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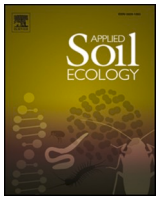

Progressive belowground soil development associated with sustainable plant establishment during copper mine waste revegetation

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ABSTRACT

Critical to the environmental sustainability of hard rock mining is the reclamation of disturbed lands following mine closure through revegetation. Improved understanding of associations between above- and belowground processes that characterize successful plant establishment is critical to the implementation of more efficient revegetation strategies for nutrient-poor mine waste materials. The specific objective of this five-year temporal study was to identify progressive biotic and abiotic indicators of primary soil development on mine waste rock (WR) on a slope hydroseeded with native plant species, and to quantify comparative effects of plant lifeform on soil development. Aboveground plant diversity and belowground substrate properties were measured annually at 67 m intervals along transects following the slope contour. Seeded WR was compared to unseeded WR and the adjacent native ecosystem. A temporal increase in WR microbial biomass was observed in seeded WR relative to unseeded areas. Microbial community analysis found the unseeded WR to be defined by oligotrophic microbes, whereas targeted grass and shrub root zone samples demonstrated significant increases in specific cellulose and lignin degrading and N-cycling phylotypes. More extensive chemical and biological fertility development was observed in shrub root zones relative to grass. Ten chemical and biological indicators increased significantly in shrub WR relative to unseeded WR, whereas grass WR was only enriched in bacterial 16S rRNA gene copy number/g substrate and bacterial/archaeal and fungal diversity. In addition, the shrub root zone had significantly higher nitrogen-cycling potential than grass root zones or unseeded WR. Thus, both grasses and shrubs improve belowground WR development; however, shrub establishment had greater fertility outcomes. Concurrent belowground fertility development is critical to sustainable plant establishment. Coupled evaluation of above- and belowground metrics provides an improved quantitative assessment of revegetation progress and a valuable tool to guide management decisions.

1. Introduction

Metals sourced from hard rock mining, such as copper, are critical for building infrastructure and advancing technology. Indeed, copper consumption in the United States alone is projected to increase 17 % by 2070 (He and Small, 2022). Although crucial to our society, hard rock mining radically disturbs natural ecosystems and degrades thousands of hectares of land following mineral extraction and waste material storage (Borden, 2011). Environmentally sustainable mining practices advocate for revegetation of the vast waste disposal areas following mine closure. Revegetating waste piles is a valuable and widely accepted strategy to reintegrate mine sites into the surrounding environment (Sheoran et al., 2010; Tordoff et al., 2000). Establishment of native plants reduces sediment erosion from wind and water; controls dust emissions; stabilizes waste dumps; and transforms disturbed land to productive ecosystems (Gil-Loaiza et al., 2016; Sheoran et al., 2010; Tordoff et al., 2000). However, in arid and semiarid regions, revegetation outcomes following hard rock mining can be unpredictable. Improved understanding of coupled above- and belowground quantitative indicators associated with sustainable plant establishment on semiarid degraded lands is critical to guide management practices that enhance revegetation success.

Waste rock (WR), a vast component of mineral mine waste, is generated from blasted rock that is discarded in large piles (dumps) due

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to mineral concentrations too low for economic extraction (Borden, 2011; Orman et al., 2011). WR dumps typically lack heavy metals and acid-generating contaminants. WR revegetation is distinctly challenging due to the coarse particle size and low organic matter levels of the materials, which limit moisture-holding capacity and reduce nutrient storage (Bradshaw, 1997). In addition, WR lacks a developed microbiome to facilitate the nutrient cycling that is essential to the development of a healthy ecosystem (Borden and Black, 2005). As such, WR revegetation provides an excellent opportunity to characterize primary stages of soil development that are associated with successful plant establishment.

Common methods for revegetating WR dumps include application of a cover-soil cap or directly seeding the waste material (Gil-Loaiza et al., 2016; Tordoff et al., 2000). The cover-soil cap enhances revegetation efforts because soil has greater fertility than WR (Kneller et al., 2018; Muñoz-Rojas et al., 2016); however, this technology is more expensive and environmentally destructive than direct seeding because the soil must be sourced from elsewhere (Gil-Loaiza et al., 2016; Honeker et al., 2017) or stockpiled during mine development. Additionally, the fine particles of cover soils (comprised of fines) are likely to erode quickly when applied to the steep slopes of WR dumps, rendering the soil cover ineffective (Leavitt et al., 2000; Williams, 2001). Hence, mining companies are increasingly interested in developing methods for directly seeding WR. The long-term success of direct seeding is dependent on accelerated incipient soil development of poor-quality WR material. Target soil properties must also be identified that define ecosystem resiliency: the presence of a soil matrix capable of sustaining a diverse, stable ecosystem under the environmental stresses of semiarid regions (i.e., frequent drought and extended wet-dry climate cycles).

Transitions in soil microbiome development that associate with plant establishment (Blackmore et al., 2018; Fierer et al., 2013; Garris et al., 2016; Guo et al., 2017; Honeker et al., 2019) during degraded land recovery are poorly understood. Soil quality indicators have traditionally been disproportionately skewed towards chemical and physical indicators such as soil texture, pH, electrical conductivity, total nitrogen, total organic carbon, macronutrients and micronutrients (Ca, S, Cu, Zn, Mn) (Asensio et al., 2013; Blecker et al., 2012; Mukhopadhyay et al., 2016). Recent reviews have emphasized the need for improved understanding of the association between substrate microbiome development and plant growth and productivity (Bandyopadhyay and Maiti, 2019; Blackmore et al., 2018; Coban et al., 2022). Previous research has used fatty acid methyl ester (FAME) (Mummey et al., 2002) and phospholipid fatty acid (PLFA) extraction (Blecker et al., 2012; Dangi et al., 2012) biotic indicators during ecosystem restoration, along with general metrics such as heterotrophic plate counts (Gil-Loaiza et al., 2016); however, these indicators provide limited understanding of the microbial community structural development that associates with successful plant establishment. Mghazli et al. (2021) characterized the structure and composition of bacterial and archaeal communities in phosphate mine waste but were unable to identify associations between microbial community composition and plant establishment. Such analyses have been traditionally associated with plant establishment in acid mine waste (Honeker et al., 2017, 2019; Moynahan et al., 2002; Tordoff et al., 2000). In addition, most studies capture a single time point in the process of reclamation (Blecker et al., 2012; Moynahan et al., 2002; Mukhopadhyay et al., 2016; Mummey et al., 2002) or use a chronosequence approach (Asensio et al., 2013; Dangi et al., 2012), both of which do not capture critical changes in mine waste development.

The specific aim of this research is to (1) identify specific belowground biological, chemical, and physical substrate properties that associate with self-sustaining plant establishment as indicators of soil development; and (2) analyze the relative impacts of grass and shrub establishment on indicators of soil development. We hypothesize that successful plant establishment over five years is correlated with significant increases in specific indicators of belowground soil quality. In this study, we compare the development of hydroseeded WR to both unseeded WR and undisturbed native soil. Abiotic and biotic substrate properties were quantified through soil chemistry and microbiome analysis. Microbiome development was assessed through quantification of total soil DNA biomass, bacterial abundance (16S rRNA gene qPCR), bacterial nitrification potential (*amoA* bacterial gene qPCR), and microbial community diversity analysis using amplicon sequencing of the bacterial/archaeal 16S rRNA gene and fungal rRNA gene ITS region.

2. Materials and methods

2.1. Site description

This study was conducted at a copper mine located near Miami, Arizona, USA (elevation 1036 m). The region is classified as a semiarid desert (AI = 0.218; Trabucco and Zomer, 2019) with an average annual temperature of 16.5 °C (Fick and Hijmans, 2017). The natural ecosystem is a shrub/grassland desert chaparral with vegetation dominated by *Eragrostis intermedia* (plains lovegrass) and *Hilaria belangeri* (curly mesquite) grasses and shrubs including *Gutierrezia sarothrae* (broom snakeweed), *Quercus turbinella* (Sonoran scrub oak), *Juniperus monosperma* (Oneseed juniper) and *Prosopis velotina* (velvet mesquite). The copper mine waste rock (WR) stockpile is composed of friable dacite that is graded to a 3:1 slope angle. This study focuses on the west and southwest aspects of the WR slopes, which were divided into an unseeded upper portion and a lower portion hydroseeded in 2012 (Fig. S1). The seed mix was composed of perennial grasses (6 species; 67 % of seed mix by seeds/m^2), perennial forbs (3 species; 28 % of seed mix by seeds/m^2) and shrubs (4 species; 5 % of seed mix by seeds/m^2) (Table S1).

2.2. Experimental design

The experiment includes two components: 1) the temporal study and 2) the comparative root zone study of grass versus shrub establishment. For the temporal study, the WR slope was monitored annually for five years (2014–2018), beginning two years after hydroseeding in 2012. Annual sampling occurred during mid-May to early June (undisturbed soil, $n = 4$; seeded WR, $n = 11$; unseeded WR, $n = 10$) as described below. Unseeded WR and seeded WR areas were sampled at regular intervals along transects following the slope contour. Sample sites were located 67 m apart along parallel transects. Two transects were established for the seeded WR portion of the slope, one on the upper seeded slope and a parallel transect on the lower portion of the seeded slope (Fig. S1). One transect bisected the unseeded WR slope and was parallel to the seeded WR transects. Four reference plots were randomly selected in native vegetation areas undisturbed by mining activity located adjacent to the WR dump. The mine is located in a mountainous region; thus, all undisturbed reference sites were located on rocky slopes surrounding the WR dump. Sites one and two were located at the north end of the WR dump on southwest-aspect slopes; site three was located at the south end of the WR dump on a southeast aspect slope; and site four was on the crest of a hill east of the WR dump with a western aspect. Native soil properties are compared to the WR substrate in Table 1.

Each sample site was marked with rebar to ensure annual sampling occurred at the same location. A 1-m^2 quadrat was placed on the ground at each sample site centered on the rebar marker. Grab samples of substrate (soil or WR) were collected to a depth of 15 cm at two opposite corners of the quadrat. The substrate from the two holes within the quadrat was combined, sieved (2 mm), and dried at 45 °C prior to chemical analysis. Following sample collection for chemical analysis, samples for microbial analysis were collected from the same two holes within the quadrat along the 15 cm depth of the soil hole using sterile technique. Microbial samples were transported at 4 °C and then stored at −80 °C. The gravimetric moisture content was determined on each microbial sample prior to storage at −80 °C.

The comparative root zone study was conducted in May 2018. This study targeted root zone samples from dominant grasses and shrubs

Table 1
Temporal study: initial 2014 conditions of undisturbed soil, seeded WR, and unseeded WR (mean ± SD).

	Undisturbed	Seeded	Unseeded
pH	6.83 ± 0.32 b	9.22 ± 0.44 a	9.28 ± 0.184 a
EC[†]	0.251 ± 0.157	0.144 ± 0.031	0.162 ± 0.053
TN[†]	1.562 ± 0.644 a	0.065 ± 0.033 b	0.049 ± 0.015 b
DNA[†]	6822 ± 2628 a	31 ± 84 b	11 ± 20 b
Percent fines	34 ± 8	30 ± 7	29 ± 7
Percent pebbles	37 ± 13	41 ± 8	43 ± 9
Percent cobbles	29 ± 6	31 ± 7	28 ± 13

Letters across rows indicate significant differences between substrate type (ANOVA and Tukey HSD for normally distributed metrics; Kruskal-Wallis and Dunn for non-normally distributed metrics; $p < 0.05$).
EC, electrical conductivity (dS/m); TN, total N (mg/g), DNA, DNA biomass (ng/g). Fines are defined as particles <2 mm in diameter; pebbles, 2–16 mm; and cobbles, 64–256 mm.
[†] Kruskal-Wallis and Dunn tests used to determine significant differences between means.

along the transects to compare the capacity of plant lifeforms as drivers of substrate fertility development. The objective of this study was to quantify the total plant lifeform effect on root zone substrate development; the plant lifeform effect included cumulative effects of aboveground plant biomass, plant litter, and the root architecture. The relative contributions of litter and plant root and shoot structure to soil development were not evaluated separately for this study. Plant leaf litter and roots were removed from the substrate prior to analysis. A subset of the seeded and unseeded WR transect sites was retained for this analysis to relate the biogeochemistry of targeted root zone substrate to the transect sites of the temporal study. Undisturbed soil data from 2016 to 2018 ($n = 9$) were included in the analysis to capture the annual variability of the natural ecosystem in the temporal study. In summary, sample sites for this study were labeled as undisturbed soil ($n = 9$), seeded WR ($n = 10$), grass WR ($n = 10$; collected from grass-associated root zones), shrub WR ($n = 6$; collected from the shrub-associated root zones), and unseeded WR ($n = 5$). The seeded WR samples reflect both bare and vegetated areas along the seeded WR transect. Shrub WR samples were collected to a depth of 15 cm under the plant canopy and close to the stem. Grass WR samples were collected immediately beneath the grass stem within the root zone to a depth of 15 cm. The shrub and grass WR samples consisted of a composite of root-zone substrate from two 15 cm deep holes within the quadrat at each site; two grass plants were sacrificed for grass root-zone samples, and two samples were collected beneath a single plant canopy for shrub root-zone samples. Leaf litter was scraped away prior to collection of the root zone samples to quantify plant effect on root zone substrate fertility. The samples were collected, processed, and stored using the same methods as those employed for the temporal study described above.

2.3. Soil chemical analysis

Substrate pH and electrical conductivity (EC) were measured in a 1:2 slurry of soil to dH_2O following 30 min of mixing, according to the method described by Honeker et al. (2017). Samples were milled prior to analysis for total organic carbon content (TOC; McGee et al., 1999) and total nitrogen (TN). Prior to TOC analysis, carbonates were removed using HCl fumigation following the protocol outlined by Harris et al. (2001), with the following modifications: samples were fumigated in ceramic crucibles instead of Ag-foil capsules, and 1–1.5 g of sample was used instead of 30 mg. Acid fumigated samples were then combusted with a Vario Max Cube to measure carbon content, in accordance with the manufacturer's protocol (Elementar, Hesse, Germany). Bioavailable phosphorus (P) was measured using the Olsen P Method (Olsen et al., 1954) with modifications for extraction with buffered alkaline solution (Kuo, 1996).

TN was measured by dry combustion of milled, solid samples concurrent with the Dumas method (Bremmer, 1996) using an Elemental Combustion System 4010 CHNSO Analyzer in accordance with the manufacturer's protocol (Costech Analytical Technologies, Valencia, CA). The standard curve for TN analysis was generated using LECO soil standards (LECO Corporation, St. Joseph, MI) at seven concentrations. Ammonium content (NH_4^+-N) was measured by colorimetric detection with ammonia salicylate and ammonia cyanurate HACH reagents (Hach Company, Loveland, CO) in a 1:2 weight/volume ratio using KCl (Kushwaha et al., 2020). Absorbance readings were completed with an Epoch 2 Microplate Spectrophotometer (BioTek Instruments, Winooski, VT). Nitrate content (NO_3^--N) was measured using a 1:5 weight to volume ratio in KCl according to the cadmium reduction technique (Dorich and Nelson, 1984); nitrate analysis was completed by Motzz Laboratory, Inc. (Phoenix, AZ).

2.4. Soil microbial analysis

DNA was extracted from 0.5 g of substrate using the FastDNA Spin Kit for Soil in accordance with the manufacturer's protocol (MP Biomedicals, Santa Ana, CA), with modifications to increase DNA yield as described in Kushwaha et al. (2020), and with further modifications during the initial lysis step: cells were lysed using a vortex at high speed for 15 min. DNA extraction samples were quantified using a Qubit® 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA) and Qubit® dsDNA High Sensitivity Assay Kit (Life Technologies, NY, USA). A reagent blank was run with all DNA extractions, and only DNA extractions performed with a reagent blank that registered below the fluorometer DNA detection limit of 0.015 ng/µl were used in further analysis. Technical replicates were processed by two different technicians to determine the coefficient of variation (CV) for the soil biomass quantification by DNA extraction using the FastDNASpin Kit for Soil. The average % CV was 10 %.

Total bacterial abundance and abundance of ammonia-oxidizing bacteria (AOB) were quantified using qPCR to amplify the 16S rRNA gene and *amoA* functional gene, respectively. qPCR was carried out using a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The 10 µl reactions consisted of 5 µl of SsoFast EvaGreen Supermix, 1 µl of DNA template, and 0.4 µM or 0.45 µM concentration of 16S rRNA or *amoA* primers, respectively. Samples and standards were run in triplicate. Interplate calibration was performed to normalize for plate variations in amplification efficiencies between separate runs (Bio-Rad Laboratories, Hercules, CA). Amplification specificity was confirmed by melt-curve analysis and gel electrophoresis of qPCR products. qPCR details (primers, reaction conditions, and standard curve) are summarized in Table S2.

Paired-end amplicon sequencing was performed by the Microbiome Core at the University of Arizona's Steele Children's Research Center using Illumina MiSeq (Illumina, Inc., San Diego, CA). The V4 region of the 16S rRNA gene was amplified using 515F and 806R primers for bacteria and archaea, and the ITS1 region of the rRNA operon was amplified using ITS1-F and ITS2 primers for fungi (Walters et al., 2015). Amplicon sequence reads were demultiplexed using *idemp* (https://github.com/yhwu/idemp), and analyzed using DADA2 (Callahan et al., 2016) and taxonomy databases SILVA v138 for bacteria/archaea (Quast et al., 2013) and UNITE for fungi (UNITE, 2019) according to the workflow outlined in Kushwaha et al. (2020). For bacteria/archaea, 4,644,329 sequence reads remained after quality filtering, with an average of 119,085 ± 79,203 reads per sample and a median sequence length of 233 bp; for fungi, 3,973,599 sequence reads remained after quality filtering, with an average of 101,887 ± 81,320 reads per sample and a median sequence length of 213 bp. After removing contaminants, a total of 26,075 ASVs for bacteria/archaea and 5064 ASVs for fungi were used for analysis.

2.5. Plant cover and diversity analysis

Plant cover at each sampling site was determined using a point-intercept methodology. A laser point bar was situated above and parallel to the ground at the sampling site to record ten readings along ten rows (total of 100 readings) within a 3 × 3 m quadrat centered on the sample site location. Intercepted laser hits were recorded as vegetation (classified by species), litter, rock (>5 mm), or bare soil. One hundred readings were taken per quadrat at each sample site; thus, each intercept reading represented 1 % of ground cover.

A complete diversity survey of all the sample sites included in the targeted sample study was conducted in 2018, wherein every plant individual present within a 9 m^2 area was identified by species. Plant species richness was recorded.

2.6. Statistical analysis

Statistical analyses were completed in R v4.0 (R Core Team, 2020). Plant and substrate values for each site were evaluated for normal distribution using a Shapiro-Wilk test. Means were compared using a one-way ANOVA test and post-hoc Tukey HSD test for data with normal distribution, and the Kruskal-Wallis test followed by post-hoc Dunn's test for non-normally distributed data ($p < 0.05$). Spearman's correlation was used to quantify associations between above- and belowground indicators at four significance levels from $p < 0.0001$ to $p < 0.05$; significance was corrected for multiple comparisons using the Benjamini-Hochberg test to control the false discovery rate. Shannon diversity of the bacterial/archaeal and fungal communities were analyzed using the *vegan* package in R (Oksanen et al., 2019). Community similarity patterns between different sampling locations were analyzed using Bray-Curtis distance non-metric multidimensional scaling (NMDS) ordination. A linear discriminant analysis effect size (LEfSe) method (Segata et al., 2011) was used to identify taxa that explained the variability in microbial community composition between substrate-types. Data were visualized with R packages *ggplot2* (Wickham, 2016) and *corrplot* v0.90 (Wei and Simko, 2021).

3. Results

3.1. Plant establishment

The average plant cover for the undisturbed sites surrounding the mine WR was 45 ± 14 % for the five-year period of this study. Plant communities were dominated by perennial shrubs and grasses. The grass and shrub distributions varied by site and year with changes in annual precipitation patterns. Over the five-year period, the dominant grass species included *Eragrostis intermedia* (plains lovegrass) and *Hilaria belangeri* (curly-mesquite) and dominant shrubs/trees included *Gutierrezia sarothrae* (broom snakeweed) and *Quercus turbinella* (Sonoran scrub oak). Broom snakeweed cover progressed from 7.2 % to 14 % from 2014 to 2017, then decreased to 1.4 % in 2018. Total plant cover of seeded WR averaged 15 ± 6 % in 2014 and 28 ± 18 % in 2018; however, the increase was not significant (ANOVA, $p = 0.073$) due to the high variability in plant establishment across the slope. The seeded WR plant community composition was characterized by early increases in grass cover (2014–2016) followed by slower growth in shrub cover; shrub and grass cover were comparable by 2017. Concurrently, the plant cover of annual plants on the seeded WR decreased significantly within this period (Fig. 1). The high variability in cover was associated with a plant community structure that followed the fertility island pattern typical of arid ecosystems, described as vegetation patch and gap formation. Thus, our sampling strategy captured and averaged both vegetated and unvegetated patches along the sampling transects. Plant cover of unseeded WR remained below 10 % for all five years and the community was dominated by annual forbs (Fig. 1). No significant change was observed in the plant community composition (by plant lifeform and

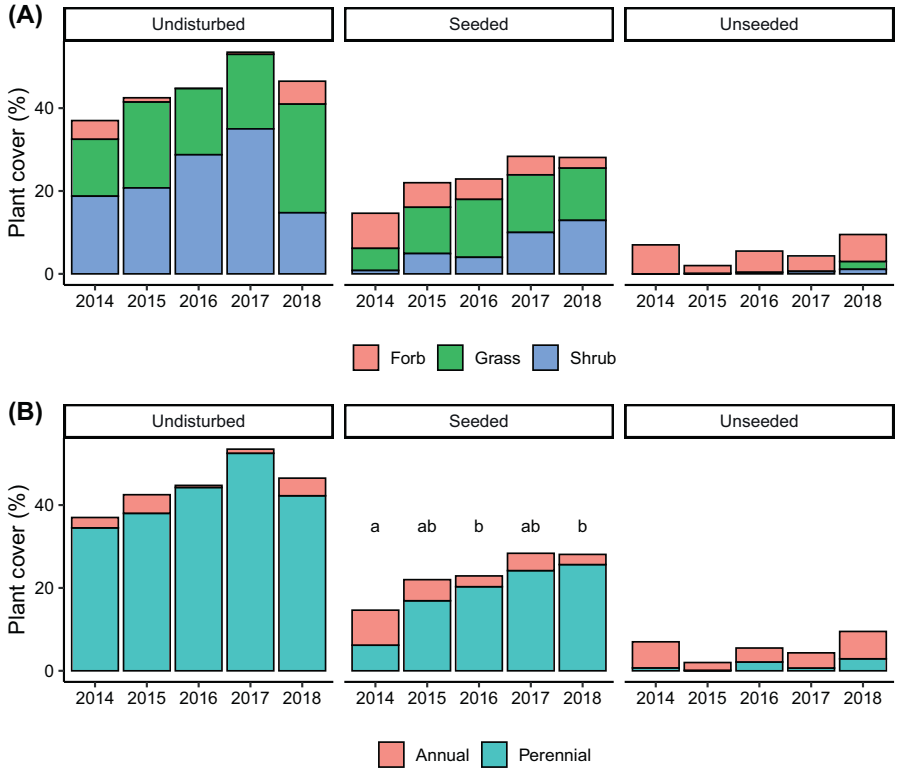

Fig. 1. Temporal study from 2014 to 2018: lant cover over time by lifeform as forb, grass or shrub (A), and annual or perennial (B) for undisturbed soil, seeded WR, and unseeded WR. Letters in seeded WR column of (B) indicate significant difference in plant cover of annual species between years (ANOVA and Tukey HSD, $p < 0.05$).

duration) for the undisturbed sites and unseeded WR from 2014 to 2018.

Plant diversity of seeded WR was assessed in 2018 to characterize 1) the establishment of seeded species; and 2) the plant community composition at the conclusion of the study. Of the species included in the original seed mix, the following were detected in 2018: four of the six perennial grasses, two of the three perennial forbs, and two of the four shrubs (Table S1). The dominant grasses of the seeded WR slope were *Bouteloua curtipendula* (sideoats grama; perennial; hydroseeded), and *Aristida purpurea* (purple threeawn; perennial; hydroseeded), which were present at eleven and thirteen of the sampling sites, respectively, from a total of sixteen sampling sites. The dominant shrub species were *Atriplex canescens* (fourwing saltbush; hydroseeded), and *Baccharis sarothroides* (desert broom; volunteer), which were present at five and eleven of the sites, respectively. The dominant forb species were *Baileya multiradiata* (desert marigold; perennial; hydroseeded), *Eriogonum polycladon* (sorrel buckwheat; annual; volunteer), and *Heterotheca subaxillaris* (camphorwood; annual; volunteer), which were present at seven, eight, and nine of the sampling sites, respectively. The dominant grasses and shrubs on the seeded slopes differed from the dominant plant species found at the undisturbed sites because the seed mix specifically incorporated common restoration plant species for semiarid ecosystems. Both total plant species richness and perennial plant richness of the undisturbed sites were significantly greater than either the seeded WR or the unseeded WR (ANOVA, $p < 0.01$) (Fig. S2). Annual species richness was significantly higher on unseeded WR than seeded WR (ANOVA, $p < 0.01$), but was similar to the undisturbed sites.

3.2. Temporal study

In the first year of the study (2014), no significant differences in belowground biogeochemistry were observed between seeded WR and unseeded WR (Table 1; recall that the WR slope was hydroseeded in 2012). The undisturbed site soils, however, were significantly different than WR. The undisturbed soil pH (pH = 6.83 ± 0.32) was significantly lower than that of the seeded and unseeded WR (pH > 9). Average undisturbed soil total nitrogen (TN) was 27-fold greater than WR sites, and DNA biomass (DNA) was over 300-fold greater than the average WR value (Table 1). During the five years monitored, TN was highly variable for seeded WR (Fig. 2a and b) with a range from 0.044 to 0.154 mg N g/WR. Average seeded WR TN values did not change significantly (0.065 ± 0.033 mg N g/WR in 2014 to 0.081 ± 0.031 mg TN g/WR in 2018). The average unseeded WR TN was 0.053 ± 0.015 mg N g/WR. Average TN values for undisturbed soils also did not change significantly and ranged from a minimum of 1.30 ± 0.361 mg N/g WR in 2018 to a maximum of 1.58 ± 0.387 mg N/g WR in 2017. In contrast, DNA biomass of seeded WR increased significantly from 2014 to 2018 with a final average value of 509 ± 447 ng DNA g/WR (Fig. 2), a change not observed for unseeded WR. Interestingly, DNA for undisturbed soil revealed significant annual fluctuation, but no significant difference was observed between the initial (2014) and final (2018) levels. Gravimetric soil moisture content for all samples ranged from 2.04 % to 5.64 % (Table S3). The average value across all sites was lowest in 2014 (2.78 %) and highest in 2017 (5.36 %). Average moisture content across treatments did not vary significantly from 2015 to 2017; however, in 2014 and 2018, the average soil moisture for the unseeded slope was significantly higher than the other substrate types (Table S3). This could be due to the lack of transpiration from the unvegetated WR.

3.3. Comparative root zone study

In 2018, a targeted plant study was conducted to better elucidate plant effects on the coupled above- and belowground ecosystem development. The comparative root zone study quantified a suite of fourteen belowground abiotic and biotic soil properties to 1) identify significant associations between plant establishment and belowground soil quality and 2) evaluate differential effects of plant lifeform on the belowground metrics. Plant cover (PC) correlated most strongly and significantly with belowground total organic carbon (TOC), TN, and DNA biomass (DNA;

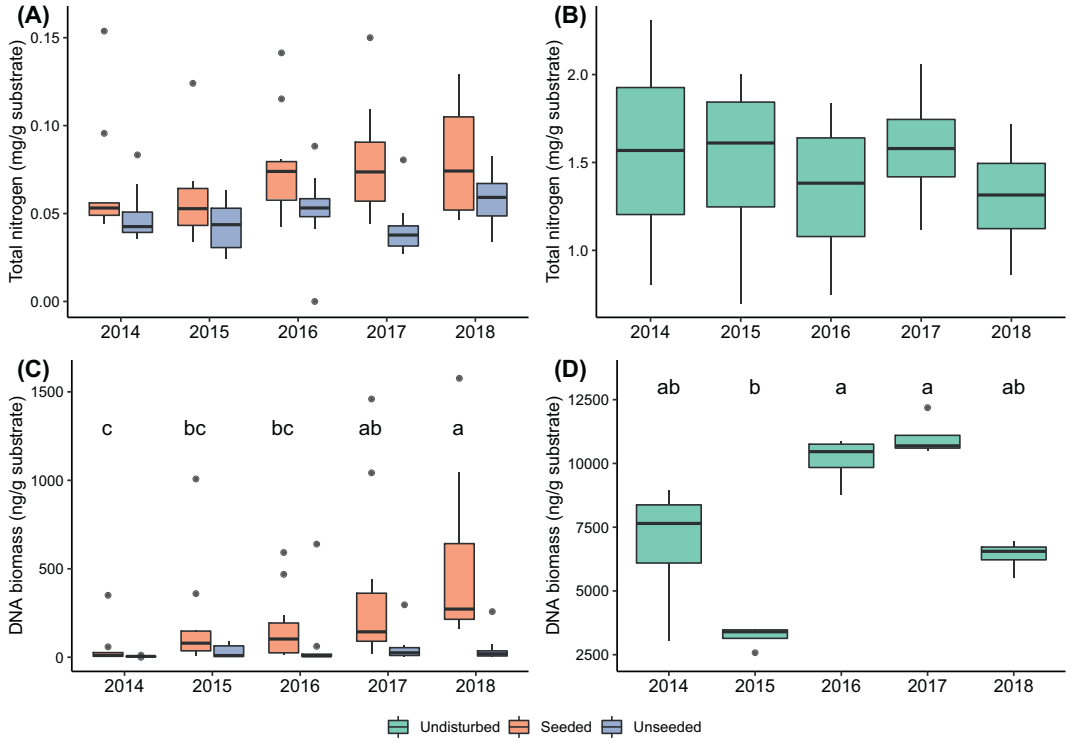

Fig. 2. Temporal study from 2014 to 2018: total N of seeded and unseeded WR (A), and undisturbed soil (B); and DNA biomass of seeded and unseeded WR (C), and undisturbed soil (D). Letters indicate significant difference of DNA biomass between years of seeded WR (C), and undisturbed soil (D) (Kruskal-Wallis and Dunn, $p < 0.05$).

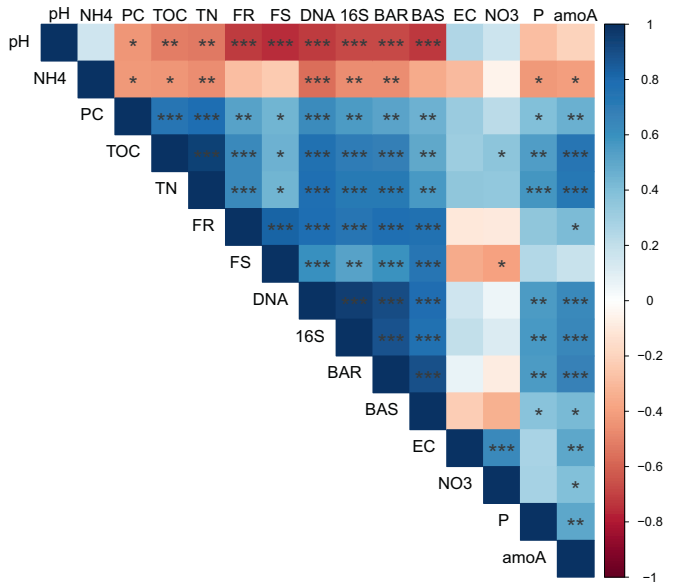

Fig. 3. Comparative root zone study: significant Spearman's correlations between plant cover, chemical and microbial substrate metrics for WR samples only (seeded, grass, shrub, and unseeded WR). PC, plant cover; TOC, total organic carbon; TN, total nitrogen; FR, fungal richness; FS, fungal Shannon diversity; DNA, DNA biomass; 16S, bacterial 16S rRNA gene copy number; BAR, bacterial and archaeal richness; BAS, bacterial and archaeal Shannon diversity; EC, electrical conductivity; P, bioavailable phosphorus; amoA, bacterial *amoA* gene copy number. Color represents strength of positive (blue) or negative correlation (red); **** $p < 0.0001$, *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3, Table S4). Strong and significant correlations were also observed between plant cover (PC) and bacterial abundance (16S), fungal and bacterial/archaeal community ASV richness (FR, BAR) and diversity (FS, BAS), and abundance of ammonia-oxidizing bacteria (AOB; amoA). Of particular significance was that DNA biomass (DNA) correlated strongly and significantly ($\rho > 0.6$; $p < 0.001$) with bacterial abundance (16S), bacterial/archaeal and fungal richness and diversity (BAR, FR, BAS, FS), and AOB abundance (amoA), demonstrating that DNA biomass is a good indicator of overall microbiome development in WR material. In addition, strong positive correlations were observed between TN and other biochemical metrics: strong and significant correlations between TN, TOC, DNA, 16S, and amoA are especially noteworthy with $\rho \geq 0.6$ and significance levels of $p < 0.001$.

The comparative root zone study revealed that shrub WR was significantly enriched in comparison to unseeded WR for ten of the biotic and abiotic soil properties measured in this study (TOC, TN, P, DNA, 16S, amoA, BAR, BAS, FR, and FS; Table 2). In contrast, grass WR enrichment relative to unseeded WR was documented for just five of the properties (16S, BAR, BAS, FR, and FS), all of which were biotic metrics. Properties in which shrub WR enrichment was significantly greater than grass WR included TN, DNA, amoA, and BAR. Further, shrub WR attained levels comparable to the undisturbed soils for TOC, 16S, amoA, BAR, and BAS ($p > 0.05$). An important difference was noted between the bacterial/archaeal and fungal microbiome development: whereas BAR and BAS were comparable in shrub WR and undisturbed soil, FR and FS were significantly higher in undisturbed soil relative to shrub WR. FR and FS were comparable in shrub and grass WR, and both were significantly higher than unseeded WR.

Interestingly, ammonium content (NH_4-N) and nitrate content (NO_3-N) followed a distinct pattern: NH_4-N was highest in unseeded WR and lowest in shrub WR, and NO_3-N was highest in shrub WR, but not significantly different between undisturbed soil and unseeded WR. pH was highest in unseeded WR and lowest in undisturbed soil.

Table 2
Comparative root zone study: chemical and microbial fertility metrics of undisturbed soil, seeded WR, shrub WR, grass WR, and unseeded WR.

	Undisturbed	Seeded	Shrub	Grass	Unseeded
pH	6.62 ± 0.39 c	8.58 ± 0.31 ab	8.31 ± 0.50 ab	8.19 ± 0.30 b	8.76 ± 0.08 a
EC†	0.156 ± 0.096 ab	0.106 ± 0.022 b	0.481 ± 0.279 a	0.111 ± 0.063 b	0.119 ± 0.014 ab
TOC†	14.16 ± 3.13 a	1.37 ± 0.55 c	7.52 ± 3.07 ab	1.73 ± 0.41 bc	0.78 ± 0.22 c
P	22.52 ± 5.45 a	3.19 ± 1.25 c	7.83 ± 3.06 b	3.26 ± 1.58 bc	1.98 ± 0.88 c
TN	1.300 ± 0.398 a	0.091 ± 0.047 c	0.586 ± 0.194 b	0.120 ± 0.037 c	0.050 ± 0.011 c
NH_4-N†	0.861 ± 0.925 ab	0.790 ± 0.148 b	0.400 ± 0.349 b	1.020 ± 0.793 ab	1.875 ± 0.752 a
NO_3-N†	6.7 ± 2.5 ab	4.1 ± 1.2 b	20.0 ± 13.9 a	4.2 ± 1.7 b	4.5 ± 0.8 ab
DNA	9250 ± 2123 a	671 ± 529 c	4162 ± 2780 b	1610 ± 548 c	28 ± 29 c
16S†	8.53 ± 0.32 a	7.36 ± 0.54 bc	8.24 ± 0.42 a	8.11 ± 0.20 ab	5.20 ± 1.88 c
amoA†	5.15 ± 2.17 ab	3.34 ± 2.38 c	6.52 ± 0.82 a	4.52 ± 1.62 bc	0.91 ± 2.03 c
BAR	2761 ± 488 a	1293 ± 564 b	2190 ± 491 a	2080 ± 555 b	221 ± 177 c
BAS	7.14 ± 0.19 a	6.41 ± 0.44 a	6.66 ± 0.17 a	6.85 ± 0.27 a	4.40 ± 1.40 b
FR	634 ± 157 a	164 ± 109 bc	262 ± 143 b	280 ± 72 b	12 ± 6 c
FS	4.74 ± 0.35 a	3.53 ± 0.58 b	3.73 ± 0.61 b	3.94 ± 0.22 b	1.57 ± 0.70 c

Letters across rows indicate significant differences between substrate type (ANOVA and Tukey HSD for normally distributed metrics; Kruskal-Wallis and Dunn for non-normally distributed metrics; $p < 0.05$).
EC, electrical conductivity (ds/m); TOC, total organic carbon (mg/g substrate); P, bioavailable phosphorus (μg/g substrate); TN, total nitrogen (mg/g substrate); NH_4-N, ammonium (mg/g substrate); NO_3-N, nitrate (mg/g substrate); DNA, DNA biomass (ng/g substrate); 16S, bacterial 16S rRNA gene copy number (log copies/g substrate); amoA, bacterial *amoA* gene copy number (log copies/g substrate); BAR, bacterial and archaeal richness; BAS, bacterial and archaeal Shannon diversity; FR, fungal richness; FS, fungal Shannon diversity.
† Kruskal-Wallis and Dunn tests used to determine significant differences between means.

The microbial community composition was analyzed to characterize plant effects on the development of the belowground microbiome. Bray-Curtis NMDS ordination revealed that substrate type explained 33 % and 24 % of the differences in community composition of bacteria/archaea and fungi, respectively (Fig. 4). The unseeded WR relative abundance profiles for both bacterial/archaeal and fungal communities clustered separately from all vegetated sites indicating a significant impact of vegetation on the community structure of both communities. Specifically, for the bacterial/archaeal community the greatest difference was observed between the unseeded WR and the undisturbed soils. WR shrub and grass bacterial/archaeal community profiles were intermediate to these two extremes and distinct from each other. Taken together, the data indicate that shrub and grass establishment have distinct, but positive impacts on bacterial/archaeal community development relative to the unseeded WR sites. In contrast, the fungal profiles for all vegetated areas were similar. Thus, grass and shrub establishment have similar impacts on fungal community development.

Actinobacteria and *Proteobacteria* were the dominant phyla in all substrates; however, their comparative relative abundances varied by substrate type (Fig. S3). *Actinobacteria* relative abundance was highest in the undisturbed soils (35.6 %), whereas *Proteobacteria* relative abundance was highest in shrub WR (41.1 %). Interestingly, both phyla had the same relative abundance in the unseeded WR; *Protobacteria* (29.2 %) and *Actinobacteria* (28.8 %). The unseeded WR was also distinguished by a significantly greater relative abundance of *Firmicutes* (9.3 %) relative

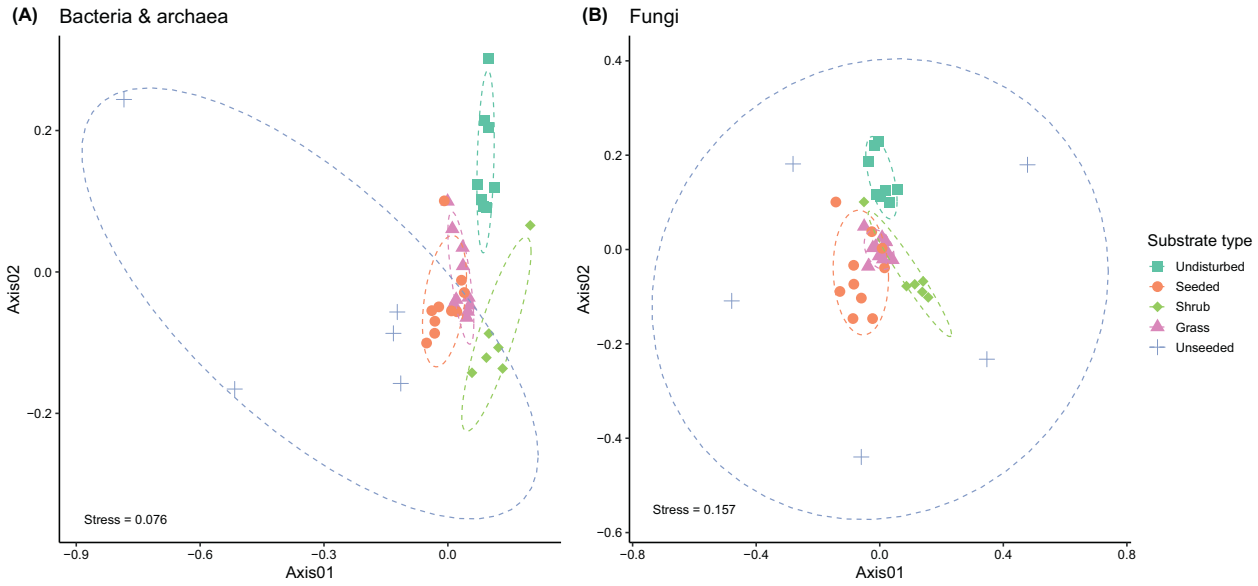

Fig. 4. Targeted sample study: Bray-Curtis distance, non-metric multidimensional scaling (NMDS) ordination plot of bacteria and archaea (A), and fungi (B) for undisturbed soil, seeded WR, shrub WR, grass WR, and unseeded WR. Substrate type explained 33 % of variation in bacteria and archaea communities, and 24 % of variation in fungi community.

to the grass, shrub, and seeded WR communities (Kruskal-Wallis, $p < 0.0001$). Other phyla present in all substrate types with relative abundances >3 % include *Bacteroidetes*, *Acidobacteria*, and *Chloroflexi*. Within the fungal communities, *Ascomycota* had the greatest relative abundance in all substrate types, followed by *Basidiomycota*.

The observed differences in microbial community composition were further explored using a linear discriminant effect size (LEfSe) analysis to identify taxa that explained significant differences between each substrate community (Fig. S4). The phylogenetic distribution of these indicator taxa varied considerably with substrate type. The undisturbed soil community was defined by eight phylotypes belonging to five phyla. Two phylotypes were associated with each of *Chloroflexi*, *Actinobacteria*, and *Proteobacteria*; one phylotype belonged to *Planctomycetes* and one to *Gemmatimonadetes*. In contrast, shrub and grass WR specific phylotypes were both dominated by *Proteobacteria* (seven of thirteen and seven of ten LEfSe-identified taxa, respectively), with the remaining phylotypes belonging to *Bacteroidetes*, *Verrucomicrobia*, *Gemmatimonadetes*, *Actinobacteria*, and *Chloroflexi*. In particular, LEfSe-identified genera for shrub WR included *Bacteroidetes* phylotypes, *Ferruginibacter* and *Edaphobaculum*, which belong to the *Chitinophagaceae* family; and *Pedobacter*, which belongs to the *Sphingobacteriaceae* family. Specific *Proteobacteria* included *Caulobactor* and *Brevundimonas*, which belong to the *Caulobacteraceae* family; and *Devosia*, which belongs to the *Devosiaceae* family. Notable LEfSe-identified genera for grass WR included *Segetibacter*, a *Bacteroidetes* which belongs to the *Chitinophagaceae* family; and *Rhodoplanes*, which is a *Proteobacteria* that belongs to the *Xanthobacteraceae* family. Unseeded WR was characterized by twelve phylotypes associated with six phyla. Half of the phylotypes were associated with *Actinobacteria*, and the others were distributed between *Armationadetes*, *Acidobacteria*, *Bacteroidetes*, *Nitrospira* and *Proteobacteria*.

Eight well characterized N-cycling phylotypes were selected to evaluate the distribution of N-cycling phylotypes in each substrate type. These bacterial and archaeal phylotypes were classified to the family level and included four N-fixing bacteria (*Azospirillaceae*, *Devosiaceae*, *Frankiaceae*, and *Rhizobiaceae*); two ammonia-oxidizing bacteria (AOB; *Nitrosococcaceae* and *Nitrosomonadaceae*), one ammonia-oxidizing archaeon (AOA; *Nitrososphaeraceae*), and one nitrite-oxidizing bacterium (NOB; *Nitrospiraceae*). The total relative abundance of all eight phylotypes was significantly higher in shrub WR than in grass, seeded, and unseeded WR (Fig. 5). Of significance was the fact that the shrub WR

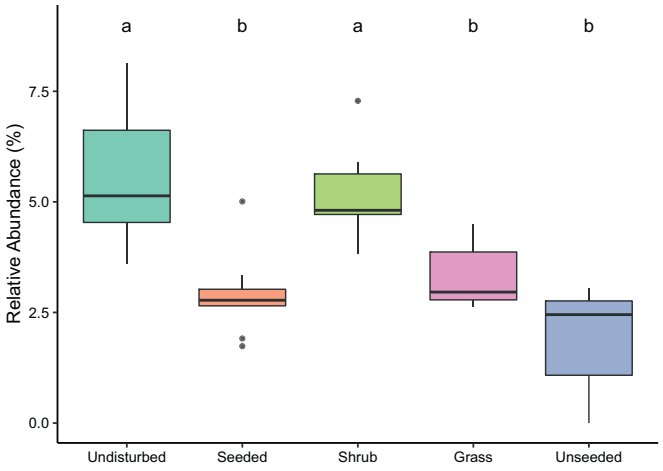

Fig. 5. Targeted sample study: um of relative abundance of eight common N-cycling bacterial and archaeal families in undisturbed soil, seeded WR, shrub WR, grass WR, and unseeded WR. Letters indicate significant difference in sum of relative abundance between substrate types (ANOVA and Tukey HSD, $p < 0.05$).

N-cycling phylotype relative abundance increased to the level of the undisturbed soils. The significant differences in *amoA* gene abundance between treatments (Fig. 6) were the same as those observed for the relative abundance of N-cycling phylotypes.

When the putative N-cycling phylotypes were separated into distinct N-cycling functional groups a different pattern was observed. The cumulative relative abundance of the N-fixing bacteria was significantly greater in shrub WR than undisturbed soil, and grass, seeded, and unseeded WR (ANOVA, $p < 0.0001$). The relative abundance of AOB phylotypes was not significantly different between substrate types (ANOVA, $p = 0.65$), but the relative abundance of AOA phylotypes was significantly higher in undisturbed soil than all types of WR (Kruskal-Wallis, $p < 0.001$). Of interest, is the fact that the significant differences observed for the *amoA* gene abundance between substrate types (Fig. 6) were not observed for the putative AOB phylotypes. This result suggests there are novel phylotypes in the plant associated WR with *amoA* genes

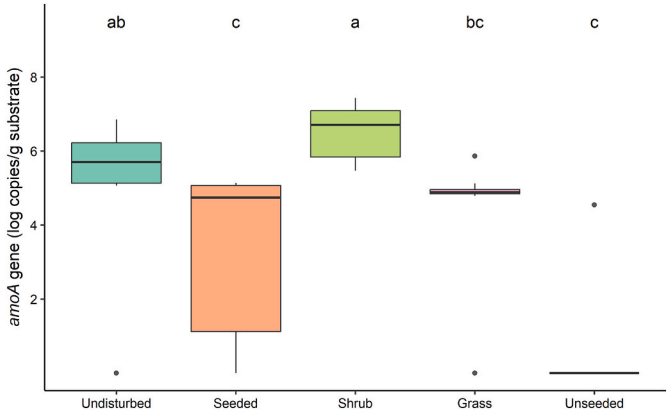

Fig. 6. Targeted sample study: *amoA* gene log copy number/g substrate in undisturbed soil, seeded WR, shrub WR, grass WR, and unseeded WR. Letters indicate significant difference in *amoA* gene abundance between substrate types (Kruskal-Wallis and Dunn, $p < 0.05$).

that have not been previously characterized as AOB. The relative abundance of NOB was not significantly different between undisturbed soil and unseeded WR.

4. Discussion

To our knowledge, this study is the first analysis of coupled above- and belowground early ecosystem development on mine waste rock (WR) during revegetation. The temporal analysis facilitated identification of belowground indicators associated with substrate fertility development that associate with progressions in plant establishment. Mine-site reclamation aims to restore lands disturbed by mine operations to self-sustaining ecosystems that support biological diversity, nutrient-cycling capacity, and dust and water erosion control; however, metrics are needed to 1) quantify progress in the early stages of ecosystem development; and 2) define properties of a resilient soil matrix to be used as target metrics. The results confirm that sustainable plant establishment is coupled with belowground substrate development of both biotic and abiotic substrate properties. In contrast, unseeded WR revealed no above- or belowground ecosystem development for the five-year duration of this study. Plant cover remained below 10 % and was generally limited to annual species. Similarly, no significant changes were observed in belowground soil fertility or microbiome development. The only belowground metric that changed consistently in unseeded WR was pH, which decreased from 9.28 ± 0.18 in 2014 to 8.76 ± 0.08 in 2018. We contend that the observed decrease in pH was driven by mineral weathering. Thus, unseeded WR did not demonstrate fertility improvement sufficient to support sustainable plant establishment under semiarid climatic conditions.

For the seeded WR, specific metrics were identified to define coupled associations in above- and belowground processes of ecosystem regeneration. Sustainable ecosystem target values were identified based on analysis of undisturbed sites surrounding the WR dump. Aboveground metrics include plant community structure and percent plant cover. The undisturbed-site plant community structure was dominated by perennial species with an even distribution between perennial grasses and shrubs. Plant cover of the seeded WR slope transitioned from predominantly annual plant cover in 2014 (two years after hydroseeding) to plant cover dominated by perennial species in 2015 and onward; a statistically significant decrease in annual cover was observed from 2014 to 2018. This shift in community composition from annual grasses and forbs to perennial grasses and shrubs is well-documented in the literature (Bonet, 2004; Lesschen et al., 2008), with the greatest changes in both the belowground microbial community and aboveground plant community occurring during the first fifteen years of revegetation (Bonet, 2004; Dangi et al., 2012; Martínez-Ruiz and Fernández-Santos, 2005). Total plant cover for seeded WR was characterized by high variability on an annual basis. This variability was partially driven by annual and seasonal inconsistencies in bimodal precipitation patterns characteristic of the southwestern US (summer monsoons and winter rains). In addition, plant establishment followed a fertility island pattern that is typical of semiarid ecosystems in which well-established plant islands are separated by large unvegetated spaces (Alday et al., 2014). Taken together, the results indicate that self-sustaining plant community establishment is possible on hydroseeded WR in the absence of a soil cap; however, environmental conditions require more effective seeding techniques to reach a sustainable percent cover comparable to undisturbed areas.

Belowground development coupled with aboveground plant establishment was document for biotic and abiotic indices through analysis of the rootzone for seeded WR. Final plant cover correlated most strongly and significantly with TN, TOC, and DNA biomass. The temporal transect study demonstrated a significant increase in DNA biomass over the five-year sampling period, but TN did not increase significantly. Thus, this analysis revealed that the DNA biomass response preceded abiotic increases in TN and TOC. Further, recall that DNA biomass correlated strongly with diverse microbiome metrics including bacterial abundance (16S), bacterial/archaeal and fungal richness and diversity (BAR, FR, BAS, FS), and AOB gene abundance (*amoA*), demonstrating that DNA biomass is a good indicator of overall microbiome development, and specifically the development of key nitrogen nutrient cycling capacity.

The comparative root zone analysis facilitated a more specific investigation of the specific coupled associations between perennial grass and shrub species and belowground microbiome development. Both grasses and shrubs significantly impacted bacterial abundance, richness and diversity, and fungal richness and diversity. NMDS analysis of the microbial community composition documented a significant, but distinct shift in the grass- and shrub-WR microbiomes relative to the unseeded WR microbiome. The twelve specific taxa defining the unseeded WR communities were predominantly oligotrophic and autotrophic phylotypes, many of which were previously identified in extreme deserts such as hyperarid regions of the Atacama Desert, Chile. Among the *Actinobacteria*, two phylotypes belonged to the class *Acidimicrobiia*, one to the order 0319-7L14, and one each to the families *Euzebyaceae* and *Sporichthyaceae*. These phylotypes were previously found to represent 36 %, 45 %, 15 %, 11 %, and 9 % relative abundance, respectively, of the soil communities in five distinct hyperarid locations of the Atacama Desert (Neilson et al., 2017). The Nitrospira phylotype belonged to the genus *Nitrospira*, a group of autotrophic nitrite-oxidizers, also known to survive under extreme, oligotophic desert conditions (Neilson et al., 2012, 2017).

In contrast, LEfSe analysis revealed that both shrub and grass plant lifeforms were associated with microbiome development leading to enhanced nutrient cycling capacity. LEfSe-identified taxa that characterized grass and shrub WR nutrient cycling capacity included phylotypes belonging to the following families: *Chitinophagaceae* (shrub and grass) described as cellulose degraders; *Sphingobacteriaceae* (shrub) characterized as cellulose and lignin degraders (Bailey et al., 2013; Eichorst and Kuske, 2012; Wilhelm et al., 2019); *Caulobacteraceae* (shrub), described as lignin degraders (Wilhelm et al., 2019); and *Devosiaceae* (shrub) and *Xanthobacteraceae* (grass), which are nitrogen fixers (Franche et al., 2009; Oren, 2014). Thus, both grass and shrub WR establishment were associated with statistically significant increases in C- and N-cycling bacteria that enhanced the nutrient cycling potential of these microbiomes relative to that of unseeded WR.

Amplicon sequencing analysis of the belowground bacterial, archaeal, and fungal communities enabled the characterization of the coupled association between plant establishment and the WR microbiome transition from an oligotrophic, autotrophic community to one with cellulose and lignin degradation and broad N-cycling potential. The

relative abundance of the specific C- and N-cycling phylotypes identified in this study can be used to track improved belowground ecosystem functional potential. This approach improves on previous studies using phospholipid fatty acid (PLFA) analysis (Blecker et al., 2012; Dangi et al., 2012), or fatty acid methyl ester (FAME) analysis (Mummey et al., 2002) that documented belowground microbial community changes associated with revegetation, but lacked the taxonomic resolution (Chen et al., 2019; Nkongolo and Narendrula-Kotha, 2020; Schwab et al., 2017) to characterize the coupled development of microbial nutrient-cycling capacity associated with different plant lifeforms during mine waste revegetation.

The study also revealed that shrub establishment is associated with greater belowground fertility development than grasses. Shrubs enriched all chemical and microbial metrics relative to unseeded WR, whereas grass driven WR enrichment was limited to select microbial metrics. Shrubs also developed a greater belowground potential for N cycling than grasses, as indicated by the significantly higher relative abundance of common N-cycling bacterial and archaeal phylotypes detected in this study, the higher TN and nitrate (NO_3-N) content; and the greater total abundance of bacterial ammonia-oxidation potential as measured by *amoA* gene quantification. This is of notable importance because nitrogen is typically the most limiting nutrient in terrestrial systems and controls net primary production. In semiarid and arid deserts, nitrogen limitations are second only to water scarcity (Vitousek and Howarth, 1991). AOB gene abundance, rather than ammonia-oxidizing archaea (AOA) gene abundance was selected as a microbial indicator of ammonia oxidation potential in this study because AOA can access the ammonium substrate in the soil at lower concentrations than AOB. Studies have shown AOB abundance increased following soil nitrogen enrichment, whereas AOA abundance did not (Di et al., 2009, 2010), suggesting that AOB responses to nitrogen levels are a better indicator of plant nitrogen availability than AOA responses. In our study, quantification of AOB using *amoA* gene abundance was more informative than assessing the relative abundance of the putative ammonia-oxidizers, *Nitrosococcaceae* and *Nitrosomonadaceae* (two bacterial families that contain the *amoA* functional gene).

Previous research has similarly reported that shrubs enhance soil fertility by forming microsites with increased organic matter and moisture (Alday et al., 2014) that offer protection from UV rays and cool the soil (Call and Roundy, 1991). Indeed, Rodríguez Rodríguez et al. (2005) found that areas where shrubs have been removed through human intervention and disturbance are more degraded than those with higher shrub abundance in the arid climate of the Canary Archipelago. Maestre et al. (2001) demonstrated that grasses facilitate shrub establishment on degraded drylands, but Pierce et al. (2019) found that in the Chihuahuan Desert grasses and shrubs compete for belowground nutrients and that grasses growing near shrubs have lower productivity than grasses growing alone. It is unclear in this study whether the early establishment of grass cover with the associated grass-driven enrichment of the soil microbiome enhanced successful shrub establishment.

The coupled analysis of above- and belowground plant establishment revealed a progressive pattern in belowground substrate development that began with microbial community development. Significant increases in both biotic and abiotic substrate fertility were only observed in the shrub root zone. Plant community establishment over five years was characterized by an initial rapid establishment of perennial grasses in 2014 followed by a gradual increase in shrub cover by 2017. We contend that this plant establishment pattern explains the observed progression in belowground substrate development, in which early grass establishment was coupled with a significant increase in DNA biomass. Significant increases in TN and TOC were coupled with shrub rather than grass establishment. Variability was also observed in bacterial/archaeal versus fungal community development. By 2018 bacterial abundance and diversity for both grass and shrub WR attained levels comparable to the undisturbed soils, whereas fungal richness and diversity remained significantly lower for all vegetated WR than in undisturbed sites. Similarly, shrub WR TOC reached undisturbed soil levels, whereas TN and P did not. Taken together, the results suggest that bacterial indicators can serve as more sensitive indicators of early substrate fertility development, whereas FR and TN can function as stable, long-term belowground development indices that indicate resilient substrate development. Previous research also determined that chemical fertility indicators represent later stages in soil development (Mummey et al., 2002). Data from the undisturbed sites also found TN to be a more stable native-soil metric, whereas biomass was more variable and highly sensitive to annual fluctuations in plant cover. Despite the observed fluctuation in the DNA biomass for the undisturbed soil, no significant difference was observed between the initial and final average values, suggesting that DNA biomass fluctuation is part of stable ecosystem dynamics. In fact, the decrease in biomass from 2017 to 2018 was associated with an observed broom snakeweed die off.

5. Conclusions

This study demonstrated coupled plant establishment and belowground fertility development under semiarid conditions without the addition of a soil cap. Importantly, the data emphasize the need to assess revegetation as a continuous progression in metrics of above- and belowground ecosystem development. As the plant structure matures from annual to perennial plant communities and grass to grass-shrub cover, a parallel progressive development occurs belowground. Early bacterial community development characterized by increases in total DNA biomass, bacterial/archaeal abundance and richness, and the relative abundance of specific cellulose and lignin degrading and N-cycling bacterial phylotypes is succeeded by fungal and chemical fertility enrichment. Heavily disturbed sites in arid and semiarid regions can lag behind their natural and undisturbed equivalents even after 40 years of recovery, as demonstrated by underdeveloped plant communities (Lesschen et al., 2008), thus the use of belowground metrics of ecosystem development is critical to assessing the progress of distinct revegetation management strategies. Deviations from positive trajectories in either the biotic or abiotic belowground metrics identified by this study can alert land managers of the need for interventions such as re-seeding, alternative seed mixes, or additional amendments. Utilization of the comprehensive suite of belowground metrics identified in this study enables the quantification of the rate and degree of ecosystem development and provides tools that allow practitioners to fine tune revegetation practices for more effective land reclamation.

CRediT authorship contribution statement

Lia Q.R. Ossanna: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Karen Serrano:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Lydia L. Jennings:** Conceptualization, Methodology, Investigation. **Jesse Dillon:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Raina M. Maier:** Funding acquisition. **Julia W. Neilson:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jesse Dillon is the owner of Cedar Creek Associates, an ecological consulting firm with contracts with the mining industry focused on environmental permitting, regulatory compliance, and closure.

Research for this manuscript was conducted at a copper mine that is a contributing member to the University of Arizona Center for Environmentally Sustainable Mining Industry-Academic Revegetation Research Cooperative as described in the Acknowledgements.

Data availability

Raw sequence data for the 16S rRNA gene and ITS region were submitted to the NCBI BioProject under the accession number PRJNA796645.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104813.

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