel et al. 2017a; Varga et al. 2020). When added alone or in combination these endophyte strains can improve plant growth in a wide range species (e.g., tree, grass, and crop) and growing conditions (e.g., nutrient and drought stress) (Khan et al. 2012, 2015; Knoth et al. 2013; Kandel et al. 2015, 2017b; Aghai et al. 2019). 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 *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 [Analytical methods](#) section). Control and endophyte-coated seeds were stored separately at 4 °C until planting.

Experimental setup

Growth Chamber

The experiment was conducted in an indoor growing complex. Plants of each experimental treatment were grown in separate chambers under a LED grow light (HIGROW 600 W full spectrum light; 400–760 nm) set on a 12 h diurnal cycle (Online Resource 1). The average photosynthetically active radiation reaching the top of the plant pots, measured using a line quantum sensor (MQ-306; Apogee Instruments, Logan UT), was $1100 \pm 219 \mu\text{mol m}^{-2} \text{s}^{-1}$ (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 $29 \pm 1.7\%$ with the lights on and $51 \pm 1.9\%$ with the lights off (logged every 15 min with a HOBO U23 Pro v2 Temperature/Relative Humidity Data Logger; Onset Computer Corporation, Bourne, MA). Each pot was watered with 55 mL of deionized water per day using a gravity-fed drip irrigation system (Online Resource 1). Although irrigation was similar, the water content was higher without compost ($P < 0.001$; $52 \pm 2\%$ with compost versus $57 \pm 2\%$ without). The DI water contributed negligible quantities of organic carbon (< 0.5 ppm).

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 dolomite-amended tailings ($n=8$) or with dolomite-amended tailings and compost ($n=8$). Although *B. curtipendula* was the primary grass established in mineralized soils at the tailings collection site (Lewis 2022), *B. curtipendula* has relatively low germination (10%) and high mortality (50%) in the tailings without soil amendments (Creamer et al. 2022a). 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 filled with 240 g of dolomite-amended tailings that were wet to $54 \pm 3.4\%$ of saturation with deionized water. Fifteen *B. curtipendula* seeds, with or without endophytes (depending on the treatment), were inserted vertically into the tailings and 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 separate chambers of growing complex. Every 3 days we measured gravimetric water content. 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 = $0.0305 * \text{shoot area in mm}^2$; 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).

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 (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. curtispindula* roots and shoots were removed from the tailings by gently loosening the tailings around the roots, and then pulling the plant free. The plant roots and shoots were rinsed with DI water on a 63 μm sieve to remove adhering fragments of tailings and compost. Root and shoot biomass yields were determined gravimetrically while surface area and length was quantified with WinRHIZO™ Regular software after image acquisition with a 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.

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 h) 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 h shaking in 1:40 m/v of 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$. In

addition, fifty-one water-extractable elements were similarly quantified after shaking the tailings for 2 h 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) as well as 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 to 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.

Total DNA extraction, quantification, and amplicon generation

We extracted total genomic DNA from the tailings (0.70 g), compost (0.50 g), and *B. curtispindula* roots (0.20 g) to assess added microbial endophyte colonization. Total genomic DNA was also extracted from surface sterilized, uncoated *B. curtispindula* seeds to identify native endophytes. There was insufficient material (<0.20 g) for reliable extraction of genomic DNA from *B. curtispindula* roots grown without compost, and for *B. curtispindula* 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. 2023). Mock community DNA standards (ZymoBIOMICS Microbial Community DNA Standard, Zymo Research, Irvine, CA) were sequenced and subjected to bioinformatic processing as well. All 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 $\geq 99\%$ 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.

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. curtispindula* seeds planted, or endophyte coated *B. curtispindula* 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). Differences in the normalized abundance of endophyte sequences identified via local BLAST+ search were analyzed with a two-way ANOVA with compost addition and seed type as fixed effects. Data that were 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. All analyses were conducted in R and R studio (version 4.2.2; R Core Team 2023). Values shown in text, tables, and figures are means and 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. curtispindula* 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.

Results

Seedling establishment and growth was highest with combined amendments

In the absence of a compost topdressing, an endophyte seed coating did not improve shoot growth or germination of *B. curtispendula* seedlings grown in dolomite-amended tailings. However, endophyte coated seedlings grown without compost had marginally smaller roots (P values ranged from 0.06 to 0.1) than uncoated seedlings also grown without compost (Fig. 1). Adding compost increased the germination

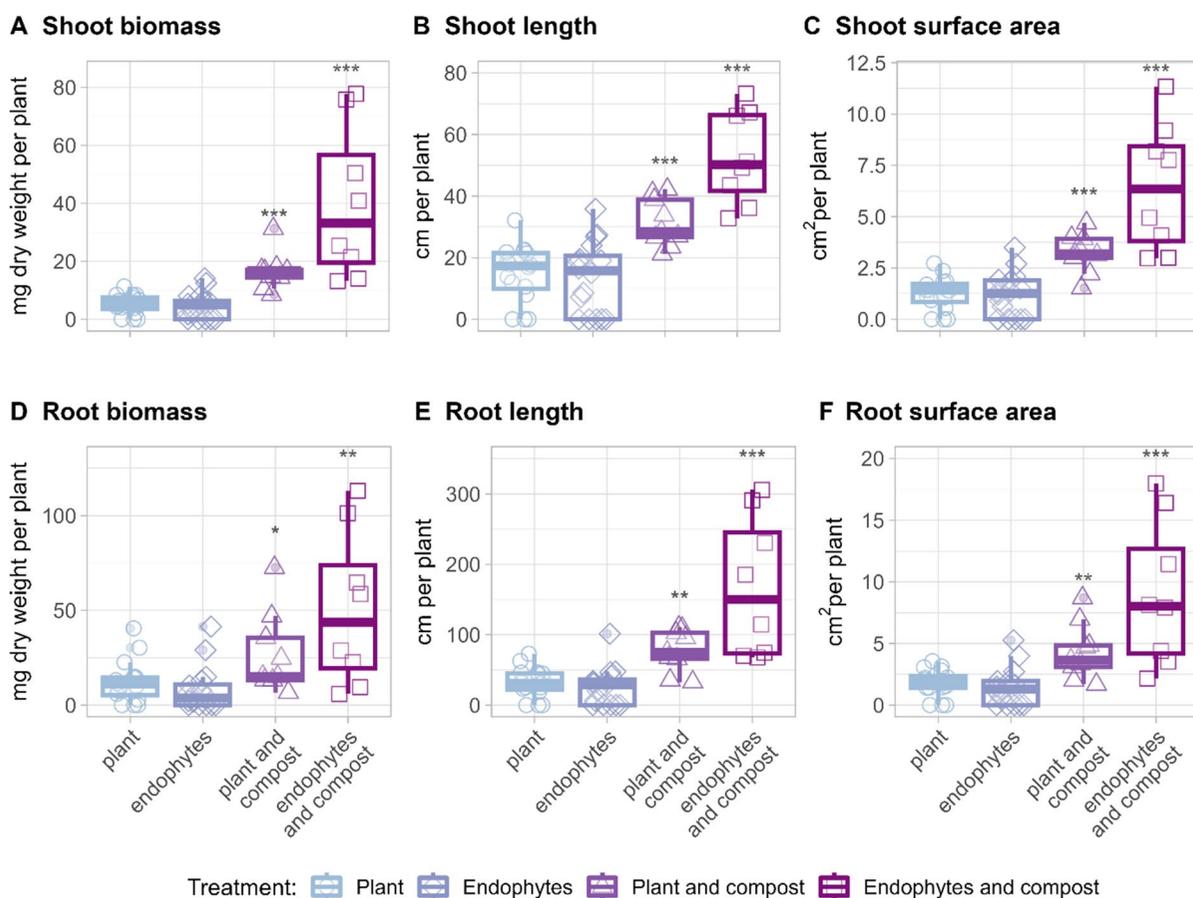


Fig. 1 Improved plant shoot and root biomass with soil amendments. (a,d) Biomass yields (mg dry weight per plant) and (b, c, e, f) growth characteristics (length, surface area) for each plant are shown as open symbols overlaid by a box-plot. The boxplot is drawn from the first to third quartiles with a horizontal line at the median. Whiskers show the minimum and maximum values while outliers are plotted as dots. Sig-

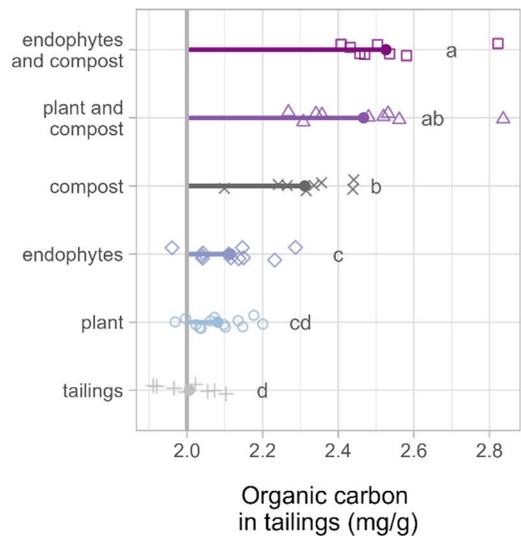
nificant increases in plant growth with endophyte and/or compost treatments relative to the plant-only controls are shown by asterisks (* $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$). The marginal ($P < 0.1$) decreases in root biomass, length, and surface area for between plants grown from endophyte coated vs. uncoated seeds without compost are not shown. Tailings were amended with dolomite in all treatments

of *B. curtispindula* seedlings independent of endophyte additions; about 50% of seeds germinated with compost but only 30% germinated without compost ($P < 0.01$). Although an endophyte seed coating, when added without compost, had minor negative impacts on root growth, the greatest above ($P < 0.001$) and belowground ($P < 0.01$) *B. curtispindula* growth (i.e., biomass, length, surface area) was observed with the combined dolomite, endophyte, and compost treatment (Fig. 1). Shoot growth rates were also the highest when *B. curtispindula* seeds were grown with this treatment ($P < 0.001$; figure in Online Resource 1).

Organic carbon was highest with combined amendments

Compost significantly increased the concentrations of organic carbon and nitrogen in the tailings ($P < 0.001$; Fig. 2). Like plant growth (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. The highest nitrogen concentrations in the tailings were found when a compost topdressing was present, regardless of whether the seeds were coated with endophytes and even regardless of whether there were plants (Fig. 2). Unlike the tailings, concentrations of organic carbon and nitrogen in the compost layer were independent of *B. curtispindula* 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 of the compost (12.5 ± 0.40) compared to the starting C:N ratio (ca. 18.9) indicates a mass loss of carbon and gain in nitrogen during the experiment that is consistent with microbial decomposition and downward mobilization of organic carbon (Creamer et al. 2013). If we include contributions from both the compost and tailings layer, 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. This is roughly 2–3 times the organic carbon and nitrogen stocks in the top 20 cm of grassland ecosystems near our field site (Wheeler et al. 2007).

A Organic carbon



B Total nitrogen

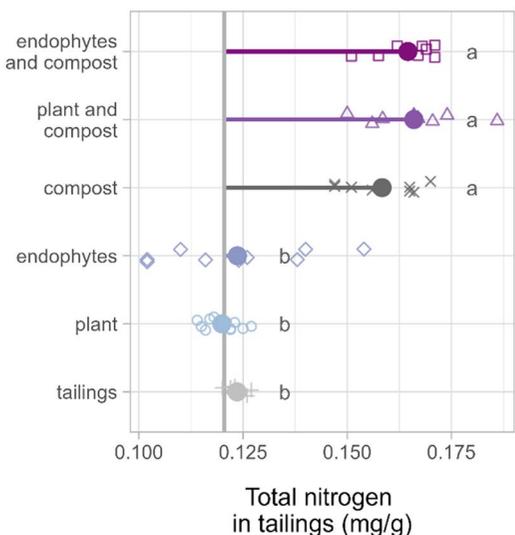


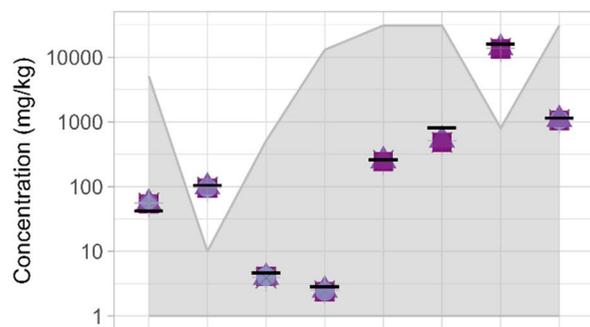
Fig. 2 **a** Organic carbon concentrations and **b** total nitrogen concentrations in tailings. The mean for each treatment is shown as a larger dot relative to pre-treatment carbon and nitrogen concentrations in the tailings (gray vertical line). All measured values for each treatment are shown with an open symbol distinguished by color and shape to show the distribution of the data. Significant differences ($P < 0.05$) between treatments are shown as lowercase letters. Tailings were amended with dolomite in all treatments

Metal(loid)s were mobilized during the experiment

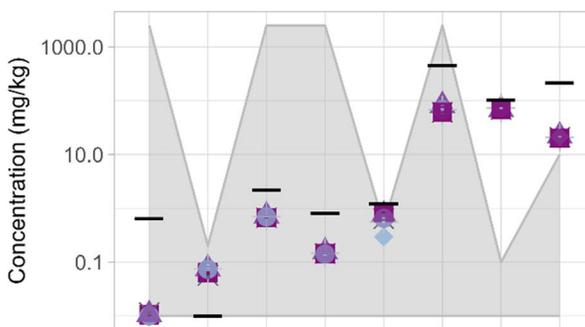
We quantified major (>5%), minor (1–5%), and trace (<1% by mass) elements in the compost, tailings, water extracts, and in *B. curtispindula* seedlings to determine if they were solubilized or immobilized during the experiment. Changes in the solid phase elemental composition of the tailings during the experiment were minor, with typically less than a 25% difference between starting and ending concentrations. Notable exceptions were the losses of Mn from ca. 800 to 500 ± 30 mg kg⁻¹, of Pb from ca. 1.6

to $1.3 \pm 0.04\%$, and of S from ca. 1.4 to $1.1 \pm 0.04\%$ (Fig. 3a). The decreases in solid phase Pb concentrations (as well as minor changes in solid phase As concentrations) were not large enough to lower concentrations below soil remediation limits (Fig. 3a). Compost and/or endophyte additions only modestly changed solid phase concentrations of most of the measured potentially toxic elements (i.e., changed by less than 10% between treatments). However, solid phase concentrations of Pb in the tailings—along with Cu, Fe, and Zn—were lower in pots with endophyte-coated *B. curtispindula* seeds ($0.001 \leq P \leq 0.01$;

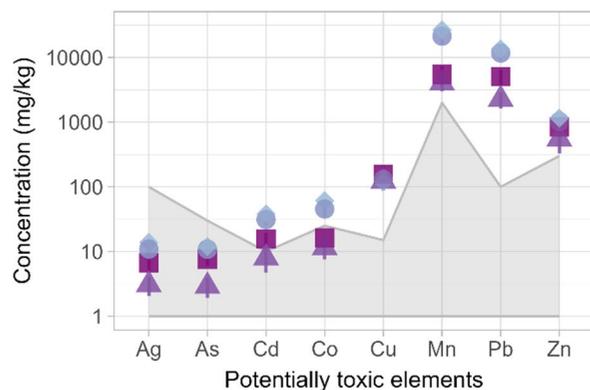
A Tailings



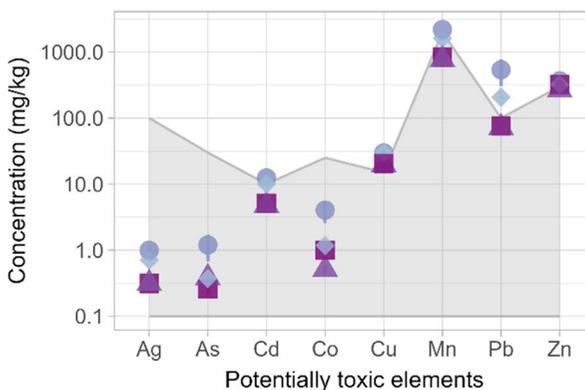
B Water



C Roots



D Shoots



Treatment: + Tailings ◆ Plant ▲ Plant and compost
 × Compost ● Endophytes ■ Endophytes and compost

Fig. 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 means \pm standard error. 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 (Arizona Department of Environmental Quality 2009), **b** drinking water for livestock

(Arizona Department of Environmental Quality 2022), and **c**, **d** forage limits for grazing livestock (National Research Council 2005) are shown as a gray fill and values that exceed regulatory limits are above the gray fill. Element concentrations, shown as a log scale, are similar between the tailings and roots, and are 10 times higher than measured concentrations in the water extracts and shoots. Tailings were amendment with dolomite in all treatments

figure in Online Resource 1). The concentrations of these elements are highly correlated (Pearson's $r \geq 0.75$) because Cu, Pb, and Zn-bearing mineral phases also contain Fe and/or S in these tailings (e.g., 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 ($P[\text{perm}] \leq 0.82$), 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 (Pearson's r ranged from 0.1 to 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 (Pearson's $r = 0.83$). After the experiment, all measured 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 water regulatory limits for grazing livestock (Arizona Department of Environmental Quality 2022) in pots without endophytes or compost (Fig. 3b). The extractability of Cu was correlated with organic carbon (Pearson's $r = 0.60$) but only marginally with tailings pH ($r = 0.30$), due to the higher pH values and organic carbon concentrations in compost treated pots.

Phytoremediation of potentially toxic trace elements was improved by compost and endophyte additions

B. curtispindula roots had higher concentrations of all potentially toxic elements than the shoots ($P[\text{perm}] = 0.0001$; 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. Therefore, only Mg and Ca were efficiently translocated from *B. curtispindula* roots into the shoots, with shoot: root concentration ratios > 1 (i.e., the translocation factor; Thakur et al. 2016). Although the translocation factors of measured elements were similar between treatments, compost lowered the concentration of all potentially toxic trace elements, except Cu and Zn, in plant shoots ($P[\text{perm}] < 0.001$; Fig. 3d). As a result, growing *B. curtispindula* with compost lowered the shoot concentrations of Cd, Mn, and Pb below the forage limits concentrations for sheep and cows, although Cu and Zn concentrations were still above forage limits regardless of treatment (National Research Council 2005). However, foliar yields, which were calculated by multiplying biomass yields by element concentrations, were higher in plants with endophyte coated seeds for Co ($P = 0.003$), Mn ($P = 0.03$), and Pb ($P = 0.05$). The highest foliar yields of Cd and Zn were measured in plants grown with a combined endophyte and compost treatment (Fig. 4). Increased foliar yields of these elements indicates an improvement in phytoremediation, specifically phytoextraction, by *B. curtispindula* if planted with compost and an endophyte seed coating.

Root concentrations of all potentially toxic trace elements, except Cu, were decreased by growing *B. curtispindula* with compost ($P[\text{perm}] < 0.0001$; Fig. 3c). Because the plants were larger with compost and Cu concentrations were similar, Cu root yields were higher for plants grown with compost ($P = 0.03$; Online Resource 1). However, for most elements 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 and a proxy of phytostabilization efficiency; Yoon et al. 2006), was decreased by compost additions ($P < 0.001$), especially 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). Root yields of Cd, Co, and Mn were also marginally lower ($P = 0.10$) for plants grown with just one addition (i.e., compost or endophytes) than plants grown without either amendment due the smaller roots (endophytes only) or lower root concentrations (compost only; Online Resource 1). However, the root yields of these elements were similar for plants grown with all amendments or with just dolomite, because although concentrations were lower, roots

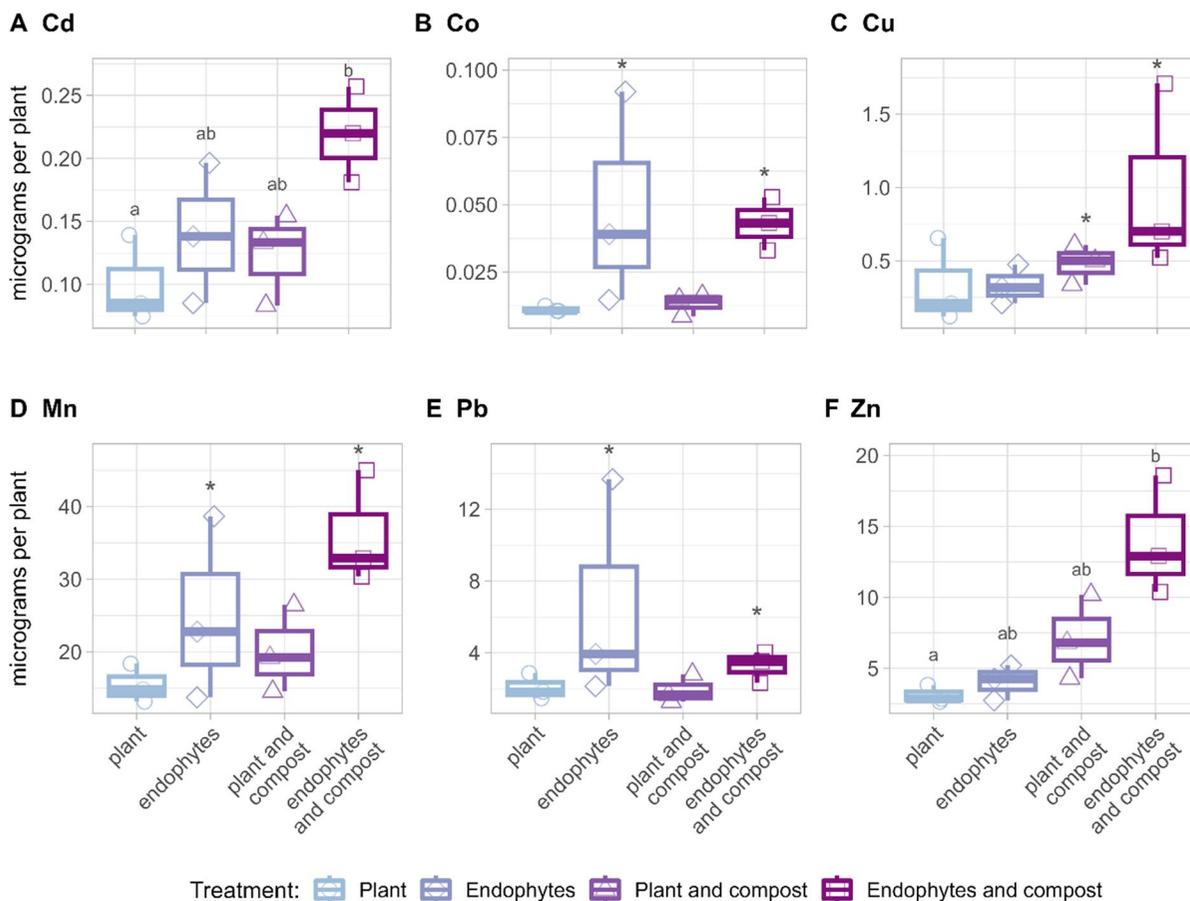


Fig. 4 Foliar yields of minor and trace elements quantified in *Bouteloua curtipendula* shoots after 45 days of growth. Asterisks indicate significant increases in Co ($P=0.003$), Mn ($P=0.03$), Pb ($P=0.05$), or Cu ($P=0.05$) with endophyte or

compost addition (main effect). Letters indicate differences in Cd and Zn between treatments, with the highest yields measured in plants grown with both compost and endophytes. Tailings were amended with dolomite in all treatments

were bigger if grown with a combined compost and endophyte addition. Because phytostabilization aims to immobilize contaminants belowground (e.g., in and on roots), the similar root yields of toxic elements among the treatments indicates that compost and endophyte amendments did not measurably improve belowground phytostabilization of metals by *B. curtipendula* compared to plants grown without amendments (which had high bioconcentration factors for Cd, Co, and Mn).

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

insufficient shoot biomass. 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 similar ($\geq 99\%$) 16S rRNA gene amplicons as the added endophytes. We detected 16S rRNA gene sequences of a total of seven out of the ten 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 $\geq 99\%$ similarity to the added

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

Treatment	Compost layer	Tailings layer	<i>B. curtipendula</i> roots	Surface sterilized seeds
Pre-treatment	<i>Sphingomonas</i> (0.02 %)	None		<i>Pseudomonas</i> (0.81 %) <i>Curtobacterium</i> (0.16 %) <i>Rahnella</i> (0.28 %) <i>Acinetobacter</i> (0.06 %) <i>Burkholderia</i> (0.02 %) <i>Herbaspirillum</i> (0.005 %)
Tailings		None		
Plant		None		
Endophytes		<i>Acinetobacter</i> (0.02%) <i>Burkholderia</i> (0.004%)		
Compost	<i>Herbaspirillum</i> (0.04 %)	<i>Herbaspirillum</i> (0.02%)		
Plant and Compost	None	None	<i>Pseudomonas</i> (0.16 %) <i>Curtobacterium</i> (0.06 %) <i>Acinetobacter</i> (0.05 %)	
Endophytes and compost	None	<i>Rhizobium</i> (0.002%)	<i>Pseudomonas</i> (0.2 %) <i>Curtobacterium</i> (0.06 %) <i>Rahnella</i> (0.05 %) <i>Acinetobacter</i> (0.03 %)	

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 $\geq 99\%$ similarity to the added *Sphingomonas*, *Rhodotorula*, and *Burkholderia* sp. (Genbank ID KU523562) were not identified in any material. *Rhizobium* sp. 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

Pseudomonas sp. were identified in the pre-treatment compost and seeds. This 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).

Discussion

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 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 seed coating 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 poly-metallic 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. Additionally, Pb toxicity may be reduced with more compost because Pb (and other phytotoxic elements) sorbs onto compost (Geebelen et al. 2002; Smith 2009; Forján et al. 2016), as supported by the immobilization of Pb in the compost layer during our experiment.

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. The high metal concentrations in the tailings may have suppressed endophytes and prevented colonization 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, 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, a consortium of eight out of the ten endophytes we used in our experiment colonized and improved growth of herbaceous (e.g., rice, corn) and woody plants (e.g., Kandel et al. 2015, Khan et al. 2015, 2016). Despite effective colonization in other plants, it is possible that the indigenous endophytes in *B. curtipendula* seeds (i.e., those identified in surface sterilized seeds), or certain strains within our added consortia competitively inhibited colonization by the added endophytes (Gaiero et al. 2013). But even if *B. curtipendula* was colonized by at least some of added endophytes, as suggested by Table 2, improved growth is not always realized after endophyte colonization. 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 environmental stress like nutrient limitation, endophytes may only improve plant growth when that environmental stress is alleviated (Cheplick et al. 1989). Additionally, whether endophytes improve plant growth is specific to the combination of plant type and added endophyte, or consortia of added endophytes, with some plant-endophyte inoculants showing similar or lower growth than uninoculated plants (Knoth et al. 2013, 2014; Khan et al. 2015).

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 $\geq 99\%$ sequence identity with the inoculants. Identifying endophytes in plants grown from field-harvested seeds was expected. 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 the added endophytes 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), and its unique presence in tailings with added compost was likely due to compost-assisted changes in tailings chemistry that allowed this strain to colonize. Alternatively, the endophytes that were identified in *B. curtipendula* roots (regardless of treatment) and field-harvested seeds (e.g., *Pseudomonas* sp., *Curtobacterium* sp., *Acinetobacter* sp.) may have improved plant growth.

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) and phytoremediation. 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). In our study, compost was critical to forming organic matter and allowing for microbial growth in the tailings. There was an additional increase in soil carbon with endophyte-coated seeds and compost, likely due to the larger developing root network (Zhao et al. 2022). 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). Root networks also impact the desorption and mobilization of metal(loid) contaminants due to local changes in pH (Yang et al. 2006; Schwab et al. 2008). 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). If the poor hydraulic conductivity and compacted structure of mine tailings is improved after growth of pioneer plants like grasses, other plant species can 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 dolomite, endophyte, and municipal waste compost additions—should be expected to provide the best long-term outcomes for restoring belowground ecosystems (Cooke and Johnson 2002).

Endophyte additions mobilized potentially toxic elements

Bacterial endophytes can enhance the mobilization of metal(loids) in mine waste by chelating iron (siderophores) or solubilizing minerals through phosphate solubilization (Ma et al. 2016). The endophyte consortium added in our experiment produces siderophores and solubilizes calcium phosphate (Doty et al. 2009; Khan et al. 2015; Kandel et al. 2017a), suggesting a potential for increased metal(loid) mobilization. And indeed, the seed coating of endophytes had three primary effects independent of compost addition suggesting changes in

metal(loid) solubility: decreasing water extractability, increasing *B. curtispendula* foliar yields of Cd, Co, Mn, Pb, and Zn (Fig. 4, Online Resource 1), and marginally lower solid phase concentrations of Cu, Pb, and Zn (Online Resource 1).

The production of siderophores by endophytes in the seed coating likely mobilized Cu, Pb, and Zn from Fe-bearing minerals in these tailings (e.g., plumbojarosite and sphalerite; Creamer et al. 2022b), resulting in their loss from the solid phase. Production of siderophores by endophytes has been associated with higher plant metal uptake due to enhanced solubility (Ma et al. 2011b), as we observed for Pb and Zn. Increased mobilization of Cd, Co, and Mn with endophyte additions is supported by the depletion of these elements from the water-extractable pool (Online Resource 1) with corresponding increases in foliar yields (Fig. 4). Additionally, phytoextraction and phytostabilization have been directly linked to plant growth promoting activities of endophyte inoculants (Wang et al. 2020; Alves et al. 2022), and for the combined compost and endophyte addition increased plant growth combined with increased mobilization led to the highest foliar yields of Cd, Cu, Mn, and Zn. Enhanced metal(loid) mobilization can be beneficial if there is corresponding plant uptake and stabilization of the mobilized contaminants (Kumar et al. 2021; Liu et al. 2022a, b). Although foliar yields of Cd, Co, Mn, Pb, and Zn were higher with endophyte additions in our experiment, solid phase concentrations of Pb and Zn decreased to a greater extent (Creamer et al. 2023), so that the net effect of the endophyte treatment was enhanced export rather than immobilization. This highlights the importance of measuring all potential fates of immobilized and solubilized contaminants during phytoremediation to capture potential risks resulting from increasing metal mobilization (Burgess et al. 2018). However, for mine waste with appreciable amounts of Cd, Co, Mn, or Zn, applying endophytes with similar Fe-chelating and mineral solubilizing functions may improve foliar yields from native plants to aid with extraction focused remediation (i.e., phytoextraction) provided potential hazards of mobilized elements are mitigated (e.g., through compost addition).

Although rhizosphere concentrations and yields of potentially toxic trace elements were not increased by endophyte and/or compost additions, *B. curtispendula*

bioconcentrates potential contaminants—particularly Cd, Co, and Mn—in its roots (root: soil ratios $\gg 1$), suggesting it is appropriate for phytostabilization and phytoremediation of these sulfidic mine tailings. However, root concentrations and bioconcentration factors (i.e., root accumulation) of Cd, Co, Mn, and Pb were lower for plants grown with compost (Fig. 3c) likely due to immobilization in the compost layer (Geebelen et al. 2002; Smith 2009; Forján et al. 2016). Due to the combination of lower toxic element concentrations for plants grown with compost and the smaller rhizosphere of plants grown with added endophytes in the absence of compost, the greatest root yields of Cd, Co, and Mn were found in plants grown either with no amendments (outside of dolomite) or with dolomite, compost, and endophytes (Online Resource 1). Chronic abiotic stress can select for endophytes that resist the stressors of the local environment (e.g., salinity but not heat resistance in coastal vegetation; Rodriguez et al. 2008). The native endophytes found in *B. curtipendula* seeds (Table 2) are likely adapted to the naturally elevated concentrations of Mn, Pb, and Zn in soils where the seeds were collected (Lewis 2022) and may have conferred metal resistance to *B. curtipendula* seedlings grown without added endophytes or compost. Although root concentrations were highest with just a dolomite amendment, a combined amendment resulted in similar root yields with the added benefits of higher germination, lower seedling mortality, and greater soil fertility which would translate to better phytoremediation of mine wastes in the field.

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 30% of the Mn and about 50% of the Zn and, 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 (Ma et al. 2011a; 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 from 0.2 to 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).

Conclusion

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 commercially available seed coat of diazotrophic endophytes, improves the growth of a widespread perennial grass, *Bouteloua curtipendula*, and provides the greatest increases in soil fertility and the highest foliar yields of Cd, Co, Mn, and Zn. As a result, this combined treatment is the best approach for phytoremediation of these tailings, which addresses ecosystem degradation in addition to contaminant stabilization and removal (Thomas et al. 2022). A similar approach employed in the field, focused on increasing pH and providing a source of microorganisms and nutrients, would likely improve remediation results (e.g., Gil-Loaiza et al. 2016). This combination is particularly suitable for improved phytostabilization of Cd, Mn, or Zn into *B. curtipendula* roots, or for targeted foliar concentration of Cd, Co, Mn, and Zn. Compared to other studies, the exceedingly 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)—is a barrier to effective re-vegetation of these barren tailings piles. A combination of compost and

endophytes apparently reduced the phytotoxicity of Pb in the tailings by enhancing stabilization (compost) and mobilization (endophytes). Direct planting of *B. curtispindula* seedlings into tailings with similarly elevated Pb concentrations may require additional amendments to immobilize Pb both to prevent offsite transport and improve plant growth or selection of endophyte strains targeted for metal(loid) immobilization, while our presented approach is appropriate for sulfidic tailings with substantial Cd, Mn, and Zn contamination.

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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.

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

Declarations

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 Intrinsic Environmental to perform his work.

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