Restoration on magnetite mine waste substrates using Western Australian native plants only marginally benefited from a commercial inoculant

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ABSTRACT

Post-mining landscapes often lack self-sustaining plant communities and functional belowground microbial communities. Inappropriate management of soil can hinder ecological restoration of mine sites. However, the potential role of microbial inoculants and plant nutrient-acquisition strategies in improving mined substrates and facilitating mine-site restoration remains relatively unexplored. An eight-month glasshouse experiment was conducted to test: (1) whether a commercially-available microbial inoculant was effective in restoring biological properties of mined substrates, and (2) the effect of plant nutrient-acquisition strategies on improving the chemical properties of mined substrates for the growth of native plants. There was no significant improvement in plant growth by adding a commercially-sourced microbial inoculant. Soil microbial biomass carbon and phosphorus increased significantly after plant growth (main effect of species * substrates * inoculation interaction). The non-mycorrhizal disturbance-specialist plant species *Maireana georgei* was effective in improving hostile conditions of the mine waste. Our results highlight that re-vegetating stockpiled topsoil using local keystone species is a desirable practice that can improve soil biological properties and benefit mine-site restoration. Commercially-available agricultural-based microbial inoculants may not be compatible for mine-site restoration using native plants, but future research using indigenous soil microbes is warranted.

1. Introduction

Increasing demand for mineral resources has led to ever-increasing mining footprints globally, resulting in large quantities of mined materials that often require rehabilitation or ecological restoration (Aznar-Sánchez et al., 2019). Australia is a major producer and exporter of mineral resources, particularly iron ore, producing about 900 million tonnes of iron ore in 2020 (Senior et al., 2021). Significant iron ore extraction occurs in remote areas of Western Australia, many areas of which harbour high biodiversity and high rates of endemism (Gibson et al., 2012). In Western Australia, as in many other jurisdictions in the country, there is often a legal requirement that post-mining landscapes are returned to a functioning, biodiverse native ecosystem comprising native plant communities through the process of ecological restoration (Stevens and Dixon, 2017).

Mining operations are among the most ecologically damaging

activities humans impose on ecosystems. Usually, large areas of natural vegetation have to be cleared and topsoil has to be stripped during mining operations. Although the value of topsoil for post-mining ecological restoration is widely acknowledged, including as a reservoir of seeds, soil biota and available plant macronutrients (Fowler et al., 2015), it remains a common practice to stockpile stripped topsoil for long periods (months to years, or even decades) because often the direct transfer of topsoil to the recipient restoration site is not feasible (Roche and Mudd, 2014). Long stockpiling periods in this manner degrade the biological quality of topsoil mainly due to lack of plant carbon inputs (Zheng et al., 2019). For example, Kumaresan et al. (2017) found that long-term topsoil stockpiling killed most of the original microbial community, in particular, archaeal and bacterial microbiome diversity. Additionally, the surface area of post-mining land is often much greater than the area cleared, resulting in a large topsoil deficit (Ghose, 2001). In addition, along with the lifetime of mining operations, large

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quantities of mine tailings are produced. Tailings are usually very challenging for the re-establishment of most plants due to hostile physiochemical properties, including a lack of soil physical structure, extreme pH, low organic matter content, and absence of available nitrogen (Cross et al., 2021b). These can severely hamper the potential for successful mine-site restoration. So, it is often necessary to ameliorate the extreme physiochemical conditions of tailings while trying to re-establish the native vegetation.

Many approaches have been proposed to facilitate and promote successful mine-site restoration, including organic amendments and biochar application (Larney and Angers, 2012; Park et al., 2014), inoculation with plant growth-promoting bacteria (Padda et al., 2021), and seed-based enablement and coating technologies (Erickson et al., 2017). These approaches have the potential to improve seed germination, early plant growth and establishment, but in many cases plant root systems cannot further develop into the un-amended substrates, or early-established plant communities could not proceed with positive ecological succession (Huang et al., 2012), and therefore a self-sustaining plant community is not formed. Therefore, more innovative ways need to be trialled to improve the ecological restoration of mined substrates. To overcome these issues, firstly the health of stockpiled topsoil needs to be retained, if freshly deposited, or restored, if already stockpiled (Merino-Martín et al., 2017); secondly, using mine tailings as a novel soil-forming parent material to "eco-engineer" additional growth substrates could be considered (Santini and Banning, 2016; Zhong et al., 2021).

On the one hand, the biological integrity of soil, namely microbial community structure, has increasingly become a critical component for ecological restoration of degraded land (Hart et al., 2020). It is well documented that a healthy community of soil microorganisms can benefit soil aggregation and nutrient cycling, plant growth and abiotic stress tolerance (Singh et al., 2011). Application of commercially-available microbial inoculant has been proposed for mine-site restoration (Perkins and Hatfield, 2016), although often such selected microorganisms also pose threats to local soil biodiversity via changing the microbial communities (Hart et al., 2017). However, the effectiveness of applying commercial microbial inoculants for improving the biological properties of mined substrates, hence promoting plant growth, remains relatively unclear. On the other hand, the importance of considering resource acquisition of plants during early succession on man-made waste substrates was pointed out by Marrs and Bradshaw (1993); however, this aspect has been little studied (Cross, 2022). Plant nutrient-acquisition strategies refer to different plant groups that evolved specialised belowground traits to overcome nutrient limitation, such as carboxylate exudation and/or cluster-root formation and forming symbiotic relationships with microorganisms in the rhizosphere (Lambers, 2022). For example, using plant traits to screen potential nurse plant species for promoting the restoration of ecosystem functions, such as soil fertility and stress resilience, was applied in restoration of mine tailings in Spain (Navarro-Cano et al., 2018). However, what has been less investigated is whether plants with different nutrient-acquisition strategies have different effects on remediating biogeochemical properties of mined substrates, for example, alleviating extreme pH, building up organic C, and increasing nutrient pools. Also, the effectiveness of applying commercial microbial inoculants and their interaction with different plant nutrient-acquisition strategies on the remediation of mined substrates is still unclear. Therefore, in the present study, through a glasshouse trial we investigated: (1) whether the biological properties of mined substrates can be improved through applying a commercially-available microbial inoculant, and (2) whether plant species with contrasting nutrient-acquisition strategies and the interactions with microbial inoculant will affect remediation of mined substrates.

2. Material and methods

2.1. Study area

Native plant species and growth substrates were sourced from a magnetite mining operation in the mid-west region of Western Australia, located 400 km northeast of Perth. This area experiences a semi-arid Mediterranean climate with mild wet winters (mean monthly maximum 19 °C) and hot dry summers (mean monthly maximum 37 °C). The study area receives 311 mm annual rainfall, about 65% of which falls between May and September (winter; Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/, Station 10195). This area also experiences 2400 mm of potential annual evapotranspiration, leading to an annual water balance of approximately −2100 mm.

The mining area is located in the Yalgoo Bioregion according to the Interim Biogeographic Regionalisation for Australia (version 7; http://www.environment.gov.au/land/nrs/science/ibra). The local plant communities are typical Eremaean sclerophyll shrublands (Beard, 1990). Plant communities are generally low to open woodlands, predominantly comprising shrubs or trees of *Acacia* spp. (e.g., *A. sibina*, *A. ramulosa* var. *ramulosa*), *Eucalyptus* spp. (e.g., *E. leptopoda*, *E. kochii*), *Melaleuca leiocarpa*, *Allocasuarina acutivalvis*, *Callitris columellaris*, and *Hakea recurva* subsp. *recurva*, with an understorey of shrubs, grasses and herbaceous annuals (Markey and Dillon, 2008).

Natural topsoil at the study area comprises highly-weathered red earth, from stony red loamy sand to loamy clay soil (Leptic Rudosol, Orthic Tenosol or Red Kandosol, according to Australian Soil Classification) containing abundant fragments (usually 2–20 mm) of ironstone gravel (Payne et al., 1998). All rocky material (>40 mm) and all large woody debris (branches and twigs) were removed prior to experimental use. Stockpiled topsoil used in the present study was sourced from stripped natural topsoil (top 15 cm) but was stockpiled for ca. 4–5 years. Dry-stacked magnetite tailings (hereafter referred to as tailings) were generated as fine-textured (processed by high-pressure grinding to <4 mm) waste materials at the end of magnetite-ore processing.

2.2. Plant species selection and growth conditions

The experiment was conducted in a glasshouse at the University of Western Australia, Perth, between May and December 2018. Six plant species native to the region were selected for the study, including *Acacia ramulosa* (Fabaceae), *Allocasuarina acutivalvis* (Casuarinaceae), *Austrostipa scabra* (Poaceae), *Eucalyptus loxophleba* (Myrtaceae), *Hakea recurva* (Proteaceae), and *Maireana georgei* (Chenopodiaceae) (Table 1). These species are common components of native vegetation assemblages adjacent to the storage facility of the tailings at the study site, and represent a wide range of taxonomic and functional diversity including a variety of nutrient-acquisition strategies and symbiotic associations with

Table 1
Nutrient-acquisition strategies of plant species investigated in this study.

Species	Family	Growth form	Life history	Nutrient-acquisition strategy
Acacia ramulosa	Fabaceae	Shrub/tree	Perennial	AM/NF
Allocasuarina acutivalvis	Casuarinaceae	Tree	Perennial	ECM/AM/NF/CR
Austrostipa scabra	Poaceae	C3 grass	Perennial	AM
Eucalyptus loxophleba	Myrtaceae	Tree	Perennial	ECM/AM
Hakea recurva	Proteaceae	Shrub/tree	Perennial	CR/NM
Maireana georgei	Chenopodiaceae	Shrub	Perennial	NM

Note: AM, arbuscular mycorrhizal; CR, cluster roots; ECM, ectomycorrhizal; NF, nitrogen-fixing; NM, non-mycorrhizal.

soil biota. These species were selected based on metrics of physiological performances (e.g., seedling survival, plant height, shoot and root biomass, root length) presented in a study of 40 native plant species grow on iron ore mine tailings (Cross et al., 2021a).

Seeds were purchased from Nindethana Seed Service Pty Ltd. (Albany, Western Australia) and sown in plastic tapered square pots (18 cm × 18 cm × 44 cm) containing the experimental substrates. For *A. ramulosa*, *H. recurva* and *M. georgei*, 15 seeds were sown per pot, whereas equal weights of seeds were sown for the smaller-seeded species, *A. acutivalvis* (0.10 g per pot, ~35 seeds); *A. scabra* (0.10 g per pot, ~20 seeds); and *E. loxophleba* (0.02 g per pot, ~100 seeds). Plants of each species were grown in three treatment substrates including capped tailings, tailings, and stockpiled topsoil with 10 replicate pots for each substrate treatment. Capped tailings pots contained about 4.8 kg of tailings overlain by 1.2 kg of mixed topsoil and tailings (1:1 *w/w*), so as to mimic the proposed substrate design in the mine-closure plan. Topsoil and tailings pots contained about 6 kg pure stockpiled topsoil and tailings, respectively. Selected chemical properties for stockpiled topsoil and tailings are presented in Table S1, based on Cross et al. (2021b). Soil mineralogical characteristics were presented in Wu et al. (2019).

Within each substrate treatment, half of the pots were provided with a microbial inoculum (hereafter, referred to as inoculated). Each inoculated pot was treated with 100 ml of inoculant containing 2 g of a commercial freeze-dried microbial mixture (Troforte® Microbe Blend – Cropping, Sunpalm Australia Pty Ltd., Wangara, WA, Australia; https://troforte.com.au/content/pdf/Troforte-Microbe-Blend-Cropping-Plus-TDS.pdf). Appendix 2 in the Supplemental Information shows the chemical properties of this inoculant. This inoculant was reconstituted in deionised (DI) water, and applied at one and two months after seeds were sown, as described in Wong et al. (2022). The fertilisation effect of the added inoculant can be neglected, given that it was diluted first by 100 ml of DI water and then by 6 kg of substrate. Additional unseeded pots were established as controls to compare changes in soil properties over time and in contrast with seeded substrates and inoculation treatment. This ended up with 70 pots in total. Water content of each pot was maintained at approximately 15–20% (water to filled pot weight) for all pots by manual watering through irrigation spikes (Products of Excellence Pty Ltd., Brookvale, NSW, Australia) placed in the middle of each pot.

It is claimed that this microbial blend contains many plant-growth promoting microorganisms, including free-living N fixing bacteria (e. g., *Azotobacter* species), arbuscular mycorrhiza fungi, and P-solubilising bacteria. Previous sequencing data of this product indicated that the most abundant OTUs included *Bacillus*, Streptophyta I and II, unclassified Enterobacteriaceae, Bacillariophyta, *Marinomonas*, *Pediococcus*, *Cobetia*, *Streptococcus*, unclassified Lactobacillaceae, Alcaligenaceae, and *Pseudomonas* (Moreira-Grez et al., 2019). *Azotobacter* and *Bradyrhizobium* were detected, but present at low abundance (Moreira-Grez et al., 2019). This microbial inoculum has also been trialled previously with pigeon pea (*Cajanus cajan*) and was suggested to improve plant performance (i.e. enhance water-use efficiency) using mined overburden materials from a Pilbara mine site in the semi-arid region of Western Australia (Wong et al., 2022). However, due to lack of viable propagules, a very low success rate (one out of 25 inoculants) of mycorrhizal root colonisation was observed in non-sterilized soil, while in sterilized soil five out of 25 inoculants increased the crop biomass, in a study involving commercial inoculants from Australia, Europe and North America (Salomon et al., 2022).

Plants were harvested at eight months after sowing. Shoots were harvested by severing at about 0.5 cm above the soil surface, and lightly washed to remove any soil. Roots were retrieved by washing away the substrates under gently running water over a mesh grid (2 mm) to prevent loss of biomass. Roots were not harvested for treatment groups with poor survival, mainly in tailings and mix pots. Harvested shoot and root mass was determined after drying the plant material to a constant weight at 70 °C for at least 72 h. Dry biomass is expressed on a per-pot basis.

2.3. Soil analyses

A homogenised soil sample (ca. 500 g) was collected after shoot biomass was harvested from each pot. Soil was sieved to remove large debris, roots and small gravel (2 mm stainless steel sieve) before being stored in zip-lock polyethylene bags at 4 °C for up to one week prior to analysis of microbial biomass and mineral N measurements. For each sample, about 250 g subsamples were air-dried (35 °C for up to one week) and stored in zip-lock polyethylene bags at room temperature for analysis of other chemical properties.

Soil microbial biomass carbon (MBC) and nitrogen (MBN) was measured by $CHCl_3$ fumigation and 0.5 M K_2SO_4 extraction of fresh soil as described in Vance et al. (1987) and Brookes et al. (1985). Soluble organic carbon (C) in extracts was analysed using an Aurora O·I 1030 W wet oxidation total organic carbon analyser (College Station, Brazos, TX, USA). A conversion factor (Kc) of 0.45 was applied to the MBC results as described in Wu et al. (1990). A conversion factor (Kn) of 0.54 was applied to the MBN results as described in Brookes et al. (1985). Soil microbial biomass P (MBP) was measured by $CHCl_3$ fumigation and 0.5 M $NaHCO_3$ extraction of fresh soil, as described in Brookes et al. (1982). Phosphorus concentrations were determined colorimetrically using a UV160A spectrophotometer at 880 nm (Shimadzu, Kyoto, Japan) after reaction with molybdate blue (Murphy and Riley, 1962; Blakemore et al., 1987). A conversion factor (Kp) of 0.4 was applied to the MBP results as described in Brookes et al. (1982). Soil mineral N (NH_4-N and NO_3-N) was extracted with 2 M KCl from fresh soil (Clough et al., 2001), and determined by LACHAT FIA QuikChem 8500 Series 2 (Loveland, CO, USA).

Soil pH (both in DI water and in 0.01 M $CaCl_2$) and electrical conductivity (EC) (deionised water) were measured (using a 1:5 soil to solution ratio) for each sample using soil pH and EC probes calibrated with pH 4 and 7 buffer solutions (Orion 720a, Beverly, MA, USA). Soil total organic C was estimated by the dichromate oxidation procedure (Heanes, 1984). Total N was measured by the combustion method via a Leco analyser (FP628, St. Joseph, MI, USA).

Exchangeable cations were determined by extraction in 0.1 M $BaCl_2$ (2 h, 1:30 soil-to-solution ratio), with detection by inductively coupled plasma optical-emission spectrometry (ICP–OES) (Thermo iCAP 6000 series ICP-OES, Freemont, CA, USA) following the methods of Blakemore et al. (1987). Effective cation exchange capacity (ECEC) was calculated as the sum of Al, Ca, K, Mg and Na concentrations. Colwell-P and -potassium (K) were extracted with 0.5 M $NaHCO_3$ (adjusted to pH = 8.5) from air-dried soil (16 h, 1:40 soil-to-solution ratio). Phosphorus and K concentrations ([P] and [K]) in the extracts were determined colorimetrically (Rayment and Lyons, 2010), using a UV160A spectrophotometer at 880 nm (Shimadzu, Kyoto, Japan) and atomic absorption spectroscopy (Varian SpectrAA 55A AAS, Palo Alto, CA, USA), respectively. All soil data are expressed on a dry-mass basis.

2.4. Statistical analyses

Several statistical methods were taken to assess the potential effects of microbial inoculation and plant nutrient-acquisition strategies on plant growth and soil biogeochemical changes. Three-way analysis of variance (ANOVA) with a general linear model was used to assess the full factorial interactions among plant species, inoculation, and substrates, and their effects on selected soil parameters. Three assumptions were tested for three-way ANOVA, including normality (using a normal quantile plot and Shapiro-Wilk normality test) and equal variances (using Bartlett's test) on the residuals, as well as independence. This was followed by Kruskal-Wallis test (a non-parametric equivalent of one-way ANOVA) by substrates to test the concatenated effects of plant species and inoculation on soil parameters, because residuals of log- or square root-transformed data were not normally distributed for each model.

Student's *t*-tests were conducted between inoculated and non-inoculated plant biomasses within substrate type according to the data sets' distribution normality and variance homogeneity. For each species, the differences in plant biomass produced in different substrates were tested statistically for those species that had at least three surviving individuals in all substrates to ensure the statistical power. A non-parametric equivalent, the Wilcoxon test, was used when needed due to the nature of the dataset. Data and statistical analyses were performed using JMP® 15 (SAS Institute Inc., Cary, NC, USA). All model results are summarised in Tables S2 and S3 in the Supplementary material.

3. Results

3.1. Plant growth

While plant growth varied greatly among species, we observed a clear pattern that plants grown in stockpiled topsoil grew better than those grown in capped tailings and tailings (Table 2). Regardless of inoculant application, all plant species produced the greatest biomass in stockpiled topsoil, followed by capped tailings, apart from *A. scabra* with inoculation. Tailings clearly limited plant growth and produced the

Table 2

Plant total biomass harvested after six months of growth and results of Student's *t*-test comparing inoculated and non-inoculated plants within substrate type. Plant total biomass includes above- and belowground biomass. Data are mean values with standard errors in parentheses, $n = 3$–5; species with less than three surviving samples do not have standard errors but are marked with dashes; *p* value in each row indicates statistical significance between inoculated and non-inoculated treatments per substrate. Different letters next to the values indicate significant differences in plant biomass per species between substrates ($p < 0.05$).

| Species | Substrate | Total biomass (g) | | | |
| | | Treatment | | Statistics | |
		Inoculated	Non-inoculated	t	p
Acacia ramulosa	Topsoil	5.68 (1.67)[a]	2.72 (1.01)[a]	−1.511	0.177
	Capped tailings	1.25 (0.30)[b]	0.65 (0.12)[b]	−1.822	0.125
	Tailings	0.32 (0.18)[c]	0.07 (0.04)[c]	−2.07	0.174
Allocasuarina acutivalvis	Topsoil	4.33 (0.63)[a]	3.19 (0.59)[a]	−1.316	0.225
	Capped tailings	0.84 (0.19)[b]	0.58 (0.09)[b]	−1.19	0.278
	Tailings	0.29 (0.15)[b]	0.35 (0.26)[b]	0.57	0.669
Austrostipa scabra	Topsoil	3.40 (0.19)[a]	2.43 (0.57)	−1.620	0.167
	Capped tailings	3.07 (0.28)[a]	0.10 (−)	–	–
	Tailings	0.16 (0.05)[b]	0.01 (−)	–	–
Eucalyptus loxophleba	Topsoil	8.53 (1.15)[a]	2.45 (0.88)	−4.203	**0.003**
	Capped tailings	2.53 (0.43)[b]	0.25 (−)	–	–
	Tailings	0.09 (−)	0.01 (−)	–	–
Hakea recurva	Topsoil	5.92 (0.54)[a]	4.58 (0.51)[a]	−1.796	0.110
	Capped tailings	1.71 (0.21)[b]	2.48 (0.44)[b]	1.570	0.170
	Tailings	1.59 (0.33)[b]	0.85 (−)	–	–
Maireana georgei	Topsoil	10.99 (0.56)[a]	6.82 (0.38)[a]	−6.182	**0.001**
	Capped tailings	2.39 (0.13)[b]	0.05 (0.01)[b]	−17.590	<0.001
	Tailings	0.57 (0.19)[c]	0.08 (−)	–	–

smallest biomass for all species. Specifically, when we analysed the effect of microbial inoculant, significantly more biomass was produced only by *E. loxophleba* ($p = 0.003$) and *M. georgei* ($p = 0.001$) with inoculation in stockpiled topsoil than on the same substrate without inoculation. However, inoculation tended to be less effective in the capped tailings as no statistically significant difference was observed in biomass produced for any species, apart from *M. georgei* ($p < 0.001$) with significantly more biomass produced in inoculated pots. Mixing stockpiled topsoil into the surface of tailings (without inoculation) had a minor effect on improving plant growth as roots mostly grew in the topsoil-mixed layer, with few penetrating into the tailings layer. Due to insufficient numbers of surviving plants, statistical analysis was only conducted for *A. ramulosa* ($p = 0.174$) and *A. acutivalvis* ($p = 0.669$) in tailings between inoculated and non-inoculated pots. Overall, the produced biomass tended to be marginally greater in inoculated pots in tailings. There were no root nodules (either fresh or dead) found on the putatively N-fixing species, *Acacia ramulosa* and *Allocasuarina acutivalvis*, on any of the substrates and treatments.

3.2. Changes in substrate chemical and biological properties

In general, the chemical properties of tailings showed relatively minor changes in response to plant species and microbial inoculation, compared with capped tailings and stockpiled topsoil. Despite the overall pattern, soil pH values in capped tailings were all higher in planted pots than in un-planted controls (Fig. 1a), while soil pH remained mostly unchanged in tailings (ca. 9.7) and topsoil (ca. 6.3) compared with controls (Fig. 1b and c). Substrate types ($p = 0.0204$) and species*substrates interactions ($p = 0.0005$) had significant effects on soil pH (Table S2), but the concatenated effects of inoculation and species were only evident in capped tailings pots ($p = 0.0377$) (Table S3).

Soil organic C concentrations declined slightly with plant grown in capped tailings compared with controls (Fig. 2a), while they were mostly unchanged in tailings (ca. 1.9 mg g^{-1}) (Fig. 2b). *Eucalyptus loxophleba* with microbial inoculation had a significantly higher organic C concentration than that without inoculation. In topsoil pots, all plants had an increased organic C level of about 7 mg g^{-1}, compared with controls (ca. 4.4 mg g^{-1}) (Fig. 2c). This was also reflected in greater plant biomass produced in topsoil pots. However, neither inoculation nor interactions of factors (species, substrates, and inoculation) had significant effects on the changes in soil organic C (Table S2). The concatenated effects of inoculation and species were only evident in capped tailings pots ($p = 0.014$) (Table S3).

Soil total N concentrations was relatively unchanged in capped tailings, irrespective of inoculation or plant species, apart from that of *E. loxophleba* (Fig. 3a). Planted pots with inoculant all exhibited marginally increased N levels (ca. 0.6 mg g^{-1}) in tailings, compared with unplanted controls (Fig. 3b). This effect was relatively evident in *A. ramulosa*, *E. loxophleba*, *H. recurva*, and *M. georgei*. However, all planted topsoil pots had slightly lower total N levels than controls, while only *A. ramulosa* with inoculation showed no change (ca. 1.1 mg g^{-1}) (Fig. 3c). Three-way ANOVA analysis showed inoculation ($p = 0.0113$) had a significant effect on total N changes (Table S2).

Soil ECEC levels were significantly higher in *A. ramulosa*, *A. scabra* and *M. georgei* without inoculation in capped tailings than in inoculated pots (Fig. 4a). Tailings' ECEC values were mostly unchanged, while topsoil pots showed all much higher values than controls (Fig. 4b and c). Three-way ANOVA showed three factors and their interactions had significant effects on soil ECEC ($p < 0.0001$), apart from inoculation only ($p = 0.1627$) (Table S2).

Regarding measured soil biological properties, overall, plant growth increased the soil microbial biomass C, N and P concentrations, compared with unplanted controls (Figs. 5, 6 and 7). This was particularly evident in capped tailings and topsoil pots. However, this was less evident in tailings due to low numbers of plant seedlings that survived
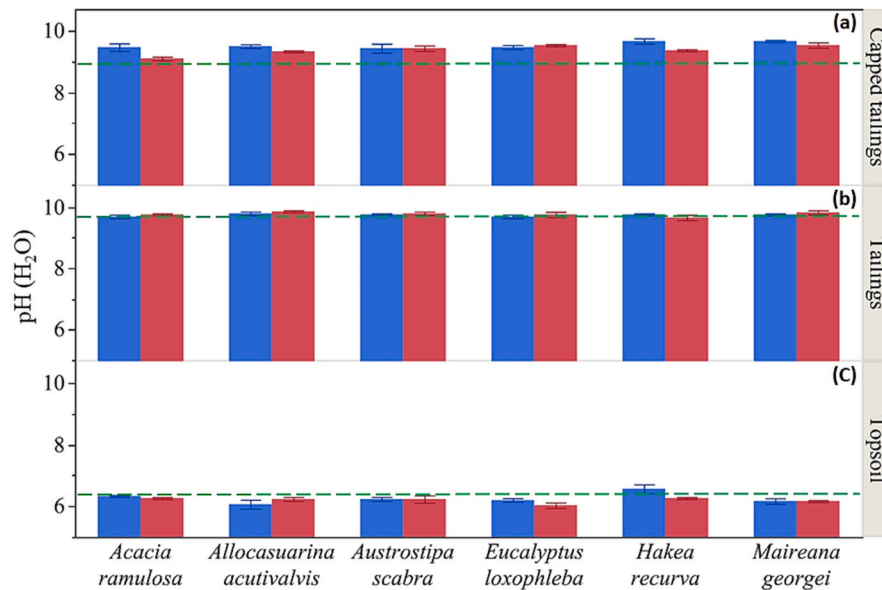
Fig. 1. Substrate pH (H$_2$O) as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (n = 5). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
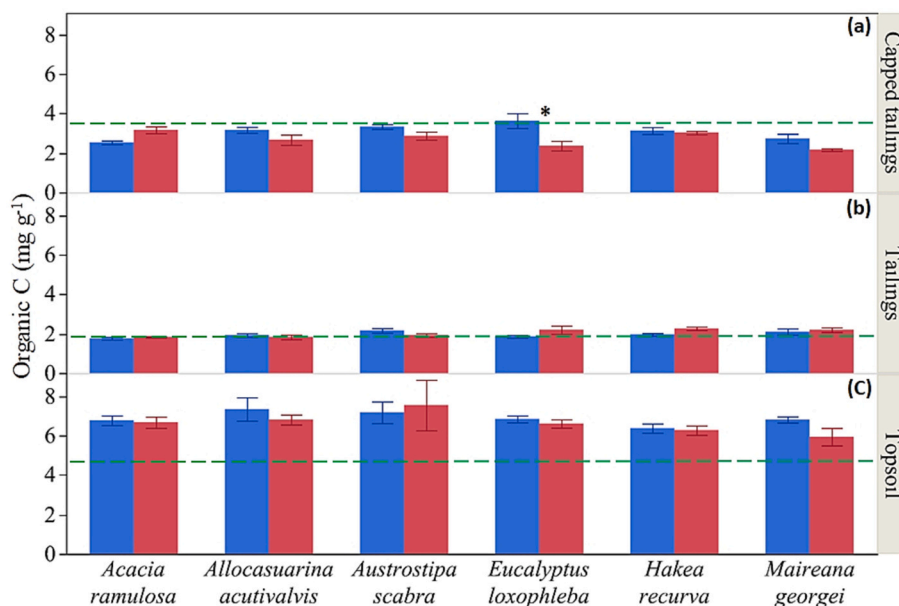

Fig. 2. Substrate total organic carbon (C) concentration as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (*n* = 5). Asterisks indicate statistical significance using the Wilcoxon test (* *p* < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during this glasshouse experiment. In particular, *A. ramulosa* with inoculation had significantly higher MBC and MBP concentrations in capped tailings than in un-inoculated ones, but not MBN concentrations (Figs. 5a, 6a and 7a). *Acacia ramulosa*, *A. acutivalvis* and *E. loxophleba* without inoculation in topsoil had higher MBC concentrations than inoculated ones (Fig. 5c). However, *A. acutivalvis* and *A. scabra* in topsoil with inoculation had significantly higher MBN concentrations than without (Fig. 6c). Three-way ANOVA showed three factors and their interactions all had significant effects on soil MBC and MBP (*p* < 0.001), but not on MBN (Table S2). The concatenated effects of inoculation and species were evident for microbial biomass properties in all capped tailings and tailings pots (*p* < 0.05) (Table S3). Surviving *M. georgei* seedlings in inoculation treatments markedly enhanced soil microbial biomass properties in tailings (Figs. 5b, 6b and 7b). Several additional soil chemical properties, including inorganic N, EC, Colwell P (measured

for potentially available P) and K, exchangeable cations are presented in Supplemental material (Figs. S1 to S11), as well as model results in Tables S2 and S3.

The results of the principal component analysis suggest that the first principal component contributed more than half (58.5%) to the coordination of data (Fig. 8). There was no clear separation between inoculated and non-inoculated pots. However, the topsoil pots (cluster on right-hand side) were completely separated from the tailings and capped tailings pots (cluster on the left-hand side) (Fig. S12). This separation was mainly driven by the total N, inorganic N, available P, and microbial biomass to the right, while pH and Colwell K appeared to the left. This separation also indicates the major differences between stockpiled topsoil and tailings even after eight months of plant growth in terms of soil biological properties and fertility.
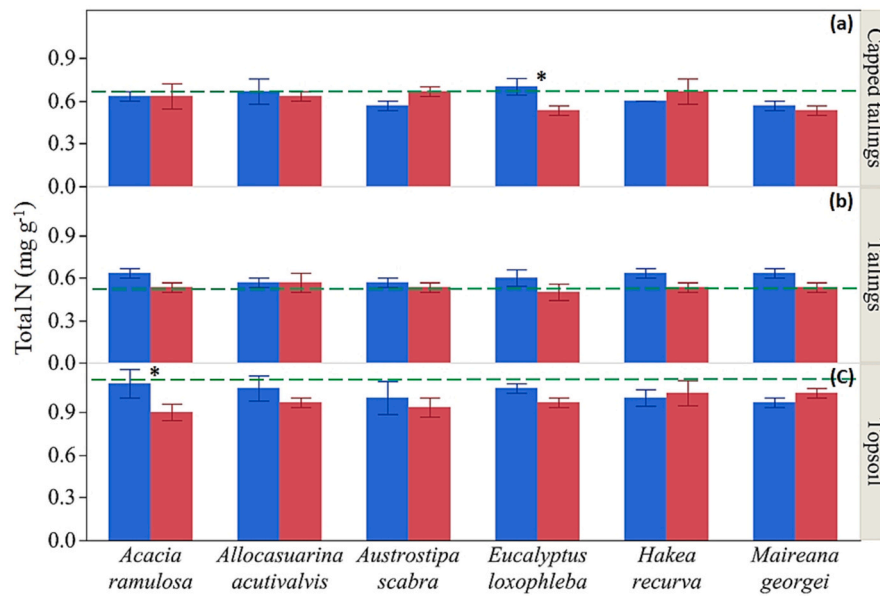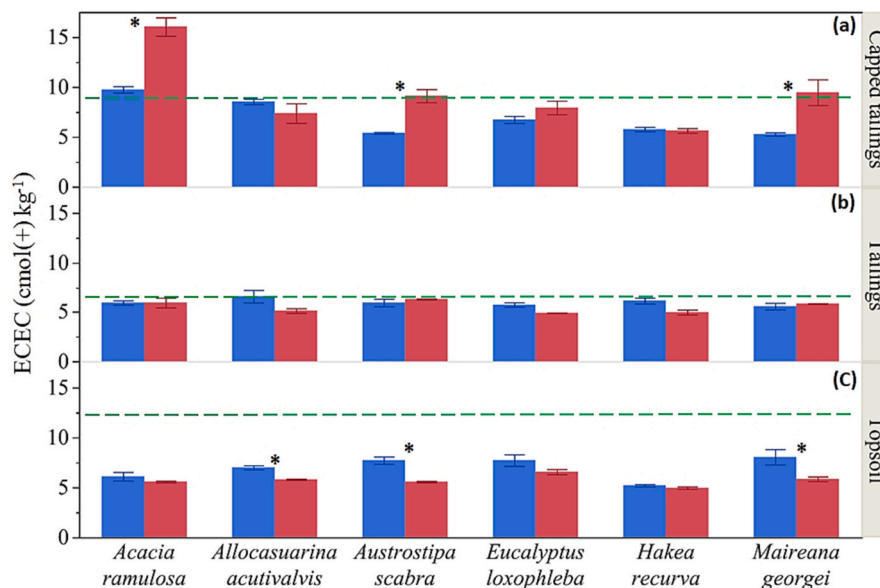
Fig. 3. Substrates total nitrogen (N) concentration as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (n = 5). Asterisks indicate statistical significance using the Wilcoxon test (* $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Substrate effective cation exchange capacity (ECEC) as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (n = 5). Asterisks indicate statistical significance using the Wilcoxon test (* $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Our results, firstly, indicate that the beneficial effect of adding a commercially-available microbial inoculant appear relatively minimal, despite usually advertised claims that these inoculants are effective in promoting plant growth. One possible reason might be incompatibility between the selected native plant species and the microbial community present in the broad-spectrum commercial inoculant. This warrants further research in isolating and selecting locally sourced soil microbes to assist post-mining land restoration, as these activities are often highly site-specific. Secondly, we highlight the importance of re-vegetating the stockpiled topsoil if direct transfer or short-term storage of stripped natural topsoil is not feasible. Re-vegetation using local keystone species, including both legumes and non-legumes, can significantly improve the health of stockpiled topsoil. This will benefit future restoration of post-mining land. Third, the potential alleviation of chemical properties of tailings as affected by plant growth was relatively limited, but effective in improving soil microbial biomass, although it varied

greatly among plant species. In particular, the non-mycorrhizal species *M. georgei*, a disturbance-specialist species (Nano and Clarke, 2008), was very effective at improving the biological activities of tailings.

4.1. Effect of commercially-available microbial inoculant

Known benefits of having healthy soil microorganisms in plant growth have driven studies in reconstructing soil microbial communities of degraded ecosystems. Unlike decades of application of soil microbial inoculants (for example, plant growth-promoting rhizobacteria) in the agricultural sector (Chaparro et al., 2012; Vishwakarma et al., 2020), this practice is far less common and only recently explored in mine-site restoration. For instance, Moreira-Grez et al. (2019) aimed to recondition the microbial communities of stockpiled topsoil sourced from a mine site in an arid region via adding a commercially-available agricultural-based soil microbial inoculant. Wong et al. (2022) aimed to improve the performance of a crop species, pigeon pea (*Cajanus cajan*), on mined overburden material via adding the same agricultural-based
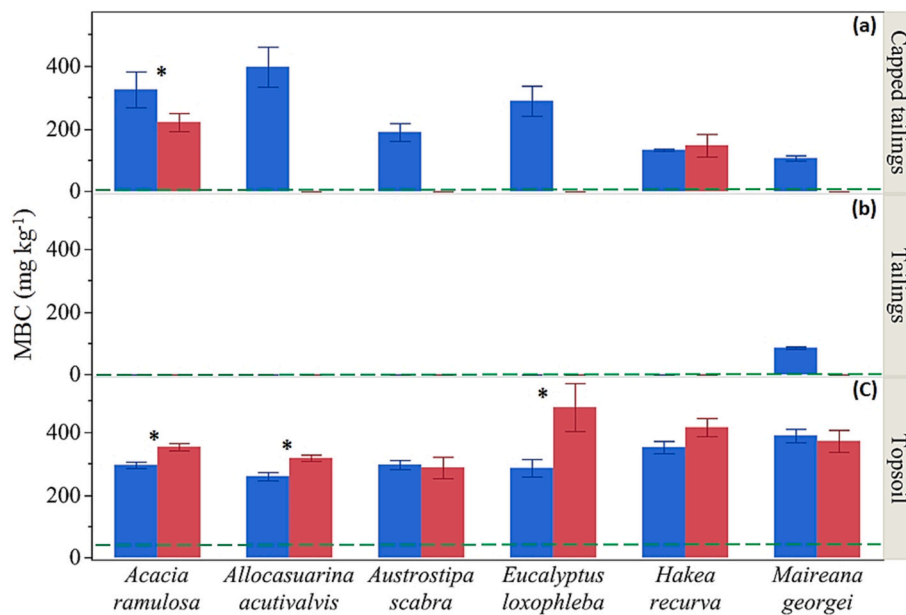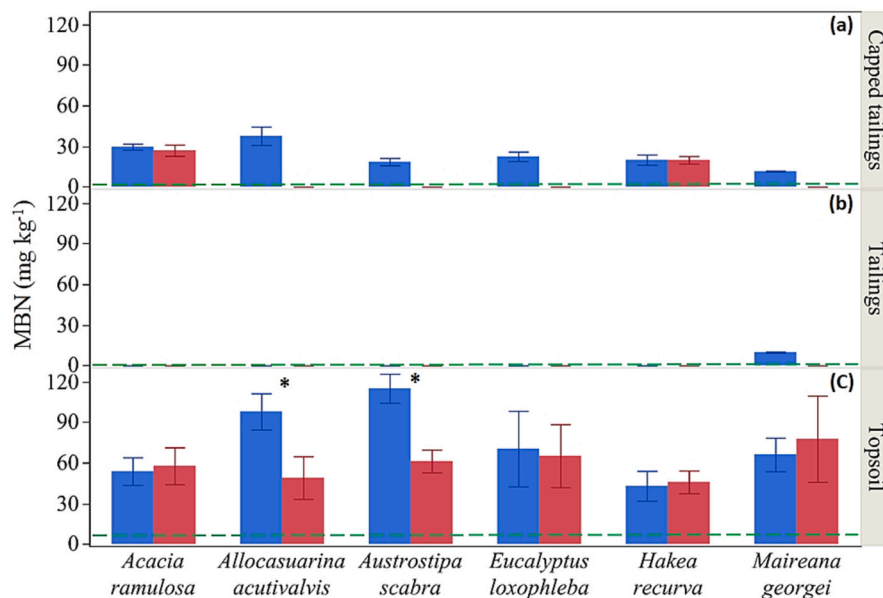
Fig. 5. Substrate microbial biomass carbon (MBC) as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (n = 5). Asterisks indicate statistical significance using the Wilcoxon test (* $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Substrate microbial biomass nitrogen (MBN) as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (n = 5). Asterisks indicate statistical significance using the Wilcoxon test (* $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

product. Similar to their findings, we show limited benefits from inoculation with an agricultural-based microbial mixture. Possible reasons are the incompatibility between inoculated mixtures of exogenous microorganisms, local soil conditions and native plant species. We surmise that significantly higher biomass produced in *Eucalyptus loxophleba* might be due to benefits from potential mycorrhizal colonisation, although we did not specifically assess mycorrhizal colonisation of *E. loxophleba* roots. More recently, cyanobacteria sourced from local biocrusts were investigated in terms of promoting plant emergence and growth for arid-region mine-site restoration in a mesocosm study (Chua et al., 2020) and restoring microbial communities of stockpiled topsoil (Jiménez-González et al., 2022); both showed promising results. Therefore, we recommend conducting further research testing the effectiveness of local soil biota for mine-site restoration under different climatic conditions.

4.2. Importance of re-vegetating stockpiled topsoil

The impact of topsoil stockpiling is largely the loss of the biological integrity of the topsoil. This has been reported in native plant bioassay experiments using stockpiled topsoil of different ages, showing that stockpiling alters soil bacterial communities and reduces microbial diversity (Birnbaum et al., 2017; Gorzelak et al., 2020). This impact on native plant growth and physiological performance was further investigated by using stockpiled and natural topsoil across different ages (up to 29 years) and ecosystems (Valliere et al., 2022). The authors concluded that plants respond mostly to biological soil differences, instead of minor difference in soil physio-chemistry across mine sites. This was also particularly evident by the absence of successful nodulation in two putatively N-fixing species in the stockpiled topsoil (both with and without commercial inoculant) in the present study. This indicates that the applied microbial inoculant likely did not contain viable
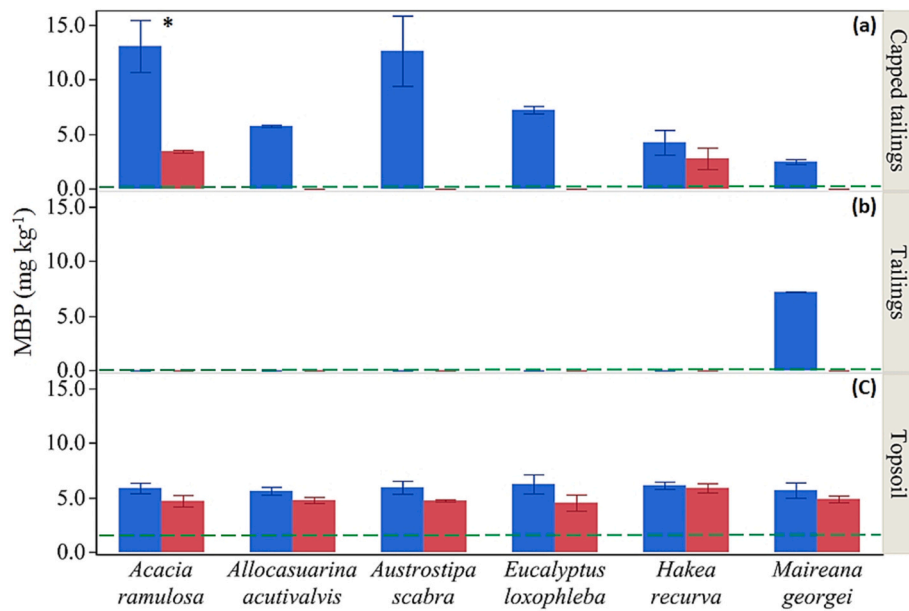
Fig. 7. Substrate microbial biomass phosphorus (MBP) as affected by six selected species grown in three substrates at the end of a glasshouse trial. Blue and red bars refer to plant growth with and without addition of inoculant, respectively. Each green broken line indicates value of each substrate control. Data are mean values with standard errors (n = 5). Asterisks indicate statistical significance using the Wilcoxon test (* $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
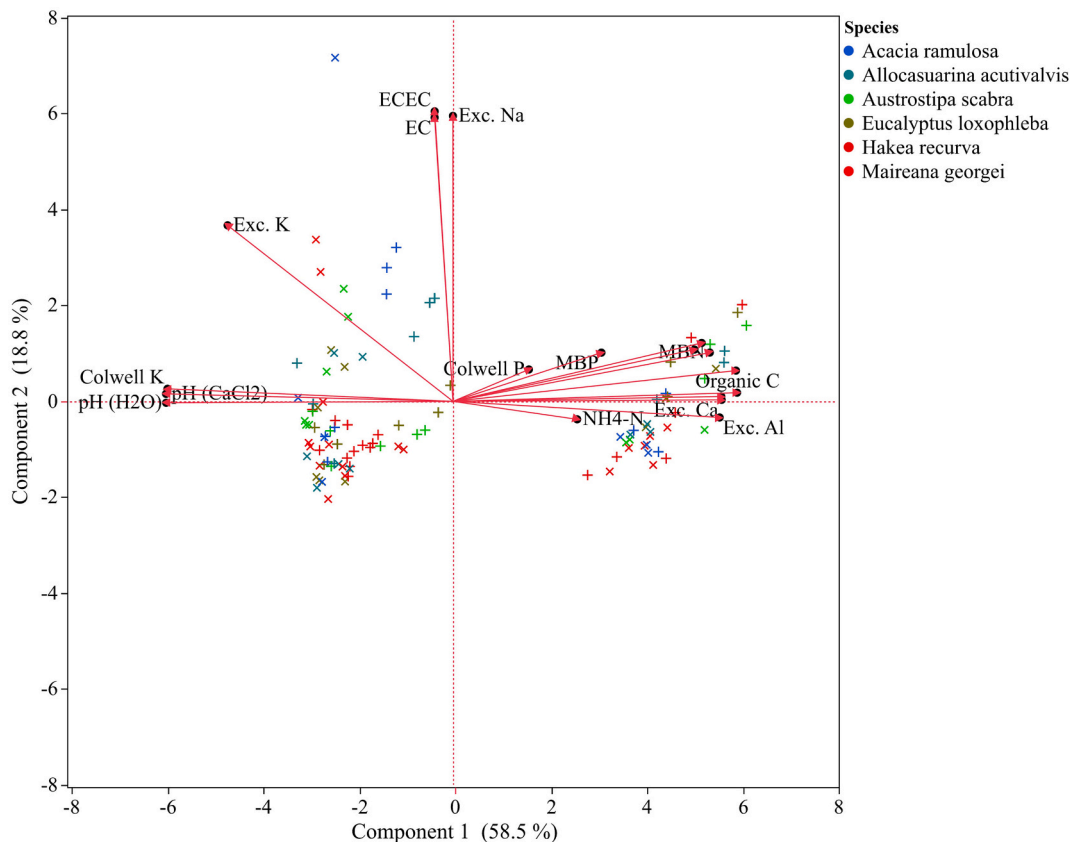

Fig. 8. Principal component analysis coordination plot for soil parameters in different mined substrates grown with different plant species for eight months. Note: plus "+" symbols represent pots with inoculation, while cross "X" symbols represent pots without inoculation. Variable abbreviations: MBC, MBN, and MBP: microbial biomass carbon, nitrogen, and phosphorus; ECEC: effective cation exchange capacity; Exc. Na, K, Ca, Mg, and Al: exchangeable sodium, potassium, calcium, magnesium, and aluminium.

strains of rhizobium or *Frankia*, even though *Bradyrhizobium* was detected in sequencing data (Moreira-Grez et al., 2019). Clearly, long-term topsoil stockpiling had negative effects on soil N-fixing microbes. Gorzelak et al. (2020) found that one of the key rhizobial taxa, *Bradyrhizobium*, was gradually lost during long-term soil storage at a mine

site. This is at odds with a previous suggestion that N-fixing bacteria have better resilience to disturbance than other soil microorganisms (Jasper, 2007). It is also worth noting that in the present study the chance of nitrate (up to 0.73 mM; Table S4) in the topsoil to suppress nodulation was low. Rawsthorne et al. (1985) suggested nitrate

concentrations >1.4–3 mM suppress nodule formation in chickpea. It is also possible that the present Western Australian native N-fixing species can function at low N supply and may not invest resources in biological N fixation (Pate et al., 1998). In the present study, the measured soil chemical properties remained mostly unchanged due to plant growth, while, more importantly, we clearly demonstrated that soil microbial biomass properties can be significantly improved via re-vegetating the stockpiled topsoil with local keystone plant species, compared with unplanted control topsoil. Soil microbial biomass measurements estimate the living component and provide good indicators of soil biological activities (Wardle, 1998). Healthy soil microbial communities contribute to above- and belowground interactions, which will benefit the ecological restoration of mined lands (Kardol and Wardle, 2010). Therefore, where feasible, it is highly recommended to at least re-vegetate topsoil stockpiles using keystone native species, including legumes and non-legumes, to restore and maintain biological functions of the soil which will facilitate future mine-site restoration.

4.3. Effects of plant nutrient-acquisition strategies

Ecological restoration utilising plant functional trait-based approaches has become increasingly popular (Carlucci et al., 2020), while studies specifically investigating the role of plant nutrient-acquisition strategies in ecological restoration have received little attention, in particular in the context of mine-site restoration. For example, plant-plant facilitation traits have been considered, such as canopy structure, resistance to soil erosion, water-use strategies, tolerance to environmental stressors, when screening for species for rehabilitating metalliferous tailings in a semi-arid region of Spain (Navarro-Cano et al., 2019) and specifically plant nutrient-acquisition strategies, such as mycorrhizal and non- mycorrhizal, in mid-west Western Australia (Cross, 2022). Mined overburden materials or processed tailings are often physically and chemically inhospitable to most plants due to non-soil like structure, extreme pH, and low organic matter content and nutrient (N and P) availability (Cross et al., 2021b). In the present glasshouse experiment, the non-mycorrhizal disturbance-specialist *M. georgei* was effective in improving the microbial biomass of tailings (without adding inoculant), while the arbuscular mycorrhizal or ecto-mycorrhizal *E. loxophleba* led to the greatest increase in organic C in tailings. The interactions between microbial inoculant and N-fixing species *A. ramulosa* have driven the significant increases in microbial biomass C, N and P. The N concentration was not increased in tailings by two putatively N-fixing species, *A. ramulosa* and *A. acutivalvis*, because their symbiotic microbes were most likely absent in tailings. This warrants future research about rhizobia (exogenous or indigenous) and testing their efficacies with local N-fixing species on targeted mined substrates. Cross et al. (2021a) tested the performance of 40 local plant species and found that N-fixing species and cluster-root forming P-mobilising species from a calcicole group grew better than species without specialised nutrient-acquisition strategies from a calcifuge group on magnetite tailings. Recently, we used narrow-leaf lupin (*Lupinus angustifolius*), capable of fixing N and mobilising P (releasing carboxylates without cluster-root formation), as a model eco-engineering plant to ameliorate magnetite tailings for subsequent growth of native species (Zhong et al., 2021). In this case, an equivalent eco-engineering native plant species would be an ideal candidate to test. If unavailable, selecting local plant species with complementary traits, such as mixed culture of N-fixing and P-mobilising species, would potentially promote the establishment of pioneer vegetation on hostile mined substrates. On the other hand, *A. ramulosa*, *A. scabra*, and *M. georgei* in the capped tailings without inoculum showed significantly increased ECEC concentrations in the substrate, mainly attributed to increased exchangeable K and Na concentrations (Figs. S10 and S11). This might be explained partially by their greater biomass and enhanced weathering of mica-group primary minerals in the tailings, which were likely biotite and muscovite (Wu et al., 2019). However, the underlying mechanism is unknown. The above unresolved findings warrant further studies.

In summary, our results have important implications for the management of stripped topsoil and restoration of post-mining landscapes. However, the study also has limitations in terms of the transferability of results from a glasshouse experiment to restoration practitioners, including (1) no test was conducted to check the viability of this inoculum (i.e. Petri dish culturing), (2) it is unsure whether the current application rate of microbial inoculum was sufficient, because only a single dose was applied, calling for further studies including multiple doses, (3) plants in the field are exposed to multiple biotic and abiotic stresses, and varied conditions, (4) the length of an eight-month glasshouse experiment may not be long enough to reveal the potential effects of microbial-inoculation-mediated restoration on mine waste. Therefore, longer-term controlled experiments and field-scale trials will be crucial for future restoration practices.

5. Concluding remarks

This study suggests that commercially-sourced, agricultural-based, broad-spectrum microbial inoculants may not be effective in facilitating plant re-establishment in ecological restoration, at least on highly-altered substrates such as magnetite tailings. Therefore, future studies should explore the use of local soil biota in inoculum development. Additionally, the management of topsoil stockpiles deserves more attention, with explicit consideration needing to be paid to restoring soil biological activities via re-vegetation of local keystone plant species. Finally, where mixed cultures are possible, we recommend selecting local plant species facilitating interactions among native species, including disturbance-specialists and species with complementary nutrient-acquisition strategies, in post-mining restoration under a variety of environmental and edaphic stressors.

CRediT authorship contribution statement

Hongtao Zhong: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft. **Wei San Wong:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Jun Zhou:** Conceptualization, Methodology, Investigation, Writing – original draft. **Adam T. Cross:** Conceptualization, Writing – review & editing. **Hans Lambers:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2023.106991.

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