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Characterization of root-associated fungi and reduced plant growth in soils from a New Mexico uranium mine

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ABSTRACT

Characterizing the diverse, root-associated fungi in mine wastes can accelerate the development of bioremediation strategies to stabilize heavy metals. Ascomycota fungi are well known for their mutualistic associations with plant roots and, separately, for roles in the accumulation of toxic compounds from the environment, such as heavy metals. We sampled soils and cultured root-associated fungi from blue grama grass (*Bouteloua gracilis*) collected from lands with a history of uranium (U) mining and contrasted against communities in nearby, off-mine sites. Plant root-associated fungal communities from mine sites were lower in taxonomic richness and diversity than root fungi from paired, off-mine sites. We assessed potential functional consequences of unique mine-associated soil microbial communities using plant bioassays, which revealed that plants grown in mine soils in the greenhouse had significantly lower germination, survival, and less total biomass than plants grown in off-mine soils but did not alter allocation patterns to roots versus shoots. We identified candidate culturable root-associated Ascomycota taxa for bioremediation and increased understanding of the biological impacts of heavy metals on microbial communities and plant growth.

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root-associated Ascomycota

INTRODUCTION

Fungi are well known for their mutualistic associations with plants (Clay 1988; Rodriguez et al. 2009) and, separately, for their roles in the accumulation of toxic heavy metals in the environment (Burgess et al. 2017; Deng and Cao 2017; Harms et al. 2011; Hassan et al. 2019). Most studies on heavy metal toxicity study fungi in isolation (e.g., in laboratory cultures; Yang et al. 2012; Zhang et al. 2008), whereas studies on plant-fungal symbioses focus on fungal benefits to plants rather than the synergistic potential for plants to influence how fungi interact with their soil environment. For example, the best candidate fungi from laboratory studies on bioremediation of heavy metals (phylum Ascomycota) are also separately documented to grow commonly in plant roots (Loro et al. 2012; Mandyam and Jumpponen 2005), especially in arid regions (Herrera et al. 2010; Knapp et al. 2012). Some arbuscular mycorrhizal (AM) fungi alleviate stress for plants in mine soils by accumulating heavy metals such as cadmium (Cd) at 10–20 times higher rates than plant roots (Janousková et al. 2007). In stressful systems, AM fungi

can be stress alleviators, increase increasing overall plant growth and enhancing heavy metal bioaccumulation and translocation into the plant shoot (Miransari 2010). However, recent studies show that Ascomycota fungi may be better suited for bioremediation than AM fungi, in part because they have been found to be the dominant phylum in areas of high contamination, such as wastewater sludge and mine sites (Evans and Seviour 2012; Jia et al. 2018; Rosenfeld et al., 2018). Thus, non-mycorrhizal fungal endophytes may feature as key microbes in interactions between heavy metals and plants through their influences on soil biochemistry.

Plant-fungal interactions may provide unrealized microbial potential to stabilize metal mixtures, but few studies have characterized fungal communities in roots of plants in metal-contaminated sites, particularly for nonmycorrhizal fungi. Taxa in the Ascomycota have not been well studied in plants under heavy metal exposure, even though they are equally or more abundant in plant roots than arbuscular mycorrhizal (AM) fungi, particularly in dryland grasses (Jumpponen et al. 2017; Márquez et al. 2012). Specifically, Ascomycota root endophytes can have primary roles in alleviating plant

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drought and nutrient stress (Colpaert et al. 2011; Govarthan et al. 2019; Kivlin et al. 2013; Newsham 2011). Plant-associated Ascomycota with high melanin production may rank among the best candidates for stress resistance in plants. Melanin pigments can adsorb radionuclides, including uranium (U) (Keith et al. 2013; McLean et al. 1998; Yang et al. 2012), and melanin fungi commonly dominate contaminated sites as well as dryland ecosystems (Cordero et al. 2017; Dighton et al. 2008; Khidir et al. 2009). Thus, contaminated sites in drylands could provide rich biorepositories to prospect for fungal taxa that maximize bioremediation. Common Ascomycetes with potential to stabilize metal mixtures include *Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium*, *Talaromyces*, and *Trichoderma* (Akhtar et al. 2007; Iram et al. 2009; Lh et al. 2019; Mohd et al. 2017; Segura et al. 2018). These taxa also commonly occur in symbiosis with plant roots as reported in published literature and biogeographic surveys (Jumpponen et al. 2017; Lagueux et al. 2021; Porras-Alfaro and Bayman 2011).

Mining legacies can not only alter microbial communities but also directly impact plant productivity and human health. For example, heavy metals in untreated wastewater can increase metal concentrations in plant tissues (Hussain et al. 2019; Lu et al. 2015). Especially in the Southwest of the United States, abandoned uranium mines disproportionately impact Native American communities, including agricultural lands in which produce is primarily bought by and sold to local community members (Arnold 2014; Yazzie et al. 2020). Heavy metal toxicity has major human health impacts, including lung and bone cancers, kidney damage, high blood pressure, autoimmune disease, and loss of reproductive function (Keith et al. 2013; Zhang et al. 2020). Expanding our understanding of how heavy metals affect plants and plant-microbe interactions can help improve both agricultural and health outcomes for local communities.

We aimed to identify candidate fungi for bioremediation by sampling replicated fungal communities in plant roots across sites with versus without mining legacies. We focused on U mining-contaminated areas in the semiarid drylands of central New Mexico. Here, the Grants mineral belt contains the second largest known U ore reserve in the United States, where average U concentrations average 5 ppm higher than the national average (Brookins 1979; Keith et al. 2013). High levels of U as well as other toxic metals (V, As) surround abandoned mine sites (Blake et al. 2017; Hoover et al. 2017). We surveyed the composition, abundance, and diversity of root-associated endophytic fungi. In addition, we used greenhouse experiments with the focal plant species, blue grama grass, to determine

the effects of contaminated soils on plant production. We addressed the following questions.

- (i) *Do plants growing in mine sites have different root fungal communities than plants outside of mine sites?* We hypothesized that fungal communities associated with plants growing in mine soils will differ from fungi in plants growing in off-mine soils, possibly driven by greater relative abundance of stress-tolerant species.
- (ii) *Do soils from mine sites reduce plant growth and fungal colonization relative to soils outside of mines?* We predicted that plants grown in soils from mine sites would have lower germination and survival, produce less biomass, and allocate more biomass to roots (larger root:shoot ratio) than plants grown in off-mine soils, possibly due to the toxic impacts of heavy metal contamination and associated stress-tolerant microbial communities.

The information obtained from this study provides new insights about fungal community composition and plant productivity in drylands affected by mining legacies, with implications for the improvement of remediation strategies for toxic metals.

MATERIALS AND METHODS

Study sites.—Samples were collected from three mine sites and three off-mine sites in the Pueblo of Laguna on 25–26 Aug 2020. Mine sites were sampled from the interior pit of the Jackpile-Paguate uranium mine. This mine was the largest open-pit uranium mine of its time, consisting of two main pits affecting 1271 hectares of land in operation from 1953 to 1982; it was backfilled in 1985 (Zehner 1985). Radiation at mine sites ranged from 20 to 77 cpm (counts per minute) during the root collection period. Off-mine sites were sampled from the wild landscape of the nearby Pueblo of Laguna in the village of Paguate. Mine soils ranged in U concentration from 4.4 to 48.4 ppm and off-mine soils ranged in U concentration from 1.5 to 1.9 ppm, compared with the average soil U concentration in the United States of 3 ppm (Keith et al. 2013) (SUPPLEMENTARY TABLE 1). All sites had similar soil pH (8.3–8.9) and shared similar concentrations of Ba, Ca, Cr, Cu, Fe, Mn, Na, Ni, P, Sr, Zn, As, U, V, and Pb. All sites were within a 3-km radius of off-mine site 1; thus, we assumed that climate was consistent across sites (SUPPLEMENTARY FIG. 1). Off-mine sites, rather than fully noncontaminated reference sites, were chosen in order to maintain results relevant to the impacted Native

American community and to provide controls with similar geology, climate, and community composition to the mine sites.

Sample collection.—Root samples were taken from the most widespread native grass species in North America, blue grama grass (*Bouteloua gracilis*). We selected three mine and three off-mine sites for a total of six sites (SUPPLEMENTARY FIG. 1). At each site, we randomly selected nine plants for root sampling at ~5 m spacing. We also collected ~1667 g of soil from the rooting zone of each plant (soil depth 0–15 cm), for use in bioassay experiments in the greenhouse. Blue grama roots are shallow and primarily occupy 0–20 cm soil depth in desert grasslands, although roots have been reported as deep as 1.8 m for this species, for which the geographic range extends north into Canada (Anderson 2003; Weaver 1958). Due to COVID-19 and limited access to supplies and laboratory facilities, sample processing was prolonged; roots were surface-sterilized and plated within 23 days after sampling, with $n = 12$ root samples plated within 48 h after sampling (2 plants from each site) and $n = 42$ plated within 17 days after sampling ($N = 54$). Because sample processing was randomized over time, the delay in plating is unlikely to have influenced the detection of differences in fungi among sites but may have increased or reduced absolute species richness across the sample set.

Fungal community composition.—

Root sterilization

We stored root samples at 4 C before processing. Roots were washed with deionized (DI) water to remove excess soil then cut into ~10 3-cm segments, placed into tissue cassettes, and stored in 70% ethanol (EtOH) for future microscopy. The remaining ~10 5-cm segments were surface-sterilized as follows: washed 1 min in 95% EtOH, transferred to 1% sodium hypochlorite for 2 min, transferred to 70% EtOH for 2 min, and rinsed three times with DI H₂O (Higgins et al. 2011; Massimo et al. 2015). We placed roots in small strainers to transfer them among solutions to avoid contamination.

Root culturing/subculturing

We surface-sterilized roots and cut them into ~3-cm sections, using sterile technique, and then placed root segments into 10-cm malt extract agar Petri plates amended with antibiotics (streptomycin and penicillin, 1 mg/L) (“MEA plates” hereafter) to prevent bacterial contamination. We inserted 10 3-cm segments of root into each MEA plate using surface-sterilized forceps,

burying both ends of the root segment into the medium. We organized culture plates such that one plant from each site was processed at the same time so that a full representation of sample types was cultured on any one day. Once fungi began to grow from the roots, individual isolates were subcultured and transferred, using sterile technique, onto a 5-cm MEA plate. Isolates received unique IDs tracking their plot and individual plant of origin. We separated isolates into morphotypes, which were then processed for DNA extraction and Sanger sequencing for taxonomic identification.

Root staining and microscopy

Roots were cleared and stained using the modified Vierheilig method (Herrera et al. 2010; Vierheilig et al. 1998). At 200× on a bright-field microscope, we estimated the proportion of 100 intercepts that intersected fungal structures (McGonigle et al. 1990), including melanized (dark) septate hyphae, hyaline septate hyphae, arbuscles and vesicles of arbuscular mycorrhizal fungi, and aseptate hyphae of Glomerales and basal fungal lineages.

Fungal identification

We extracted DNA from a subset of isolates (96 of 181 isolates) representing each fungal morphotype using the QIAGEN DNeasy 96 Plant Kit (Germantown, MD, USA). Isolates were amplified via polymerase chain reaction (PCR) using standard fungal primers ITS1-FL and TW13 (Gardes and Bruns 2008; Taylor et al., 2016) and Sanger sequenced (Genewiz, South Plainfield, New Jersey; Lyons et al. 2021). We acknowledge that the internal transcribed spacer (ITS) sequencing region cannot parse some Ascomycota genera, including *Fusarium* and *Darksidea* (Romero-Jiménez et al. 2022; Torres-Cruz et al. 2022). We lacked additional resources necessary to sequence these loci due to the large size of our study. Given our questions and the robustness of community composition metrics to differing cutoffs for clustering (because additionally splitting typically produces more rare species) (Matute and Sepúlveda 2019), the impact of additional resolution on species identifications on pairwise dissimilarity scores is minimal. The forward and reverse reads were assembled using Geneious, sorted by length using the sortbylength command, and clustered into operational taxon units (OTUs) at 97% similarity via the cluster_smallmem command in USEARCH (11.0.667, 32 bit) (Edgar 2010). Each isolate was matched to an OTU at 97% similarity using usearch_global from USEARCH. The taxonomic identity for each OTU was determined with SINTAX (Edgar 2016) set to a cutoff of 0.8 and using the UNITE database (8.2, general FASTA release for fungi) (Abarenkov et al.

2010). Sequence data were archived in GenBank, accession numbers ON815377–ON815468.

Soil chemistry.—At each site, we collected a composite soil sample by combining ~1 kg of soil in equal amounts by volume from the rooting zones (soil depth 0–15 cm) of 12 focal plants, including the nine plants used for culturing. Soils were stored at 4 C then subjected to the following chemical analyses. Soil samples were dried overnight in an oven at 60 C in a controlled-temperature oven (Devore et al. 2019). Samples were sieved with a US Standard no. 230 mesh (63 μ m) then analyzed for total elements content (Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Sr, Zn, As, U, V, and Pb) (Blake et al. 2015). Triplicate of homogenized samples (1 g each) were weighed in 50-mL polypropylene tubes for acid digestion. Soil samples were digested by adding 2 mL of concentrated nitric acid (HNO_3), 4 mL of concentrated hydrochloric acid (HCl), and 2 mL of concentrated hydrofluoric acid (HF) (Rahman et al. 2018; Velasco et al. 2019). All reagents used were trace metal grade and high purity. Following heating, acid digested solutions were filtered and diluted with 18 M Ω water to 25 mL to be analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer Optima 5300 DV; Waltham, MA, USA) and inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 300D). Total organic and inorganic carbon content in soil samples was estimated by loss-on-ignition (LOI). The moisture was first removed from the samples by drying them overnight at 105 C. Then the samples were heated at 1100 C for 3 h in a muffle furnace to measure total organic and inorganic (carbonates) content percentage by weight (Dean 1974). The calculation of the total organic and inorganic content in mass was conducted by subtracting the mass of the burned sample from the initial mass of the dried sample (details on LOI calculations are provided in the supplementary information). LOI ranges between 2% and 6.8% across all sites.

Plant bioassay experiment.—

Soil treatments

We grew plants in a total of 36 pots in the greenhouse. Half of the pots in the bioassay received soils from mine sites; the other half were inoculated with off-mine soils. We collected soil samples from the base of each blue grama plant where a corresponding root sample was obtained; thus, each pot received rooting-zone soil from a unique plant individual from the field sites (SUPPLEMENTARY FIG. 1). We stored all soils at 4 C prior to setting up the experiment. During 16–17 Oct 2020, we sieved soils with 2 mm mesh sieves and

transferred them to pots (6.8 $\times$ 17.7 cm) in the greenhouse. Pots were set up from bottom to top: autoclaved paper towel (4 cm) to hold soil in place, $\frac{1}{3}$ volume of sterile soil (Pavestone Natural Play Sand; Tyrone, GA, USA; autoclaved twice for 180 min, at 121 ± 2 C on a gravity cycle), $\frac{2}{3}$ volume of living field-collected soil (~840 g), then 1 cm of sterile soil. Pots were then watered to water holding capacity before seeds were planted.

Greenhouse setup

We placed plants in a greenhouse bay with daytime temperatures of 21–24 C and night temperatures of 15–18 C. We surface-sterilized blue grama seeds, sourced from Curtis & Curtis Seed (Clovis, New Mexico), in 70% EtOH for 3 min and washed them in DI H_2O for 15 min. Eight seeds were carefully placed in each pot and monitored for germination. Plants were hand-watered to water holding capacity for 1 month to avoid overwatering and seed loss.

Bioassay: Response variables

Seedling emergence data were collected daily beginning on day 5, the first day of seedling emergence from the soil, until day 16. At day 16, plants were thinned to one healthy individual per pot to avoid intraspecific competition. A sterile seedling germinated on an autoclaved paper towel in a closed, clear plastic chamber was transplanted into any pot that had no seedling emergence at day 16 ($n = 18$ pots). On day 31, we installed a drip irrigation system, and plants were watered Monday to Friday for 2 min at 7 AM. Plants were censused for survival, plant height, number of leaves, and additional germination every 5 days, beginning on day 18, until harvest on day 85. During harvest, we collected and dried shoots, weighed the total amount of wet roots, cut off a portion of roots for staining, and dried the remaining roots. Root colonization was estimated using the modified Vierhling method as described above for field-collected roots. However, among the subset of roots saved for microscopy, only seven samples had detectable fungal hyphae. Thus, root fungal colonization results from the bioassay were inconclusive.

Data analysis.—

Fungal community structure

To compare fungal community composition between mine and off-mine sites, we used the matrix of taxonomically resolved isolates from Sanger sequencing data (OTU matrix), with the abundance of each taxon (number of isolates that grew in culture) from each plant as the unit of observation. From the OTU matrix, we determined the Bray-Curtis similarity using PRIMER 6.0 (Clarke and Gorley 2006). We applied permutational multivariate

analysis of variance (PERMANOVA) with a nested mixed effects model using replicate site as a random effect nested within the fixed factor of site type (mine vs. off-mine) to account for the statistical non-independence of plants located at the same site. PERMANOVA models were run with 100 randomized restarts and 9999 randomizations, and the effect of site type was tested over variation among sites ($n = 3$) to obtain the pseudo- F ratio. We tested for multivariate homogeneity of variances and differences between site types in dispersion using Permutational analysis of multivariate dispersions (PERMDISP). Then, we used SIMPER (Clarke 1993) to identify which fungal taxa contributed the most to differences among sites.

Fungal diversity, richness, and abundance

All significance reporting is relative to a significance cutoff of $P < 0.05$. We used raw counts from the OTU matrix to calculate several metrics of fungal diversity, including OTU richness, the Shannon diversity index, and the inverse of Simpson's evenness, using the VEGAN package in R (Oksanen et al. 2019; R Core Team 2020). We used mixed effects general linear models on each diversity metric as well as fungal abundance, determined as the total number of isolates cultured and as percentage root colonization. Models used the lmer function in R package LME4 (Bates et al. 2015) to test for the effects of the fixed factor of site type (mine/off-mine) on fungal diversity metrics and abundance, using site as a random effect nested within the site type factor (R Core Team 2020). We present analysis of deviance results using chi-square log-likelihood ratio tests from R package CAR (Fox and Weisberg 2019). All models met assumptions of homogeneity of variances and normality of residuals. Graphics were produced in GGLOT2 (Wickham 2016).

Soil chemistry and fungal communities

We combined the matrix of data on soil chemistry with the root fungal composition matrix in exploratory analysis of relationships between soil chemical properties and fungal community composition. We used Mantel tests in PRIMER 6.0 (RELATE function) for matrix correlation (Spearman R) and distance-based linear models (DistLM function, PRIMER 6.0) to examine correlations between fungal community composition and individual soil properties. Using R, we also examined correlations between fungal diversity metrics and abundance against each soil chemical property, by replacing the factor of site type (mine/off-mine) in the general linear models described above with each soil chemical property as a continuous variable.

Bioassay experiment

We evaluated soil inoculum treatment effects on plant responses using general linear mixed effects models on the following response variables: proportion of seedlings emerged, total plant biomass, root:shoot ratio, and fungal colonization of roots. These models included the fixed effects of soil origin (mine/off-mine) and the random effect of site nested within the soil inoculum origin treatment. We used generalized linear mixed effects models of the same form, but with a binomial distribution of the response variable, to assess soil origin effects on plant mortality (live = 1, dead = 0).

RESULTS

Soil chemistry diverged between mine and off-mine sites. Mine soils contained more than 22 times the amount of U than off-mine sites on average; mine soils also contained 2.5 times more vanadium (V) (FIG. 1). Soil nutrients important for plant production, including P, K, Mg, Ca, Cu, and Z, were lower in mine sites compared with off-mine sites (SUPPLEMENTARY TABLE 1). Specifically, mine sites had 23% less P (marginally nonsignificant, $P = 0.06$), but other soil chemical differences between mine and off-mine soils were not statistically significantly different. Mine and off-mine sites did not differ in soil pH.

(i) Do plants growing in mine sites have different root fungal communities than plants outside of mine sites?

Root-associated fungal abundance, diversity, and community composition

We detected 28 unique OTUs from 181 individual fungal isolates collected across all sites. Thirty-three percent of isolates were identified to genus level with >90% confidence level. Across the full data set, 99% of isolates were Ascomycota and 1% were Basidiomycota, and the top five most common genera were *Fusarium* sp. 1 (OTU3), *Fusarium* sp. 2 (OTU8), *Darksidea* sp. (OTU6), *Pleosporales* sp. 1 (OTU12), and *Xylariales* sp. 1 (OTU10).

Root fungal richness and diversity diverged between mine and off-mine sites, but the total abundance of fungal isolates obtained did not significantly differ, indicating that there was no need to rarefy diversity metrics (Gotelli and Colwell 2001). Fungal OTU richness ($P = 0.007$) was 25% lower on-mine than off-mine, and Shannon diversity was 20% less ($P = 0.016$) in roots from mine soils compared

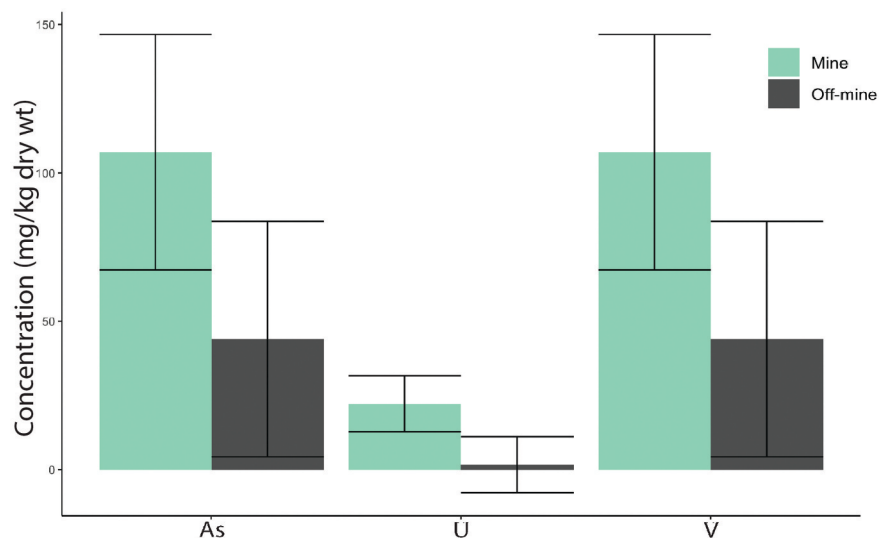


Figure 1. Heavy metal concentrations of arsenic (As), uranium (U), and vanadium (V) in soil collections from mine and off-mine sites. Differences were not significant due to small sample size ($n = 3$ soil samples per bar). Bars show estimated marginal means with SE.

with off-mine (FIG. 2). Differences in evenness were nonsignificant. Fungal abundance cultured from roots and root colonization by fungi estimated via microscopy also showed no significant difference between mine and off-mine sites (FIG. 2).

Mine and off-mine sites did not significantly diverge in overall culturable fungal OTU community composition (perMANOVA, pseudo- $F = 2.44$,

$df = 1, 46$, $P = 0.127$) (FIG. 3). Average dissimilarity between mine and off-mine sites was 87%, whereas within mine sites dissimilarity was 86% and within sites off-mine dissimilarity was 80%.

The majority of fungi cultured from mine sites were classified to the phylum Ascomycota (99%, with the other 1% classified to phylum Basidiomycota). Of the Ascomycota, 40% of OTUs were classified into order

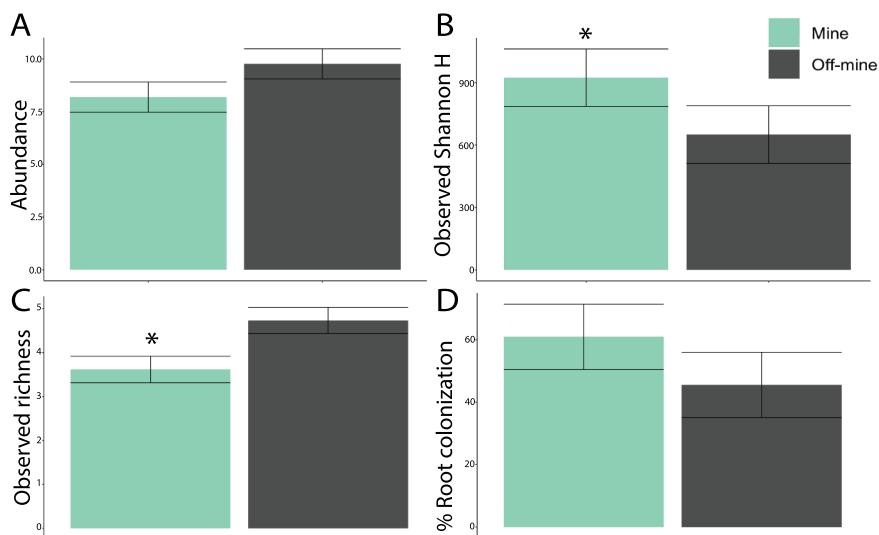


Figure 2. Differences in fungal communities between mine and off-mine sites. (A) Abundance measured as the total number of fungal isolates cultured per plant, (B) Shannon Diversity (H') of cultured isolates (C) Richness of cultured isolates, (D) Abundance measured as % root colonization. Bars show estimated marginal means with SE. Sample size was $n = 9$ plants per site $\times$ 3 sites per soil type = 27 plants per soil type.

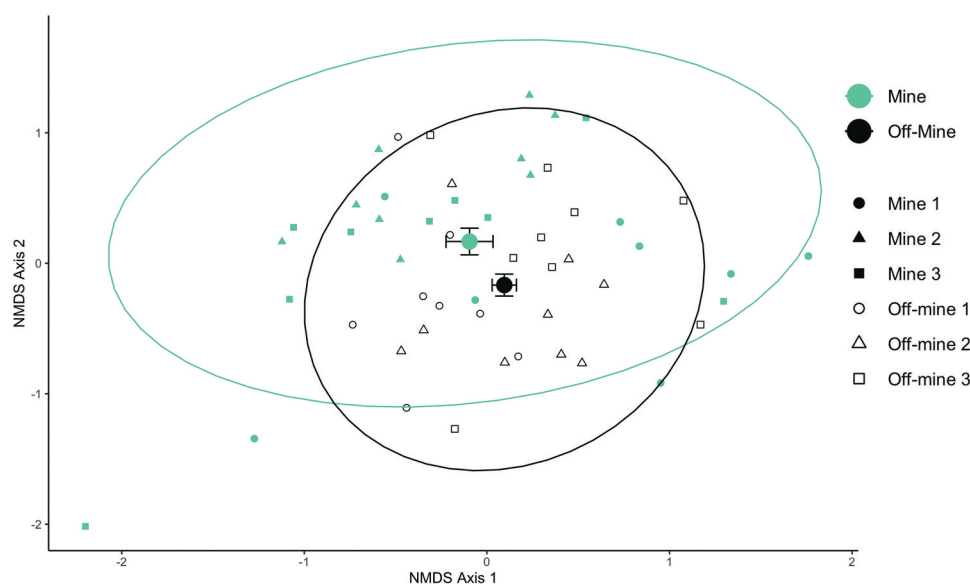


Figure 3. Results from nonmetric multidimensional scaling analysis (NMS) visualizing the similarity in overall fungal community composition among site types. Each dot represents an individual plant assigned among two site types (mine/off-mine), $n = 54$. All root-associated fungi were isolated from blue grama grass (*Boutelloua gracilis*) inside the Jackpile mine or in nearby off-mine sites.

Pleosporales, 39% were order Hypocreales, 11% were order Xylariales, and 10% were order Sordariales (FIG. 4). Of these taxa, 13% were categorized as dark septate endophytic (DSE) fungi. *Fusarium* and *Darksidea* were the most common genera in mine sites. Fungal taxa that diverged most between mine and off-mine sites included *Pleosporales* sp. 1 (OTU12), which was 90% less abundant in mine sites, *Fusarium* sp. 2 (OTU8), which was 45% more abundant in mine sites, and *Xylariales* sp. 1 (OTU10), which was 83% less abundant in mine sites compared with off-mine sites. Other notable fungal taxa that were more abundant in mine than off-mine sites included *Fusarium* sp. 1 (OTU3; 65% more abundant), *Darksidea* sp. (OTU6; 74% more abundant), and uncultured fungus (OTU11; 130% more abundant). OTUs that contributed >5% to overall dissimilarity are listed according to SIMPER classification in TABLE 1.

Soil chemistry and fungal community composition

Comparison of biological OTU matrix data with soil chemistry data showed no significant matrix correlation between the fungal community and soil. However, potassium (K; $P = 0.001$), magnesium (Mg; $P = 0.001$), and sulfur (S; $P = 0.007$) were each significant predictors of fungal community composition through distance-based linear univariate models. Negative correlations of fungal abundance, richness, and diversity metrics against each soil chemical property indicated that community richness was correlated to phosphorus (P; $P = 0.025$, $r = -0.986$), manganese (Mg; $P = 0.043$, $r = -0.959$), and zinc (Zn; $P = 0.054$, $r = -0.991$). Diversity was negatively correlated with

phosphorus concentration (P; $P = 0.028$, $r = -0.986$), but no other characterized element; abundance was not negatively correlated with soil chemistry composition.

(ii) Do soils from mine sites reduce plant growth and fungal colonization relative to soils outside of mines?—

Plant biomass

Mine soils reduced overall plant production (FIG. 5, SUPPLEMENTARY TABLE 1). Both aboveground biomass and belowground biomass were smaller for plants grown in mine soils relative to off-mine. Aboveground plant biomass was 70% lower ($P = 0.0028$) and belowground root biomass was 60% lower ($P = 0.0194$) in mine soils compared with off-mine soils. Total plant biomass was 65% lower ($P = 0.0077$) in mine soils. Mine soils did not alter plant allocation between aboveground and belowground tissue types, as supported by the nonsignificant differences in root:shoot ratio between mine and off-mine soils (FIG. 5C). Additionally, mine soils had 52% lower seedling emergence after 16 days ($P = 0.0435$), and plant mortality doubled for plants grown in mine soils ($P < 0.001$).

DISCUSSION

Uranium mining legacy reduced culturable fungal diversity and richness in plant roots.—Roots of blue grama from mine sites had 20% lower fungal biodiversity than in soils outside of the mine, with no net difference in the abundance of isolates cultured. Previous

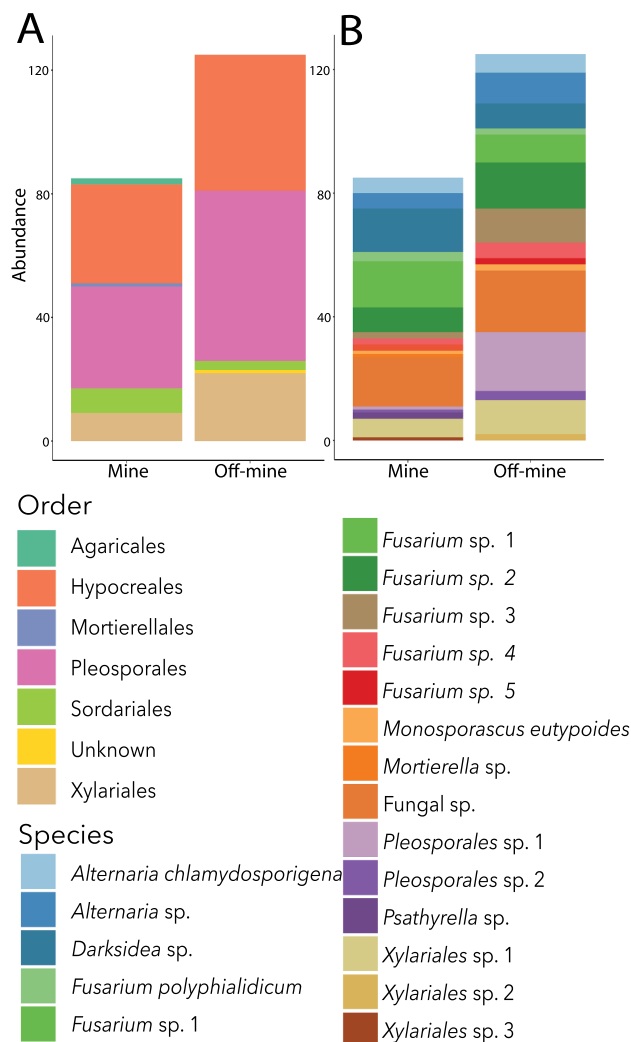


Figure 4. Relative abundance of fungal taxa in mine and off-mine sites separated by order (A) and by species (B). Each colored bar indicates the proportion of taxa belonging to order or genus/species indicated in the key.

studies have focused mainly on soil fungi and arbuscular mycorrhizal (AM) fungi in soils with mine legacies (Gagnon et al. 2020; Wang 2017). Our finding of low diversity of root-associated Ascomycota in uranium mine soils was consistent in magnitude with the low diversity of soil bacteria and soil fungi in soils with

heavy metals in other studies (Rosenfeld et al., 2018). Similarly, root-associated fungi in mercury-contaminated soils had 8–10% lower diversity and 33–41% lower richness in mine soils compared with reference soils, where species were identified through culture-based sequencing (Pietro-Souza et al. 2017). Ascomycota were consistently the most prevalent phylum across metal-contaminated sites, constituting between 97% and 99% of all root-associated fungi across grass species as determined by culture-independent DNA analysis (Narendrula-Kotha and Nkongolo 2017). We found no overall significant divergence in overall composition of culturable root fungi between mine and off-mine sites, although some specific taxa did strongly differ in abundance (see next section). In our prior work, culture-based methods accounted for far fewer fungal taxa (~2.2%) as compared with culture-independent methods for grass root-associated microbes in similar environments, with 7608 OTUs from Illumina sequencing of IT2 compared with 1033 isolates of 166 OTUs from Sanger sequencing of pure cultures (Rudgers et al. 2021). However, because our intent was to characterize fungi for use in bioremediation, it was necessary to use culture-based methods to catalog and archive the isolates for future experiments; our past work importantly showed that we successfully cultured 56% of the host-plant indicator taxa that were identified by ITS2 environmental sequences from roots, and that general patterns in diversity, composition, and richness were robustly similar between culture-based and culture-independent methods (Rudgers et al. 2021).

Microbial populations in arid ecosystems are not typically sensitive to small nutrient shifts, as demonstrated through phosphorous and nitrogen soil nutrient addition studies (Chen et al. 2019; Chung et al. 2017; Sinsabaugh et al. 2015). Similar levels of congruence in overall fungal community composition between contaminated sites and reference sites were reported by (Kerfahi et al. 2020), in which soil chemistry did not predict overall differences in root fungal community composition. Notable differences in root fungal community composition are often correlated to soil pH

Table 1. SIMPER results of fungal taxa that differed most strongly (>5% dissimilarity).

Species	OTU	Average abundance		Average dissimilarity	Contribution %
		Mine	Off-mine		
<i>Pleosporales</i> sp. 1	OTU12	0.04	0.73	9.97	11.41
<i>Fusarium</i> sp. 2	OTU8	0.31	0.58	8.66	9.91
<i>Fusarium</i> sp. 1	OTU3	0.58	0.35	8.64	9.88
<i>Darksidea</i> sp.	OTU6	0.54	0.31	8.23	9.42
<i>Xylariales</i> sp. 1	OTU10	0.23	0.42	6.75	7.72
<i>Alternaria</i> sp.	OTU4	0.19	0.38	6.26	7.16
<i>Fusarium</i> sp. 3	OTU1	0.08	0.42	5.4	6.18
<i>Alternaria chlamydosporigena</i>	OTU5	0.19	0.23	4.47	5.12

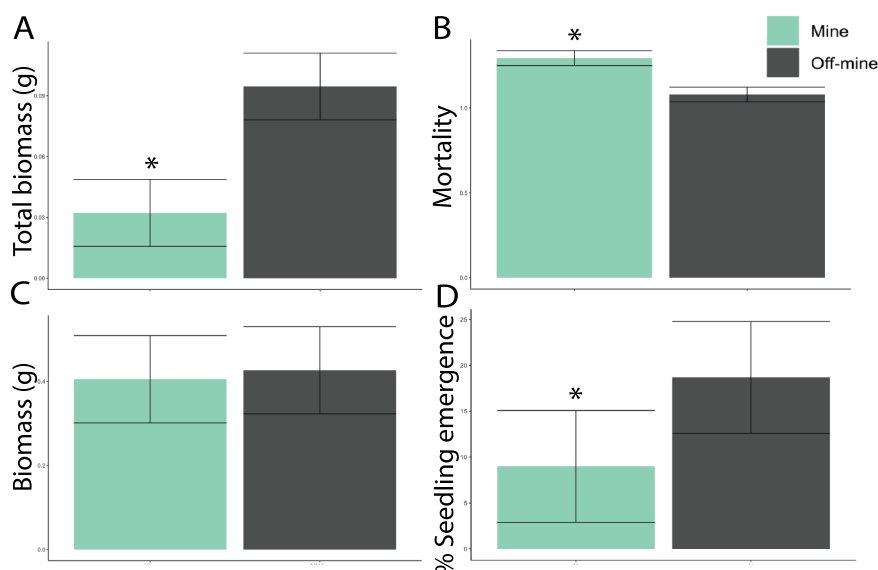


Figure 5. Differences in biomass and emergence of *Bouteloua gracilis* in greenhouse bioassay comparing mine soils ($n = 18$) and off-mine soils ($n = 18$). A. Total plant biomass. B. Plant mortality. C. Root-to-shoot ratio of biomass. D. Percentage seedling emergence out of eight originally planted seeds. Results of seedling emergence and mortality were limited by sample size. Bars show estimated marginal means with SE.

(Rudgers et al. 2021; Siciliano et al. 2014). Our sites did not differ in soil pH, which may explain the lack of dissimilarity between fungal communities. More work will be needed to understand the impact of soil chemistry on root-associated fungal abundance. Our research indicates no significant markers contributing to shifts in abundance, but our results are limited by sample size ($n = 3$ sites of each type for soil chemistry data). However, fungal community diversity and richness were correlated to soil phosphorus, which is known to impact nitrification and denitrification potential (Cui et al. 2020).

Targeting *Fusarium* and *Darksidea* for bioremediation studies.

—The finding that our isolates consisted of 40% *Fusarium* spp. and 13% dark septate endophytic (DSE) taxa are consistent with common reports of *Fusarium* spp. and DSE fungi in other high-stress environments. Of the fungal species identified in this study, *Fusarium oxysporum*, *Fusarium* sp., *Darksidea* sp., and *Setophoma terrestris* are promising targets to test for fungal bioremediation capacity. These taxa were more abundant in mine sites than off-mine and have been linked to stress reduction in other systems. *Fusarium oxysporum* have been linked to stress resistance in chromium-contaminated soils, landfill-contaminated soils in China, coal mining legacy sites, and effective degradation of the harmful pesticide pentachloronitrobenzene (PCNM) (Amatussalam et al. 2011; Ertit Taştan 2017). DSE colonization has also been correlated with

increased concentrations of Pb and decreases in organic matter in soils (Regvar et al. 2010). Both *Darksidea* sp. and *Setophoma terrestris* are known DSE fungi, with melanized, septate hyphae. Melanins, located near cell walls, are thought to reduce toxicity of Cu, Zn, Cd, and Pb (Fogarty and Tobin 1996). Melanin is also known to have a high adsorption capacity for U (McLean et al. 1998). With the cultured isolates from this study, we are now poised to test whether translocation or bioprecipitation of heavy metals plays a role in endophyte plant-fungal symbioses.

Improvements to OTU taxonomy that utilize additional gene regions to more precisely classify fungal taxa will improve our ability to focus on specific species of *Fusarium* and identify other DSE taxa from our isolate collection. To our knowledge, there is no current data on threshold for U or V toxicity for either *Fusarium* or *Darksidea*. Further study of the stress capacity of these species and their functional roles in plants under stress will improve our ability to identify mechanisms involved in symbiotic plant relationships and their effects on heavy metals.

Mine soils reduced plant production.

—Plants exposed to soils from mine sites had reduced seedling emergence, biomass, and survival relative to those in soils collected off-mine that were grown in the same greenhouse environment. This result has important biogeographic consequences because our focal plant is the most widespread native grass in North America, blue grama (*Bouteloua*

gracilis). A combination of depletion of nutrients and greater concentrations of toxic heavy metals is the most plausible cause of the observed shifts in the root fungal community as well as declines in overall plant fitness. However, limited sample size caused by compositing soils from each site for soil chemical analyses made it difficult to detect strong positive or negative correlations between soil chemistry, fungal communities, and plant production.

Reduced plant growth in soils from mine sites could be caused by the direct effects of heavy metals in soil inocula or the indirect effects of a mining legacy on soil microbial communities. Our bioassay experiment indicated that plant biomass was most strongly linked to the U concentration of the soil (FIGS. 1, 5). However, it remains unclear whether U concentration is directly linked to plant health or whether impacts of U on microbial communities caused the plant declines in our simple bioassay. Previously, exposure of roots to heavy metals decreased plant growth, and separately, certain microbial communities were shown to dramatically increase plant growth (Aydinalp and Marinova 2009; Nihorimbere et al. 2011; Rudgers et al. 2020). Fungi are well known to mediate plant responses to soil environment and respond to changes in soil environment (Johnson et al. 2005; Kivlin et al. 2013; Lynn et al. 2019; Rodriguez-Ramos et al. 2021; Rudgers et al. 2014). Thus, soil sterilization treatments in future experiments could help disentangle soil chemistry from soil biological/microbial mechanisms. We did not sterilize soils because our bioassay experiment was a first test to determine whether mine soils had negative effects on plants. Because sterilizations could dramatically alter soil chemistry, including the chemistry of heavy metals (Kraufse et al. 2019), such additional manipulations should be applied cautiously.

Further testing of mine soils on specific plant-fungal symbiosis can help determine the degree to which plant responses are mediated by individual fungal species. Inoculating plants with one of the fungal isolates, now cataloged in our fungarium, and testing for responses in mine and off-mine soils will allow us to track differences in bioremediation functions among different fungal species. To explore plant-fungal symbioses further, plant function could be assessed along U concentration gradients to identify the toxicity threshold for soil U on specific plant-fungal partners.

CONCLUSION

In our study comparing roots and soils collected from mine and off-mine sites, U mining legacies had the strongest influence on culturable root fungal endophyte biodiversity and richness, with weaker declines in fungal abundance and no significant shift in overall fungal community

composition. We identified *Fusarium oxysporum*, a second *Fusarium* sp., a *Darksidea* sp., and *Setopoma terrestris* as focal species to test as potential bioremediators because of their dominance in roots of *Bouteloua gracilis* in mine soils. Our results broaden general understanding of shifts in plant-associated fungal community structure under heavy metal contamination stress.

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LITERATURE CITED

- Abarenkov K, Henrik Nilsson R, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjølner R, Larsson E, Pennanen T, et al. 2010 Apr. The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytol.* 186(2):281–285. doi:10.1111/j.1469-8137.2009.03160.x. PMID: 20409185.
- Akhtar K, Akhtar MW, Khalid AM. 2007. Removal and recovery of uranium from aqueous solutions by *Trichoderma harzianum*. *Water Res.* 41(6):1366–1378.
- Amatussalam A, Abubacker MN, Rajendran RB. 2011 Dec. In situ Carica papaya stem matrix and *Fusarium oxysporum* (NCBT-156) mediated bioremediation of chromium. *Indian J Exp Biol.* 49(12):925–931. PMID: 22403866.
- Anderson MD. 2003. *Bouteloua gracilis*. Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). [accessed 2022 July 14]. <https://www.fs.fed.us/database/feis/plants/graminoid/bougra/all.html>.

- Arnold C. 2014. Once upon a mine: the legacy of uranium on the Navajo Nation. *Environ Health Perspect.* 122(2):A44–A49.
- Aydinalp C, Marinova S. 2009. The effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*). *Bulgarian J Agric Sci.* 15:347–350.
- Bates D, Machler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw.* 67(1):1–48.
- Blake JM, Avasarala S, Artyushkova K, Ali A-MS, Brearley AJ, Shuey C, Robinson W, Nez P, Bill C, Lewis S, et al. 2015. Elevated concentrations of U and co-occurring metals in abandoned mine wastes in a Northeastern Arizona Native American community. *Environ Sci Technol.* 49(14):8506–8514.
- Blake JM, Vore CLD, Avasarala S, Ali A-M, Roldan C, Bowers F, Spilde MN, Artyushkova K, Kirk MF, Peterson E, et al. 2017. Uranium mobility and accumulation along the Rio Paguete, Jackpile mine in Laguna Pueblo, NM. *Environ Sci Process Impacts.* 19(4):605–621.
- Brookins DG. 1979. Uranium deposits of the Grants, New Mexico mineral belt (2), report, July 1979; Washington D.C.: University of North Texas Libraries, UNT Digital Library; crediting UNT Libraries Government Documents Department. [accessed 2023 January 31]. <https://digital.library.unt.edu/ark:/67531/metadc784595/>
- Burges A, Epelde L, Blanco F, Becerril JM, Garbisu C. 2017 Apr 15. Ecosystem services and plant physiological status during endophyte-assisted phytoremediation of metal contaminated soil. *Sci Total Environ.* 584-585:329–338. doi:10.1016/j.scitotenv.2016.12.146. Epub 2016 Dec 29. PMID: 28040210.
- Chen D, Xing W, Lan Z, et al. 2019. Direct and indirect effects of nitrogen enrichment on soil organisms and carbon and nitrogen mineralization in a semi-arid grassland. *Funct Ecol.* 33:175–187.
- Chung YA, Sinsabaugh RL, Kuske CR, Reed SC, Rudgers JA. 2017. Spatial variation in edaphic characteristics is a stronger control than nitrogen inputs in regulating soil microbial effects on a desert grass. *United States.* doi:10.1016/j.jaridenv.2017.03.005.
- Clarke K. 1993. Non-parametric multivariate analyses of changes in community structure. *Austral J Ecol.* 18(1):117–143.
- Clarke KR, Gorley RN. 2006. PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research). Plymouth: PRIMER-E.
- Clay K. 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology.* 69(1):10–16.
- Colpaert J, Wevers J, Krzmaric E. 2011. How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. *Ann For Sci.* 68(1):17–24.
- Cordero R, Vij R, Casadevall A. 2017. Microbial melanins for radioprotection and bioremediation. *Microb Biotechnol.* 10(5):1186–1190.
- Cui Y, Zhang Y, Duan C, Wang X, Zhang X, Ju W, Chen H, Yue S, Wang Y, Li Set al. 2020. Ecoenzymatic stoichiometry reveals microbial phosphorus limitation decreases the nitrogen cycling potential of soils in semi-arid agricultural ecosystems. *Soil Tillage Res.* 197:104463.
- Dean WE. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods. *J Sediment Res.* 44:242–248.
- Deng Z, Cao L. 2017. Fungal endophytes and their interactions with plants in phytoremediation: a review. *Chemosphere.* 168:1100–1106.
- Devore CL, Rodriguez-Freire L, Mehdi-Ali A, Ducheneaux C, Artyushkova K, Zhou Z, Latta DE, Lueth VW, Gonzales M, Lewis J, et al. 2019. Effect of bicarbonate and phosphate on arsenic release from mining-impacted sediments in the Cheyenne River watershed, South Dakota, USA. *Environ Sci Process Impacts.* 21(3):456–468.
- Dighton J, Tugay T, Zhdanova N. 2008. Fungi and ionizing radiation from radionuclides. *FEMS Microbiol Lett.* 281(2):109–120.
- Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinform.* 26(19):2460–2461.
- Edgar RC. 2016. SINTAX, a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. doi:10.1101/074161.
- Ertit Taştan B. 2017. Clean up fly ash from coal burning plants by new isolated fungi *Fusarium oxysporum* and *Penicillium glabrum*. *J Environ Manage.* 200:46–52.
- Evans TN, Seviour RJ. 2012. Estimating biodiversity of fungi in activated sludge communities using culture-independent methods. *Microb Ecol.* 63(4):773–786.
- Fogarty RV, Tobin JM. 1996. Fungal melanins and their interactions with metals. *Enzyme Microb Technol.* 19(4):311–317.
- Fox J, Weisberg S. 2019. An R companion to applied regression. 3rd ed. Sage, Thousand Oaks CA. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Gagnon V, Rodrigue-Morin M, Tremblay J, Wasserscheid J, Champagne J, Bellenger J-P, Greer C, Roy S. 2020. Life in mine tailings: microbial population structure across the bulk soil, rhizosphere, and roots of boreal species colonizing mine tailings in northwestern Québec. *Ann Microbiol.* 70(1):10.1186/s13213-020-01582-9.
- Gardes M, Bruns T. 2008. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol Ecol.* 2(2):113–118.
- Gotelli NJ, Colwell RK. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett.* 4(4):379–391.
- Govarthanan M, Mythili R, Kamala-Kannan S, Selvankumar T, Srinivasan P, Kim H. 2019. In-vitro bio-mineralization of arsenic and lead from aqueous solution and soil by wood rot fungus, *Trichoderma* sp. *Ecotoxicol Environ Saf.* 174:699–705.
- Harms H, Schlosser D, Wick LY. 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat Rev Microbiol.* 9(3):177–192.
- Hassan A, Pariatamby A, Ahmed A, Auta HS, Hamid FS. 2019. Enhanced bioremediation of heavy metal contaminated landfill soil using filamentous fungi consortia: a demonstration of bioaugmentation potential. *Water Air Soil Pollut.* 230(9):1–20.
- Herrera J, Khidir HH, Eudy DM, Porras-Alfaro A, Natvig DO, Sinsabaugh RL. 2010. Shifting fungal endophyte communities colonize *Bouteloua gracilis*: effect of host tissue and geographical distribution. *Mycologia.* 102(5):1012–1026.
- Higgins KL, Coley PD, Kursar TA, Arnold AE. 2011. Culturing and direct PCR suggest prevalent host generalism among diverse fungal endophytes of tropical forest grasses. *Mycologia.* 103(2):247–260.
- Hoover J, Gonzales M, Shuey C, Barney Y, Lewis J. 2017. Elevated arsenic and uranium concentrations in

- unregulated water sources on the Navajo Nation, USA. *Expos Health*. 9(2):113–124.
- Hussain A, Priyadarshi M, Dubey S. **2019**. Experimental study on accumulation of heavy metals in vegetables irrigated with treated wastewater. *Appl Water Sci*. 9(5):122.
- Iram S, Ahmad I, Javed B, Yaqoob S, Akhtar K, Kazmi MR. **2009**. Fungal tolerance to heavy metals. *Pak J Bot*. 41(5):2583–2594.
- Janousková M, Pavlíková D, Vosatka M. **2007**. Potential contribution of arbuscular mycorrhiza to cadmium immobilization in soil. *Chemosphere*. 65(11):1959–1965.
- Jia T, Wang R, Fan X, Chai B. **2018**. A comparative study of fungal community structure, diversity and richness between the soil and the phyllosphere of native grass species in a copper tailings dam in Shanxi Province, China. *Appl Sci*. 8(8):1297.
- Johnson NC, Wolf J, Reyes MA, Panter A, Koch GW, Redman A. **2005**. Species of plants and associated arbuscular mycorrhizal fungi mediate mycorrhizal responses to CO₂ enrichment. *Glob Chang Biol*. 11(7):1156–1166.
- Jumpponen A, Herrera J, Porras-Alfaro A, Rudgers J. **2017**. Biogeography of root-associated fungal endophytes. In: Tedersoo L, editor. *Biogeography of mycorrhizal symbiosis*. Vol. 230. Springer International Publishing. p. 195–222. doi:10.1007/978-3-319-56363-3_10.
- Keith S, Faroon O, Roney N, Scinicariello F, Wilbur S, Ingerman L, Lladós F, Plewak D, Wohlers D, Diamond G. **2013**. Toxicological profile for uranium. Agency for Toxic Substances and Disease Registry (US).
- Kerfahi D, Ogwu MC, Ariunzaya D, Balt A, Davaasuren D, Enkhmandal O, Purevsuren T, Batbaatar A, Tibbett M, Undrakhbold S, et al. **2020**. Metal-tolerant fungal communities are delineated by high zinc, lead, and copper concentrations in metalliferous gobi desert soils. *Microb Ecol*. 79(2):420–431.
- Khidir H, Eudy D, Porras-Alfaro A, Herrera J, Natvig D, Sinsabaugh R. **2009**. A general suite of fungal endophytes dominate the roots of two dominant grasses in a semiarid grassland. *J Arid Environ*. 74(1):35–42.
- Kivlin SN, Emery SM, Rudgers JA. **2013**. Fungal symbionts alter plant responses to global change. *Am J Bot*. 100(7):1445–1457.
- Knapp DG, Pintye A, Kovács GM. **2012**. The dark side is not fastidious – dark septate endophytic fungi of native and invasive plants of semiarid sandy areas. *PLoS ONE*. 7(2):e32570.
- Krauß T, Schütze E, Phieler R, Fürst D, Merten D, Büchel G, Kothe E. **2019**. Changes in element availability induced by sterilization in heavy metal contaminated substrates: a comprehensive study. *J Hazard Mater*. 370:70–79.
- Lagueux D, Jumpponen A, Porras-Alfaro A, Herrera J, Chung YA, Baur LE, Smith MD, Knapp AK, Collins SL, Rudgers JA. **2021**. Experimental drought re-ordered assemblages of root-associated fungi across North American grasslands. *J Ecol*. 109(2):776–792.
- Loro M, Valero-Jiménez CA, Nozawa S, Márquez LM. **2012**. Diversity and composition of fungal endophytes in semiarid Northwest Venezuela. *J Arid Environ*. 85:46–55.
- Lu Y, Yao H, Shan D, Jiang Y, Zhang S, Yang J. **2015**. Heavy metal residues in soil and accumulation in maize at long-term wastewater irrigation area in Tongliao, China. *J Chem*. 2015:e628280.
- Lynn JS, Duarte DA, Rudgers JA. **2019**. Soil microbes that may accompany climate warming increase alpine plant production. *Oecologia*. 191(3):493–504.
- Lyons KG, Mann M, Lenihan M, Roybal O, Carroll K, Reynoso K, Kivlin SN, Taylor DL, Rudgers JA. **2021**. Culturable root endophyte communities are shaped by both warming and plant host identity in the Rocky Mountains, USA. *Fungal Ecol*. 49:101002.
- Mandyam K, Jumpponen A. **2005**. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Stud Mycol*. 53:173–189.
- Márquez SS, Bills GF, Herrero N, Zabalgoitia I. **2012**. Non-systemic fungal endophytes of grasses. *Fungal Ecol*. 5(3):289–297.
- Massimo NC, Nandi Devan MM, Arendt KR, Wilch MH, Riddle JM, Furr SH, Steen C, U'Ren JM, Sandberg DC, Arnold AE. **2015**. Fungal endophytes in aboveground tissues of desert plants: infrequent in culture, but highly diverse and distinctive symbionts. *Microb Ecol*. 70(1):61–76.
- Matute DR, Sepúlveda VE. **2019**. Fungal species boundaries in the genomics era. *Fungal Genet Biol*. 131:103249.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. **1990**. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytol*. 115(3):495–501.
- McLean J, Purvis OW, Williamson BJ, Bailey EH. **1998**. Role for lichen melanins in uranium remediation. *Nature*. 391(6668):649–650.
- Miransari M. **2010**. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biol*. doi:10.1111/j.1438-8677.2009.00308.x.
- Mohd S, Shukla J, Kushwaha AS, Mandrah K, Shankar J, Arjaria N, Saxena PN, Narayan R, Roy SK, Kumar M. **2017**. Endophytic fungi *piriformospora indica* mediated protection of host from arsenic toxicity. *Front Microbiol*. 8:8.
- Narendrula-Kotha R, Nkongolo KK. **2017**. Bacterial and fungal community structure and diversity in a mining region under long-term metal exposure revealed by metagenomics sequencing. *Ecol Genet Genom*. 2:13–24.
- Newsham KK. **2011**. A meta-analysis of plant responses to dark septate root endophytes. *New Phytol*. 190(3):783–793.
- Nihorimbere V, Ongena M, Smargiassi M, Thonart P. **2011**. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotech Agron Soc Env*. 15:327–337.
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, et al. **2019**. *Vegan: community ecology package*. CRAN.R. <http://CRAN.R-project.org/package=vegan>
- Pietro-Souza W, Mello IS, Vendruscullo SJ, Silva GFD, Cunha CND, White JF, Soares MA. **2017**. Endophytic fungal communities of *Polygonum acuminatum* and *Aeschynomene fluminensis* are influenced by soil mercury contamination. *Plos ONE*. 12(7):e0182017.
- Porras-Alfaro A, Bayman P. **2011**. Hidden fungi, emergent properties: endophytes and microbiomes. *Annu Rev Phytopathol*. 49(1):291–315.
- Rahman A, El Hayek E, Blake JM, Bixby RJ, Ali A-M, Spilde M, Otieno AA, Miltenberger K, Ridgeway C,

- Artyushkova K, et al. **2018**. Metal reactivity in laboratory burned wood from a watershed affected by wildfires. *Environ Sci Technol*. 52(15):8115–8123.
- R Core Team. **2020**. R: the R project for statistical computing. <https://www.r-project.org/>
- Regvar M, Likar M, Piltaver A, Kugonič N, Smith JE. **2010**. Fungal community structure under goat willows (*Salix caprea* L.) growing at metal polluted site: the potential of screening in a model phytostabilisation study. *Plant Soil*. 330(1):345–356.
- Rodriguez-Ramos JC, Cale JA, Cahill JF, Simard SW, Karst J, Erbilgin N. **2021**. Changes in soil fungal community composition depend on functional group and forest disturbance type. *New Phytol*. 229(2):1105–1117.
- Rodriguez RJ Jr, White Jr JF, Arnold AE, Redman RS. **2009**. Fungal endophytes: diversity and functional roles. *New Phytol*. 182(2):314–330.
- Romero-Jiménez MJ, Rudgers JA, Jumpponen A, Herrera J, Hutchinson M, Kuske C, Dunbar J, Knapp DG, Kovács GM, Porras-Alfaro A. **2022**. *Darksidea phi*, sp. nov., a dark septate root-associated fungus in foundation grasses in North American great plains. *Mycologia*. 114(2):254–269.
- Rosenfeld CE, James BR, Santelli CM. **2018**. Persistent bacterial and fungal community shifts exhibited in selenium-contaminated reclaimed mine soils. *Appl Environ Microbiol*. 84(16):e01394–18. /aem/84/16/e01394–18.atom.
- Rudgers JA, Afkhami ME, Bell-Dereske L, Chung YA, Crawford KM, Kivlin SN, Mann MA, Nuñez MA. **2020**. Climate disruption of plant-microbe interactions. *Ann Rev Ecol Evol Syst*. 51(1):561–586.
- Rudgers J, Fox S, Porras-Alfaro A, Herrera J, Reazin C, Kent D, Souza L, Chung Y, Jumpponen A. **2021**. Biogeography of root-associated fungi in foundation grasses of North American plains. *J Biogeogr*. 49. doi:10.1111/jbi.14260.
- Rudgers JA, Kivlin SN, Whitney KD, Price MV, Waser NM, Harte J. **2014**. Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. *Ecology*. 95(7):1918–1928.
- Segura FR, Cavalheiro Paulelli AC, Leite Braga GU, Pedreira Filho WDR, Silva FF, Batista BL. **2018**. Promising filamentous native fungi isolated from paddy soils for arsenic mitigation in rice grains cultivated under flooded conditions. *J Environ Chem Eng*. 6(4):3926.
- Siciliano SD, Palmer AS, Winsley T, Lamb E, Bissett A, Brown MV, van Dorst J, Ji M, Ferrari BC, Grogan P, et al. **2014**. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. *Soil Biol Biochem*. 78:10–20.
- Sinsabaugh RL, Belnap J, Rudgers J, Kuske CR, Martinez N, Sandquist D. **2015**. Soil microbial responses to nitrogen addition in arid ecosystems. *Front Microbiol*. 6. doi:10.3389/fmicb.2015.00819.
- Soares Guimarães LH, Segura FR, Tonani L, Reginavon-Zeska-Kress M, Lisboa Rodrigues J, Augusto Calixto L, Ferreira Silva F, Lemos Batista B. **2019**. Arsenic volatilization by *Aspergillus* sp. and *Penicillium* sp. Isolated from rice rhizosphere as a promising eco-safe tool for arsenic mitigation. *J Environ Manage*. 237:170–179.
- Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T. **2016**. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for illumina amplicon sequencing. *Appl Environ Microbiol*. 82(24):7217–7226.
- Torres-Cruz TJ, Whitaker BK, Proctor RH, Broders K, Laraba I, Kim HS, Brown DW, O'Donnell K, Estrada-Rodríguez TL, Lee YH, et al. **2022**. FUSARIUM-ID v.3.0: an updated, downloadable resource for *Fusarium* species identification. *Plant Dis*. 106(6):1610–1616.
- Velasco CA, Artyushkova K, Ali A-MS, Osburn CL, Gonzalez-Estrella J, Lezama-Pacheco JS, Cabaniss SE, Cerrato JM. **2019**. Organic functional group chemistry in mineralized deposits containing U(IV) and U(VI) from the Jackpile Mine in New Mexico. *Environ Sci Technol*. 53(10):5758–5767.
- Vierheilig H, Coughlan AP, Wyss U, Piché Y. **1998**. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl Environ Microbiol*. 64(12):5004–5007.
- Wang F. **2017**. Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: mechanisms and applications. *Crit Rev Environ Sci Technol*. 47(20):1901–1957.
- Weaver JE. **1958**. Summary and interpretation of underground development in natural grassland communities. *Ecol Monogr*. 28(1):55–78.
- Wickham H. **2016**. ggplot2: elegant graphics for data analysis. 2nd ed. Springer International Publishing. doi:10.1007/978-3-319-24277-4.
- Yang HB, Tan N, Wu FJ, Liu HJ, Sun M, She ZG, Lin YC. **2012**. Biosorption of uranium(VI) by a mangrove endophytic fungus *Fusarium* sp. #ZZF51 from the South China Sea. *J Radioanal Nucl Chem*. 292(3):1011–1016.
- Yazzie SA, Davis S, Seixas N, Yost MG. **2020**. Assessing the impact of housing features and environmental factors on home indoor radon concentration levels on the Navajo Nation. *Int J Environ Res Public Health*. 17(8):2813.
- Zehner HH. **1985**. Hydrology and water-quality monitoring considerations, Jackpile uranium mine, Northwestern New Mexico. US Geological Survey.
- Zhang W, Liu W, Bao S, Liu H, Zhang Y, Zhang B, Zhou A, Chen J, Hao K, Xia W, et al. **2020**. Association of adverse birth outcomes with prenatal uranium exposure: a population-based cohort study. *Environ Int*. 135:105391.
- Zhang Y, Zhang Y, Liu M, Shi X, Zhao Z. **2008**. Dark septate endophyte (DSE) fungi isolated from metal polluted soils: their taxonomic position, tolerance, and accumulation of heavy metals In Vitro. *J Microbiol*. 46(6):624–632.