



Adding Agricultural Topsoil and Growing Perennial Ryegrass (*Lolium perenne* L.) Enhanced the Rehabilitation of Metal/loids Enriched Iron Ore Mine Tailings

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Abstract

Revegetating iron-ore mine tailings is difficult due to their poor physicochemical and microbial properties. This experiment aimed to improve tailings properties for better plant growth and rhizosphere activities. First, a pot experiment was conducted to assess the influence of topsoil amendment on the remediation of tailings collected from two sites (coded A and B) in Western Australia to promote the growth of perennial ryegrass (*Lolium perenne* L. cv Ausvic). Each pot was filled with pre-defined ratios of topsoil to mine tailings (0, 10, 20, 30, 50 and 100% w/w). Perennial ryegrass was harvested 60 days after sowing and analysed for plant growth, root morphological characteristics and metal uptake by shoots. Second, a study on biological attributes and an incubation study over 84 days were carried out. The addition of topsoil significantly decreased pH, but EC and total organic carbon (TOC) increased with an increased proportion of topsoil. Total concentrations of As, Co, Cr, Cu, Ni, Pb, Zn, Fe and Mn decreased with an increased ratio of topsoil in growth medium. The highest root dry biomass was observed in the treatments with 30% and 50% topsoil addition to tailings, whereas shoot dry biomass only increased with 50% topsoil. Total root length increased significantly with 50% topsoil addition to site A tailings. A significant increase in microbial biomass carbon (MBC) was found with 50% addition of topsoil compared with the control. Microbial quotient (MBC/TOC) decreased significantly with the addition of topsoil to both types of tailings. Metal contents in perennial ryegrass shoots decreased with the increasing rate of topsoil, but perennial ryegrass was an effective phytoextractor of Cu, Zn and Mn. Further, the incubation study revealed that 50% topsoil application increased cumulative respiration emissions in both A and B tailings after 42 days. Iron ore mine tailings can be improved in terms of physicochemical and biological attributes by adding topsoil. However, further investigation should be carried out to determine the speciation of metal/loids in amended iron ore tailings.

Keywords Metal · Perennial ryegrass · Phytoextraction · Tailings · Topsoil

1 Introduction

Metal-contaminated mine tailings are mostly slurries of waste material generated after processing and extracting a valuable portion of ore (Li et al. 2015). Tailings pose a substantial environmental risk, because they are deposited in open-air tailing ponds without further treatment (Benidire et

al. 2021), potentially causing contamination of surface and groundwater and eventually leading to the loss of biodiversity, amenities and economic wealth (Wong 2003).

Several remediation techniques have been developed during the past decade to solve the environmental concerns caused by mining operations (Benidire et al. 2021). Among those methods, the establishment of vegetative cover to stabilise mine tailings is considered an important, viable (Phillips and Courtney 2022), and long-term solution (Mendez and Maier 2007; Tordoff et al. 2000). It is the initial step of a progressive and final closure of ore-processing residues and tailings facilities (Tordoff et al. 2000; Ye et al. 2002). A vegetative cover maintains an improved visual environment and initiates a new ecosystem for local flora and fauna

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(Phillips and Courtney 2022). However, mine tailings are materials modified by human interventions, so they are devoid of the most ecological services inherent to natural soils, such as soil carbon sequestration and nutrient supply (Barrette et al. 2022). Moreover, the physicochemical properties of tailings negatively affect the restoration (Sun et al. 2018). Typically, the coarser parts in the tailings have poor buffering capacity, lack organic matter and nutrients, and have low water holding capacity (Sun et al. 2018). Furthermore, it is virtually impossible to build vegetation because seed germination and seedling growth are sporadic at best (Sun et al. 2018) due to poor water holding capacity and a high evaporation rate, indicating that the vegetation restoration on coarse-grained tailings is limited by water deficit (Mouazen et al. 2014). Mine tailings commonly exhibit adverse properties such as extreme pH, high concentration of metals, and a lack of organic matter (Cheng et al. 2023; Qian et al. 2023). Previous research efforts thus focused on the addition of organic amendments to compensate for these deficiencies, induce some biotic soil functions and establish the microbiome environment; in particular, biosolids, composts, wood chips, municipal and pulp and paper sludge showed promising results in mine tailings reclamation (Li and Fung 1998).

Salvaged topsoil (up to 50 cm depth) is a valuable amendment in mine tailings reclamation (Proto and Courtney 2023) to increase the speed of the ecosystem recovery and function (Barrette et al. 2022). If the topsoil amendment is available, the post-mining reclamation can be achieved faster than with other amendments (Cooke and Johnson 2002). The topsoil material, including logging residues, is a soil “living resource” inoculum with seed banks, organic matter and microorganisms (Barrette et al. 2022). It is important to note that many researchers have used topsoil as a reference (positive control) to determine the impact of amended mine tailings, or to compare the vegetation response in the topsoil to mine tailings supplemented with other amendments (Guitttonny-Larchevêque and Pednault 2016; Shrestha et al. 2009).

Most grass species produce large above- and below-ground biomass that enriches tailings in organic matter (Cooke and Johnson 2002). Further, Barrette et al. (2022) observed that microaggregates (2–20 µm) were formed by perennial ryegrass (*Lolium perenne* L.) roots associated with mineral particles due to root gel, root fragments, and microbial extra-cellular polysaccharides. Perennial ryegrass (*Lolium perenne* L.) belongs to one of the 11 dominating genera of *Poaceae*, which has the capacity to establish rapidly in substrates with high concentrations of some metals and in unfavourable conditions, which makes it a good candidate for phytoremediation (Masotla et al. 2023). For example, *Lolium perenne* L. efficiently took up Cd, and

harvesting and removing aboveground plant biomass multiple times contributed to the rehabilitation of Cd-contaminated soil (Li et al. 2023).

This study had two main objectives: first, we aimed to measure the effects of amending iron ore tailings with topsoil on selected physicochemical properties, including pH, CEC, and total elemental concentrations. Second, we aimed to identify the impact of the different topsoil tailings treatments on (i) the survival, growth, root architecture and metal uptake of perennial ryegrass and (ii) microbial activities such as microbial biomass carbon and nitrogen and microbial respiration. It was hypothesised that (1) topsoil amendment at a high ratio would have a significant impact on mine tailings’ physicochemical properties and microbial activities, and (2) perennial ryegrass would act as a phytoextractor of some metals from tailings amended with the increasing proportions of topsoil.

2 Materials and Methods

2.1 Site Description and Sample Collection

Iron ore mine tailings were collected from two mining sites (coded A and B) (22° 14' 46" S, 119° 36' 34" E and 22° 03' 53" S, 117° 53' 57" E, respectively) located in the Pilbara region, Western Australia. One part of each tailings was air-dried at 40 °C for 1 week and passed through 2 mm sieve for further physicochemical analyses. The other part of the tailings was stored at 4 °C for measuring microbial activity.

The topsoil was collected from Shenton Park field station (31°96' 09"S, 115°81'49"E) of the University of Western Australia.

2.2 Glasshouse Pot Experiment

The glasshouse pot experiment was performed with perennial ryegrass (*Lolium perenne*) that can accumulate high concentrations of metals, and has been reported as a good candidate for the revegetation of mining areas (Benidire et al. 2021; Rossini-Oliva et al. 2017). The randomised complete block design with factorial arrangements of treatments (untreated two mine tailings; 10, 20, 30 and 50% w/w topsoil-amended mine tailings; and 100% w/w topsoil (Table 1) was replicated three times. The rock mineral fertiliser (Troforte Innovations Pty Ltd, WA, Australia) was added to the growth medium in each pot at 1 g kg⁻¹. Thoroughly mixed substrates were kept under glasshouse conditions at 60% of the water holding capacity for 1 week before planting.

The *L. perenne* seeds were provided by Irwin Hunter Pty. Ltd, Western Australia. The seeds were surface sterilised in 1% v/v NaOCl for 5 min and then rinsed thoroughly

Table 1 Experimental treatments

Tailings source	Topsoil rates (%, w/w)	Acronyms
Tailings A	0%	A+0
	10%	A+10
	20%	A+20
	30%	A+30
	50%	A+50
Tailings B	0%	B+0
	10%	B+10
	20%	B+20
	30%	B+30
	50%	B+50
Topsoil	100%	Ag100

with deionised water. Seeds were placed in river sand in a tray and moistened with deionised water until the seedlings reached 3 cm in height. The sprouted seedlings were transferred to 2 L plastic pots (17 cm top diameter × 18 cm depth × 13.5 cm bottom diameter) lined with polythene cover containing 2 kg of topsoil or untreated or amended mine tailings. Pots were watered daily with deionised water to maintain moisture content at 60% of the water holding capacity until harvesting.

The experiment was carried out in a glasshouse (26/15 °C day/night temperatures, 74% humidity) at the University of Western Australia (31°59'9.6"S, 115°49'19.2"E) for 60 days.

2.3 Changes in Physicochemical Attributes of Tailings after Topsoil Application

Some physicochemical characteristics of the tailings A and B used in this study have been described by in detail elsewhere (Sarathchandra et al. 2022). Briefly, tailings A had a higher clay particle percentage with lower TOC and CEC than tailings B. By contrast, tailings A had higher pH, EC, water holding capacity and air porosity than tailings B. The topsoil was used as a source of organic matter and nutrients to improve the tailing quality and fertility. Once the amendment was applied in pre-defined ratios (0, 10, 20, 30, 50 and 100% w/w), the amended tailings were thoroughly mixed and incubated in glasshouse conditions for 1 week before analysing physicochemical properties.

The pH was measured using a pH meter (ORION 5 star series meter, Thermo Electron Corporation, Massachusetts, USA) with a glass electrode in a 1:5 substrate suspension. The electric conductivity was measured using the saturated tailings-paste extract (ORION 5 star series meter, Thermo Electron Corporation, Massachusetts, USA) (Rayment and Lyons 2010). Cation exchange capacity (CEC) was measured by mixing with silver thiourea (AgTU^+) for 24 h, and the exchangeable base concentrations were then determined

by analysing the centrifuged and filtered extracts using an inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 5300DV, Perkin Elmer, Shelton, Massachusetts, USA) (Rayment and Lyons 2010). Total organic carbon (TOC) was determined by the wet oxidation/ K_2CrO_4 titration method (Rayment and Lyons 2010). Dry combustion was used to determine the total nitrogen with an Elementar instrument (Vario MACRO; Elementar; Analysen-Systeme GmbH, Hanau, Germany). Total elemental concentrations (As, Co, Cu, Cr, Fe, Mn, Pb, Ni and Zn) of unamended and amended tailings and topsoil were determined using ICP-OES after digestion in the mixture of HNO_3 and HClO_4 (3:1 ratio) (Rayment and Lyons 2010).

2.4 Plant Growth and Analysis

After 60 days of growth, perennial ryegrass was harvested and separated into shoot and roots. Shoots were washed in distilled water and dried to constant weight at 70 °C. The dried shoots were ground and analysed for total metal (Cu, Co, Fe, Mn, Pb and Zn) and As concentrations. The 0.2 g of dried shoot material was placed in a 50 mL pyrex conical flask and digested in a mixture of acids ($\text{HNO}_3/\text{HClO}_4$; 3:1, v/v) as described by (Simmons 1978). The digests were then analysed by ICP-OES. Every digestion and analysis batch included blank samples and two standard reference materials to check the accuracy and provide quality assurance.

The separated roots were washed under running tap water and then rinsed with deionised water. Approximately 0.2–0.5 g of fresh root subsamples were stored in 50% v/v ethanol with ink until scanning on a root scanner (Epson Perfection V 800 Photo, Digital ICE Technologies, Nagano-ken, Japan). Total root length and average root diameter were determined using the commercial software package winRHIZO 2008a (Regent Instruments Canada Inc., Quebec, QC, Canada). Total root dry weight was determined by drying roots at 70 °C for 72 h.

2.5 Biological Attributes– Incubation Study

The microbial biomass carbon and nitrogen (MBC and MBN) in the substrate treatments after incubating in a sealed desiccator in the dark at 25 °C for 24 h were evaluated using the chloroform fumigation-extraction method (Lim and Choi 2014). This method measured dissolved organic carbon in the extracts with a Shimadzu TOC Analyser (TOC-5000 A, Tokyo, Japan). Microbial biomass C in the substrate (MBC) = extractable C (fumigated substrate – non fumigated substrate) / K_C , and microbial biomass N in the substrate (MBN) = extractable N (fumigated substrate – non-fumigated substrate) / K_N ; where K_C (=0.45) and K_N (=0.68) represent the efficiency of extraction of microbial

Table 2 Physicochemical properties of unamended and topsoil-amended tailings and topsoil

Tailings Source (S)	Topsoil rate (w/w %) (R)	Treatment	pH (H ₂ O)	EC (dS m ⁻¹)	CEC (cmol ₍₊₎ kg ⁻¹)
Tailings A	0%	A+0	6.65±0.00a	2.54±0.23de	2.03±0.12c
	10%	A+10	6.55±0.01b	2.53±0.03de	2.15±0.12c
	20%	A+20	6.24±0.01c	2.89±0.01d	2.28±0.12c
	30%	A+30	6.15±0.01d	3.65±0.00c	2.40±0.13bc
	50%	A+50	5.89±0.01 fg	4.84±0.00b	2.65±0.14bc
Tailings B	0%	B+0	6.11±0.01d	0.19±0.04 g	4.01±0.18a
	10%	B+10	6.01±0.02e	0.29±0.01 g	3.94±0.15a
	20%	B+20	5.94±0.02ef	0.59±0.01 fg	3.86±0.11a
	30%	B+30	5.84±0.02 g	1.07±0.03f	3.79±0.08a
	50%	B+50	5.75±0.02 h	2.28±0.03e	3.64±0.04a
Topsoil		Ag100	5.65±0.01i	48.8±0.00a	3.26±0.20ab
<i>P</i> values					
S × R			<0.0001	<0.0001	<0.0001

EC = electrical conductivity; CEC = cation exchange capacity. Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. (Means $\pm$ s.e., $n = 3$)

biomass C and N, respectively. The microbial quotient was calculated for each treatment as microbial biomass carbon (MBC) / total organic carbon (TOC) (Haynes 1999).

Microbial respiration rates were determined by placing 50 g of a substrate in a glass jar (650 mL) sealed with air-tight rubber stoppers (silicone septum, 3.3 mm VWR, Germany). After 5 days of pre-incubation at 25 °C, the accumulated CO₂ in the headspace of the glass jars was determined by an infrared CO₂ analyser (Q-S151, CO₂ Analyser, Qubitsystems, Kingston, Canada). Triplicate analyses were carried out for each treatment every second day for the first 2 weeks followed by weekly measurements until the end of the incubation period of 84 days (12 weeks). Finally, the data were processed using the Q mini CO₂ embedded software to calculate the respiration rate.

2.6 Statistical Analysis

The reported data are means $\pm$ standard error of three true replicates ($n=3$). All data were subjected to two-way ANOVA by Genstat software (19th Edition, VSN International Ltd, Rothamsted, Harpenden, UK). Two types of tailings and the amended rate of topsoil and their interaction were the tested factors. In addition, PCA was performed to assess the factor loadings of physicochemical, plant and microbial parameters.

3 Results

3.1 Physicochemical Properties of Amended and Unamended Tailings

Physicochemical properties of mine tailings changed significantly ($P < 0.001$) after the addition of different rates of topsoil (Table 2). Mine tailings showed slightly acidic pH

Table 3 Change of total organic carbon (TOC) and total nitrogen (TN) (g kg⁻¹) with application of topsoil

Topsoil rate (% w/w) (R)	TOC	TN
0	0.89±0.11f	0.19±0.02f
10	6.61±0.1e	0.38±0.01e
20	12.33±0.1d	0.58±0.01d
30	18.05±0.1c	0.78±0.02c
50	29.49±0.1b	1.17±0.03b
100	58.09±0.1a	2.14±0.06a
<i>P</i> value	<0.0001	<0.0001
R		

Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. Two-way ANOVA showed the significant main effect of topsoil rate on total organic carbon and total nitrogen, but not the interaction (tailings source $\times$ topsoil rate). For each rate, the data were averaged across two tailing sources (Means $\pm$ s.e., $n = 6$)

(H₂O) (6.65 and 6.11 in A and B, respectively) which was significantly ($P < 0.001$) reduced by the addition of the topsoil (Ag). Indeed, after 7 days of topsoil (Ag) incorporation, both substrates showed pH values similar to those found in control (non-contaminated topsoil). The incorporation of 50% topsoil amendment (A+50) and (B+50) significantly (by 91% and 110%, $P < 0.001$) increased EC of mine tailings A and B than control [(A+0) and (B+0)] respectively (Table 2).

The CEC levels (Table 2) determined in amended substrates (20%, 30%, 50%) were not significantly ($P < 0.001$) affected by topsoil addition in both tailings A and B individually. However, B+50 showed significant higher impact on CEC levels than A+50 by 31%.

The interaction effect of tailings source $\times$ topsoil rate was not significant for TOC and total N (TN). However, the main effect of topsoil rate was significant for TOC and TN (Table 3), and the main effect of tailing source was significant only for TN (Table 4). With the application of different

Table 4 Changes in total nitrogen (TN g kg⁻¹) with tailings source

Tailings source (S)	TN
Tailings A	0.9±0.15a
Tailings B	0.8±0.15b
<i>P</i> value	0.042
S	

Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. Two-way ANOVA showed the significant main effect of tailings source on total nitrogen, but not the interaction (tailings source $\times$ topsoil rate). For each rate, the data were averaged across six topsoil rates (Means $\pm$ s.e., $n = 18$)

rates of topsoil amendment, the highest TOC value was recorded at 50% topsoil addition. Compared with the non-amended tailings, the increases observed in mine tailings A supplemented with topsoil (10%, 20%, 30% and 50%) were 7-, 13-, 20- and 33-fold, respectively. Total N levels (Table 3) were low in untreated mine tailings but tended to increase by an average of about 2-, 3-, 4- and 6-fold in respectively 10%, 20%, 30% and 50% topsoil rates in both

mine tailings and significantly higher total nitrogen content was observed in tailings A than B by 11% (Table 4).

Total concentration of As, Co, Cr, Zn and Mn showed tailings source $\times$ topsoil rate interaction (Table 5). The highest concentration of As and Zn was found in unamended B (B+0) tailings, whereas Co, Cr and Mn were high in unamended A (A+0) tailings. A significantly ($P \leq 0.05$) lowest metal/loid concentrations (As, Co, Cr, and Mn) were found in 50% topsoil application in tailings A: As (by 42%), Co (by 43%), Cr (by 35%), and Mn (by 57%) than A+0, whereas in mine tailings B concentrations were reduced by 49% for As, 40% for Co, 25% for Cr, and 45% for Zn. Total metal/loid concentrations were very low in topsoil compared to both tailings (Table 5).

Total concentrations of Cu, Ni, Pb and Fe were influenced by the significant main effect of topsoil rate (Table 6), those of Cu and Ni showed the significant main effect of tailings source (Table 7), whereas interaction of tailings source $\times$ topsoil rate was not significant ($P \leq 0.05$). Total metal concentrations were highest in unamended tailings

Table 5 Total concentration of As, Co, Cr, Zn (mg kg⁻¹); Mn (g kg⁻¹) in unamended and amended tailings

Tailings Source (S)	Topsoil rate (w/w%) (R)	Treatment	As	Co	Cr	Zn	Mn
Tailings A	0%	A+0	7±2c	16±1a	26±1a	48±3de	7±0a
	10%	A+10	7±2c	14±1a	24±1a	44±2de	6±0a
	20%	A+20	6±1d	13±1b	22±1ab	41±2de	6±0b
	30%	A+30	6±1d	11±1b	20±1ab	37±2e	5±0c
	50%	A+50	4±1e	9±1c	17±1b	30±1ef	3±0d
Tailings B	0%	B+0	45±4a	10±1b	16±0c	109±6a	0.4±0e
	10%	B+10	41±4a	9±1c	15±0c	99±5ab	0.4±0e
	20%	B+20	36±3ab	9±1c	15±0c	89±5ab	0.3±0e
	30%	B+30	32±3ab	8±1d	14±d	80±4bc	0.3±0e
	50%	B+50	23±2b	6±0f	12±0e	60±3 cd	0.2±0e
Topsoil		Ag 100	1±0d	1±0 g	8±0f	12±0f	0±0f
<i>P</i> value			<0.0001	0.013	<0.0001	<0.0001	<0.0001
S $\times$ R							

Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. Two-way ANOVA showed the significant interaction effect of tailings source $\times$ topsoil rate. (Means $\pm$ s.e., $n = 3$)

Table 6 Changes in total concentrations of Cu, Ni and Pb (mg kg⁻¹) and Fe (g kg⁻¹) in tailings amended with different rates of topsoil

Topsoil rate (% w/w) (R)	Cu	Ni	Pb	Fe
0	15±1a	28±4a	22±1a	453±4a
10	14±1ab	25±3a	21±1a	410±4b
20	13±1bc	22±3ab	20±1ab	370±3c
30	11±1c	20±2bc	19±1ab	323±3d
50	9±0d	15±2b	17±1b	236±2e
100	3±0e	2±0c	12±0c	20±2.1f
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
R				

Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. Two-way ANOVA showed the significant main effect of topsoil rate on total metal concentrations, but not the interaction effect (tailings source $\times$ topsoil rate). For each rate, the data were averaged across two tailing sources (Means $\pm$ s.e., $n = 6$)

Table 7 Changes in total concentrations of Cu and Ni (mg kg⁻¹) with tailings sources

Tailings source	Cu	Ni
Tailings A	11.5±1.0a	23.1±2.8a
Tailings B	10.0±1.0b	14.0±1.5b
<i>P</i> value	0.001	<0.0001

S
Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. Two-way ANOVA showed the significant main effect of tailings source on Cu and Ni, but not the interaction (tailings source $\times$ topsoil rate). For each rate, the data were averaged across six topsoil rates (Means $\pm$ s.e., $n = 18$)

(0%) and decreased gradually with an increasing rate of topsoil amendment. The lowest concentration was in 50% topsoil application, with the metal concentration decreased by 40% for Cu, 46% for Ni and 23% for Pb and 48% for Fe. The Cu and Ni concentrations were higher in tailings A (by 14% and 49%, respectively) than in tailings B (Table 7).

3.2 Influence of Topsoil on Plant Growth in Glasshouse Conditions

The root dry biomass (Fig. 1) productivity of perennial ryegrass grown in amended B treatments showed a statistically significant increase in B+30 (by 85%) and B+50 (by 111%) treatments compared with unamended tailings (B+0). However, topsoil application at various rates to tailings A did not significantly change perennial ryegrass root dry biomass (Fig. 1).

After 60 days of growth, tailings source $\times$ topsoil rate was not significant for shoot dry biomass of perennial ryegrass. However, the main effects of tailings source and topsoil rate were significant at $P \leq 0.05$. The results revealed that the significantly highest shoot biomass reported in perennial ryegrass grown in tailings B (2-fold higher than tailings A) (Fig. 2a). Topsoil application at 50% rate showed 2-fold higher shoot dry biomass than in the 0% topsoil application (Fig. 2b).

Fig. 1 Root dry biomass of perennial ryegrass grown in mine tailings A and B amended with various proportions of topsoil (see Table 1 for treatment notations). Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD test. The interaction effect of tailings source $\times$ topsoil rate was significant at $P \leq 0.05$ (Means $\pm$ s.e., $n = 3$)

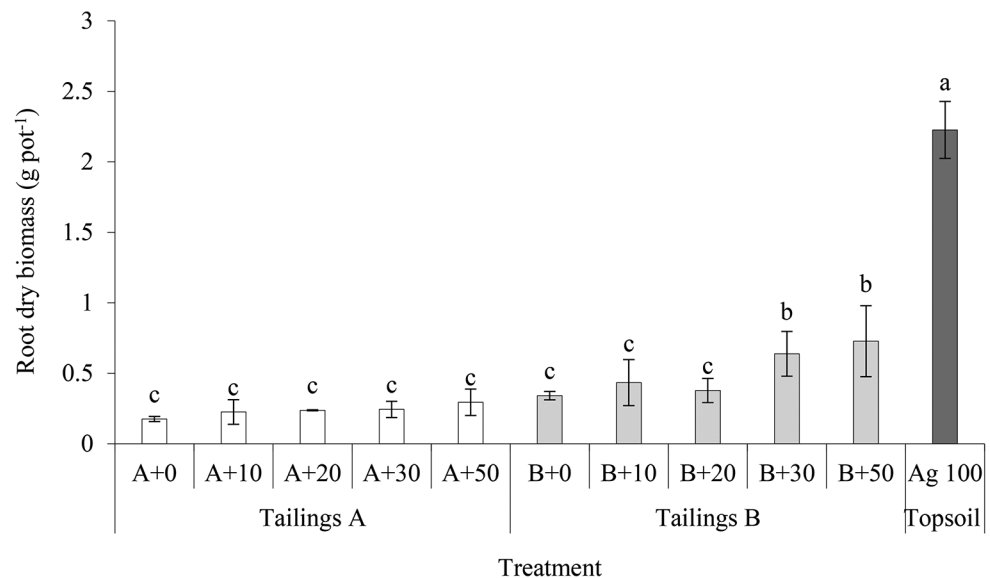
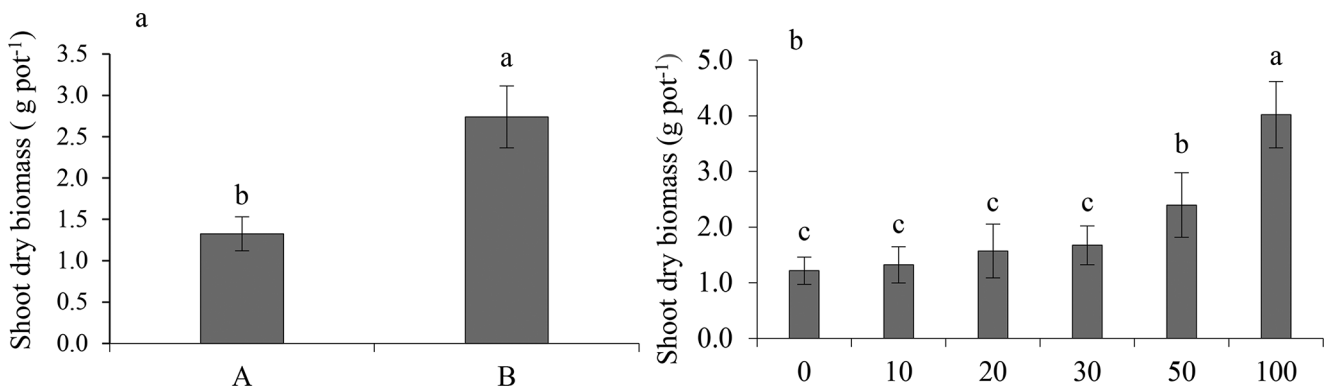


Fig. 2 The main effect of tailings source (a) and topsoil rate (b) on shoot dry biomass of perennial ryegrass. The data were averaged across the tailings source ($n = 18$) (a) and topsoil rate ($n = 6$) (b). Dif-

ferent letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD test (Means $\pm$ s.e.)

Fig. 3 Effect of topsoil addition on total root length of perennial ryegrass grown in mine tailings A and B (see Table 1 for treatment notations). Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD test. The tailings source $\times$ topsoil rate interaction was significant at $P \leq 0.05$ (Means $\pm$ s.e., $n = 3$)

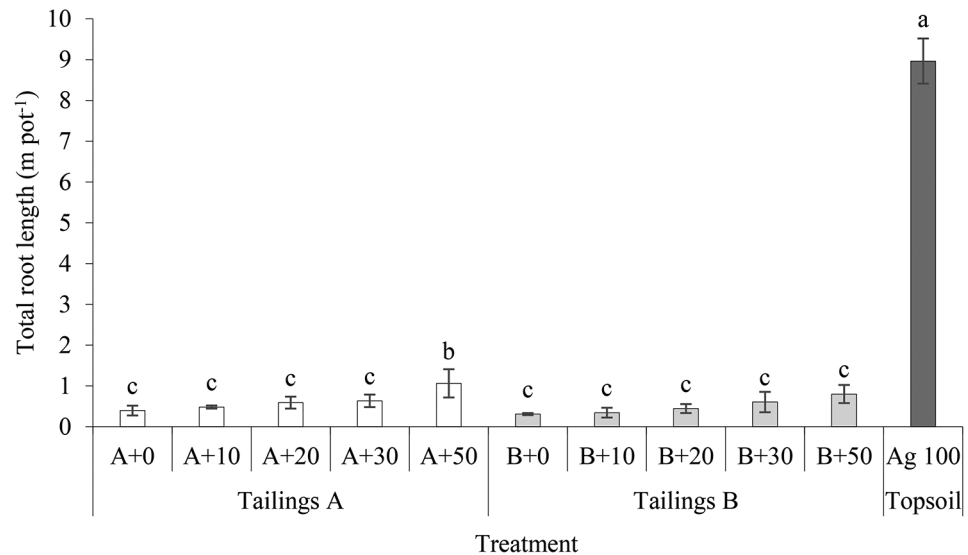
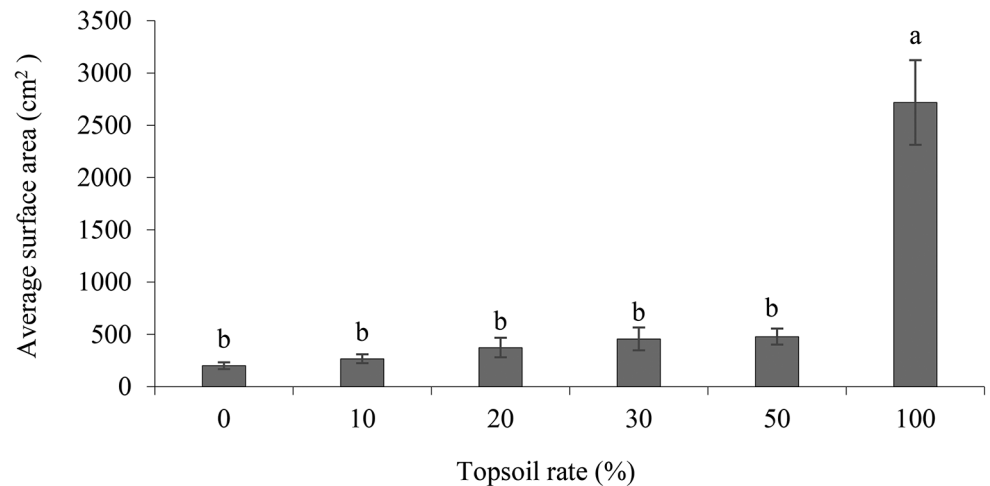


Fig. 4 The main effect of topsoil rate on average root surface area of perennial ryegrass. The data were averaged across the tailings source ($n=6$) because tailings source $\times$ rate interaction was insignificant. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD test (Means $\pm$ s.e., $n = 6$)



The total root length (Fig. 3) of perennial ryegrass was influenced by the tailings source $\times$ topsoil rate interaction at $P \leq 0.05$. The total root length in the A+50 treatment was significantly (by 3-fold) higher than in the A+0. By contrast, the treatments with mine tailings B did not differ significantly. However, the highest total root length (8.96 m pot⁻¹) was observed in topsoil and was 30-fold higher than in both unamended mine tailings (A+0 and B+0).

The average root surface area (Fig. 4) did not show a significant interaction effect of tailings source $\times$ topsoil rate ($P \leq 0.05$), but the main effect of topsoil rate was significantly higher with 100% topsoil application rate to 0% treatment by 13-fold whereas, root surface area was not significantly affected between 0%, 10%, 20%, 30% and 50% topsoil application rates (Fig. 4).

3.3 Metal Uptake by Perennial Ryegrass Grown in Amended Tailings

The main effect of topsoil rate on metal accumulation in shoots of perennial ryegrass is shown in Table 8 because the interaction tailings source $\times$ topsoil rate was not significant. The initial measurable decrease in metal content (Cu, Zn, Fe, Mn) in perennial ryegrass shoots was observed at 10% topsoil application rate (by 67%, 50%, 62% and 44%, respectively). However, the shoot Mn content was higher (by 11%) at 50% topsoil application than in unamended tailings. The lowest metal uptake occurred in 30% topsoil application rate; compared to unamended tailings, the decreases were 67% for Cu, 50% for Zn, 88% for Fe, 44% for Mn, and 100% for Cr and Ni. The extraction [(substrate metal concentration — plant metal uptake)/substrate metal concentration] of Cu, Fe, Mn, Zn, Cr and Ni increased by 0.3%, 2.4%, 41%, 0.2%, 0.1% and 0.1%, respectively, at 50% topsoil application compared with control 0% topsoil

Table 8 Effect of topsoil rate on metals Cu, Pb, Zn, Cr, Ni (mg kg⁻¹) and Fe, Mn (g kg⁻¹) uptake in perennial ryegrass shoots

Topsoil rate (w/w%) (R)	Cu	Pb	Zn	Cr	Ni	Fe	Mn
0	0.03±0a	0.0±0a	0.2±0b	0.01±0a	0.01±0a	30.7±11a	0.9±0a
10	0.01±0c	<D.L	0.1±0b	0.02±0a	0.01±0a	11.7±5ab	0.5±0b
20	0.02±0b	<D.L	0.1±0a	0.01±0b	0.01±0a	13.5±9ab	0.7±0a
30	0.01±0c	<D.L	0.1±0b	0.00±0b	0.00±0b	3.6±1b	0.5±0b
50	0.03±0a	<D.L	0.2±0a	0.01±0a	0.01±0a	10.1±2ab	1.0±0a
100	0.02±0b	<D.L	0.2±0a	0.01±0b	0.00±0b	1.9±1b	1.0±0a
P value	0.04	0.03	0.05	0.05	0.05	0.05	0.05
R							

D.L= detection limit

Different letters in the same column indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test. Two-way ANOVA showed the significant main effect of topsoil rate. For each rate, the data were averaged across six topsoil rates (Means $\pm$ s.e., $n = 18$)

application. Further, with topsoil application shoot As and Pb uptake was below the detection limit (Table 8).

3.4 Determination of Microbial Biomass Carbon/Nitrogen and Microbial Quotient

Microbial biomass carbon and nitrogen showed the significant ($P \leq 0.05$) main effects of topsoil rate (Fig. 5a) and tailings source (Fig. 5b) but not the interaction effect of tailings source $\times$ topsoil rate. Generally, MBC mean values were significantly higher in the amended treatments (20%, 30% and 50%) (Fig. 5a) than in the control. Significantly, the highest MBC value (529% higher than the control) was reported at 50% topsoil application rate. The MBN showed a similar trend of changing with topsoil application as MBC (576% increase with 50% topsoil rate compared with the non-amended tailings) (Fig. 5a). Both microbial biomass carbon and nitrogen were 16% and 11% significantly higher in mine tailings B than mine tailings A respectively (Fig. 5b). Hence, interaction of tailings source $\times$ topsoil rate was not significant in microbial quotient and highest was shown in unamended tailings (Fig. 5c) resulting a significant reduction by 81% on average with the topsoil rates (10, 20, 30 and 50% w/w) than control. However, in this study tailings B showed a significantly higher q mic value (39%) than tailings A (Fig. 5d).

3.5 Incubation Studies

The cumulative CO₂ released over 84 days was highest at 100% topsoil rate (Fig. 6a), and a slow increasing trend was observed in the periods 0–14 days and 14–42 days. The cumulative respiration increased around 90% from 42 days to 70 days in 100% topsoil application (Fig. 6a). Cumulative respiration of the unamended tailings was shown to be very low. In contrast, a significant increase in cumulative CO₂ evolution was observed in amended tailings (Fig. 6b)

than control. The rate of increase was considerably greater in the period 42–56 days compared to the preceding 0–42 days period. The treatments B+30 and B+50 showed the highest increase rate of all treatments in the period of 42–56 days. Further, as it is shown in Fig. 6, C-CO₂ tends to approach a steady state rate after 70 days in all treatments, including control.

3.6 Principal Component Analysis

The first two principal component axes explained 74.9% of the total data variance (Fig. 7). From the PCA, six main clusters were identified based on natural groupings in the F2 versus F1 plot (Fig. 7). The clusters A+0, A+10, A+20 and A+30 were plotted with positive F1 and negative F2. The clustering of Ag100 showed negative F1 and F2 scores, interlinking with MBC, MBN, TOC, TC, root length and root dry weight. However, clusters B+30, B+20 and A+50 were characterised by neutral F1 and F2, whereas average root diameter and surface area corresponded with that. It is important to note that B-0 and B+10 clusters showed a strong positive relationship with both F1 and F2 axes. Moreover, CEC, shoot dry weight and shoot content of metals such as Zn, Mn, Cu, Cr and Ni were positively related to F2 and negatively associated with F1, whereas shoot content of Fe and Pb showed near-neutral scores with both F1 and F2 axis (Fig. 8).

4 Discussion

The lowest values of pH in the 50% topsoil treatment indicated it is the effective rate for stabilising pH of iron ore mine tailings. It is known that a decreased pH enhances the mobility and availability of most metals (Cele and Maboeta 2016), such as Pb, Zn and Cu (Tordoff et al. 2000), which indirectly enhances the extraction of metal/oids by

Fig. 5 Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in mine tailings A and B amended with topsoil. Main effect of topsoil rate on MBC and MBN (a); Main effect of tailings source on MBC and MBN (b); Main effect of topsoil rate on microbial quotient ($q_{mic} = \log_{10}$ of MBC/TOC) (c); Main effect of tailings source on microbial quotient (d). Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD test. The data were averaged across the tailings source (a and c) ($n = 6$) and topsoil rates (b and d) ($n = 18$) because the interaction (tailings source $\times$ topsoil rate) was not significant (Means $\pm$ s.e.). For treatment notations, see Table 1

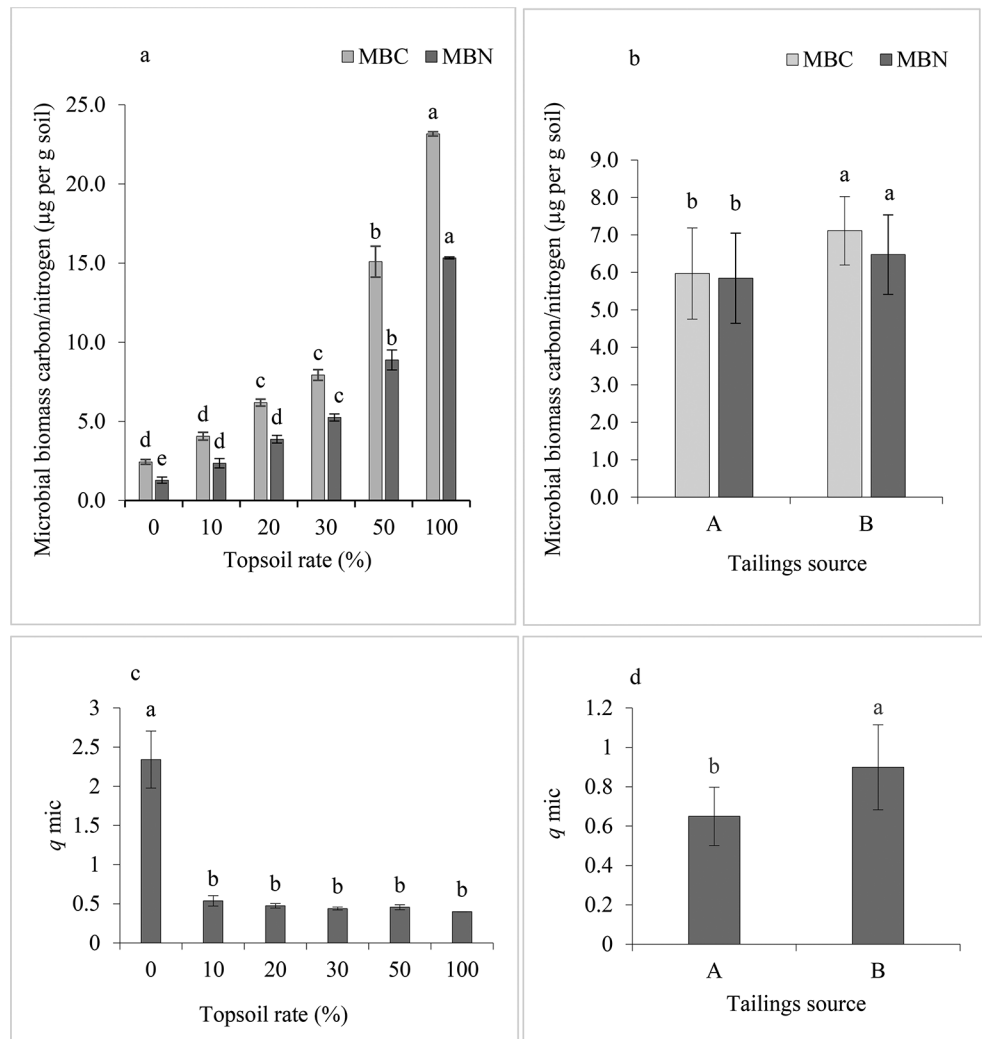
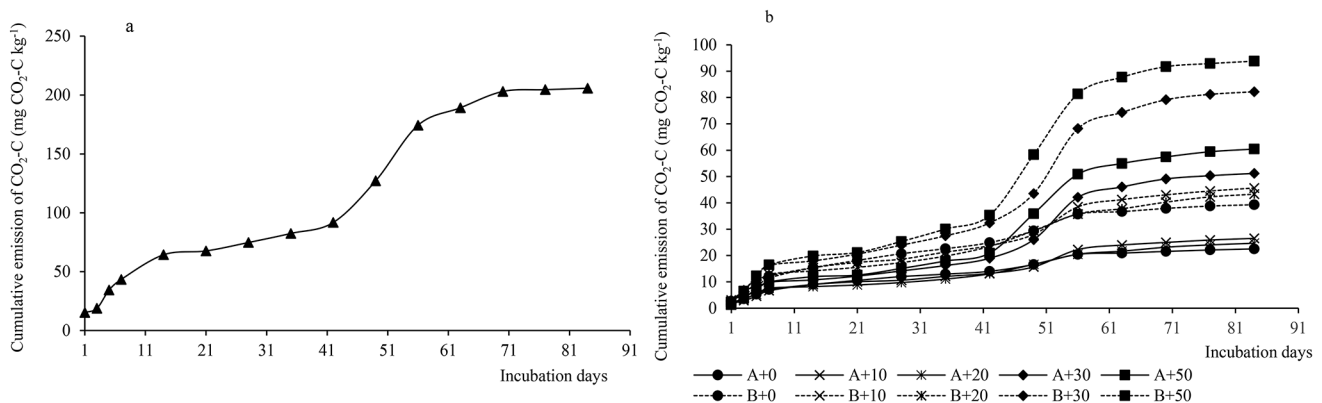


Fig. 6 Cumulative CO_2 emission in topsoil only (a) unamended and amended mine tailings A and B with various proportions of topsoil (b) (see Table 1 for treatment notations). Tukey's HSD test shows that tailings source $\times$ topsoil rate interaction is significant at each point ($P \leq 0.05$)

perennial ryegrass. According to Meeinkuirt et al. (2012), 0.2 dS m^{-1} is the standard level of acceptable salinity for plant growth. In contrast, soils with electrical conductivity values greater than 4 dS m^{-1} are considered saline (Cele and

Maboeta 2016). In the present study, the addition of topsoil (50%) resulted in a significant increase in EC compared with the unamended tailings, which can be ascribed to the presence of soluble salts in the topsoil (Cele and Maboeta 2016).

Fig. 7 Results of principal component analysis. Clustering of treatments considering tailings type and topsoil rate with measured variables. Each treatment is composed of three replicates

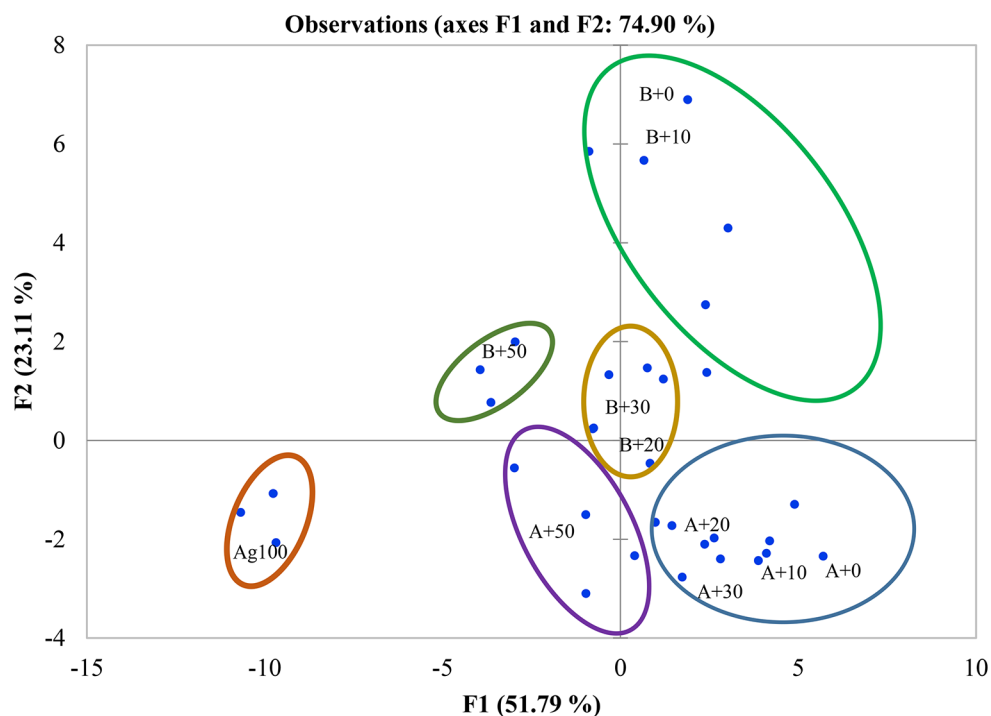
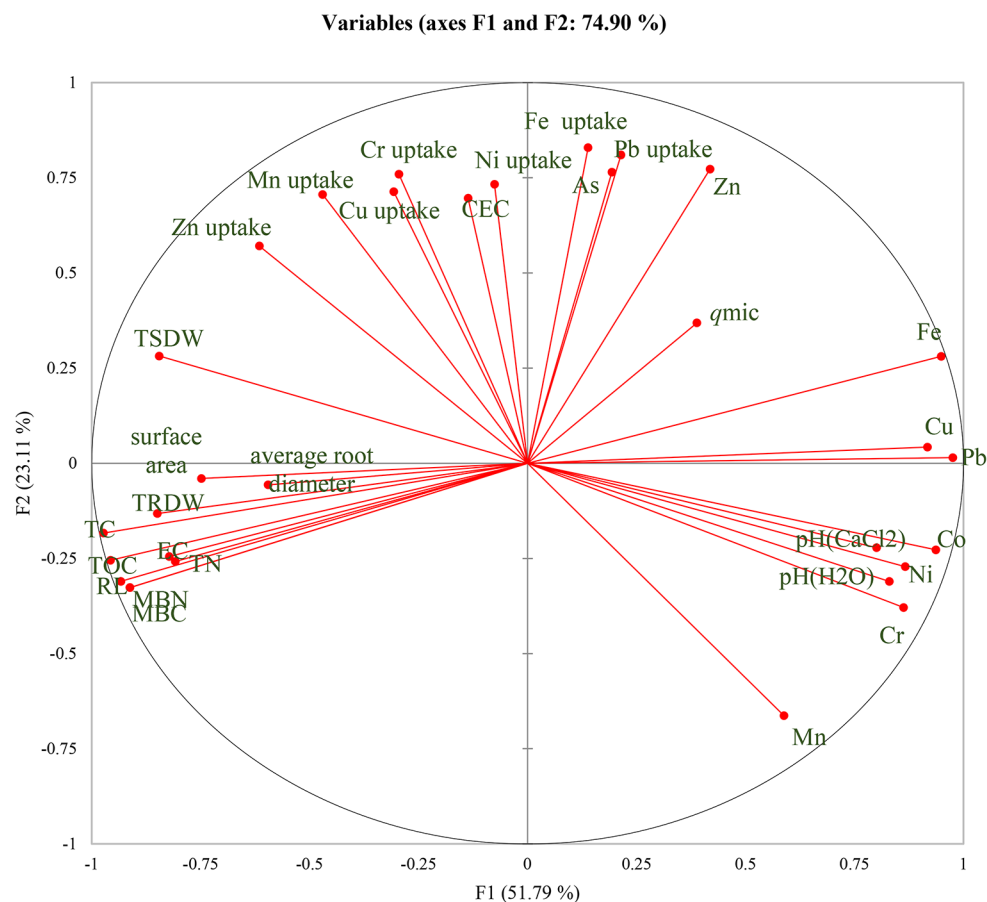


Fig. 8 Principal component analysis of root architecture parameters (average root diameter, root surface area, total root dry weight, total root length), physicochemical substrate properties (CEC, EC, TOC, pH CaCl₂, pH H₂O, TC and TC), total metal concentrations (Cu, Cr, Fe, Pb, Ni, Mn and Zn), microbial parameters (MBC, MBN and q_{mic}) and plant metal accumulation (Cu, Cr, Fe, Pb, Ni, Mn and Zn)



The diluted concentrations of metal/loids in substrates can be attributed to low concentrations found in topsoils. Notably, studied metals exhibited the highest concentrations in unamended tailings. Further, topsoil addition may improve the nutrient status and physicochemical properties of the mine tailings and provide soil microorganisms (Burke 2003; Cele and Maboeta 2016; Rate et al. 2004; Rivera et al. 2014; Sydnor and Redente 2002) that contribute to the mine tailings reclamation. Additionally, the application of topsoil adds some organic matter that could enhance soil quality and provides beneficial soil organisms to increase microbial activity and respiration in tailings (Rivera et al. 2014). Further, a fine tailings texture and no organic matter may lead to high compaction that can be altered with topsoil application (Bulot et al. 2017). According to Santini and Banning (2016), mine site restoration may be achievable if disturbances are carefully managed and minimised by adding topsoil. Still, it could be technically challenging, expensive, and prone to failure, which has prompted reconsideration of approaches to and alternatives for restoring mine sites. Further, the authors could not identify rehabilitation performance indices or criteria to monitor and evaluate iron ore mine tailings, despite the studies that were carried out to understand the properties and behaviour of tailings and related strategies of establishing vegetation cover via organic amendment (Asensio et al. 2013; Di Carlo et al. 2019). However, further to Di Carlo et al. (2019) mentioned that based on standard soil quality criteria guidelines, ‘rehabilitation goals’ for several key physicochemical properties such as pH, EC, bulk density and CEC are already being established for bauxite residues, in order to support the development of ecosystems on closed-down disposal sites (Gräfe and Klauber 2011).

In this study, microbial biomass carbon significantly increased with an increasing rate of topsoil, suggesting that microorganisms started proliferating with access to readily available organic substrates (Moreno-Barriga et al. 2017). Topsoil may act as a microbial inoculant by providing C sources, improving soil structure and drainage, and providing a viable microbial community to perform vital soil ecosystem functions such as nutrient cycling (Santini et al. 2015). Addition of topsoil containing native microbial communities may not only ameliorate the tailings’ physicochemical conditions but also speed up the shift toward soil-like microbial communities (Li et al. 2015). Further, the topsoil added available nutrients and distinct microorganisms into tailings, stimulating the microbial biomass and significantly boosting microbial activities in the topsoil-amended tailings (20%, 30% and 50%). The percentage of TOC present as MBC (microbial quotient) is considered a more sensitive indicator of soil fertility than either MBC or TOC individually (Laudicina et al. 2011). In the present study, microbial

quotient was relatively high in the non-amended tailings and declined in the treatments with topsoil amendment. The increase in available organic C in the tailings amended with increasing proportions of topsoil was matched by the increased MBC; consequently, microbial quotient remained unaltered over a broad range of topsoil additions.

Microbial respiration is commonly used to quantify metabolic activity (Chodak et al. 2009; Mingorance et al. 2017). The cumulative respiration reflects the metabolic activity of the microbial biomass in degrading organic substances (Marabottini et al. 2013). In the present study, comparatively, topsoil had higher cumulative respiration than non-amended and amended tailings and the cumulative respiration of unamended tailings was relatively low, totalling 22.5 mg C kg⁻¹ and 39.3 mg C kg⁻¹ over the entire incubation period of 84 days in tailings A and B, respectively due to low organic matter content in tailings limiting the energy and food sources for the existing microbial populations. However, following amendments of the tailings with topsoil (adding organic matter), microbes gradually started to proliferate and enhance their metabolic activities, which was particularly pronounced after around 42 days. In the period 42–56 days after incubation started, there was an increasing trend of cumulative respiration, indicating elevated microbial abundance and stimulated microbial activity that would correspond to increased organic matter decomposition and time-dependent nutrient release proportional to the rate of organic amendment (Yazdanpanah et al. 2016). The presence of metals in the tailings might have significantly reduced the capacity of bacteria to decompose organic substrates, decreasing the amount of CO₂ respired (Rost et al. 2001). Still, dilution of metal concentration due to increasing rates of topsoil addition resulted in a proportional increase in microbial respiration. After 70 days, respiration reached a steady state in all treatments, suggesting that the incubation period chosen was appropriate with the selected conditions.

In this study, the highest metal uptake by perennial ryegrass was shown at 50% topsoil rate, with extraction ranging between 1 and 41% for different metals. By contrast, As and Pb concentrations in shoots were below the detection limit. A possible explanation for Pb is that it can form insoluble complexes with organic matter in the amended substrate that decrease its mobility (Weng et al. 2002); in addition, plant physiological characteristics (e.g. root cation exchange capacity) may restrict transport of Pb from roots to shoots. The As could be present in the tailings as arsenite, and generally arsenite is more toxic than arsenate because the former reacts with sulfhydryl groups in enzymes and tissue proteins in roots. This may lead to inhibition of cellular function and failure to transport arsenic into shoots (Rahman et al. 2016). Further, the cluster analysis suggests that

the application of topsoil improves physicochemical characteristics such as pH, CEC, TOC and reduces the impedance of metal uptake by plant shoots.

5 Conclusion

The physicochemical and biological attributes of iron ore tailings can be improved by applying topsoil. In this study, 50% topsoil application resulted in the highest perennial ryegrass growth and the highest phytoextraction capacity of Cu, Fe, Mn, Pb, Zn, Cr and Ni. Shoot concentrations of As and Pb were below the detection limit, suggesting that these elements could be stabilised by applying organic matter-rich topsoil or that the physiological properties of roots may inhibit the transport to shoots.

Applying a low rate of soil amendment is likely to be an economically feasible scenario. Initially, such a low amendment rate may not be completely effective in rehabilitating iron ore tailings (even when enhanced by planting perennial ryegrass), but over time a compounding effect of natural phenomena may contribute to improved structure and other properties of tailings. These suggestions should be tested in long-term field experiments with topsoil amendment and perennial ryegrass on tailings.

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Declarations

Conflict of Interest The authors declare no competing interests.

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