



Metal uptake from iron ore mine tailings by perennial ryegrass (*Lolium perenne* L.) is higher after wheat straw than wheat straw biochar amendment

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Abstract

Background and aims Mine tailings often contain high concentrations of metals that may lead to serious environmental concerns. Phytoremediation by suitable plant species, coupled with organic amendments, represents an intriguing way of improving the quality of mine tailings.

Methodology We conducted a glasshouse pot study to assess the impact of biochar derived from wheat straw (BC) and wheat straw (WS) at different application rates (w/w: 0%, 1%, 2%, 5%, 10%) in enhancing physicochemical and biological attributes of iron ore mine tailings. Perennial ryegrass (*Lolium perenne* L.) growth and uptake of metals (Co, Cu, Fe, Mn, Zn, Cr, Ni, Pb) and As were assessed.

Results The pH_(H2O) of iron mine tailings (6.62 with 0% amendment) significantly increased to 8.01 in BC 10% but decreased to 5.33 in WS 10%. However, electrical conductivity, total carbon, total nitrogen and cation exchange capacity increased significantly with application of either amendment. Improvement in physicochemical parameters of tailings by amending with WS was associated with significantly greater plant growth compared with BC amendment. Shoot

As and Pb accumulation by perennial ryegrass was low (below detection limit), whereas that of Co, Cu, Fe, Mn, Zn, Cr and Ni increased with an increase in both BC and WS application. Microbial biomass carbon and respiration were enhanced more in the WS than in BC treatments.

Conclusion This study suggests wheat straw is a better amendment than biochar for the iron ore mine tailings.

Keywords Biochar · Iron ore · Mine tailings · Metals · Perennial ryegrass · Wheat straw

Introduction

Mining activities frequently contaminate the surroundings due to mine tailings deposition in open piles (Visconti et al. 2020). The high levels of potentially toxic elements, such as As, Cd, Cu and Zn resulting from mining activities may persist in the environment for decades or centuries, threatening public and ecosystem health (Boechat et al. 2016; Cui et al. 2020; Lajayer et al. 2017). Iron ore mining is one of the most significant activities in the resource sector in Western Australia (WA), and it accounts for about 47% of the total value of all resources in the state (Kuranchie et al. 2016). Based on current production levels, it can be estimated that about 632 million tonnes of iron ore mine tailings are produced yearly in WA (Kuranchie et al. 2015). Therefore, iron

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ore tailings have an increasingly significant impact on global landscapes, including both legacy (abandoned) mines and the current tailings-producing operations around the world in major mining regions such as Australia (Cross et al. 2019). In addition, the iron ore tailings often pose a significant management problem due to the volumes generated and the presence of pollutants, mainly metals Fe, Mn, Cr, Cd, Pb, Zn, Ni, Cu, etc. (Banerjee et al. 2016). Further, physicochemical limitations to plant growth on mine tailings include extreme pH, high salt content, lack of nutrients, metal toxicities, high bulk density, lack of structure, slow water infiltration, poor water retention, and low air permeability (Lee et al. 2014). Hence, a lack of vegetation cover is frequent at mining sites (Benidire et al. 2021; Benidire et al. 2022), emphasising the importance of developing sustainable strategies for pollution control and amelioration of physicochemical conditions in mine tailings. The use of alternatives to expensive and time-consuming in situ remediation techniques, such as the application of organic and/or mineral amendments, is gaining momentum (Nejad et al. 2018) because these amendments enhance the chemical and biological processes in the tailings that lead to providing suitable conditions for a stable vegetative cover.

Biochar is a porous carbonaceous material derived from biomass combustion under high temperatures and low oxygen conditions (Rodríguez-Vila et al. 2015). It exhibits a range of physicochemical properties depending on feedstock, pyrolysis conditions, and modification techniques such as activation, magnetic modification, and acid/base treatments (Palansooriya et al. 2019). As an amendment, biochar has shown potential in metal immobilisation and improved soil fertility and carbon sequestration (Yin et al. 2016). In comparison with a wide range of low-cost sorbents, biochar generally exhibits a slightly acidic to highly alkaline pH (5.9–12.3) and relatively high cation exchange capacity ($14\text{--}44\text{ cmol}_{(+)}\text{ kg}^{-1}$) (Yin et al. 2016). Biochars lead to efficient immobilisation of metal cations mainly via metal adsorption and surface complexation (Atkinson et al. 2010; Gaskin et al. 2007). In addition, the application of biochar to soil can alter organic matter decomposition, which is linked to the release of nutrients such as nitrogen (N) (Solaiman et al. 2012).

Organic farms widely use straw incorporation to improve soil fertility (Zhang et al. 2018). Organic

substrates with high C:N ratios, such as wheat straw (C:N 50–100) promote microbial N immobilisation in the short-term (Garnier et al. 2003; Zhang et al. 2018). However, over long-term, the application of straw can significantly increase soil active organic carbon pools, macroaggregates, porosity and water content (Liu et al. 2014; Zhang et al. 2018). The changes in soil properties after applications of straw can be beneficial to soil microbes both long- and short-term (Flieûbach and MaËder 2000; Garnier et al. 2003); accordingly, higher soil microbial biomass and activity are frequently observed after straw amendment. As Ibrahim and Goh (2007) reported, wheat straw addition initiated microbial activity in mine tailings and increased macroaggregate formation. Fresh wheat straw contains a high percentage of readily available carbon and is readily used by soil microorganisms; during straw decomposition by microorganisms, extracellular polysaccharides are deposited in soil, which leads to aggregate formation (Ibrahim and Goh 2007). However, how the interaction between straw and indigenous soil microorganisms contributes to degrading the recalcitrant components of wheat straw is not well understood. Similarly, the contributions of straw and soil microorganisms to the immobilisation of trace metals in soil remains unclear. There is also a research gap regarding the performance of biologically treated straw (supplemented with a complex mixture of microbial agents used to improve efficiency of biodegradation) to remediate soil and water contaminated with trace metals (Muhammad et al. 2021).

Phytoremediation is one of the best eco-friendly management practices to vegetate the tailings areas. Phytoremediation uses plants and their associated soil microorganisms, soil amendments, and agronomic techniques to remove environmental contaminants or render them harmless (Wang et al. 2012). This can serve the purpose of both stabilising mine tailings dumps and providing a pleasing landscape (Banerjee et al. 2016). Therefore, selecting appropriate plant species for phytoremediation and successful site reclamation is critical (Banerjee et al. 2016). The plants selected for phytoextraction should develop an extensive root system and a large amount of aboveground biomass with high concentrations of metals, whereas in phytostabilisation the translocation of metals from roots to shoots should be as low as possible (Alvarenga et al. 2008). The most effective approaches

are to identify plants that are (i) easy to harvest, (ii) have certain heavy metal tolerance, fast growth, large biomass, and strong adaptability and (iii) can absorb and remove heavy metals from the soil (Zhang et al. 2019).

Several authors have reported perennial ryegrass (*Lolium perenne* L.) as a suitable species for revegetation of metalliferous wastes (Alvarenga et al. 2008; Arienzo et al. 2004; Pitchel and Salt 1998), producing high dry matter yields and accumulating moderate to high levels of metals in its shoot biomass (Alvarenga et al. 2008; Zhou et al. 2023). However, there are still knowledge gaps on how wheat straw and biochar derived from straw influence growth of perennial ryegrass and metal accumulation in remediation of iron ore mine tailings. Therefore, this work was aimed at assessing how amendments (wheat straw and biochar derived from wheat straw) influence (i) germination and early growth of perennial ryegrass, (ii) physicochemical and microbial attributes of iron ore tailings, and (iii) plant growth and metal/loid uptake.

Materials and methods

Tailings

Tailings samples were collected from a mining site in Pilbara, Western Australia as stated in Sarathchandra et al. (2022). The tailings were air-dried at 40 °C for approximately 1 week, crushed and sieved (2 mm). Tailings had sandy loam properties [particle size distribution: 20–2000 µm (65%), 2–20 µm (20%) and <0.002–2 µm (15%)]. The chemical properties were: pH_(H₂O) 6.62, electrical conductivity 0.16 dS m⁻¹ and cation exchange capacity (CEC) 5.5 cmol₍₊₎ kg⁻¹.

Physicochemical analysis of the biochar, wheat straw and tailings

Biochar (BC) was produced from wheat straw by pyrolysis at 550 °C (Pacific Pyrolysis Pty Ltd., NSW, Australia). Before starting the glasshouse experiment, mine tailings were mixed with river sand (1:1) to reduce the clay proportion of the tailings, followed by addition of biochar and wheat straw into the tailings/sand mixture at pre-determined rates at 1%, 2%,

5% and 10% (w/w). All the substrates were air-dried, crushed to pass through a 2-mm sieve, homogenised thoroughly, and stored for substrate analysis. Substrate pH and EC were measured (ORION 5 star series meter, Thermo Electron Corporation, Massachusetts, USA) in water at 1:10 substrate/deionised water ratio after shaking at 25 °C for 24 h. Total carbon (Pitchel and Salt 1998) and total nitrogen (Courtney and Mullen 2008) were measured using an elemental analyser (Vario MACRO; Elementar; Analysen-Systeme GmbH, Hanau, Germany). Cation exchange capacity (CEC) was measured by the 0.01 M Ag-thiourea method (Rayment and Lyons 2011). Total trace metal/metalloid concentrations (Co, Cd, Cu, Pb, Fe, Mn, Ni, Zn, and As) in the unamended and amended tailings were measured by acid digestion using a mixture of HNO₃ and HClO₄ (3:1 v/v) and determination by inductively coupled plasma - optical emission spectrometry (ICP-OES) (Optima 5300DV, PerkinElmer, Shelton, Connecticut, USA).

Germination assay and phytotoxicity assessment

In brief, 10 g of substrate from each unamended and mixed treatment was mixed with deionised water (ratio of 1:10 w/v), shaken on a rotary shaker at 25 °C in the dark for 17 h, centrifuged at 1211 g for 10 minutes, and filtered through a 0.45 µm Fitropur filter. Toxicity was assessed using germination of perennial ryegrass (*Lolium perenne* L.) seeds (Irwin and Hunter Pty. Ltd., Belmont, WA) were surface-sterilised using 1% v/v HClO₄ for 5 minutes and then immersed in deionised water with bubbling air for 2–3 h. The seeds were laid in sterile Petri dishes (9 cm diameter) lined with a No. 42 Whatman filter paper; then, 10 mL of treatment extract was added. Ten millilitres of deionised water served as the control. Germination tests included three replicates (each with 10 seeds) for each of the nine treatments (Table 3). Germination was recorded 1, 3, 5 and 7 days after placement of seeds, based on the emergence of radicle (at least 1–2 mm long) and plumule (first visible appearance of the plumule from the coleoptile). The filter paper was moistened with 5 mL of the corresponding extract every second day to maintain sufficient hydration of the filter paper. Phytotoxicity of various treatments was also assessed using the longest leaf length and the longest root length.

Glasshouse experimental setup and plant growth

The treatment substrates were fertilised with a basal dose (1 g kg⁻¹) of rock mineral fertiliser (N 10%, P 7%, K 4.50%, Fe 2.70% and Mn 0.43% w/w) provided by Troforte Innovations Pty Ltd., WA, Australia. The treatment substrate (2 kg) was added to each pot (17 cm top diameter × 18 cm depth × 13.5 cm bottom diameter) lined with a polythene bag. All pots were irrigated with deionised water to 60% of the field water holding capacity and allowed to equilibrate for about one week.

The pot experiment was set up in a randomised complete block design (RCBD) in triplicate. The experiment was conducted in a temperature-controlled glasshouse at the University of Western Australia (31° 59' 9.6" S, 115° 49' 19.2" E). The average temperature was 26/15 °C (day/night) during the growth period, with 74% relative humidity.

Perennial ryegrass (*Lolium perenne* L.) was chosen as an indicator plant to evaluate the effect of biochar and wheat straw on metal phytoextraction. Perennial ryegrass seeds were surface-sterilised in a solution of NaOCl (1% v/v) for 5 min, rinsed with deionised water 3–4 times, and soaked overnight in deionised water with air bubbling. Preliminary studies showed that perennial ryegrass could not survive in the tailings with direct seeding. Therefore, perennial ryegrass seeds were germinated in river sand until the seedlings reached a shoot length of up to 3 cm and were then transplanted into pots (five seedlings per pot). Watering was done daily with deionised water to maintain 60% of the water holding capacity. The treatments lasted for 60 days after transplanting (i.e., mid-November 2020 to mid-January 2021).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured on three random leaves per experimental unit for every replicate using a portable efficiency analyser (Hansatech Instruments, Pentney, UK) in the morning on the harvest day. The leaves were dark-adapted (~45 min) before a fluorescence response was induced by a 3 s flash of 650 nm light at an intensity of 1500 μmol m⁻² s⁻¹, provided by an array of three light-emitting diodes covering a 4 mm diameter circle on the leaf surface. The actual quantum yield of photosynthetic system II (PSII) was determined with

the parameter $F_V/F_M = ((F_M - F_0)/F_M)$, where F_V is the variable fluorescence yield, F_M is the maximal fluorescence yield, and F_0 is the minimal fluorescence yield. The maximum quantum yield and variation in F_0 (related to the chlorophyll *a* content of the light-harvesting complex) were evaluated to quantify plant viability and PSII efficiency (Lorenz et al. 2022).

Plant analysis

After 60 days of treatments, perennial ryegrass shoots were harvested by cutting off at the base of individual plants. For plant analyses, the shoots were rinsed thoroughly with deionised water and were placed in a 70 °C oven for 2–3 days until they reached a constant weight. The dried shoot samples were then finely ground (<100 μm) in a stainless steel grinder, and plant samples weighing 0.2 g were digested in a mixture of HNO₃ and HClO₄ according to the method described elsewhere (Ma et al. 2010) to determine metal/metalloid concentrations (As, Cd, Cr, Co, Cu, Ni, Fe, Mn, Pb, Zn) using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 5300DV, Perkin Elmer, Shelton, Massachusetts, USA).

The roots in each pot were first gently separated from the bulk substrate, and then rhizosphere substrate was carefully collected from the root samples using a paint brush. Rhizosphere substrates were stored at 4 °C for microbial biomass carbon analysis. The root samples were washed under running tap water to remove any attached substrate particles. The roots were subsampled for (1) root biometric analyses such as total root length, surface area and average diameter by root scanning (WinRhizo Pro Vision 5.0 software (Regent Instruments Inc., Quebec, Canada) and (2) to assess mycorrhizal colonisation using the gridline intersection method (Abbott and Robson 1981). Root dry biomass was determined by weighing the dry (70 °C for 48 h) roots (combining scanned sub-sample, sub-sample used for mycorrhizal assessment and the remaining roots).

Microbial biomass carbon determination

Microbial biomass carbon (MBC) was measured by the chloroform fumigation-extraction method. Briefly, the moist sub-samples (10 g dry weight) were fumigated for 24 h with alcohol-free CHCl₃ and were

then extracted with 0.5 M K_2SO_4 (pH 8.5). Non-fumigated sub-samples (10 g dry weight) were also extracted (Hargreaves et al. 2003; Vance et al. 1987). The soil extracts were passed through the syringe filters (0.45 μm , Filtropur S) and were determined by an automated TOC Analyser (Shimadzu, TOC-500, Tokyo, Japan). All the results are expressed on an oven-dry soil basis (105 °C, 24 h) and are mean of three replicates of each treatment analysis.

Incubation of tailings/treatment substrates

Microbial CO_2 respiration

The unamended tailings and treatment substrates were pre-incubated at 25 °C for 7 days by maintaining 45% of water holding capacity before starting the incubation trial. At the end of 7-day pre-incubation, 50 g of substrate was placed in a mason jar and incubated at 25 °C for 105 days (15 weeks). The jars were placed in an incubator room, and constant soil moisture content was maintained by weighing each sample twice weekly and adjusting the water content to the target mass. Air samples from the headspace of the sealed mason jars were drawn through septa by a syringe, and CO_2 concentrations were measured using an infrared CO_2 gas analyser (Q-S151, CO_2 Analyser, Qubit systems, Kingston, Canada). Headspace CO_2 of each jar was measured every second day until 14 days and once a week until the end of incubation. After each sampling, the jars were opened for 30 s and were then sealed again. The mean values of the rate of CO_2 emission (expressed as the amount of CO_2 -C per gram of substrate per hour) after 15 weeks of incubation were taken to represent the basal respiration (BR) because the rate of soil CO_2 emission reached a constant value at that time (data not shown). The metabolic quotient (qCO_2) was determined as the ratio between BR and MBC (Merlo et al. 2022). There were three replicate jars for each treatment substrate as well as the control (unamended tailings) plus three blanks.

Statistical analysis

Statistical analyses were performed using software packages Genstat (19th Edition, VSN International Ltd., Rothamsted, Harpenden, UK). The experimental treatments were factorised into organic amendment

types (biochar and wheat straw) and amendment rate (0%, 1%, 2%, 5% and 10%). Data were treated for normal distribution, and log transformation was conducted if the data were not normally distributed. A two-way analysis of variance was followed by multiple comparisons using Tukey's HSD test. A significance level of $P \leq 0.05$ was used throughout the study.

Results

General properties of tailings and treatment substrates

Changes to tailings physicochemical characteristics with biochar and wheat straw treatments after 7 days of equilibration are stated in Table 1. Soil $pH_{(H_2O)}$ increased in tailings with biochar additions (Table 1), but significant differences were only detected at the BC 5% and BC 10% addition levels. By contrast, a significant decrease in $pH_{(H_2O)}$ was noted with WS 5% and WS 10% (the interaction amendment type $\times$ rate was significant at $P < 0.0001$). Similar patterns were observed in $pH_{(CaCl_2)}$. Electrical conductivity (EC) of the tailings increased significantly with increasing rates of biochar and wheat straw additions, but the increase was greater in case of wheat straw than biochar (the interaction amendment type $\times$ rate was significant at $P = 0.008$) (Table 1). There was an interaction effect ($P = < 0.0001$) of amendment type and rate regarding total carbon content. Total carbon content in unamended tailings was 200 times and 150 times lower than in biochar and wheat straw (Table 1). Total carbon content in the treatment mixtures increased with the increasing rate of both amendments. The highest carbon content was observed in 10% BC and 10% WS. Similar relationships were noted for total nitrogen content in the treatment mixtures (Table 1).

Untreated tailings had relatively low cation exchange capacity (CEC), and adding amendments resulted in a significant interaction ($P < 0.0001$). Biochar-amended treatments had higher CEC values than the equivalent rates of wheat straw-amended tailings, with the difference between the two amendment types increasing with the increasing rate of addition (Table 1). There was no significant interaction effect (amendment type $\times$ rate) observed for C:N ratio, however, significant main effects of amendment type

Table 1 Physicochemical attributes of unamended and amended mine tailings, biochar and wheat straw after 7 days of equilibration (just before the experiment commenced)

Amendment	Treatments	pH _(H₂O)	P pH _(CaCl₂)	EC (dS m ⁻¹)	TC (g kg ⁻¹)	TN (g kg ⁻¹)	CEC (cmol(+) kg ⁻¹)
Control (unamended)		6.62 ± 0.02 cd	6.27 ± 0.02c	0.16 ± 0.00e	2.31 ± 0.01 k	0.20 ± 0.01e	5.54 ± 0.03 fg
Biochar	BC (100%)	8.43 ± 0.02a	7.59 ± 0.03a	3.79 ± 0.03a	567 ± 0.94a	20.83 ± 0.77a	30.63 ± 0.05a
Wheat straw	WS (100%)	5.09 ± 0.07e	4.89 ± 0.02f	4.39 ± 0.12a	392 ± 0.59b	15.17 ± 0.69b	10.30 ± 0.02b
Biochar amended	BC 1%	6.61 ± 0.07 cd	6.43 ± 0.03c	0.31 ± 0.02de	7.96 ± 0.01i	0.40 ± 0.02de	5.79 ± 0.03 fg
	BC 2%	6.80 ± 0.22c	6.65 ± 0.02b	0.48 ± 0.02c	13.6 ± 0.01 g	0.61 ± 0.08de	6.05 ± 0.03e
	BC 5%	7.47 ± 0.09b	6.73 ± 0.04b	0.77 ± 0.01c	30.9 ± 0.30e	1.23 ± 0.05cde	6.80 ± 0.03d
	BC 10%	8.01 ± 0.12a	7.15 ± 0.08a	1.49 ± 0.11b	58.8 ± 0.08c	2.26 ± 0.09c	8.05 ± 0.03c
Wheat straw amended	WS 1%	6.47 ± 0.22 cd	6.05 ± 0.06d	0.48 ± 0.05d	6.20 ± 0.02j	0.35 ± 0.02e	5.59 ± 0.03 h
	WS 2%	6.14 ± 0.05d	5.89 ± 0.03d	0.57 ± 0.02 cd	10.1 ± 0.02 h	0.50 ± 0.03de	5.64 ± 0.03gh
	WS 5%	5.39 ± 0.05e	5.40 ± 0.03e	1.35 ± 0.10b	21.8 ± 0.04f	0.95 ± 0.05de	5.78 ± 0.03f
	WS 10%	5.33 ± 0.04e	5.31 ± 0.05e	2.06 ± 0.15a	41.0 ± 0.06d	1.69 ± 0.08 cd	6.02 ± 0.03e
<i>P</i> values		<0.0001	<0.0001	0.008	<0.0001	<0.0001	<0.0001
<i>A</i> × <i>R</i>							

A Amendment, *CEC* cation exchange capacity, *EC* electrical conductivity, *TC* total carbon, *TN* total nitrogen, *R* rate (in % w/w). Means ± s.e. (*n* = 3). For each parameter, means followed by different letters are significantly different according to Tukey's HSD (*P* ≤ 0.05)

(*P* = <0.0001) and rate (*P* = 0.012) were observed (data not shown).

Total metal concentration in unamended and biochar- and wheat straw-amended tailings

Total metal concentration of Cu, Mn, Pb and Zn was influenced by the significant interaction amendment type × rate (*P* < 0.001), whereas total concentration of As, Co, Fe, Cr and Ni was influenced only by amendment rate (Table 2). A significant decrease in Cu, Mn, Pb and Zn concentrations was observed in the WS 10% treatment compared with unamended tailings.

Seed germination and phytotoxicity assay

Germination percentage showed a significant interaction effect of amendment type × rate at all three intervals due to generally greater germination in the biochar- than wheat straw-amended treatments (Table 3). Five and 7 days after the commencement of seed imbibition, there was a significant decline in percent germination with an increase in the rate of WS (but not BC) treatments.

Seedling growth (leaf and root lengths) showed a significant interaction effect (*P* ≤ 0.05), except for longest leaf length 7 days after imbibition, in which

case only the main effects (amendment and rate) were significant (Table 3). The length of the longest root showed no significant difference among the biochar rates on all measurement days, whereas in wheat straw-amended treatments, a significant smaller length was recorded in the 10% treatment on all measurement days.

Chlorophyll fluorescence

Neither interaction (*P* ≤ 0.05) nor the main effects (amendment type and rate) were significant regarding Fv/fm (data not shown).

Changes of plant dry biomass with amendments

Perennial ryegrass shoot growth was influenced a significant interaction (amendment type × amendment rate), with greater shoot growth produced in the WS than BC treatment, but only when applied at the rates ≥ 2% (w/w) (Fig. 1). Perennial ryegrass grown on WS 5% and WS 10% had biomass more than 8 times greater than the non-amended control.

Root dry biomass was not influenced by a significant interaction (amendment type × rate), but the main effect of amendment rate was significant. All amendment rates yielded more root biomass than the

Table 2 Total metal(loid) concentrations (mg kg⁻¹) in unamended tailings, and biochar- and wheat straw-amended tailings after 7 days of equilibration (just before the experiment commenced)

Treatment	As	Co	Cu	Fe	Mn	Pb	Zn	Cr	Ni
Control (unamended)	45 ± 4.1a	10 ± 0.8a	14 ± 0.2b	456,832 ± 5587a	389 ± 5a	21 ± 0.8a	109 ± 6a	16 ± 0.3a	21 ± 1.2a
BC (100%)	0.4 ± 0.0b	0.6 ± 0.0b	16 ± 0.1a	2450 ± 402b	242 ± 1b	0.7 ± 0.2c	57 ± 5c	11 ± 0.6a	7 ± 0.3b
WS (100%)	0.4 ± 0.0b	0.3 ± 0.0b	4 ± 0.1d	342 ± 8c	80 ± 1c	0.3 ± 0.0c	13 ± 0d	11 ± 0.4a	5 ± 0.3b
BC 1%	44 ± 4.0a	10 ± 0.8a	14 ± 0.2b	452,289 ± 5533a	387 ± 5a	21 ± 0.8a	108 ± 6a	16 ± 0.3a	20 ± 1.2a
BC 2%	44 ± 4.0a	10 ± 0.8a	14 ± 0.2b	447,745 ± 5480a	386 ± 5a	21 ± 0.8a	108 ± 5a	16 ± 0.3a	20 ± 1.2a
BC 5%	43 ± 3.9a	10 ± 0.8a	14 ± 0.3b	434,113 ± 5319a	382 ± 5a	20 ± 0.8a	106 ± 5a	16 ± 0.3a	20 ± 1.1a
BC 10%	40 ± 3.7a	9 ± 0.8a	14 ± 0.4b	411,394 ± 5051a	374 ± 5a	19 ± 0.7b	104 ± 5a	16 ± 0.3a	19 ± 1.1a
WS 1%	44 ± 4.0a	10 ± 0.8a	14 ± 0.2b	452,267 ± 5531a	386 ± 5a	21 ± 0.8a	108 ± 6a	16 ± 0.3a	20 ± 1.2a
WS 2%	44 ± 4.0a	10 ± 0.8a	13 ± 0.1b	447,703 ± 5475a	383 ± 5a	21 ± 0.8a	107 ± 6a	16 ± 0.3a	20 ± 1.2a
WS 5%	49 ± 4.4a	10 ± 0.8a	13 ± 0.1b	434,008 ± 5308a	374 ± 5a	20 ± 0.8a	104 ± 5a	16 ± 0.3a	20 ± 1.2a
WS 10%	40 ± 1.0a	9 ± 0.8a	13 ± 0.1c	411,183 ± 5029a	358 ± 5a	19 ± 0.7b	99 ± 5b	16 ± 0.3a	19 ± 1.1a
<i>P</i> values	0.973	0.995	<0.001	0.998	<0.001	<0.001	<0.001	0.874	0.619
<i>A</i> × <i>R</i>									
<i>A</i>	0.678	0.736		0.779				0.476	0.314
<i>R</i>	<0.001	<0.001		<0.001				<0.001	<0.001

A Amendment, *R* rate (in % w/w). Means ± s.e. (*n* = 3). For each parameter, means followed by different letters are significantly different according to Tukey's HSD (*P* ≤ 0.05)

Table 3 Germination percentage (G%), longest root length (cm) and shoot length (cm) of perennial ryegrass in iron ore tailings (unamended control and tailings amended with biochar and wheat straw) at different time intervals after commencement of imbibition

Treatment	After 3 days			After 5 days			After 7 days		
	G%	LRL	LLL	G%	LRL	LLL	G%	LRL	LLL
unamended tailings	77 ± 7b	2.5 ± 0.2a	1.1 ± 0.1abc	80 ± 0a	5.6 ± 0.5a	3.3 ± 0.4cde	83 ± 3a	6.5 ± 0.1a	6.1 ± 0.2ab
BC 1%	70 ± 5b	2.7 ± 0.2a	1.2 ± 0.1ab	83 ± 5a	5.2 ± 0.2a	3.9 ± 0.3abcd	97 ± 3a	6.7 ± 0.2a	5.9 ± 0.3ab
BC 2%	87 ± 5a	2.8 ± 0.1a	1.2 ± 0.1abc	93 ± 3a	5.1 ± 0.3a	4.2 ± 0.1abcd	97 ± 3a	6.7 ± 0.3a	6.1 ± 0.2ab
BC 5%	70 ± 0b	2.7 ± 0.1a	1.5 ± 0.3a	87 ± 3a	4.7 ± 0.2a	4.6 ± 0.2a	87 ± 3a	6.7 ± 0.5a	6.0 ± 0.1ab
BC 10%	57 ± 0c	2.9 ± 0.2a	1.0 ± 0.1bcd	80 ± 3a	5.8 ± 0.1a	4.1 ± 0.1abc	80 ± 4a	6.5 ± 0.5a	6.5 ± 0.1a
WS 1%	60 ± 5c	2.4 ± 0.6a	1.3 ± 0.2abc	80 ± 8a	5.0 ± 0.2a	3.5 ± 0.3cde	83 ± 7a	5.9 ± 0.4a	5.5 ± 0.2ab
WS 2%	67 ± 3c	2.1 ± 0.2a	0.9 ± 0.4bcd	80 ± 0a	5.0 ± 0.2a	3.4 ± 0.1bcde	83 ± 3a	5.9 ± 0.1a	5.6 ± 0.2ab
WS 5%	53 ± 3c	2.1 ± 0.2a	0.7 ± 0.1 cd	67 ± 7c	4.9 ± 0.2a	3.4 ± 0.1bcde	70 ± 5b	5.4 ± 0.5a	5.1 ± 0.3 b
WS 10%	47 ± 7d	1.0 ± 0.2b	0.6 ± 0.2d	53 ± 3c	2.3 ± 0.3b	2.8 ± 0.3e	57 ± 7c	3.0 ± 0.6b	5.0 ± 0.5 b
DI water	60 ± 8c	2.2 ± 0.3a	0.6 ± 0.1d	70 ± 5b	5.7 ± 0.3a	3.0 ± 0.3de	80 ± 0a	6.6 ± 0.5a	5.5 ± 0.5ab
<i>P</i> values	0.001	0.023	0.004	0.009	0.000	0.014	0.005	0.002	0.228
<i>A</i> × <i>R</i>									
<i>A</i>									0.000
<i>R</i>									0.000

A Amendment, *LRL* longest root length, *LLL* longest leaf length, *R* rate (in % w/w). Means ± s.e. (*n* = 3). For each parameter, means followed by different letters are significantly different according to Tukey's HSD (*P* ≤ 0.05)

non-amended control (*P* ≤ 0.05) (Fig. 2). Regarding the R:S ratio, neither interaction (amendment type × rate) nor the main effects were significant (data not shown).

Metal accumulation by perennial ryegrass shoots

Shoot concentration of specific metals (Co, Cu, Fe, Mn, Zn, Cr and Ni), the interaction (amendment

Fig. 1 Effects of application of wheat straw biochar (BC) and wheat straw (WS) at different rates on the shoot dry biomass of perennial ryegrass grown for 60 days in iron ore tailings. Means $\pm$ s.e. ($n=3$). Different letters indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test

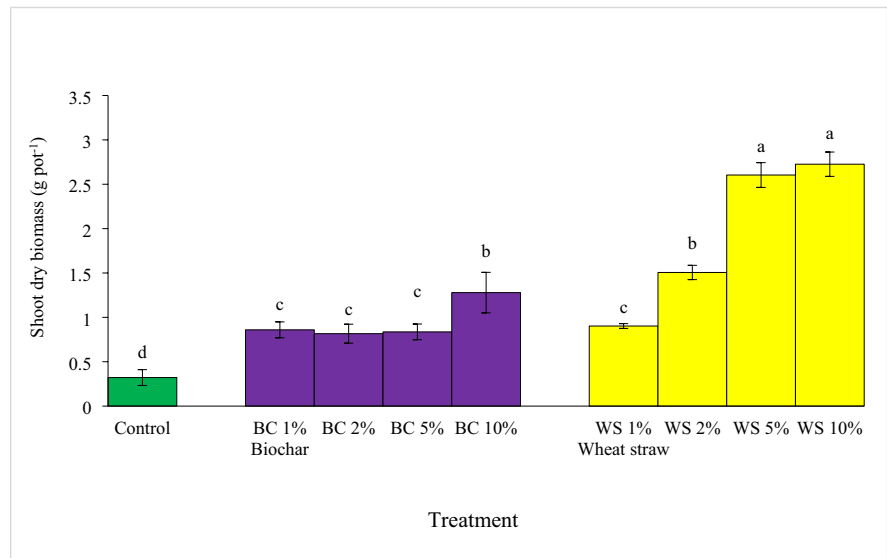
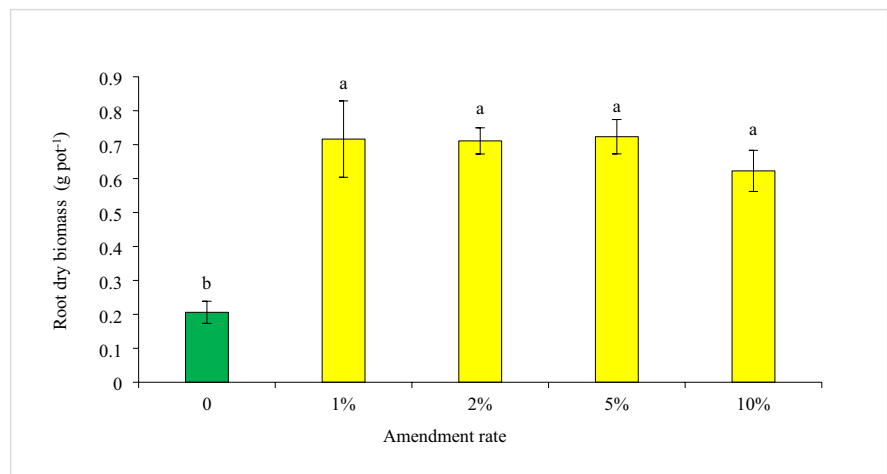


Fig. 2 The main effects of amendment rate (% w/w) on perennial ryegrass root dry biomass grown in iron ore tailings. The data were averaged across amendment type (hence, $n=6$) because the interaction amendment type $\times$ amendment rate was not significant. Means $\pm$ s.e. Different letters indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test



type $\times$ amendment rate) was significant in all cases except Mn (Table 4). For Mn, the treatment WS 10% resulted in higher shoot concentration than WS 1% and all BC treatments. The shoot concentrations of Co, Cu, Fe, Zn, Cr and Ni were higher in the WS 5% and WS 10% than in the other treatments (Table 4). Shoot concentrations of Co, Cu, Fe and Mn in the WS 10% treatment were 6- to 8-fold greater than the non-amended control. Interestingly, all the metals (except Fe) in shoots of perennial ryegrass did not differ between the non-amended control and all the BC treatments; for Fe, shoot concentration in the BC 10% was 22% higher than in the non-amended control (Table 4). The As and Pb accumulation in shoots

was considerably decreased by the application of both biochar and wheat straw amendments, falling below the detection limit ($<0.4 \text{ mg kg}^{-1}$).

Changes in root architecture and mycorrhizal colonisation with biochar and wheat straw amendments

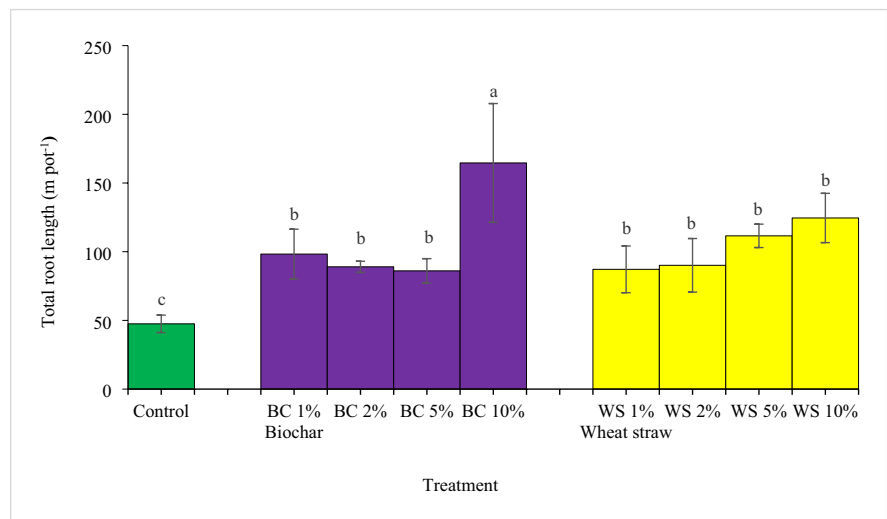
Amendments (biochar and wheat straw) and rates (0, 1, 2, 5, 10% w/w) had a significant interaction effect on *L. perenne* total root length (Fig. 3). All amendment treatments resulted in greater total root length than the non-amended control, with the longest total root length in the BC 10% treatment.

Table 4 Effect of biochar and wheat straw on metal uptake (mg g^{-1}) in perennial ryegrass shoots grown in iron ore mine tailings

Treatment	Co	Cu	Fe	Mn	Zn	Cr	Ni
Control	$0.4 \pm 0.1\text{bc}$	$2.7 \pm 0.7\text{c}$	$245 \pm 69\text{c}$	$98 \pm 30\text{c}$	$30 \pm 8\text{c}$	$5.4 \pm 1.3\text{b}$	$2.9 \pm 0.7\text{b}$
BC 1%	$0.4 \pm 0.1\text{bc}$	$3.9 \pm 0.6\text{bc}$	$318 \pm 65\text{b}$	$151 \pm 13\text{bc}$	$52 \pm 8\text{bc}$	$3.5 \pm 0.5\text{b}$	$2.0 \pm 0.2\text{b}$
BC 2%	$0.1 \pm 0.0\text{c}$	$2.4 \pm 0.4\text{c}$	$202 \pm 30\text{c}$	$76 \pm 5\text{c}$	$33 \pm 3\text{c}$	$0.7 \pm 0.2\text{b}$	$1.1 \pm 0.2\text{b}$
BC 5%	$0.2 \pm 0.0\text{c}$	$3.3 \pm 0.6\text{c}$	$255 \pm 47\text{c}$	$115 \pm 18\text{c}$	$38 \pm 4\text{c}$	$1.7 \pm 0.1\text{b}$	$1.5 \pm 0.0\text{b}$
BC 10%	$0.6 \pm 0.1\text{bc}$	$5.4 \pm 0.5\text{bc}$	$312 \pm 56\text{b}$	$229 \pm 50\text{bc}$	$69 \pm 12\text{bc}$	$4.0 \pm 1.3\text{b}$	$2.3 \pm 0.4\text{b}$
WS 1%	$0.5 \pm 0.1\text{bc}$	$4.6 \pm 1.0\text{bc}$	$338 \pm 23\text{b}$	$168 \pm 17\text{bc}$	$61 \pm 7\text{bc}$	$2.4 \pm 0.7\text{b}$	$1.8 \pm 0.2\text{b}$
WS 2%	$1.0 \pm 0.2\text{b}$	$8.4 \pm 1.0\text{b}$	$631 \pm 117\text{b}$	$302 \pm 17\text{abc}$	$102 \pm 9\text{b}$	$4.2 \pm 0.2\text{b}$	$4.6 \pm 0.3\text{b}$
WS 5%	$2.0 \pm 0.3\text{a}$	$19 \pm 2.2\text{a}$	$1601 \pm 299\text{a}$	$545 \pm 68\text{ab}$	$197 \pm 15\text{a}$	$27 \pm 4\text{a}$	$15 \pm 2\text{a}$
WS 10%	$2.2 \pm 0.3\text{a}$	$21 \pm 2.2\text{a}$	$1619 \pm 165\text{a}$	$730 \pm 182\text{a}$	$253 \pm 14\text{a}$	$37 \pm 9\text{a}$	$21 \pm 4\text{a}$
<i>P</i> values	0.005	0.001	0.001	0.058	<0.0001	0.002	0.001
A x R							
A				0.000			
R				0.010			

A Amendment, R rate (in % w/w). Means $\pm$ s.e. ($n=3$). For each parameter, means followed different letters are significantly different according to Tukey's HSD ($P \leq 0.05$)

Fig. 3 Effects of application of wheat straw biochar (BC) and wheat straw (WS) at different rates on the total root length of perennial ryegrass grown in iron ore tailings. Means $\pm$ s.e. ($n=3$). Different letters indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test



The mycorrhizal colonisation of perennial ryegrass roots was influenced by neither significant interaction (amendment type $\times$ rate) nor the main effects (data not shown).

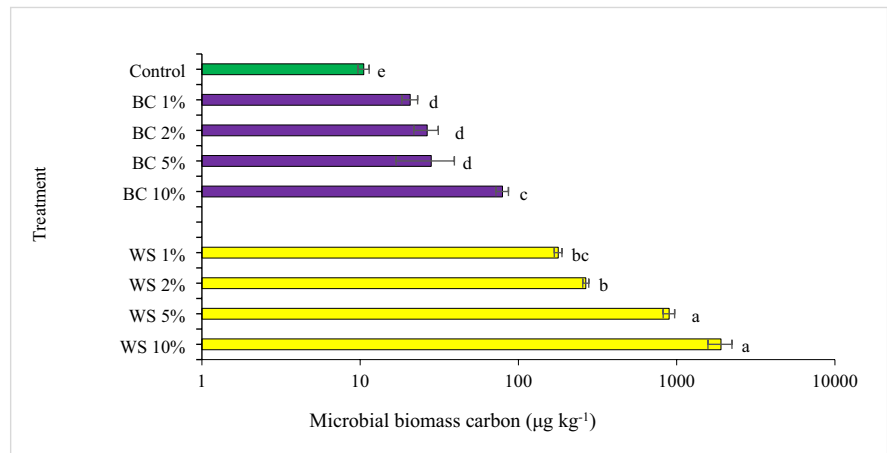
Microbial biomass carbon (MBC) and metabolic quotient ($q\text{CO}_2$) changed with the addition of biochar and wheat straw

The MBC in the tailings increased with the incorporation of organic amendments (Fig. 4), with a

significant interaction of amendment type and rate ($P \leq 0.05$). Wheat straw was more effective in promoting MBC than biochar, with WS 1% showing the same result as BC 10%, and WS 5% and WS 10% resulting in greater MBC than in any other treatment. Compared with the control, BC at 1%, 2%, 5% and 10% increased MBC by 13, 14, 14 and 19 times compared to the non-amended control.

The metabolic quotient values ($q\text{CO}_2$) were not significantly influenced by the interaction or the main effects (data not shown).

Fig. 4 Effect of application of wheat straw biochar (BC) and wheat straw (WS) at different rates on microbial biomass carbon in rhizosphere tailings at the harvest of perennial ryegrass after 60-day growth. Means $\pm$ s.e. ($n=3$). Different letters indicate significant differences at $P \leq 0.05$ according to the Tukey's HSD test



CO₂ respiration as influenced by biochar and wheat straw amendments

The cumulative CO₂ emissions during the 105-day incubation period in control, BC 1%, BC 2%, BC 5% and BC 10% treatments were estimated to increase proportionately with biochar ratio (Fig. 5a). Cumulative CO₂ emissions in the BC 10%, BC 5% and BC 2% treatments were significantly higher than that in control by 3-, 2- and 2.3-times respectively (both $P \leq 0.05$); the BC 1% treatment did not show higher emission than the control (non-amended tailings).

The addition of WS resulted in a significant increase in the cumulative CO₂ emissions compared with the non-amended tailings (Fig. 5b). Significantly ($P \leq 0.05$) higher CO₂ emissions were observed in WS 10%, WS 5%, WS 2% and WS 1% (by 93-, 48-, 13- and 8-fold) than in control (Fig. 5b).

Discussion

The effect of biochar and wheat straw on the physicochemical properties

The increase in soil pH following biochar addition has been described previously (Nelissen et al. 2012; Reverchon et al. 2015; Van Zwieten et al. 2009) and also occurred in this study (up to 1 unit under the highest rate of biochar (BC 10%)) due to higher pH of biochar (8.43) than tailings (6.62) (Table 1). Soil pH is a critical factor controlling revegetation success (Córdova et al. 2011). Therefore, biochar could

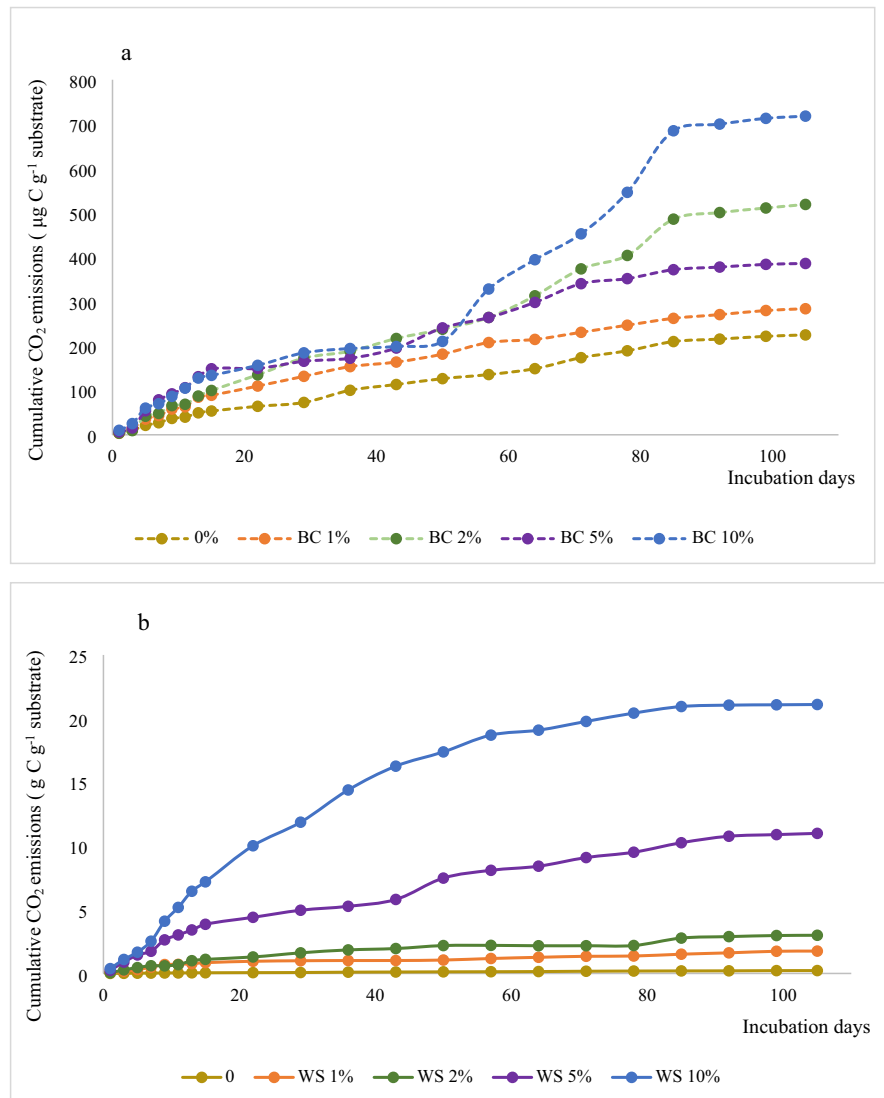
be used as a liming amendment that might alleviate soil acidity by direct and indirect effects and enhance the physicochemical and biological soil properties by increasing availability of essential nutrients for plant uptake and immobilising toxic pollutants (Bolan et al. 2023); however, systematic studies are still needed to test the liming potential of biochars. In contrast to biochar, wheat straw amendment decreased the pH of amended tailings (Table 1).

Substrate EC increased in proportion to the biochar application rates (Table 1), but only BC 2%, 5% and 10% had significantly higher EC than the control tailings. The increase in EC can be attributed to a high amount of ash in the biochars (Abrishamkesh et al. 2016), which contain soluble salts (Al-Wabel et al. 2019; Atkinson et al. 2010; Fellet et al. 2011; Nelissen et al. 2012; Smider and Singh 2014).

Total carbon was influenced by the rate of biochar and wheat straw addition (Table 1). Current studies have indicated that biochar applications can increase C through the direct and indirect effects. The direct effect is the amount of stable biochar C added, whereas the indirect effect (negative priming effect) is due to potentially decreased mineralisation of native soil organic matter and/or fresh crop residues (Blanco-Canqui et al. 2020). Wheat straw contains hemicellulose, lignin and type I cellulose that provides slowly decomposing C (Liu et al. 2005), thus increasing the total C content in the medium term.

The increase in CEC in the biochar treated tailings (Table 1) was due to a large number of negative charges at the surface of biochar. A similar trend was observed with the addition of wheat straw (Table 1),

Fig. 5 Cumulative microbial respiration as influenced by additions of wheat straw biochar (BC) (a) and wheat straw (WS) (b) to iron ore tailings during a 105-day incubation



likely due to dissociated polygalacturonic acids in the cell walls.

There was no significant difference among treatments (tailings amended with biochar or wheat straw) in total metals/metalloid after 7 days of equilibration (Table 2). It has been reported that biochar may immobilise or mobilise metals in soil depending on the types of metals and the applied biochar properties (Wu et al. 2012). Further, remediation mechanisms associated with biochar application to metal contaminated soil/tailings are physical adsorption, ion exchange, electrostatic interaction, complexation and precipitation (Wang et al. 2022). However, the minimum incubation time to initiate the appropriate

metal remediation mechanism is yet to be defined, and 7-day equilibrium (Table 2) may not be sufficient for biochar or wheat straw testing here.

Early growth of perennial ryegrass seedlings from a phytotoxicity perspective

Germination percentage and shoot and root lengths were the variables determining the plant response to phytotoxic compounds present in aqueous biochar extracts (Rogovska et al. 2012), with additional nutrients in biochar at least partly responsible for improved growth. In the present study, leaf and root lengths of perennial ryegrass germinated in BC 10%

extract were significantly greater than those germinated in DI water and the control extract (Table 3). However, the treatments with wheat straw did not show increased germination percent and improved early growth of perennial ryegrass compared to biochar treatments, highly likely due to relatively poor nutrient content extracted from wheat straw during the 17-h shaking.

Impact of amendments on root/shoot growth parameters

Biochar effects on root architecture are still poorly understood (Bruun et al. 2014). Our data suggest a positive effect of biochar on total root length (Fig. 3). It is speculated that the observed effects are associated with physical and/or chemical changes in tailings brought about by biochar. For example, biochar decreases soil density (Glaser et al. 2002) and may facilitate root proliferation by creating wider or additional pores (Bruun et al. 2014). In earlier work, Lehmann et al. (2011) reported an increase in root biomass root in the presence of biochar type materials, similar to the present study (Fig. 2). Improving key tailings properties such as nutrient and water conditions may result in enhanced root system development.

Perennial ryegrass shoot biomass increased with the increasing rates of biochar (Fig. 1), potentially due to improved physicochemical characteristics of tailings as critical factors influencing plant growth via stimulation of the microbial community and nutrient cycling (Dai et al. 2020). However, higher plant growth was observed in wheat straw amended tailings than biochar treatments (Fig. 1). Wheat straw biomass consists of complex lingo-cellulosic materials in which rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, uronic acid (as anhydrous sugar equivalents), various important cell wall phenolics (e.g. diferulic and coumaric acid) and lignin are present, making it relatively difficult to decompose (Singh et al. 2019). However, Guan et al. (2020) suggested that the addition of inorganic fertilisers may increase the decomposition of wheat straw and subsequently increase the release of nutrients essential for plant growth. This could be the reason for higher plant growth in wheat straw amended tailings than biochar amended tailings.

Metal accumulation in perennial ryegrass shoots as influenced by biochar and wheat straw application

In the present study, decreased metal uptake of Co, Cu, Fe, Mn, Zn, Cr and Ni in the BC 2% treatment compared with the control was consistent with the observations of Fellet et al. (2011). The biochar application increased the organic carbon content and pH in the tailings (Table 1), likely increasing metal complexation with organic matter and decreasing a relative proportion of the metallic cations at higher pH, with both of these processes limiting the mobility and availability of Cu (Chen et al. 2018). Further, biochar can be an effective amendment for immobilising heavy metals available in contaminated soils. But biochar has variable effects depending on its chemical and physical properties and those of the treated soil (Chen et al. 2018; Liang et al. 2016). Adsorption of Pb in the substrate through complexation with carboxyl, hydroxyl functional groups and cation exchange with ions such as Ca^{2+} , Mg^{2+} available in biochar may result in low bioavailability and thus reduce the uptake by perennial ryegrass shoots (Ahmad et al. 2014). Moreover, surface precipitation and/or co-precipitation in less soluble compounds such as phosphate may also impact on reducing Pb uptake by perennial ryegrass (Vanini et al. 2021). By contrast, shoot metal accumulation increased with increasing rate of wheat straw amendment (Table 3). Other studies also indicated that organic amendments such as wheat straw could enhance metal mobility due to a potential decrease in soil pH and an increase in the proportion of free metal cations (Xu et al. 2016). In the case of Zn, complexation with low molecular weight organic acids is available more to uptake by plant roots and transported into plant shoots (Tang et al. 2015).

Changes in microbial biomass carbon associated with the addition of biochar and wheat straw

The microbial biomass significantly increased with an increase in the biochar application rates, which was consistent with Lehmann et al. (2011), suggesting that biochar addition could positively affect microbial growth (Bashir et al. 2019). The microbial biomass carbon increase correlated with the abundance of macro- and micro-pores in biochar (Ahmad et al. 2014), allowing a balance between water- and air-filled pores acting as microbial habitats. Furthermore, a

significant change in pH of the tailings (Table 1) might enhance nutrient cycling and induce a positive change in microbial biomass carbon (Bashir et al. 2019). In addition, labile fractions of carbon and nitrogen may be released from the biochar, which may influence the microbial biomass carbon increment. It was found that the sorption of polar organic molecules and nutrients could provide an energy source to increase microbial biomass carbon (Lou et al. 2014).

In this study, an increase in microbial biomass carbon was greater in the wheat straw than in the biochar treatments (Fig. 4). During the course of the experiment, the cellulose fraction of the straw would have been decomposed, providing a food and energy source for microorganisms, whereas the breakdown of recalcitrant lignin would provide only a minimal amount of carbon to the soil microbial biomass (Allison and Killham 1988).

CO₂ release after amending tailings with biochar or wheat straw

The BC treatments showed a lower increase in CO₂–C emissions (Fig. 5a) than unamended tailings. As Zhu et al. (2017) reported, low amount of labile organic C might be the most likely scenario of decreased cumulative CO₂ emissions in the biochar-amended treatments compared to others (Fig. 5a). Wheat straw amended tailings showed higher cumulative CO₂ emissions than all the treatments (Fig. 5b). Similar results were observed by Zhu et al. (2017), that cumulative CO₂ emissions were higher following straw amendments with or without the presence of biochar throughout the incubation period (Sial et al. 2019). The labile organic C derived from the straw due to the high amount of easily mineralisable C compounds in straw (Qayyum et al. 2012). This situation creates favourable conditions such as an increase in soluble organic C content (Bibi et al. 2018) in tailings for soil microorganisms which subsequently increases soil respiration and cumulative CO₂ emissions (El-Naggar et al. 2015; Zhu et al. 2017).

Conclusion

This study suggests that the addition of wheat straw (WS 10%) or biochar (BC 10%) into iron ore mine

tailings could enhance the physicochemical (pH, EC, CEC) and biological properties (MBC and CO₂ emission) of tailings. These improved characteristics promoted plant yield and resulted in the higher metal accumulation by perennial ryegrass shoots at BC 5% and BC 10% due to increased plant growth, immobilisation of metals in soil in stable metal-organic complexes. The treatments with wheat straw performed better than those with biochar. Extensive further studies are needed to elucidate the mechanism of metal immobilisation with application of wheat straw or biochar to mine tailings. In addition, testing of biochar and wheat straw amendments under field conditions is warranted.

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Authors' contribution Sajeevee Sarathchandra: Conceptualisation, Methodology, Investigation, Formal analysis, Writing original draft. Zed Rengel: Conceptualisation, Supervision, Revision. Zakaria Solaiman: Conceptualisation, Supervision, Revision.

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Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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