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# Long-term effects of vegetation cover on the rehabilitation of lead/zinc mine tailings

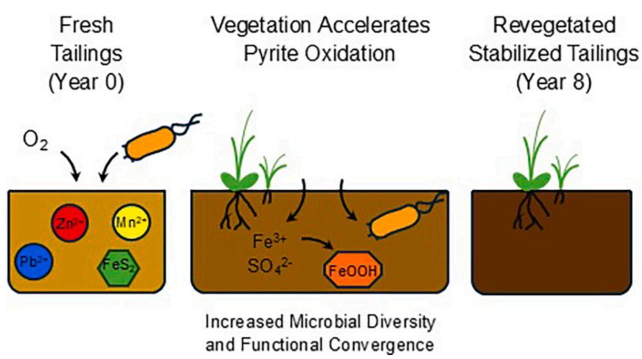
Felipe E. Sepúlveda Olea [a,1], Ian T. Burke [b], Ronan Courtney [c], William M. Mayes [d], Andrew J. Weightman [e], Gordon Webster [e], Franciszek Bydalek [f], Douglas I. Stewart [a,*]

[a] School of Civil Engineering, University of Leeds, Leeds, LS2 9JT, UK
[b] School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, UK
[c] Dept. Of Biological Sciences, University of Limerick, Limerick, V94 T9PX, Ireland
[d] School of Environmental and Life Sciences, University of Hull, Hull, HU6 7RX, UK
[e] School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK
[f] Dept. of Chemistry and the Centre of Excellence in Water-Based Early-Warning Systems for Health Protection, University of Bath, Bath, BA2 7AY, UK

## HIGHLIGHTS

- Vegetation can be established and sustained on carbonate-rich Pb/Zn mine tailings
- Oxidation of pyrite in the tailings was accelerated by vegetative cover
- Oxidation of pyrite produces ferric oxyhydroxides that reduce toxic metal mobility
- The bacterial community in revegetated tailings became more diverse over time
- Bacterial population in tailings functionally converge with similar natural sites

## GRAPHICAL ABSTRACT




## ABSTRACT

Pb/Zn sulfide ore is extracted from carbonate host rock by milling to grainsizes <120 μm and separation by flotation. This produces large volumes of near neutral pH tailings that must be carefully managed due to regulatory concern about the residual Pb and Zn concentrations (up to ~0.3 % by wt. of each) and trace concentrations of other potentially toxic elements. To prevent dust formation, vegetation cover is established on inactive areas of the tailings management facility (TMF). This paper reports the changes in the chemical and microbiological composition of the tailings as a function of both time and depth.

Over the course of eight years, there is progressive oxidation of pyrite in the tailings, and accumulation of soil organic matter in the surface layers. The mobility of most potentially toxic elements is reduced due to sorption to ferric oxyhydroxides formed as a result of pyrite oxidation, although Cu is more mobile in surface layers probably due to formation of dissolved organic carbon complexes. The microbial community diversity in the surface layer increased with the age of the tailings and was similar to natural calcareous soil after 8 years. At this age the functional profile (functional diversity) of the community was similar to that of natural calcareous soil, despite differences remaining in taxonomic composition. Development of soil-like properties in the surface layer, such as increased soil organic matter content and a soil-like microbial community, suggest that the vegetation cover will be self-sustaining. Therefore, revegetation is a viable option for TMF closure planning.

* Corresponding author.

*E-mail addresses:* f.sepulvedaolea@leeds.ac.uk (F.E. Sepúlveda Olea), i.t.burke@leeds.ac.uk (I.T. Burke), ronan.courtney@ul.ie (R. Courtney), w.mayes@hull.ac.uk (W.M. Mayes), weightman@cardiff.ac.uk (A.J. Weightman), websterg@cardiff.ac.uk (G. Webster), fab52@bath.ac.uk (F. Bydalek), d.i.stewart@leeds.ac.uk (D.I. Stewart).

[1] Formerly School of Civil Engineering, University of Leeds, Leeds LS2 9JT, UK (Currently Amphos 21, Santiago, Chile)

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## 1. Introduction

Processing of metal ores involves the separation of ore concentrates from unwanted minerals present (called gangue within the mining industry). This usually involves milling to very fine grainsizes (e.g. <200 μm) to facilitate separation (Romero et al., 2007; Gil-Loaiza et al., 2016; Burke et al., 2023). As a result, ore processing typically produces fine tailings containing the unwanted minerals (e.g. carbonates and silicates) in amounts that greatly exceed the metallic products (Burke et al., 2023). It's estimated that some 7125 Mt. of mine tailings are produced per annum (Mudd and Boger, 2013). These tailings are usually disposed of in purpose-built tailings management facilities (TMFs) near the site of production. Unfortunately, if left un-reclaimed, these TMFs can contribute to particulate dispersion into the surrounding environment and to contamination of ground and surface water via leaching of residual metal(loid)s (Mudd and Boger, 2013; Burke et al., 2023; Gil-Loaiza et al., 2016; Othmani et al., 2013).

Managed closure of a TMF commonly involves construction of an engineered cap to cover the tailings, often consisting of an inert, low permeability layer overlain by a planted surface layer. However, capping strategies can be very expensive (Gil-Loaiza et al., 2016). A more economic and sustainable alternative to capping is phytostabilization of the tailings, which involves establishing vegetation cover directly on the tailings, usually assisted by the addition of amendments such as compost, biosolids, lime and fertilizers (Gil-Loaiza et al., 2016).

The development of vegetative cover on the surface of waste deposits has two purposes. Firstly, it provides a physical protective layer that reduces dispersion of the material via wind and water erosion processes (Gil-Loaiza et al., 2016; Potysz et al., 2017). Secondly, as plants grow and a rhizosphere begins to develop, organic compounds (e.g. root exudates) are introduced into the tailings, stimulating geochemical changes, which may include pH stabilization and changes in metal (and metalloid) speciation and mobility (Potysz et al., 2017; Gil-Loaiza et al., 2016; Bray et al., 2018; Sun et al., 2021). The interaction of metals and metalloids with organic compounds, however, has the potential to reduce their bioavailability, via surface complexation, adsorption, redox changes and precipitation; or to enhance mobility via desorption, redox changes, pH dependant dissolution and formation of aqueous complexes (Sun et al., 2021; Li et al., 2014a; Potysz et al., 2017).

Previous work has shown that vegetation growth on mine and ore processing wastes can also lead to the development of soil-like conditions, amenable to a more diverse ecosystem (Bray et al., 2018; Burke et al., 2023; Gil-Loaiza et al., 2016; Guo et al., 2022). The occurrence of soil forming processes, as a consequence of vegetation growth, is both evidenced and facilitated by microbial colonisation. This is due to the significant direct and indirect effects that plants have on soil microbial communities, and vice versa (Fierer, 2017). The structure of the microbial community responds quickly to changes in an ecosystem and, in some instances, changes in the structure or activity of the community can precede detectable changes in soil physical and chemical properties (Nielsen et al., 2002; Ke et al., 2021). Thus, the soil microbial community not only accelerates soil forming processes but can also be an indicator of impending soil improvement (Nielsen et al., 2002; Ke et al., 2021; Fierer, 2017).

The Tara Boliden Mine exploits the Navan orebody, which is a carbonate-hosted (limestone and dolomite) lead-zinc sulfide (galena, PbS; sphalerite, ZnS) deposit (Drummond et al., 2023). The tailings produced are dominated by carbonate and silicate minerals, with a relatively low sulfide content, which generates neutral (pH 6–9) drainage waters (Fitzsimons and Courtney, 2022; Burke et al., 2023). Management of the Tara TMF involves the establishment of vegetation cover on inactive areas, primarily to prevent dust formation, by addition of organic compost to surface layers and seeding of perennial grasses. Over time this has resulted in the development of an increasingly robust and diverse vegetation cover (Burke et al., 2023; Fitzsimons and Courtney, 2022).

Previous studies in the Tara TMF have characterized the fresh tailings material, the tailings drainage waters, and the time and depth dependant changes in As and Sb speciation in the tailings, along with background geochemical parameters (Burke et al., 2023, Fitzsimons and Courtney, 2022). It was observed in the vegetated tailings that there was oxidation of Fe(II) in pyrite to Fe(III)-oxides over time, as well as oxidation of As(III) and Sb(III) to As(V) and Sb(V). However, the behaviour of other potentially toxic elements such as Cu, Cd, Mo, Ni, Pb and Zn was not investigated (Burke et al., 2023). Beyond the Tara TMF, there are relatively few studies of the effect of vegetation on contaminant behaviour (Jordan et al., 2008; Chambers and Sidle, 1991).

This paper reports the long-term effects of vegetation cover on the rehabilitation of the Tara Boliden lead/zinc mine tailings, as a function of both time and depth. Core or trial pit samples were taken from areas of tailings that have been managed with vegetation cover for 1, 3 and 8 years, from depths of 0 to 30–70 cm. Water extractable and total concentrations of a suite of major elements and metal(loid)s (e.g. Cu, Cd, Mo, Ni, Pb and Zn) were measured in the tailings profile, along with relevant geochemical parameters (DOC, LOI, pH, EC, %Fe as Fe (II)). Bacterial 16S rRNA gene (barcode) sequencing of DNA extracted from tailing samples was used to study the evolution of the microbial community structure. Microbial communities were also compared with those of natural soils with similar host geology. On the basis of these results, the long-term effects of direct vegetation as a management strategy for mine tailings is discussed.

## 2. Material and methods

### 2.1. Sample collection

Fieldwork was carried out at the tailings management facility at Boliden Tara, County Meath, Ireland in September 2022 (SI Table S.1 and Fig. S.1). In this facility the surface layer of the tailings was amended with spent mushroom compost and seeded with a grassland mix. Over time, perennial grasses and clovers have become established. After 8 years, there are also small, self-seeded trees and shrubs.

Replicate soil cores were taken at two locations where grassland species had been established for 1 and 3 years (see SI Figs. S.2 and S.4). The first 2 cm of each core was stored separately as the zone with immediate root influence, and from 2 cm downwards, cores were subdivided at 5 or 10 cm intervals and stored in new re-sealable sample bags. Two trial pits were excavated in two 8-year-old trial soil mesocosms, consisting of intermediate bulk container tanks containing tailings with vegetation cover and under-drainage (see SI Figs. S.3 and S.6). The excavation was undertaken in intervals of 3 to 4 cm, and samples were stored in re-sealable sample bags.

Replicate portions of each sample were transferred to 1.5 ml microcentrifuge tubes and stored at −20 °C for DNA analysis, the remainder of the samples were stored at 4 °C.

### 2.2. Solid phase analysis

Moisture content was determined on samples from each core/ trial pit by drying at 105 °C for 24 h, after which loss on ignition (LOI) was determined at 550 °C for 4 h (see SI section 1.A). As a proxy for reactive iron species, %Fe extractable as Fe (II) was determined using the ferrozine method (Lovley and Phillips, 1986) (see SI section 1.B).

X-ray fluorescence analysis (XRF) was undertaken on a Rigaku ZSX Primus II spectrometer. Samples were prepared as fused beads for major element analysis and pressed pellets for trace element analysis (see SI section 1.C). The elements Cd, Mo, S, Sb and Sn (not covered by the XRF analysis) were determined by aqua regia digestion and inductively coupled plasma - optical emission spectrometry (ICP-OES) (Thermo Scientific iCAP 7400 Radial) (see SI section 1.C).

### 2.3. Water extraction for pH, EC, DOC and water leachable elements

Approximately 15 g of each sample (dry weight, as calculated using the moisture content) was placed in a 50 ml centrifuge tube and Milli-Q water was added to produce a liquid/solid (L/S) ratio of 2. Tubes were placed on a roller mixer for 24 h and then centrifuged at 3000 rpm for 5 min. A portion of the supernatant was recovered and filtered with 0.45 μm nylon syringe filters and the pH and EC were measured in the remaining unfiltered solution (HACH HQ40D pH meter with HACH Intellical pH and EC probes).

Aliquots of the filtered supernatant were analysed for dissolved organic carbon (DOC) with an Analytik Jena Multi N/C 2100 combustion analyser, calibrated using commercially prepared stocks of organic and inorganic carbon. Further aliquots of filtered supernatant were acidified with 0.5 ml of concentrated (70 %) $HNO_3$ and water leachable elements were analysed by ICP-OES (Thermo Scientific iCAP 7400 Radial).

### 2.4. DNA extraction from tailings samples

The tailings samples from which DNA was recovered (with number of replicates) are listed in Table S.2. DNA was extracted in two batches (at the University of Leeds and Cardiff University). Batch one was extracted from ~0.3 g of tailings with a DNeasy® PowerSoil® Pro Kit (Qiagen) according to the manufacturer's instructions. DNA was recovered from the kit's spin-filter using 50 μl of elution buffer (10 mM Tris-HCl, pH 8.5), and the then stored at −20 °C. Batch two was extracted (at Cardiff University) using the FastDNA™ Spin Kit for Soil (MP Biomedicals) as previously described (Webster et al., 2003). DNA was recovered from the spin-filter by elution with 100 μl of molecular grade water (MGW; Severn Biotech Ltd.) and stored at −20 °C. In addition, DNA was recovered from soil from Malham Cove, Yorkshire, England using the DNeasy® kit so the bacterial populations of the tailings samples could be compared with a natural calcareous grassland soil (background information about this site is given in SI Table S.1). A detailed description of both extraction protocols can be found in SI section 1.D.

### 2.5. Bacterial 16S rRNA gene sequencing

Bacterial 16S rRNA gene barcodes (Caporaso et al., 2011), amplified by PCR from DNA extractions, were processed as described in SI section 1.E.

QIIME2 (Bolyen et al., 2019) was used for the 16S rRNA gene barcode analysis, setting a 97 % identity threshold for clustering operational taxonomic units (OTUs). Taxonomic classification of OTUs was undertaken with the Greengenes2 2022.10 reference tree (McDonald et al., 2024) using a confidence value of 70 % to give a reasonable trade-off between sensitivity and error rate in the taxonomy prediction. OTUs which were not classified to a bacterial phylum with a confidence >70 % (e.g. Archaea and poor reads) were not included in the diversity and statistical analyses. Shannon's diversity index of the bacterial populations and the Bray Curtis dissimilarity between the populations were calculated using QIIME2 using even sampling depths of 28,000 and 10,000 sequences per sample, respectively.

### 2.6. Statistics

Correlations and principal component analysis (PCA) were carried out in OriginPro 2024b. Statistical significance was calculated with ANOVA and two-way *t*-tests (assuming unequal variances) in Microsoft Excel. To investigate the metabolic profiles of microbial communities, the PICRUSt2 pipeline (Douglas et al., 2020) was used to explore functional potential based on enzyme classifications within the KEGG database (Kanehisa and Goto, 2000). PICRUSt2 output was processed in R environment (R 4.5.0). Differences in community function were assessed through Bray-Curtis dissimilarities between all individual replicates within and between sample groups, then these were averaged to evaluate within-group and between-group dissimilarity (vegan 2.7–1 package; Oksanen et al., 2007). Principal coordinates analysis (PCoA) was used to visualize similarities and differences among groups based on these Bray-Curtis dissimilarities (vegan 2.7–1 package). The effect of time on changes in microbial metabolic profiles across different soil depths was assessed by PERMANOVA (vegan 2.7–1 package). Differential abundance analysis was performed using DESeq2 (Love et al., 2014), incorporating a zero-inflated negative binomial (ZINB) model via the ZINB-WaVE framework (Risso et al., 2018) to account for overdispersion and excess zeros in the count data. This approach enabled the identification of significantly differentially abundant enzymes, with emphasis on enzyme groups involved in microbial responses to toxic metal transformation and detoxification.

## 3. Results

### 3.1. Site observations

A detailed description of the Tara TMF as well as the three sampling sites can be found in Fitzsimons and Courtney (2022) and Burke et al. (2023). In summary, the vegetation cover on the tailings consisted of perennial grasses, clovers, and after 8 years, small trees and shrubs. The 1-year-old site (Fig. S.2-a) was the most sparsely vegetated, with intermittent patches of grasses and trifoliate clovers (e.g. *Trifolium repens*). The cores (Fig. S.4-a) showed that the fresh tailings were grey in colour, with some subtle layered variations. At the locations where there was vegetation, the root zone was very shallow (~2 cm) and sparsely occupied. At the 3-year-old site, the vegetation cover was much more robust, effectively covering the entire plot (Fig. S.2-b). Tufts of coarse and taller grasses could be seen. The cores (Fig. S.4-b) showed a still shallow (~2 cm) but denser root zone, with occasional penetration of roots to deeper levels (≤ 20 cm). While still predominantly grey in colour, browner tones could be observed following the areas with root penetration, as well as in a few other localised regions of the core (Fig. S.5). The 8-year-old mesocosms (Fig. S.3) had denser coverage with grassland species, and a few self-seeded trees and shrubs (e.g. *Salix* spp.). The trial pits (Fig. S.6) showed a dense root mat up to ~4 cm thick, with occasional roots penetrating to deeper levels. The tailings were uniformly pale brown in colour throughout the depth of the trial pits.

### 3.2. Geochemical parameters

The pH of the tailings was near neutral at all three locations (Fig. 1). The pH was 7.1 ± 0.1 for 1-year-old samples, 7.0 ± 0.1 for 3-year-old samples and 7.4 ± 0.1 for 8-year-old samples. There was no consistent variation of pH with depth at any location. However, a two-way *t*-test indicates that there was a small but significant difference between all three ages ($p < 0.05$).

The electrical conductivity (EC) of the water extractions was 2.5 ± 0.4, 2.6 ± 0.5, and 2.3 ± 0.5 mS $cm^{-1}$ for the 1-, 3-, and 8-year-old samples, respectively. However, there was no significant trend with depth at any location, and no significant difference with the age of the tailings.

The % of acid extractable Fe in a reduced Fe(II) oxidation state (a proxy of the redox conditions) was 84.9 ± 14.1 %, 93.4 ± 12.1 %, and 26.5 ± 2.3 % in the 1-, 3-, and 8-year-old samples, respectively (Fig. 1c). There was no statistically significant difference between the 1- and 3-year-old samples, but at both ages the %Fe(II) was significantly higher than in the 8-year-old samples (i.e. the younger samples were more reducing). Only the 3-year-old site shows a correlation between %Fe(II) and depth (correlation coefficient $r = 0.70$), with more oxidising conditions closer to the surface.

There was a small but statistically significant increase in the LOI with the age of the tailings (Fig. 1d), from 1.0 ± 0.2 % in 1-year old sample, to

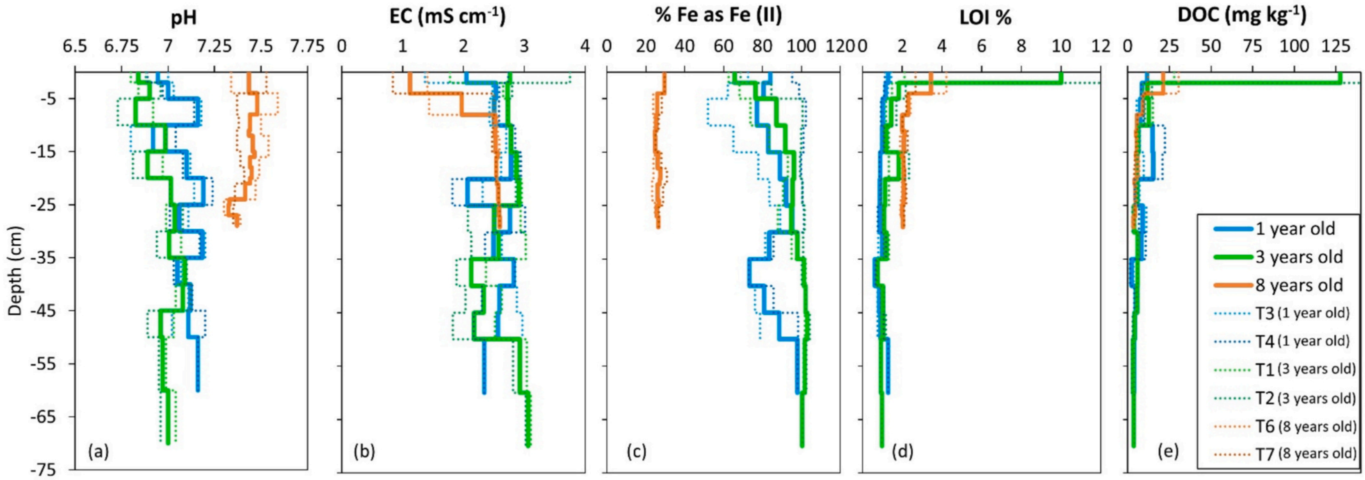


**Fig. 1.** Geochemical parameters (pH, EC, %Fe, LOI, DIC) measured on subsamples of the core (1- and 3-yr-old) and trial pit (8-yr-old) samples vs depth (all samples were analysed except for % of acid extractable Fe as Fe(II) where alternate depth samples were analysed, starting with the surface samples, and intermediate values were interpolated). Average values for replicate cores (or trial pits) shown with continuous lines, and individual replicates in dotted lines, according to the legend.

1.2 ± 0.4 % in 3-year-old samples (excluding an outlier reading of 17.9 %), and 2.3 ± 0.5 % in the 8-year-old samples. At all ages, there was a slight correlation between LOI and depth (correlation coefficient $r = -0.51$), with DOC tending to decrease from the surface over ~10 cm.

The DOC was 8.2 ± 5.1 mg DOC (kg-dry weight tailings)$^{-1}$ in 1-year-old samples, 7.9 ± 5.2 mg DOC (kg-dry weight tailings)$^{-1}$ in 3-year-old samples (excluding one outlier value of 227.3 mg kg$^{-1}$), and 7.4 ± 6.4 mg DOC (kg-dry weight tailings)$^{-1}$ in 8-year-old samples. There was no statistically significant difference in DOC with sample age, however, for each age there was a trend for DOC to decrease from the surface to a depth of ~20 cm (correlation coefficient $r = -0.55$).

### 3.3. Total and water leachable composition

Calcium was the most abundant element in the tailings at all three locations (averages of 30.7, 31.0 and 30.2 wt% CaO in 1-, 3- and 8-year-old sites respectively), followed by Si (averages of 23.9, 19.3 and 19.6 wt % $SiO_2$ in 1-, 3- and 8-year-old sites respectively). Fe, S, Al, Ba, Mg, K had average concentrations in the range 1–6 wt% oxides at all three ages (details in SI Figs. S.7, S.8 and S.12). There were elevated concentrations of both the metals being mined, with a tendency towards higher concentrations in the older tailings (the average Zn concentrations are 1966, 2681 and 2965 mg kg$^{-1}$ in 1-, 3- and 8-year-old sites respectively, and the average Pb concentrations were 1730, 1911 and 2757 mg kg$^{-1}$ in 1-, 3- and 8-year-old sites respectively; see SI Fig. S.11). Several other potentially toxic elements were detected in the tailings. Arsenic concentrations were in the range 500–750 mg kg$^{-1}$, Cr, Cu, Sb, Ni and V in the range ~ 40–100 mg kg$^{-1}$, and Cd and Mo in the range ~ 2–6 mg kg$^{-1}$ (see SI Figs. S.10 and S.12). Cd, V and Sb tended to have higher concentrations in the older sites.

The main water leachable elements were Ca (averages of 1195, 1194 and 1173 mg kg$^{-1}$ dry weight of tailings in 1-, 3- and 8-year-old sites, respectively) and S (averages of 1143, 1197 and 1061 mg kg$^{-1}$ dry weight of tailings in 1-, 3- and 8-year-old sites, respectively). The leachable Ca was ~0.55 % of the total Ca in the tailings, whereas the leachable sulfur was ~6 % of the total S in the tailings. There was no significant difference in the amount of leachable Ca and S with sample depth or age of the tailings (Fig. 2 and SI Figs. S.7 and S.8). Mg was the third most abundant leachable element with average concentrations in the range 140–200 mg kg$^{-1}$, and there was a tendency for Mg concentration to increase with depth (correlation coefficient $r = 0.70$). Water extractable K concentrations were ~ 67–80 mg kg$^{-1}$ in the 1- and 3-year-old samples, but only 19 mg kg$^{-1}$ in the 8-year-old samples. Similarly, Na concentrations were ~ 34–45 mg kg$^{-1}$ in the 1- and 3-year-old samples, but only 3 mg kg$^{-1}$ in the 8-year-old samples (see Fig. 2 and SI Figs. S.7 and S.8). Fe and Al were undetectable in almost all the water extractions, with some exceptions, mostly in surficial samples.

Zn, Ni, Pb and Mo were all leached from the tailings with water. However, considerably more were leached from the 1- and 3-year-old tailings (2.0 ± 0.9, 1.0 ± 0.5, 0.10 ± 0.06 and 0.04 ± 0.02 mg kg$^{-1}$ of tailings for Zn, Ni, Pb and Mo, respectively) than from the 8-year-old tailings (0.34 ± 0.28, 0.08 ± 0.05, 0.01 ± 0.007 and 0.013 ± 0.009 mg kg$^{-1}$ of tailings for Zn, Ni, Pb and Mo, respectively; see Figure and 3 SI Figs. S.10 and S.11). The amounts mobilised in the water leaching tests on the younger tailings represented ~2 % of the total Mo and Ni, but only ~0.1 % and < 0.01 % of total Zn and Pb. Cu was undetectable in leachates from samples from depths >15 cm in all sites, but up to ~0.1 mg kg$^{-1}$ was leached from near surface samples (Fig. 3 and SI Fig. S.10). Cd was undetectable in all leachates of the 8-year-old tailings, however, up to ~0.008 mg kg$^{-1}$ of Cd was leached from the subsurface samples of the 1 and 3-year-old tailings. As, Cr, Sb and V were mostly undetectable in the water leachate.

### 3.4. Bacterial 16S rRNA gene sequencing

Sufficient bacterial DNA was recovered for 16S rRNA gene sequencing from tailings from depths down to 60–70 cm, although the concentration of extractable DNA recovered was very low (<0.1 ng μl$^{-1}$) at depths below ~10 cm (also, many DNA extractions on samples from below this depth were unsuccessful). For each age of tailings, the Shannon diversity index of the bacterial populations decreased with depth (Table 1). Below the surface layer, most sequence reads were assigned to the *Pseudomonadota* (below 12 cm >70 % of sequence reads were assigned to the *Pseudomonadota*).

Within the surface layer, the Shannon diversity index increased with the age of the tailings (ANOVA, $p = 0.007$). Paired $t$-tests indicate that the difference between the 1- and 3-year-old surface tailings was not significant, but both have significantly lower Shannon indexes than the 8-year-old surface sample ($p < 0.02$). Shannon diversity decreased significantly with depth in both the 3- and 8-year-old tailings, so that no difference in bacterial diversity with the age of the tailings was observed below 4-5 cm (ANOVA $p \approx 0.99$, down to 8–10 cm). In the 8-year-old tailings, the Shannon diversity index for the surface layer was very similar to that of the surface layer of a calcareous grassland soil from Malham Cove (t-test $p = 0.64$).

In the younger tailings, there was significant variation in the

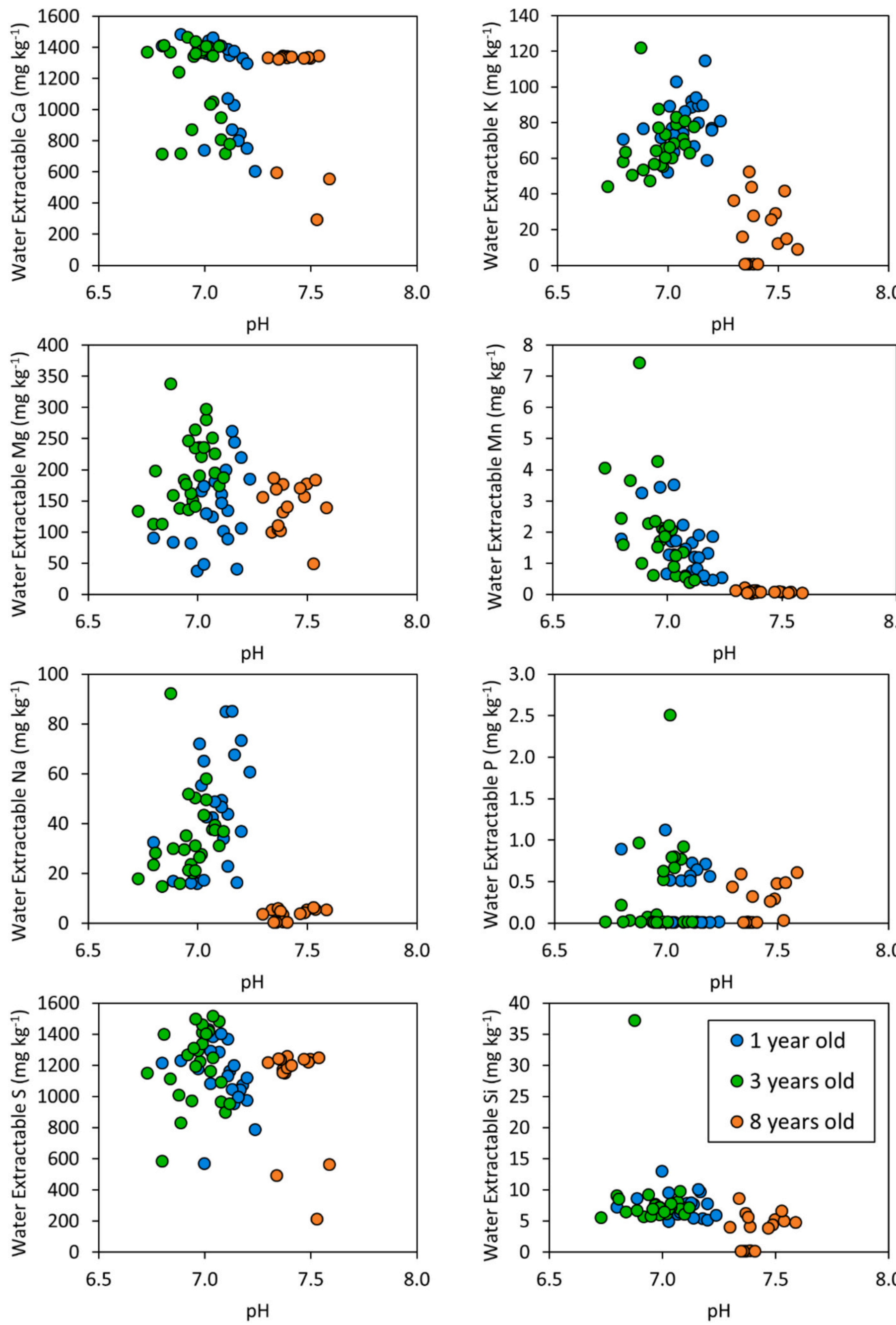


**Fig. 2.** Water extractable major elements (Ca, K, Mg, Mn, Na, P, S, Si) vs pH in all tailing samples (expressed in mg of element per kg dry weight of tailings).

populations between individual samples (as evidenced by the Bray Curtis Dissimilarity, a measure of beta diversity; Table 2). However, the beta diversity between the surface layer samples of the 8-year-old tailings was significantly lower (ANOVA $p < 0.001$) than either the 1- or 3-year-old tailing (which were not significantly different from one another). Nonetheless, while the beta diversity between samples from the surface layer of the 8-year-old tailing approached that of the calcareous grassland soil, the difference was still significant.

At phylum level, the population of the surface layer of the 1-year-old tailings was dominated by *Pseudomonadota* (59 ± 11 %), and *Bacteroidota* (15 ± 6 %), with no other phylum representing >5 % of the reads (see Fig. 4). The relative abundance of *Pseudomonadota* and *Bacteroidota* decreased, and the relative abundance of *Actinomycetota*, *Acidobacteriota*, *Chloroflexota* and *Planctomycetota* increased progressively, with the age of the tailings. In the 8-year-old tailings the population of the surface layer consisted of *Pseudomonadota* (36 ± 5 %), *Actinomycetota* (22 ± 5 %), *Acidobacteriota* (12 ± 2 %), *Bacteroidota* (8 ± 2 %), *Chloroflexota* (8 ± 2 %), and *Planctomycetota* (6 ± 2 %). The distribution of OTUs by phylum of the 8-year-old tailing was closer to the bacterial population in the calcareous grassland soil from Malham Cove, than were the populations of the younger tailings (the soil from Malham Cove contained *Pseudomonadota*, 26 ± 1 %; *Actinomycetota*, 25 ± 2 %; *Verrucomicrobiota*, 15 ± 3 %; *Acidobacteriota*, 11 ± 2 %; *Planctomycetota*, 5 ± 1 %; *Chloroflexota*, 4 ± 1 %; *Bacteroidota*, 2 ± 1 %). Similarly, OTUs in the surface layers classified to the two most abundant bacterial phyla (*Psuedomonadota* and *Actinomycetota*), exhibited differences with age of the tailings (SI Fig. S.13). At the taxonomic level of order, the distribution in the 8-year-old tailing was again closer to that in the calcareous grassland soil from Malham Cove, than were the populations of the younger tailings. However, some differences remained between the 8-

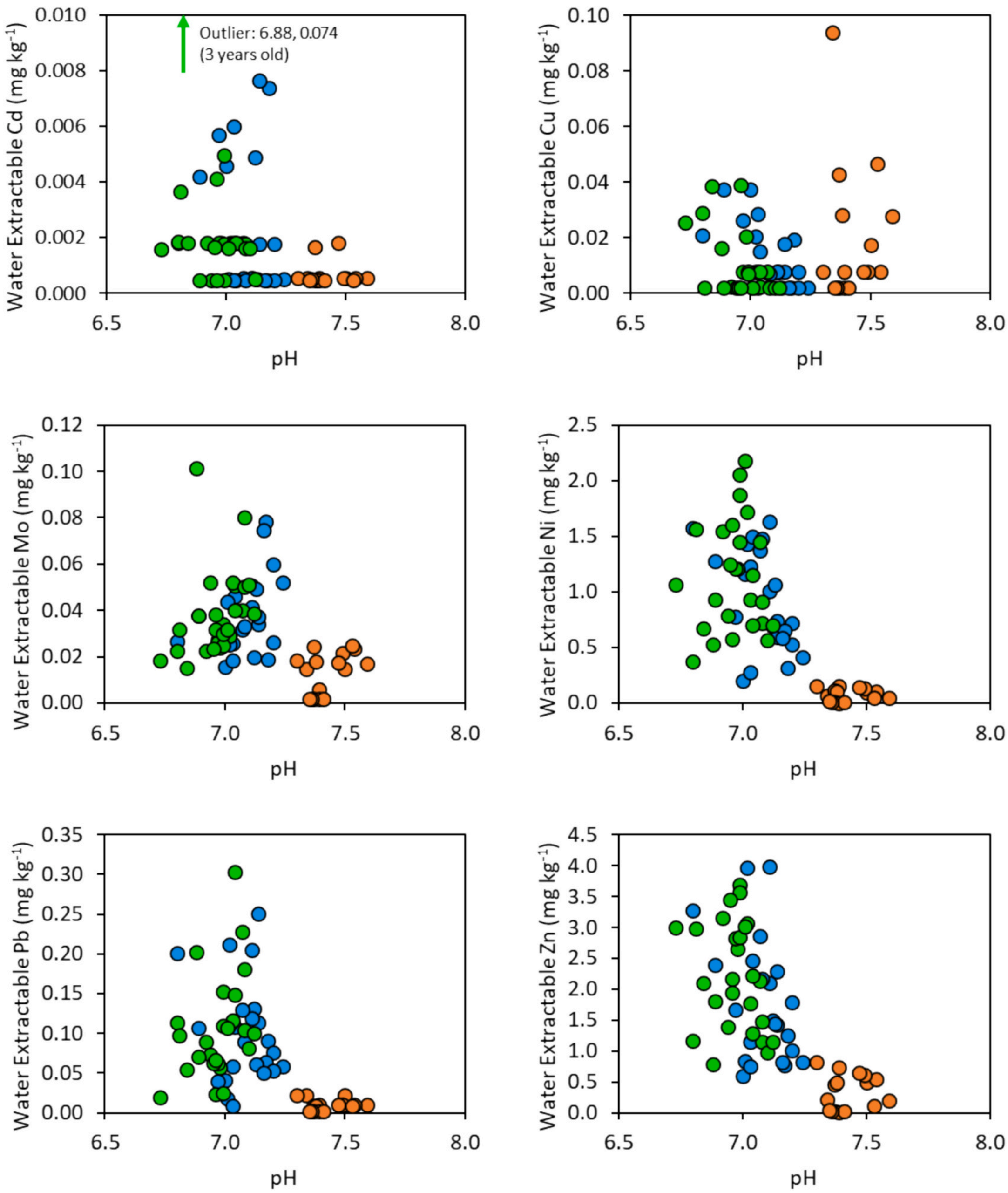


**Fig. 3.** Water extractable potentially toxic elements (Cd, Cu, Mo, Ni, Pb, Zn) vs pH in all tailing samples (expressed in mg of element per kg dry weight of tailings).

**Table 1**
Bacterial diversity in the tailings samples (Shannon's diversity index based on an even sampling depth 28,000 sequences per sample).

| 1 yr-old tailings | | 3-yr-old tailings | | 8-yr-old tailings | | Malham Cove | |
|---|---|---|---|---|---|---|---|
| Depth | Shannon Index* | Depth | Shannon Index* | Depth | Shannon Index* | Depth | Shannon Index* |
| 0-2 cm | 6.62 ± 1.29 | 0-2 cm | 7.24 ± 0.53 | 0-4 cm | 8.05 ± 0.17 | 0-3 cm | 8.01 ± 0.12 |
| 2-5 cm | 5.63 ± 1.91 | 2-5 cm | 5.24 ± 1.23 | 4-8 cm | 5.31 ± 2.12 | 3-6 cm | 7.80 ± 0.30 |
| 5-10 cm | 5.24 ± 1.59 | 5-10 cm | 5.19 ± 1.00 | 8–11/12 cm | 5.54 ± 1.61 | Overall (0-6 cm) | 7.90 ± 0.23 |
| 25-30 cm | 4.67 | 25-30 cm | 3.86 | 25-29 cm | 4.48 | | |

[*] Mean value ±1 S.D. (S.D. is omitted where there were fewer than 3 replicates).

year-old tailings and the calcareous grassland soil. Despite decreasing with age of the tailings, the relative abundances of the phyla *Pseudomonadota* and *Bacteroidota* were still significantly higher than in the natural calcareous grassland soil (*t*-test $p < 0.001$). Also, while the relative abundances of *Actinomycetota* and *Acidobacteriota* were not significantly different, the relative abundance of *Verrucomicrobiota* was significantly higher in the Malham Cove soil (t-test $p < 0.002$).

Two other features of the data differentiate the 8-year-old tailings from the calcareous grassland soil from Malham Cove. Firstly, the relative abundance of the main phyla varied less between replicate samples of the grassland soil than with the tailings. Secondly, despite similar patterns at phylum level, and convergence at order level, there

**Table 2**
Beta diversity between the surface samples based on Bray Curtis Dissimilarity between individual samples (based on an even sampling depth 10,000 sequences per sample).

| | 1-yr-old 0-2 cm | 3-yr-old 0-2 cm | 8-yr-old 0-4 cm | Malham Cove |
|---|---|---|---|---|
| 1-yr-old 0-2 cm | 0.588 ± 0.163 | 0.730 ± 0.092 | 0.824 ± 0.051 | 0.942 ± 0.025 |
| 3-yr-old 0-2 cm | | 0.530 ± 0.149 | 0.782 ± 0.029 | 0.915 ± 0.037 |
| 8-yr-old 0-4 cm | | | 0.366 ± 0.071 | 0.841 ± 0.061 |
| Malham Cove | | | | 0.309 ± 0.048 |

were very few common OTUs between the two populations.

### 3.5. Functional profiling

PICRUSt2 predicted the presence of 3039 unique enzymes across all collected soil samples. PERMANOVA analysis indicated a significant effect of sample age on the enzymatic profiles of surface soil samples ($R^2 = 0.47$, $p < 0.05$), suggesting substantial temporal shifts in predicted metabolic potential (Fig. S.14, Supplementary Material). The near-surface samples showed a weaker temporal relationship ($R^2 = 0.30$, $p < 0.05$); however, this result should be interpreted cautiously because group dispersions were unequal, making it unclear whether this pattern reflects a true temporal effect. For deeper soil samples, no significant temporal differences in projected functional profiles were detected ($p > 0.05$). Further analysis of the functionality of the studied microbial communities was done based on predicted abundance on selected enzyme classes directly associated with sulfur and iron metabolism, and toxic metal detoxification, all expected to be upregulated in the mine tailing sites. The predicted enzymes can be narrowed down to oxido-reductases that oxidize metal ions (EC 1.16) or those acting on a sulfur group of a donor (EC 1.8), and translocases responsible for the translocation of inorganic cations (EC 7.2). Similarly to the general functional profile analysis, the projected effect of sample age was strongest for surface samples (PERMANOVA $R^2 = 0.56$, $p < 0.05$), and gradually diminished at the near surface level ($R^2 = 0.26$, $p < 0.05$) and disappeared at the deeper level ($R^2 = 0.12$, $p > 0.05$) (Fig. 6A). Considering the strength of the time effect and level of exposure to environmental factors, the 1- and 8-year-old surface samples were analysed to predict differentiating enzymes which could indicate the direction of the shift of metabolic profile and associated changes in soil chemistry. DESeq2 analysis showed 29 differentiating ($p < 0.05$) enzymes from the total of 69 enzymes identified from the group of 3 enzyme classes (EC 1.16, 7.2, 1.8) (Fig. 6B). The microbial community in the 1-yr-old surface samples is projected to contain enzymes involved in respiratory sulfur and iron oxidation (i.e. ferroxidase, sulfite dehydrogenase (quinone), sulfide: quinone reductase (SQR)) and reduction (dissimilatory (DSR) and assimilatory (NADPH) sulfite reductases), proton-translocating complexes (i.e. NADH: ubiquinone reductase, complex I or $H^+$-transporting ATPase) and oxidative stress (methionine sulfoxide reductase (S)). In the 8-yr-old surface samples the community is projected to be enriched in enzymes linked to detoxification (sulfite oxidase) and redox balancing (mycothione reductase, disulfide reductases).

The co-occurrence of oxidative and reductive sulfur and iron metabolism observed in early stages of rehabilitation of mine tailings suggests the steep redox gradient in the tailings leads to development of diverse microhabitats. Additionally, the projected enrichment of proton-translocating complexes alongside methionine sulfoxide reductases providing repair of oxidatively damaged proteins might indicate generally unfavourable conditions causing energetic and oxidative stress burden for microbial communities at this stage of site rehabilitation. After 8 years, the projected functional profile had shifted. Enzymes associated with sulfur and iron respiration significantly decreased relative to 1-year-old samples. Instead, predicted enriched enzymes were primarily involved in detoxification and thiol/disulfide redox balance. The projected lack of iron-cycling enzymes after 8 years suggests a decline of iron-driven microbial metabolism as mine tailings rehabilitation progressed. The projected decrease of iron-cycling enzymes and the increase in antioxidant/redox-balancing enzymes suggest progressing environmental stabilization, with reduced reliance on lithotrophic sulfur/iron energy metabolism and increased emphasis on maintaining intracellular redox homeostasis.

It is important to note that functional predictions were generated using PICRUSt2, which derives functional profile from 16S rRNA gene profiles and thus does not lead to a direct measure of gene abundance and metabolic activity. Therefore, the described enzymatic profiles should be viewed as hypothesis-generating rather than definitive

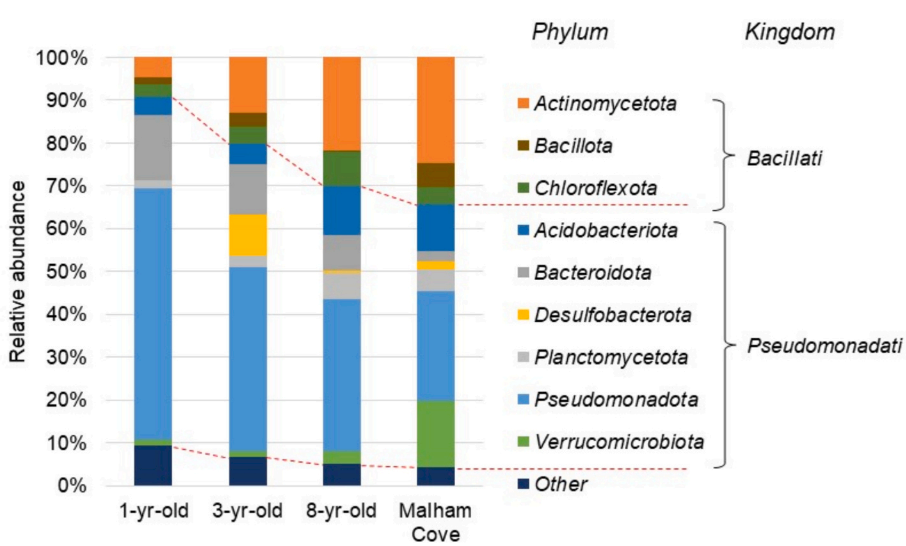


**Fig. 4.** Phylogenetic diversity of the bacteria in the surface samples of tailings determined by 16S rRNA gene sequencing ("other" represents phyla with relative abundance ≤5 %). The Kingdoms shown are defined in the List of Prokaryotic names with Standing in Nomenclature (LPSN; Freese et al., 2025).

evidence of functional profile. For a more comprehensive and quantitative understanding of microbial functional capacity in rehabilitating mine tailings, shotgun metagenomic or metatranscriptomic analyses are required (Douglas et al., 2020).

## 4. Discussion

### *4.1. Geochemistry of mine tailings*

At Tara Boliden Mine the carbonate-hosted lead-zinc sulfide ore is milled to grainsizes <120 μm to allow separation (in flotation cells) of the sulfidic ore from the gangue (Oman, 2022). The fresh tailings produced are primarily composed of carbonate minerals (49 % calcite and 11 % dolomite), silicates (16 % quartz, as well as some feldspar and mica), small amounts of pyrite ($FeS_2 \leq 3$ %) and traces of galena and sphalerite (PbS, ZnS < 0.5 %) (Fitzsimons and Courtney, 2022; Burke et al., 2023). The major element compositions of the mine tailings from all three locations are consistent with this mineralogy. At each location the composition is relatively constant with depth, and there is very little variation in major element composition between the three sites sampled. Similarly, there is only modest variation in the minor element content of the tailings (the only notable difference is that Cd, Pb and Sb show modest enrichment in the 8-year-old tailings).

Pyrite and other sulfidic minerals in the fresh tailings are relatively reactive due to newly exposed surfaces, so can oxidize quite rapidly when exposed to oxygenated rainwater (Gil-Loaiza et al., 2016; Bao et al., 2022). Pyrite oxidation produces acidity (reactions 1, Rimstidt and Vaughan, 2003), which is neutralized by calcite in the tailings (reaction 3) which leads to the formation of gypsum ($CaSO_4{\cdot}2H_2O$; reaction 4). Gypsum is found in tailings from the Tara TMF that are more than 1 year old (Burke et al., 2023).

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4{}^{2-} + 2H^+ \tag{1}$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4{}^{2-} + 16H^+ \tag{2}$$

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3{}^- \tag{3}$$

$$Ca^{2+} + SO_4{}^{2-} + 2H_2O \rightarrow CaSO_4{\cdot}2H_2O \tag{4}$$

Fe(II) produced by pyrite oxidation will also be oxidised by oxygenated rainwater.

$$Fe^{2+} + ¼\,O_2 + H^+ \rightarrow Fe^{3+} + ½\,H_2O \tag{5}$$

The $Fe^{3+}$ produced by reaction 5 can support further pyrite oxidation (and acid generation) via reaction 2. Any Fe(III) not consumed by reaction 2 will precipitate as iron oxides/hydroxides at the neutral pH of the tailings (Langmuir, 1997).

Ca and S were the most leachable elements in the tailings by an order of magnitude, which is consistent with the presence of gypsum in all the deposited tailings to a depth of 70 cm after 1 yr. The Ca/S molar ratio in the leachate was ~0.84, which is close to the stoichiometric ratio for dissolution of Gypsum ($CaSO_4{\cdot}2H_2O$) (see Fig. S.15). Mg is the next most leachable element, which suggests that magnesium sulfate may also be present in the tailings (the molar ratio of (Ca + Mg) to S was ~1). Similar aqueous Ca and S concentrations were observed in all the water leaching tests (representing 0.5–0.6 % of the total Ca and 6 % of the total S), suggesting there is sufficient gypsum in the tailings after 1 year in vegetated management for gypsum dissolution to be solubility limited. However, it is unlikely that pyrite oxidation was complete in the surface tailings even after 3 years in vegetated management, as the proportion of the acid extractable Fe that was in the Fe(II) oxidation state was on average ~ 85 and ~ 93 % in the 1 and 3-year-old tailings, respectively. Thus, any Fe(III) produced by reaction 5 (oxidation of Fe(II) produced by reaction 1) must have been consumed by the oxidation of further pyrite by reaction 2. However, in the 8-year-old tailings ~75 % of the acid extractable Fe was Fe(III), a proportion that is supported by the prevailing brown colouration observed in the trial pit. Thus, in the 8-year-old mesocosms, pyrite oxidation was largely complete to a depth of 30 cm. The pH profiles appear to confirm this deduction. The average pH of the 1-year-old tailings and 3-year-old tailings were 7.1 and 7.0, respectively, whereas the average pH of the 8-year-old tailings was 7.4 (without a depth trend at any age). This is compatible with acid generation by pyrite oxidation in the younger tailings, with recovery to slightly alkaline values more typical of calcareous soils in the 8-year-old tailings. It is notable that the tailings from this carbonate-hosted ore have sufficient buffering capacity to maintain neutral pH despite the very high acid-generating capacity of reactions 1 and 2.

Increase in the age of the tailings was associated with more verdant vegetation (SI Figs. S2 and S3). Generally, the soil organic matter (SOM) content (determined by LOI) increased with age of the tailings, reflecting the accumulation of plant litter and more complex organic molecules with time. SOM peaked in the surface samples of the 3-year-old tailings, suggesting that there was some heterogeneity in the distribution of the compost used as an initial amendment, but otherwise SOM decreased slightly with depth for each age of tailings. Except where the SOM peaked, the DOC in the water leaching tests did not vary with age of the tailings. Thus, it appears to have reached a relatively steady state, where any increase in release from vegetation and by soil organic matter mineralization is matched by increases in consumption by microbial metabolism and sorption to newly-formed ferrous oxides in the older tailings (Guo et al., 2024). Growth of plant roots can change the properties of the growth media by water uptake, and root exudation, penetration and decay, which reduce saturation, create micro-fissures and macropores and thereby increase air penetration into the root zone (Xiao et al., 2024). Near the surface of the 3-year-old tailings the colour change from grey to brown that is indicative of pyrite oxidation in these tailings is associated with the plant roots (Fig. S.5). Thus, it is likely that the presence of surface vegetation may have accelerated microbially mediated sulfide oxidation (which requires oxygen and moisture).

### *4.2. Leachability of toxic trace elements*

Despite the tailings containing 2–3000 mg $kg^{-1}$ Pb and Zn (remnants of the ore), and decreasingly lesser amounts of As, Cr, Cu, Sb, Ni, V, Cd and Mo, the water extractable concentrations of potentially toxic elements were low. Also, with the exception of Cu, water extractability was lower in the 8-year-old tailings than in the younger tailings (typically by an order of magnitude).

The relationships between toxic metal leachability and the environmental factors are elucidated by PCA analysis (Fig. 5). The most notable feature of which is that the 1- and 3-year-old samples cluster together, illustrating their similarities (although the surface samples sit above the main cluster on the PCA plot). The 8-year-old samples form a separate looser cluster to the left of the younger samples (again the surface samples sit above this loose cluster, but to the left of the younger surface samples). The variables that are most aligned with the separation of the main clusters are pH and the percentage of acid extractable Fe as Fe(II). LOI, a measure of SOM, is orientated in a direction associated with both the separation of the surface samples from the main clusters and the separation of the 8-year-old samples from the younger tailings.

The principal determinants of the mobility of most trace elements present in the tailings are pH and % Fe as Fe(II). Both are controlled by pyrite oxidation in these tailings (pyrite oxidation temporarily lowers the pH of the tailings and produces ferric oxides in the longer term). Both cation (e.g. Cu, Pb, Cd, Ni, Zn) and oxyanion (e.g. As, Cr, Sb, V) forming elements can to bind to ferric oxyhydroxides at neutral pH (Bradl, 2004; Shi et al., 2021; Bahr et al., 2022); therefore the lower metal(loid) mobility observed is likely due to the increased availability of ferric oxyhydroxide surfaces in the more oxidised samples. Fitzsimons and Courtney (2022) also observed an order of magnitude increase in trace element sorption (for Pb, Zn, Cu, Ni and Cd) as pH was varied between

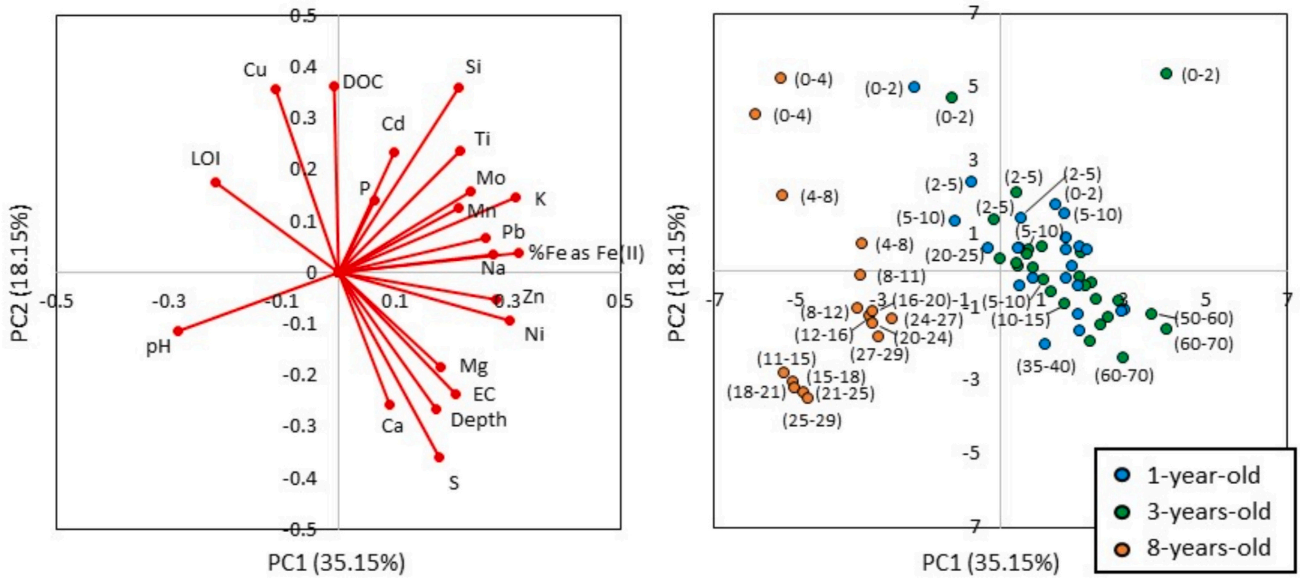


**Fig. 5.** PCA analysis of selected parameters in tailing samples, including water soluble element concentrations, according to age (colour code in legend) and depth (labels in righthand graph). Unlabelled points in the right-hand panel are samples from depths >15 cm in the 1- and 3-year-old tailings.

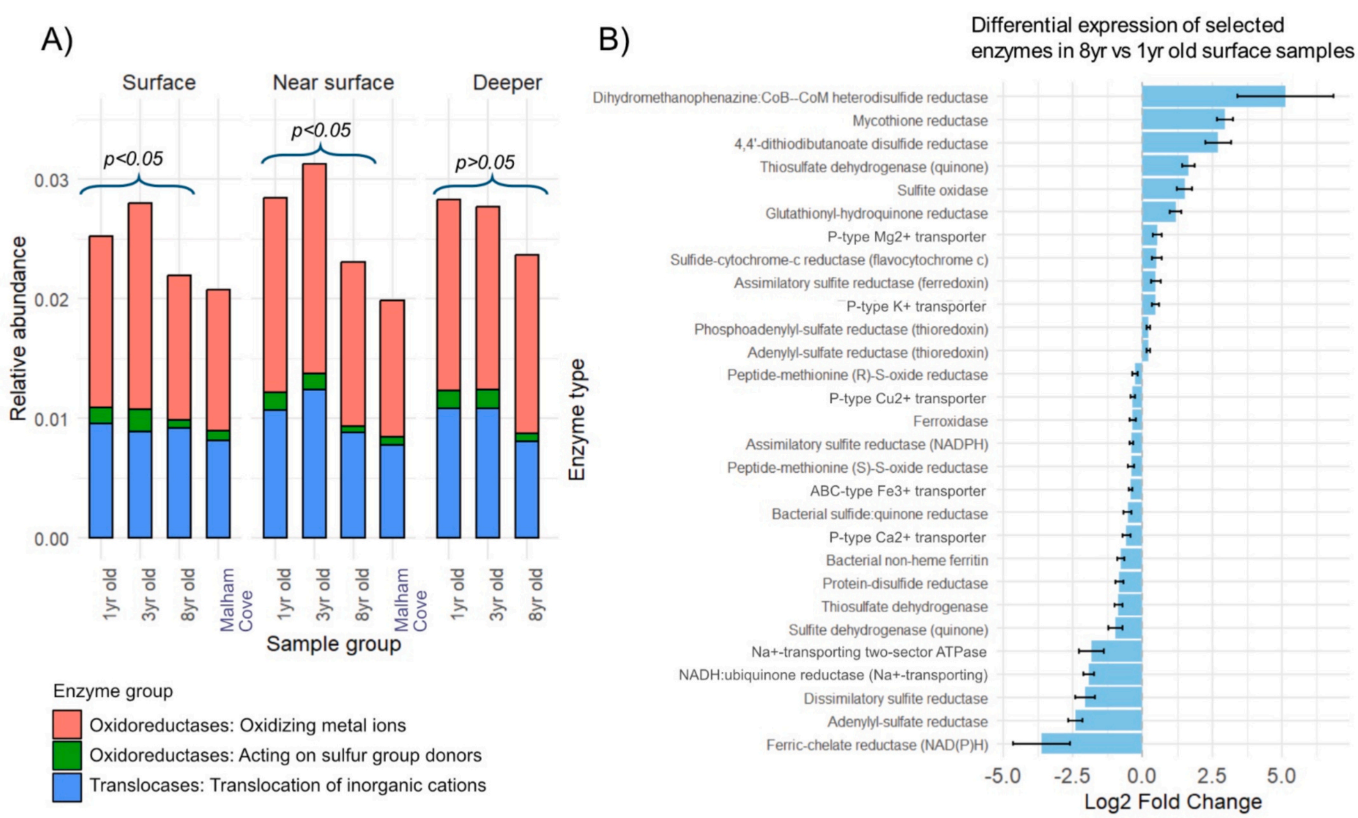


**Fig. 6.** (A) Predicted functional profiles of microbial communities based on PICRUSt2 inference from 16S rRNA gene data, showing the relative abundance of selected enzymes associated with heavy metal cycling in lead–zinc mine tailings during soil rehabilitation. Control samples from the Malham Cove were unavailable for deeper soil layers. The PERMANOVA *p*-value reflects the significance of differences in predicted functional composition within samples grouped by soil profile depth. (B) Differential abundance analysis (DESeq2) comparing enzyme abundances in surface soils from 1-year-old and 8-year-old rehabilitation plots, focusing on the pre-selected enzyme groups linked to toxic metal cycling.

pH 7 and 8 in tests using freshly deposited Tara mine tailings; therefore, the small increase in pH observed as Fe(II) content reduced will also tend to decrease the mobility of elements in contact with these materials. The leachability of several toxic metals, particularly Zn and Ni, also decrease as SOM increases (a component of the LOI vector is towards lower metal leachability). This is probably because humic-coated mineral surfaces can strongly adsorb many metal ions (Bradl, 2004).

In contrast to most other trace elements, Cu extractability showed a

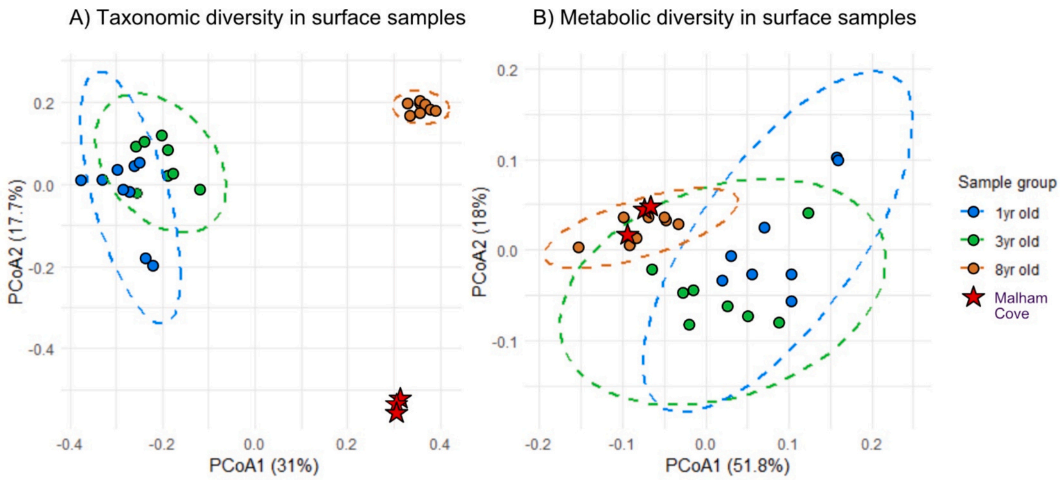


**Fig. 7.** Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity showing shifts in microbial A) taxonomy and B) metabolic diversity in surface soil layers throughout the monitoring period (8 years) of rehabilitation of lead/zinc mine tailings. Each point represents the (A) taxonomic or (B) metabolic profile of a microbial community in a sample replicate, with distances between points reflecting compositional dissimilarity. Temporal clustering indicates changes in taxonomic and functional potential over time. Malham Cove represent a reference site for natural calcareous grassland soils.

clear correlation with DOC and is relatively insensitive to changes pH and % Fe as Fe(II) (Fig. 5). Inorganic forms of Cu have low solubility at near neutral pH values (Wu et al., 2002). However, SOM and organic composts contain humic and fulvic acids (Stevenson, 1994; Lanno et al., 2022), and these ligands have high affinity for Cu, and can compete with mineral sorption sites for Cu (Weng et al., 2002; Agnello et al., 2014; Wu et al., 2002). Therefore, it is speculated that the dissolved Cu fraction in the tailings is probably associated with such dissolved organic complexes. However, even in the 8-year-old surface tailings <0.2 % of the total Cu is water extractable, so the impact on metal mobility is small.

### 4.3. Microbial diversity as an indicator of soil forming processes

It is notable that bacterial DNA could be recovered from this mineral waste to depths up to 60 cm only 1 year after it was placed in the TMF. However, DNA recovery was difficult from deeper tailings and, where successful, DNA concentrations were generally low. Also, where 16S rRNA gene sequencing was possible, deeper tailings showed large variations in the Shannon Index between replicate samples, and bacterial populations were heavily dominated by the phylum *Pseudomonadota* (formerly *Proteobacteria)*. Together this suggests that the deeper tailings are somewhat barren bacterial environments characteristic of early successional stages (Thouin et al., 2022), with the populations lacking the complexity of a natural soil horizon. Further, the preponderance of *Pseudomonadota* in the early successional stage communities in these tailings suggests that this phylum contains numerous opportunistic, faster-growing, resilient bacteria, as such species tend to dominate early successional stage communities (Ortiz-Álvarez et al., 2018).

In contrast, the bacterial populations of the surface layer of the tailings show a clear progression over time, from low diversity and high variability to populations with high diversity and much lower variability (Fig. 4 and Tables 1 and 2). During this progression there were changes in the relative abundance of OTUs within the abundant phyla (*Psuedomonadota* and *Actinomycetota*), with some bacterial orders decreasing in relative abundance, while others increased (SI Table S.13). A similar transition has been observed with revegetation of bauxite ore processing residue, where *Psuedomonadota* were abundant in both untreated and revegetated residue, but families/orders characteristic of early colonisers were relative abundant in untreated residue, whereas more soil like species were relatively abundant in revegetated residue (Schmalenberger et al., 2013). The Shannon Index increased with the age of the tailings, to the point where the mean value for the 8-year-old tailings was similar to the mean value for the surface soil from Malham Cove (both of which were close to the Shannon Index reported for extensive grasslands; Labouyrie et al., 2023). Also, the variability in the Shannon Index between replicates decreased with the age of the tailings, to the point where there was only modest variation between samples of 8-year-old tailings that was similar to variation between replicates of the surface soil from Malham Cove. Likewise, the Bray-Curtis dissimilarity values indicate that only ~40 % of OTUs are common in any pair of replicate samples from the 1-year-old tailings, whereas ~65 % of OTUs in common in the 8-year-old tailings, which is close to ~70 % of OTUs in common in the surface soil from Malham Cove (Bray-Curtis dissimilarity weights common and rare OTUs equally).

Although Shannon diversity indices were comparable between 8 yr old surface samples and the Malham Cove reference site's surface samples, the PCoA ordination revealed distinct clustering, indicating that the underlying community composition differs markedly between samples at the level of OTUs (Fig. 7A). Whether this can be attributed to differences between the locations, or it highlights the ongoing soil rehabilitation process that is not possible to fully assess as potentially a longer timeframe or more geographically localised reference site would be needed for a definitive conclusion. It should also be noted that, while the sites represent similar types of environments (calcareous grassland; see SI Table S.1), they are in different countries separated by the Irish Sea (an arm of the North Atlantic Ocean). In general however, taxonomic convergence of the rehabilitated mine tailings sites towards unmined reference sites can take decades, although the process can be significantly accelerated with active reclamation strategies such as biosolids amendment and plant selection (Li et al., 2014b; Singh et al., 2024; Zornoza et al., 2015). Taxonomic profile itself however might not be a decisive parameter determining the stage of site rehabilitation as functional profile equally reflects microbial community response to the changing chemistry of the environment (Siwek et al., 2024).

In this study, taxonomic divergence was contrasted by convergence in predicted functional profiles inferred via PICRUSt2 analysis (Fig. 7B). The predicted functional composition of microbial communities in 8-year-old rehabilitated soils overlapped with that of Malham Cove reference samples, whereas year 1 and year 3 samples occupied a distinct PCoA space. This pattern suggests that as soil rehabilitation progresses, microbial communities may achieve functional profile of undisturbed soils well before taxonomic convergence is realized. However, as a single, geographically distinct, reference site was used in this study, this needs further verification.

Such functional redundancy, where taxonomically distinct communities perform similar ecological functions, has been documented in multiple systems, including late-successional plant-associated microbial communities and chemically contaminated soils (Fukami et al., 2005; Burke et al., 2011; Louca et al., 2018; Jiao et al., 2019). The similarity observed between the predicted functional profiles of Malham Cove soils and Tara TMF soils after eight years of rehabilitation implies that closely related taxa or different taxa with overlapping ecological niches may be fulfilling equivalent roles in nutrient cycling and other ecosystem functions, even if exact species-level composition remains distinct.

## 5. Conclusion

Over the course of eight years, vegetation cover was successfully established and sustained on fine grained carbonate-rich Pb/Zn mine tailings. This period saw the progressive oxidation of pyrite, which was potentially accelerated by the presence of the vegetation cover, leading to the accumulation of ferric oxyhydroxides within the tailings. The mobility of several potentially toxic elements, including Cd, Mo, Ni, Pb and Zn, was reduced following pyrite oxidation due to their increased sorption to the neoformed ferric oxyhydroxides. In contrast, Cu was more mobile in organic matter-rich surface layers due to formation of soluble complexes with DOC. Over the same period, a more diverse, soil-like microbial community was established in tailings surface layers and taxonomic diversity increased. The functional profile (functional diversity) of the community in the surface layer of the tailings converged after eight years with that of a natural calcareous soil, despite there being differences in taxonomic composition between the two sites. Overall, this study highlights the effectiveness of revegetation as a sustainable and cost-effective alternative to traditional capping methods for the closure of Pb/Zn mine TMFs.

## CRediT authorship contribution statement

**Felipe E. Sepúlveda Olea:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ian T. Burke:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Ronan Courtney:** Writing – review & editing, Investigation. **William M. Mayes:** Writing – review & editing, Investigation, Funding acquisition. **Andrew J. Weightman:** Writing – review & editing, Supervision, Conceptualization. **Gordon Webster:** Writing – review & editing, Investigation. **Franciszek Bydalek:** Writing – review & editing, Formal analysis. **Douglas I. Stewart:** Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The Authors were granted access to Tara Mines tailings management facility by Oliver Fitzsimons (Environmental Department, Boliden Tara Mines, Ireland). The authors declare that they have no other known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.181257.

## Data availability

Sequence data is available from the European Nucleotide Archive and all other data is reported on the paper and its supplementary information files

## References

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D., Esposito, G., 2014. Enhanced phytoremediation: a review of low molecular weight organic acids and surfactants used as amendments. Crit. Rev. Environ. Sci. Technol. 44, 2531–2576.

Bahr, C., Jekel, M., Amy, G., 2022. Vanadium removal from drinking water by fixed-bed adsorption on granular ferric hydroxide. AWWA Water Sci. 4, e1271.

Bao, Z., Al, T., Bain, J., Shrimpton, H.K., Finfrock, Y.Z., Ptacek, C.J., Blowes, D.W., 2022. Sphalerite weathering and controls on Zn and Cd migration in mine waste rock: an integrated study from the molecular scale to the field scale. Geochim. Cosmochim. Acta 318, 1–18.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., Mciver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.

Bradl, H.B., 2004. Adsorption of heavy metal ions on soils and soils constituents. J. Colloid Interface Sci. 277, 1–18.

Bray, A.W., Stewart, D.I., Courtney, R., Rout, S.P., Humphreys, P.N., Mayes, W.M., Burke, I.T., 2018. Sustained bauxite residue rehabilitation with gypsum and organic matter 16 years after initial treatment. Environ. Sci. Technol. 52, 152–161.

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., Thomas, T., 2011. Bacterial community assembly based on functional genes rather than species. Proc. Natl. Acad. Sci. 108, 14288–14293.

Burke, I.T., Courtney, R., Mayes, W.M., 2023. Antimony and arsenic behaviour in lead/zinc mine tailings during storage under vegetation cover. Appl. Geochem. 158, 105806.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA 108 (Suppl. 1), 4516–4522.

Chambers, J.C., Sidle, R.C., 1991. Fate of heavy metals in an abandoned lead-zinc tailings pond: I. vegetation. J. Environ. Qual. 20, 745–751.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688.

Drummond, D., Blakeman, R., Ashton, J., Farrelly, I., Cloutier, J., Yesares, L., Boyce, A., Andrew, C.J., Stanley, G., Hitzman, M.W., 2023. Ore depositional processes at the carbonate-hosted Tara deep Zn-Pb deposit, Navan, Ireland. In: Irish-Type Zn-Pb Deposits around the World. Irish Association for Economic Geology, Dublin.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590.

Fitzsimons, O., Courtney, R., 2022. Characterisation of Pb/Zn tailings and drainage waters to inform post-closure water treatment strategies. Mine Water Environ. 41, 1118–1123.

Freese, H.M., Meier-Kolthoff, J.P., Sardà Carbasse, J., Afolayan, A.O., Göker, M., 2025. TYGS and LPSN in 2025: a Global Core Biodata Resource for genome-based classification and nomenclature of prokaryotes within DSMZ Digital Diversity. Nucleic Acids Res. gkaf1110. https://doi.org/10.1093/nar/gkaf1110. (Accessed 30 September 2025).

Fukami, T., Martijn Bezemer, T., Mortimer, S.R., van der Putten, W.H., 2005. Species divergence and trait convergence in experimental plant community assembly. Ecol. Lett. 8, 1283–1290.

Gil-Loaiza, J., White, S.A., Root, R.A., Solís-Dominguez, F.A., Hammond, C.M., Chorover, J., Maier, R.M., 2016. Phytostabilization of mine tailings using compost-assisted direct planting: translating greenhouse results to the field. Sci. Total Environ. 565, 451–461.

Guo, Y., Qin, X., Guo, X., Jiang, Y., Tian, T., Zhu, F., Xue, S., 2022. Dynamic variations of soil-formation indicators in bauxite residue driven by the integration of waste solids and microorganisms. Bull. Environ. Contam. Toxicol. 109, 202–208.
Guo, Z., Wang, Y., Liu, J., He, L., Zhu, X., Zuo, Y., Wang, N., Yuan, F., Sun, Y., Zhang, L., Song, Y., Song, C., Xu, X., 2024. Mapping turnover of dissolved organic carbon in global topsoil. Sci. Total Environ. 906, 167621.
Jiao, S., Chen, W., Wei, G., 2019. Resilience and assemblage of soil microbiome in response to chemical contamination combined with plant growth. Appl. Environ. Microbiol. 85, e02523–18.
Jordan, S.N., Mullen, G.J., Courtney, R.G., 2008. Utilization of spent mushroom compost for the revegetation of lead–zinc tailings: effects on physico-chemical properties of tailings and growth of *Lolium perenne*. Bioresour. Technol. 99, 8125–8129.
Kanehisa, M., Goto, S., 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.
Ke, W., Zhang, X., Zhu, F., Wu, H., Zhang, Y., Shi, Y., Hartley, W., Xue, S., 2021. Appropriate human intervention stimulates the development of microbial communities and soil formation at a long-term weathered bauxite residue disposal area. J. Hazard. Mater. 405, 124689.
Labouyrie, M., Ballabio, C., Romero, F., Panagos, P., Jones, A., Schmid, M.W., Mikryukov, V., Dulya, O., Tedersoo, L., Bahram, M., Lugato, E., van der Heijden, M. G.A., Orgiazzi, A., 2023. Patterns in soil microbial diversity across Europe. Nat. Commun. 14, 3311.
Langmuir, D., 1997. Aqueous environmental geochemistry *prentice hall: upper Saddle River*. NJ 600.
Lanno, M., Klavins, M., Purmalis, O., Shanskiy, M., Kisand, A., Kriipsalu, M., 2022. Properties of humic substances in composts comprised of different organic source material. Agriculture 12, 1797.
Li, H., Liu, Y., Zeng, G., Zhou, L., Wang, X., Wang, Y., Wang, C., Hu, X., Xu, W., 2014a. Enhanced efficiency of cadmium removal by *Boehmeria nivea* (L.) gaud. in the presence of exogenous citric and oxalic acids. J. Environ. Sci. 26, 2508–2516.
Li, Y., Wen, H., Chen, L., Yin, T., 2014b. Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. PLoS One 9, e115024.
Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2, 936–943.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550.
Lovley, D.R., Phillips, E.J., 1986. Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Appl. Environ. Microbiol. 52, 751–757.
McDonald, D., Jiang, Y., Balaban, M., Cantrell, K., Zhu, Q., Gonzalez, A., Morton, J.T., Nicolaou, G., Parks, D.H., Karst, S.M., Albertsen, M., Hugenholtz, P., Desantis, T., Song, S.J., Bartko, A., Havulinna, A.S., Jousilahti, P., Cheng, S., Inouye, M., Niiranen, T., Jain, M., Salomaa, V., Lahti, L., Mirarab, S., Knight, R., 2024. Greengenes2 unifies microbial data in a single reference tree. Nat. Biotechnol. 42, 715–718.
Mudd, G., Boger, D.V., 2013. The ever growing case for paste and thickened tailings - towards more sustainable mine waste management. AusIMM Bulletin 56–59.
Nielsen, M.N., Winding, A., Binnerup, S., Hansen, B., 2002. Microorganisms as Indicators of Soil Health.
Oksanen, J., Kindt, R., Legendre, P., Hara, B., Henry, M., Stevens, H., 2007. The Vegan Package.
Oman, F., 2022. Boliden Summary Report Mineral Resources and Mineral Reserves, Tara Mine Boliden.
Ortiz-Álvarez, R., Fierer, N., De Los Ríos, A., Casamayor, E.O., Barberán, A., 2018. Consistent changes in the taxonomic structure and functional attributes of bacterial communities during primary succession. ISME J. 12, 1658–1667.
Othmani, M.A., Souissi, F., Benzaazoua, M., Bouzahzah, H., Bussiere, B., Mansouri, A., 2013. The geochemical behaviour of mine tailings from the Touiref Pb–Zn district in Tunisia in weathering cells leaching tests. Mine Water Environ. 32, 28–41.
Potysz, A., Grybos, M., Kierczak, J., Guibaud, G., Fondaneche, P., Lens, P.N.L., van Hullebusch, E.D., 2017. Metal mobilization from metallurgical wastes by soil organic acids. Chemosphere 178, 197–211.
Rimstidt, J.D., Vaughan, D.J., 2003. Pyrite oxidation: a state-of-the-art assessment of the reaction mechanism. Geochim. Cosmochim. Acta 67, 873–880.
Risso, D., Perraudeau, F., Gribkova, S., Dudoit, S., Vert, J.-P., 2018. A general and flexible method for signal extraction from single-cell RNA-seq data. Nat. Commun. 9, 284.
Romero, F.M., Armienta, M.A., González-Hernández, G., 2007. Solid-phase control on the mobility of potentially toxic elements in an abandoned lead/zinc mine tailings impoundment, Taxco, Mexico. Appl. Geochem. 22, 109–127.
Schmalenberger, A., O'sullivan, O., Gahan, J., Cotter, P.D., Courtney, R., 2013. Bacterial communities established in bauxite residues with different restoration histories. Environ. Sci. Technol. 47, 7110–7119.
Shi, M., Min, X., Ke, Y., Lin, Z., Yang, Z., Wang, S., Peng, N., Yan, X., Luo, S., Wu, J., Wei, Y., 2021. Recent progress in understanding the mechanism of heavy metals retention by iron (oxyhydr)oxides. Sci. Total Environ. 752, 141930.
Singh, J.P., Bottos, E.M., van Hamme, J.D., Fraser, L.H., 2024. Microbial composition and function in reclaimed mine sites along a reclamation chronosequence become increasingly similar to undisturbed reference sites. Sci. Total Environ. 920, 170996.
Siwek, P., Jaźwa, M., Niklińska, M., Klimek, B., 2024. Soil bacterial activity and functional diversity as indicators of recultivation of alkaline settlements of a 'Solvay' process. J. Soil. Sediment. 24, 2791–2802.
Stevenson, F.J., 1994. Humus Chemistry : Genesis, Composition. Wiley, reactions., New York.
Sun, Y., Luo, T., Zhong, S., Zhou, F., Zhang, Y., Ma, Y., Fu, Q., 2021. Long-term effects of low-molecular-weight organic acids on remobilization of Cd, Cr, Pb, and As in alkaline coastal wetland soil. Environmental Pollutants and Bioavailability 33, 266–277.
Thouin, H., Norini, M.-P., Battaglia-BRunet, F., Gautret, P., Crampon, M., Le Forestier, L., 2022. Temporal evolution of surface and sub-surface geochemistry and microbial communities of Pb-rich mine tailings during phytostabilization: a one-year pilot-scale study. J. Environ. Manag. 318, 115538.
Webster, G., Newberry, C.J., Fry, J.C., Weightman, A.J., 2003. Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale. J. Microbiol. Methods 55, 155–164.
Weng, L., Temminghoff, E.J.M., Lofts, S., Tipping, E., van Riemsdijk, W.H., 2002. Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. Environ. Sci. Technol. 36, 4804–4810.
Wu, J., West, L.J., Stewart, D.I., 2002. Effect of humic substances on Cu(II) solubility in kaolin-sand soil. J. Hazard. Mater. 94, 223–238.
Xiao, T., Li, P., Fei, W., Wang, J., 2024. Effects of vegetation roots on the structure and hydraulic properties of soils: a perspective review. Sci. Total Environ. 906, 167524.
Zornoza, R., Acosta, J.A., Martínez-Martínez, S., Faz, A., Bååth, E., 2015. Main factors controlling microbial community structure and function after reclamation of a tailing pond with aided phytostabilization. Geoderma 245-246, 1–10.