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Initiation of soil formation in weathered sulfidic Cu-Pb-Zn tailings under subtropical and semi-arid climatic conditions

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Abstract

Field evidence has been scarce about soil (or technosol) formation and direct phytostabilization of base metal mine tailings under field conditions. The present study evaluated key attributes of soil formation in weathered and neutral Cu-Pb-Zn tailings subject to organic amendment (WC: woodchips) and colonization of pioneer native plant species (mixed native woody and grass plant species) in a 2.5-year field trial under subtropical and semi-arid climatic conditions. Key soil indicators of engineered soil formation process were characterized, including organic carbon fractions, aggregation, microbial community and key enzymatic activities. The majority (64-87 %) of the OC was stabilized in microaggregate or organo-mineral complexes in the amended tailings. The levels of OC and water soluble OC were elevated by 2-3 folds across the treatments, with the highest level in the treatment of WC and plant colonization (WC+P). Specifically, the WC+P treatment increased the proportion of water stable macroaggregates. Plants further contributed to the N rich organic matter in the tailings, favouring organo-mineral interactions and organic stabilization. Besides, the plants played a major role in boosting microbial biomass and activities in the treated tailings. WC and plants enhanced the contents of organic carbon (OC) associated with aggregates (e.g., physically protected OC), formation of water-stable aggregates (e.g., micro and macroaggregates), chemical buffering capacity (e.g., cation exchange capacity). Microbial community and enzymatic activities were also stimulated in the amended tailings. The present results showed that the formation of functional technosol was initiated in the eco-engineered and weathered Cu-Pb-Zn tailings under field conditions for direct phytostabilization.

Keywords

Soil formation, Organic carbon fractions, microbial community, enzymes, Cu-Pb-Zn tailings

1. Introduction

[1]Sulfidic metal mine tailings inherently possess major constraints of hydrogeochemical instability due to the presence of abundant reactive primary minerals (e.g., sulphides) and physical compaction due to fine and homogenous texture, without favourable basic physicochemical conditions for the survival and colonization of even tolerant native plant species (Huang et al., 2012). As a result, direct phytostabilization of sulfidic metal mine tailings under field conditions has so far been largely unsuccessful without substantial improvement of physical structure, and biogeochemical capacity and functions (Ye et al., 2002; Mendez and Maier, 2007; Huang et al., 2012). Organic amendments (OA) (e.g., municipal and industrial by-products, manures, biosolids) were used to improve short-term plant establishment, (Romero et al., 2007; Meeinkuirt et al., 2013). However, long-term growth and recruitment of revegetated plant species would require systematic development and rehabilitation of soil attributes and processes in the tailings towards those of functional technosols (Huang et al., 2014; Li et al., 2015a; Zornoza et al., 2017). Conventional revegetation approach for tailings is to apply a surface barrier material (30-100cm) and a suitable growth medium for plant establishment. The *in situ* technology to eco-engineer tailings into technosols would not only reduce costs related to sourcing and transporting large volumes of external capping materials and/or soils, but also reduce the environmental liability to surrounding ecosystems. Sulfidic metal mine tailings (e.g., Cu, Pb-Zn, Fe ore) are mineralogicallly and chemically different from natural soil, which are much more abundant in primary minerals (e.g., pyrite, chalcopyrites) with unstable geochemistry and inherent extreme toxicity (Li and Huang, 2015). Natural weathering of these primary minerals, especially pyrite is shown to be relatively rapid even in arid area and controls the levels of acidity and solubility of toxic metals and saline ions (Gleisner and Herbert, 2002; Hayes et al., 2011), which are fundamental causes for

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[1] WC: woodchips
OC: organic carbon
OA: organic amendments
MIM: Mount Isa Mines
TD5: Tailings dam 5

phytotoxicity and rehabilitation failure (Callery and Courtney, 2015). After an extensive weathering of sulfidic tailings over decades (>e.g., about 30 years) under semi-arid climatic conditions, the relative hydro-geochemical stability resulted from the oxidation and depletion of large amounts of sulphides (<8 wt.%) compared to the freshly deposited sulfidic tailings (>30 wt.%) (Forsyth, 2014), providing the opportunity to rehabilitate physical structure (e.g., water-stable aggregates) and soil biological properties towards functional technosols and the colonization of target plant communities (Huang et al., 2012; Huang et al., 2014). In the early phase of soil formation, water-stable aggregates and microbial metabolic functions in relation to organic matter decomposition would be indicative of the initiation of soil formation in the weathered tailings, in response to the eco-engineering inputs, such as organic inputs and pioneer plant colonization.

Tailings contain very low levels of organic matter, with little soil microbial abundance and diversity and metabolic activities (Li et al., 2015b). Although exogenous inputs of organic matter significantly overcome physical compaction of the tailings through bulking effects, it is necessary to initiate and stimulate the formation of water-stable aggregates and intrinsic physical structure for the formation of functional technosols. Aggregation of tailing particles would be results of integrated interactions of (geo)chemistry, mineralogy and biology in the eco-engineered tailings. Soil formation from parent materials is substantially accelerated by the involvement of microorganisms and plant roots in the natural soil systems (Jenny, 1941). Our previous studies showed that organic amendments and colonization of tolerant pioneer plant stimulated aggregation, microbial abundance and organic carbon sequestration in Pb-Zn tailings in glasshouse trials (Yuan et al., 2016).

It is recognized that studies conducted under glasshouse conditions had much more favourable water and temperature conditions than those in the field, leading to improved survival and growth of microorganisms and introduced plants. Field evidence of eco-engineered soil formation in sulfidic metal mine tailings has been scarce in literature. It is necessary to investigate if the eco-engineering factors (such as organic amendments and pioneer plant growth) could also effectively stimulate physicochemical and biological changes in the tailings under field conditions, in order to develop field-based eco-engineering methodology to stimulate *in situ* soil formation from tailings. This would be a prerequisite to improving success of phytostabilization of sulfidic tailings.

The present study aimed to characterize key indicators of initial phase of soil formation, including the properties of aggregates and associated organic carbon contents, microbial biomass, and activities of representative soil enzymes, in the weathered Cu-Pb-Zn tailings subject to organic amendments and/or colonization of mixed native pioneer plant species for 2.5 years under the field conditions. We sampled a 2.5-year field trial established in Northwest Queensland, Australia, under subtropical (19-32 °C) and semi-arid climatic conditions (2800 mm annual pan evaporation and 464 mm average annual rainfall mainly in November-February). In this field trial, molecular analysis revealed a shift from lithotroph- to organotroph-dominance bacterial communities in woodchips (WC) amended Cu-Pb-Zn tailings with native vegetation cover (Li et al., 2015a).

2. Materials and methods

2.1 Experimental design and sampling

Background information regarding the location and climatic conditions of experimental site were previously described (Li et al., 2014). Briefly, the tailings dam of Mount Isa Mines (MIM) (20.73 °S, 139.5 °E) is located in Northwest Queensland, Australia, under subtropical and semi-arid climatic conditions. The rainfall and temperature conditions during field incubation were in the appendices (Fig.A1).

The weathered and neutral Cu/Pb-Zn tailings were excavated in bulk from MIM Tailings dam 5 (TD5) (decommissioned), which were air-dried, crushed and mixed thoroughly before use. An appropriate volume of the tailings was mixed with designated treatments: namely, control (tailings only, TD5) and WC (ratio of organic carbon and nitrogen, OC: N = 98) at the rate of 20 % (v/v) (TD5+WC), which were loaded into intermediate bulk containers (1 x 1 x 1 m dimension) *in situ* in November 2009. Native plants (*Triodia pungens*, *Acacia chisolmii*, *Ptilotus exaltatus*) were sown/transplanted in the tailings with or without WC (i.e., TD5+P, TD5+WC+P). There were 4 treatments in total: TD5, TD5+WC, TD5+P, and TD5+WC+P, with 3 replicates in each treatment. Tailings and the amended/revegetated tailings from each replicate were sampled in April 2012 at 0-10 cm depth from the treatments described above. Each sample consisted of a composite of 5 cores taken randomly from the central area (about 5-10 cm from the edge) of each container. The fresh samples were sealed in plastic bags in the field and transported back to the laboratory in a cool container and stored at 4°C for microbial analysis within 1 week after collection. For physicochemical analyses, subsamples were dried at 40 °C and sieved through 2 mm prior to use.

2.2 Physicochemical analysis

The pH and electrical conductivity (EC) (1:5 tailings: water) in water were measured by using a pH electrode (TPS 900-P) and an EC electrode (TPS 2100), respectively. Cation exchange capacity (CEC) was quantified using the silver thiourea method (Rayment and Lyons, 2011). Water holding capacity (WHC) was measured using the saturation method (Wang et al., 2003). Total elemental concentrations (i.e., Cu, Pb, Zn, S, Fe) were determined by means of inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian) after aqua-regia digestion. A standard reference soil material (SRM 2711a Montana soil, National Institute of Standards, USA) was used to verify the accuracy of element determinations with recoveries ranging from 90 ± 10%. Water-soluble organic carbon (WSOC) was extracted by shaking fresh samples with deionized water at a ratio of 1: 2 (w/v) on an end-over-end shaker at 20 °C for 1 h and determined using the dichromate digestion method (Bremner and Jenkinson, 1960). Concentrations of water-soluble elements (i.e., Cu, Pb, Zn, S) were analysed with ICP-OES after shaking 1 g samples in 50 ml deionised water for 1 h.

2.3 Aggregates separation and organic carbon fractionation

OC in the tailings was fractionated following the procedure as shown in Fig. 1 (Six et al., 2002). In brief, 150 g of air-dried samples were submerged in deionized water on a 250 μm sieve for 5 minutes to allow slaking of water-unstable aggregates, which were separated through a nest of sieves (250 μm and 53 μm) using wet sieving method. The sieving carried out manually by moving the sieves up and down 3 cm at the rate of 50-time in 2 minutes. Fractions retained on each sieve were gently back-washed into 500 ml polyethylene evaporation containers and oven dried at 50-60 °C for 15 h. The mass of silt+clay particles (< 53 μm) was calculated by the differences between total mass of the sample used for fractionation and the aggregates collected. Mean weight diameter (MWD) was calculated using the following equation **Error! Reference source not found.**):

$MWD = \sum_{i=1}^{n} x_i \, w_i$ (Eq. 1)

where x_i is the mean diameter of any particular size range of aggregates separated by sieving, w_i is the weight of aggregates in that size range as a fraction of the total dry weight of the tailings used, and n is the number of aggregate classes separated.

Following initial separation, a 15 g sub-sample of macroaggregate (> 250 μm) and microaggregate (53-250 μm) fractions were dispersed in 45ml 0.5 % sodium hexametaphosphate (Calgon) using a mechanical end-over-end shaker for 15 h at a speed of 30 rpm at room temperature (22 ± 1 °C). The dispersed macroaggregate and microaggregate fractions were further separated by passing the tailings through 53 μm sieve. The OC in the intra-macroaggregate particulate fraction (> 53 μm) (macro-iPOC) was regarded as unprotected OC in the tailings; and that in the intra- microaggregate particulate fraction (> 53 μm) (micro-iPOC) was regarded as physically protected OC in the tailings. The OC in intra-macroaggregate silt+clay fraction (< 53 μm) (macro-iMOC), intra-microaggregate silt+clay fraction (< 53 μm) (micro-iMOC), and silt+clay particles (< 53 μm) (oMOC) were all regarded as mineral associated OC (MOC).

Fig. 1

OC and N concentrations in tailings and each fractions were determined by dry-combustion with a LECO CNS-2000 analyzer (LECO Corporation, MI, USA) after acid-removal of inorganic carbon (You et al. 2014). There was a mean of 99.6 % mass recovery in aggregate dispersion and fractionation procedure. Recovery of OC ranged from 85.5 to 115.3 % (mean, 104.1 %) in macroaggregate dispersion and fractionation, and ranged from 84.7 to 109.2 % (mean, 96.9 %) in microaggregate dispersion and fractionation, respectively. OC stock in each fraction was calculated based on OC concentrations of each fraction and mass distribution (%) of the fraction.

2.4 Microbial biomass, mineralization rates and enzyme assays

Microbial biomass carbon (MBC) in fresh samples was determined using the chloroform fumigation and extraction method. MBC was calculated as the difference of OC between fumigated and unfumigated samples with a conversion factor K_{EC} as 0.38 (Vance et al., 1987). Microbial biomass nitrogen (MBN) was calculated as the difference of ninhydrin nitrogen (Inubushi et al., 1991) between fumigated and unfumigated samples, which was multiplied with a conversion factor K_{EN} as 0.54 (Joergensen, 1996).

The N mineralization rate was assessed using an incubation method (Chen et al., 2004). In brief, 50 g fresh tailings samples were incubated aerobically at 25 °C for 28 days. Water loss from the tailings during incubation was adjusted with deionised water every two days. Subsamples were taken at day 0 and 28 after commencing incubation and extracted with 2M potassium chloride for the analysis of mineral N (the sum of ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) in the extract). The net mineralization rate was calculated from the difference of mineral N in the extracts of each incubated sample between day 28 and day 0. Concentrations of NH_4-N in the extracts were measured with the indophenol blue method (Verdouw et al., 1978) and NO_3-N with the salicylic acid colorimetric method (Cataldo et al., 1975). Activities of four enzymes were measured in this study. Dehydrogenase activity was measured using the method of (Serra-Wittling et al., 1995). Invertase activity was determined using sucrose as the substrate (Frankeberger and Johanson, 1983). Urease activity was determined with urea as a substrate for incubation (McGarity and Myers, 1967). Neutral phosphatase activity was analysed by the disodium phenyl phosphate method (Shen et al., 2006).

2.5 Statistical methods

Primary data processing was performed using Microsoft® Excel. One-way analysis of variance (ANOVA) was carried out for significant treatment effects after normality check. Two-way

ANOVA was carried out for test of significance of the effects of organic amendments (OA) and Plant. Means were compared using the least significant differences (LSD) test at $P = 0.05$. Pearson linear correlations between biogeochemical properties and microbial properties in tailings were also calculated. All statistical analyses were conducted using the SPSS software package (SPSS Statistics 20.0, Chicago, IL, USA).

3. Results

3.1 Geochemical and physico-chemical conditions among treatments

The weathered Cu-Pb-Zn tailings represents a habitat deficient in nutrients but high in levels of soluble salts and total heavy metals, which were above known tolerance thresholds. Specifically, the levels of total N (TN) and water soluble nitrogen (WSN) in the tailings TD5 were the lowest, which were 0.11 g kg^{-1} and 0.9 mg kg^{-1}, respectively (Table 1). Tailings amended with the WC with/without plant colonization (i.e., TD5+WC, TD5+P, TD5+WC+P) showed an overall improvement of the levels of N. The N levels were more than tripled in all the amended tailings, ranging from 0.34 to 0.43 g kg^{-1}, with the highest levels in the TD5+WC+P treatment. The levels of WSN increased up to 3.1 mg kg^{-1} in TD5+WC. In the tailings with plant cover, (i.e., TD5+P and TD5+WC+P), WSN increased by 5-fold (17.2 and 19.0 mg kg^{-1} respectively), compared to those in TD5+WC (Table 1).

Despite of the high degree of weathering, the weathered tailings contained high concentrations of soluble salts (EC) and water-soluble S. Cu, Pb and Zn, regardless of treatments, perhaps due to inadequeate leaching activities. However, the levels of water soluble heavy metals (such as Cu and Zn) were 1000-fold lower than their total levels, due to the presence of circumneutral pH conditions. The treatments did not significantly decrease pH in TD5+P and TD5+WC+P. In contrast, EC levels in all the amended tailings (i.e., TD5+WC, TD5+P, TD5+WC+P) were significantly elevated, compared to that in the control (tailings only) ($P < 0.001$), possibly due to enhanced dissolution of minerals and ion exchange. The levels of water-soluble S and Cu in these amended tailings were elevated to 2-3 folds of those in the control (Table 1).

In the tailings without treatments, microaggregates accounted for about 49.8 % and macroaggregates only 11.2 % across the treatments. The treatments of WC and plant growth (i.e., TD5+WC, TD5+P, TD5+WC+P) increased the proportion of macroaggregates to 19-21% (compared to 11% in the control) and MWD by 33-50% (Table 1). The WHC was the lowest in the control (TD5), but increased by 2-3% in all the treated tailings ($P < 0.01$).

Table 1

3.2 Organic carbon fractions

In the tailings without the eco-engineering inputs, the TOC levels in the tailings were as low as 1.5 g kg^{-1} (Table 1). The WC with/without plant growth treatments significantly elevated the TOC levels by as much as 2-6 folds in the amended tailings (including TD5+WC, TD5+P, TD5+WC+P) ($P < 0.05$), with the highest in the TD5+WC+P. Water-soluble organic carbon (WSOC) accounted for only 1-2 % of the TOC in the treated tailings. Compared to as low as 5.1 mg kg^{-1} in the TD5, WSOC levels were significantly increased ($P < 0.05$) in all the amended tailings, ranging from 26.1 to 78.3 mg kg^{-1}, with the highest in the TD5+WC+P. The majority (64-87 %) of the OC was stabilized in microaggregate or organo-mineral complexes in all the treatments (Fig. 2).

Specifically, the WC and plant treatments significantly ($P < 0.05$) increased OC concentrations and OC stocks in all the fractions, except for the OC in Macro-iMOC fraction of the WC treatment (i.e., TD5+WC) (Table 2). The OC concentrations associated with the Macro-iPOC fraction increased from 3.2 g kg^{-1} in the control to 11.7-13.8 g kg^{-1} in the amended tailings, regardless of plant colonization (including TD5+WC, TD5+P, TD5+WC+P). The OC stock in Macro-iPOC fraction increased from 0.19 g kg^{-1} in the control to 0.9-1.7 g kg^{-1} tailing, accounting for 22-36 % of TOC. The physically protected OC in microaggregate (Micro-iPOC) also increased significantly in all the amended tailings ($P < 0.001$), regardless of plant growth. The concentrations of Micro-iPOC increased from 2.3-3.7 g kg^{-1} in the control to 4.3-6.0 g kg^{-1} in the amended tailings. Similarly, the OC stock in Micro-iPOC fraction increased from 0.73 g kg^{-1} in the control, to 1.42-1.45 g kg^{-1} in the WC-amended tailings regardless of plant growth (i.e., TD5+WC and TD5+WC+P). The physically protected OC accounted for 13-34 % of the TOC in the amended tailings. The OC concentrations in mineral associated fractions (Macro-iMOC, Micro-iMOC and iMOC) increased from 1.8 g kg^{-1} in the control to 3.1-9.1 g kg^{-1} in the amended tailings. The corresponding OC stock was 1.1-2.0 g kg^{-1} (45-77 % of TOC) in the amended tailings, compared to that (0.6 g kg^{-1}) in the control.

In the bulk tailings, OC: N ratios ranged from 11.7-13.7 without significant differences among the treatments (Table 1); but this ratio did vary greatly among different OC fractions (Table 2). Specifically, in all the amended tailings (i.e., TD5+WC, TD5+P, TD5+WC+P), OC: N ratios were highest in the Macro-iPOC (24.5-33.0) fraction, followed by Micro-iPOC (16.8-17.7) and the lowest in MOC (4.1-6.3) (e.g., Macro-iMOC, Micro-iMOC). Furthermore, OC: N ratios in Macro-iPOC fraction significantly increased to 24.5-33.0 ($P < 0.05$) in the amended tailings, compared to 13.9 g kg-1 in the control. The ratios in physically protected OC (Micro-iPOC) elevated to16.8-17.7 ($P < 0.05$) in the amended tailings, compared to 7.3 in the control. The OC: N ratios in Macro-iMOC and Micro-iMOC in all the tailings were not different.

Table 2

Fig. 2

3.3 Microbial properties altered by WC and plant

Microbial biomass (MBC, MBN), microbial quotient (MBC: TOC), and net mineralization rate were measured in the tailings samples collected from different treatments. MBC only accounted for a small portion (0.5-1.4 %) of the TOC in all the treatments. It was not surprised to have observed the lowest the levels of MBC and MBN in the tailings without any treatments. Microbial biomass (estimated from MBC and MBN) were stimulated significantly in all the amended tailings, particularly in the WC-amended tailings colonized by the mixed native plants (TD5+WC+P). A similar trend of net N mineralization rates was observed among these treatments (Table 3). In particular, compared to the control and WC-treatments (i.e., TD5 and TD5+WC), tailings colonized by the plants (regardless of WC amendment) were characterized by higher biomass, microbial quotient (MBC: TOC) and net N mineralization rates, indicating the importance of root systems in rehabilitating active microbial biomass (Table 3).

Table 3.

In the present study, selected enzymes were examined, which are closely related to the energy transfer (dehydrogenase) and element cycling processes of C (invertase), N (urease) and P (neutral phosphatase) in the tailings (Fig. 3). Microbial biomass was positively correlated to with these enzyme activities (Table 4). Consistent with the stimulated microbial biomass in the tailings with plant colonization, the activities of dehydrogenase, invertase and neutral phosphatase were significantly elevated in these treatments (i.e., TD5+P and TD5+WC+P), with the highest in TD5+WC+P. The urease activity was significantly increased in the WC amended tailings with or without plants (i.e., TD5+WC and TD5+WC+P), but without significant difference between treatments with and without plants (i.e., TD5 and vs TD5+P. Neutral phosphatase activity showed no significant difference among the amended tailings (i.e., TD5+WC, TD5+P, and TD5+WC+P).

Fig. 3

Table 4.

4. Discussion

The present findings suggested that eco-engineering inputs (i.e., WC and plant introduction) did accelerate the early phase of soil formation in neutral and weathered Cu/Pb-Zn tailings under field conditions. The eco-engineering inputs (i.e., organic amendment and plant colonization) increased soil organic matter and N accumulation in the treated tailings, stimulated aggregation and organo-mineral interactions, and elevated microbial biomass and enzymatic functions in the amended tailings (i.e., TD5+WC, TD5+P, and TD5+WC+P), particularly in those with combined organic amendment (WC) and plant colonization. These were indicative of positive changes leading towards of rehabilitation of soil structure and functions, in response to the eco-engineering inputs under field conditions, although the rates of aggregation and carbon accumulation were lower than those examined under the glasshouse condition (Yuan et al., 2016).

In metal mine tailings deficient in organic matter, organic carbon associated with aggregates and minerals is an important indicator of the effectiveness of eco-engineering practices and the initiation of early phase soil formation in tailings. SOC is not only compositionally essential (e.g., for direct physical effects of improving compaction) but functionally critical (e.g., organo-mineral interactions and chemical buffering) within the context of soil formation (Li et al., 2015a; Yuan et al., 2016). The TOC levels were positively correlated with the levels of N (including TN, WSN), the water-stability of aggregates (WMD), hydraulic properties (WHC), ion buffering capacity (CEC), microbial biomass (MBC, MBN) and activities (net mineralization, enzymes). The rates of OC stabilization (0.5 -1.0 g OC kg^{-1} y^{-1}) (sum of OC physically protected and mineral associated) in the amended tailings under field conditions were even 5-10 folder than those reported in natural soil during primary succession where development chemical conditions would be relatively quasi-equilibrated without dynamic hydro-geochemical reactions like in metal mine tailings (Anderson, 1977; Crews et al., 2001). As a result, the accumulation of OC in aggregates and mineral complexes would be an integrated indicator demonstrating the early sign of soil formation from the fine textured tailings, in response to eco-engineering inputs.

Both WC and introduction of native plants contributed to the elevated OC availability to microbial colonizers. In all the amended tailings (i.e., TD5+WC, TD5+P, and TD5+WC+P), there were relatively higher proportions of OC in the unprotected form (22-36%) (Macro-iPOC). The OC: N ratios (24.5-33.0) (Table 2) in this fraction were also significantly higher than the bulk tailings (11.7-13.3) (Table 1) in the amended tailings. It is assumed that the Macro-iPOC would be further decomposed and stabilized in these amended tailings, continuing to enhance the formation of water-stable aggregates and overall physical properties as technosols (Bruun et al., 2010).

It is widely accepted that recalcitrance, spatial inaccessibility and organo-mineral interactions are major mechanisms contributing to OC stabilization in soils (von Lützow et al. 2008). In the present study, the majority of OC (64-77%) in the amended tailings were protected in microaggregates or in the form of organo-mineral complexes. The mineral associated OC (MOC) became increasing dominant in the amened tailings, accounting for 45-77 % of TOC, This suggests that the increasing organic matter from WC and their derivatives from decomposition would have promoted organo-mineral interactions in the tailings (Yuan et al., 2016).

Organo-mineral interactions are the fundamental step of aggregation and OC stabilization in the agricultural and natural soils (Kögel-Knabner et al., 2008; Monserie et al., 2009). Aggregate formation is a critical process of technosol formation in tailings, which is essential to the development of physical structure with sufficient hydraulic properties to support root penetration and water infiltration. In the present study, the increases in aggregate proportion and water-stability were positively correlated with the levels of stabilized OC (Table 5) in the WC-amended tailings with or without plants. From the evidence here, the combined effects of organic matter decomposition driven by the addition of WC and root effects of colonizing plants and associated microbial activities had initiated the *in situ* organo-mineral interaction, aggregation and OC stabilization under the subtropical and semi-arid climatic conditions. Further investigation may focus on how to accelerate the rate of the structural development in the rooting profile of the amended tailings.

The forms of organic matter seemed to have marked impacts on the organo-mineral interaction, aggregation and OC stabilization processes in the tailings. In the amended tailings, OC concentrations were significantly higher in the mineral associated fractions (i.e., Macro-iMOC and Micro-iMOC) with plant cover (TD5+P and TD5+WC+P), compared to those without plants (i.e., TD5 and TD5+WC). The different OC stabilized with tailings mineral particles among treatments may be partially attributed to the different forms of OC entering into tailings and the biogeochemical modification of organic compounds. The added WC was low in N (OC: N ratio of 98) with a slow decomposition rate in the tailings, since favourable OC: N ratios are required in soil OC decomposition (Aber and Melillo, 1982). In addition, the low levels of available N in the WC amended tailings was one of main negative factors resulting in the low abundance of bacterial community which was dominant with tolerant bacteria (e.g., *rubrobacter*) (Li et al., 2014). In contrast, in the tailings with plant cover, root exudates and root biomass turnover would have generated the mixture of labile OC and a range of small molecular weight compounds (e.g., carbohydrates, carboxylic acids and amino acids) in the tailings (Lynch and Whipps, 1991). Due to negative charges in organic molecules, these substances may readily interact with the mineral phase (Jones and Brassington, 1998), which was exposed with abundant reactive sites resulted from rapid weathering processes in the tailings (Yuan et al., 2014; Yuan et al., 2016).

Meanwhile, the root systems of the colonizing plants presented a favourable hosting effect on microbial communities as revealed by the increased microbial biomass and associated organic matter decomposition (Fig. 3). As decomposition proceeds, N is retained in the microbial products (e.g., organic molecules and compounds) (Quideau et al., 2000), facilitating interactions with tailings mineral particles and the stabilization of relevant pools of OC in the form of organo-mineral complexes. Functional groups of organic matter (e.g., aliphatic or phenolic OH), aliphatic acid (e.g., citric acid, malic acid) and some proteinaceous organic compounds (e.g., amines, ring-NH, heterocyclic-N) in soil are likely to strongly bind with minerals (e.g., Fe, Al, Mn oxides, edge sites of phyllosilicates, allophane, imogolite, smectite, vermiculite, illite) to form resistant organo-mineral complexes in the soil (Feng et al., 2005; Vieublé Gonod et al., 2006; Kögel-Knabner et al., 2008). Therefore, coupling with the introduction of native plants (e.g., tolerant acacia species) which are tolerant of saline and low nutrient conditions, organic amendments of active functional groups and more favourable OC: N ratios would be preferred to amend the weathered tailings for accelerating soil formation.

Table 5

The WC-amendment may have triggered enhanced dissolution of secondary minerals already formed and perhaps, further weathering of S-bearing mineral (e.g., pyrite, chalcopyrite), resulted in elevated levels of salts, S and Cu in the water extract in the amended tailings, compared to the tailings without any treatments (Table 1). The weathered and neutral Cu-Pb-Zn tailings were largely depleted in reactive minerals (e.g., pyrite) with a relatively stable hydro-geochemistry (Forsyth, 2014). Many metal mine tailings are rich in sulphidic minerals and it is a prerequisite of technosol formation to weather most of these unstable primary minerals into geochemically and relatively stable secondary minerals (Huang et al., 2012). On one hand, microbial communities present in this type of tailings are likely to be more diverse than those in the extremely acid tailings (Mendez et al., 2008). On the other hand, direct plant colonization could be achieved in the highly weathered sulfidic metal mine tailings (e.g., Cu, Pb-Zn tailings) or tailings dominant by secondary minerals (e.g, coal deposit, bauxite residues), which would further advance the progress and developmental stage of technosol formation (Courtney et al., 2013; Huot et al., 2014). Nevertheless, abundant *Thiobacillus sp.*, autotrophic sulphur oxidizers, remain in this weathered tailings (Li et al., 2014). In addition, heterotrophic bacterial community may play some role in facilitating mineral weathering processes (Pradhan et al., 2008). The induced mineral weathering associated with organic amendments in tailings has been reported in previous studies (Li et al., 2013). Although rapid geochemical reactions may be unlikely in the extensively weathered tailings, further dissolution of minerals in the tailings may have occurred in response to the treatments, leading to elevated salinity in pore water. Introduced plants and microbes may be at least initially, stressed by the elevated levels of salts and trace metals resulted from enhanced dissolution of mineral products formed from the weathering process in the tailings, during the initial stage of phytostabilization. This implies the importance of leaching and surface transportation of salts (e.g., sulphates) during seasonal rainfalls, which are important in natural soil formation (Stockmann et al., 2011).

It is important to develop a diverse and functioning microbial community to realize biogeochemical functions (e.g., OM decomposition and nutrient cycling) for sustaining the productivity of plant communities (Harris, 2003). Previous studies have indicated that initially low microbial diversity in non-vegetated tailings rapidly increased after plant establishment and later succession (Alguacil et al., 2011; Li et al., 2014), with significantly improved microbial functions (decomposition, nutrient cycling etc.) even with a relatively low plant coverage in tailings (Moynahan et al., 2002). In the present study, microbial biomass and biogeochemical processes were improved in the amended tailings with WC and/or growing plants, as indicated by the MBC and MBN and enzyme activities. Specifically, the increased OC in the amended tailings would have provided energy and nutrients for microbial colonizers, greatly stimulating development of heterotrophic bacteria and facilitating the decomposition and element cycling processes in the tailings (Table 5). The presence of surviving plants is an important factor to shift the microbial community in the tailings away from the autotrophic dominant structure (Li et al., 2014).

In general, after 2.5 years field incubation, the amended tailings is still far away from natural soil, where the greatest TOC in TD5+WC+P was only 5 g kg^{-1} (Table 1), which is 10-50 % of those in natural soils under similar climatic conditions (Bird et al., 2002; Kihara et al., 2012). Besides, bacterial communities in all the amended tailings were still dominated by tolerant species and a fungal community is hardly detected in these tailings (Li et al., 2014), with much less diverse than communities that colonize in natural soils (Berg and Smalla, 2009). However, the current evidence suggests that the combined approach with organic matter and tolerant plant introduction can initiate the process of soil formation towards the goal of functional technosol, albeit at a slow rate under the semi-arid climatic conditions.

5. Conclusions

This study suggested that the early soil formation *in situ* could be initiated in neutral and weathered Cu/Pb-Zn tailings by means of combined organic amendment and introduction of tolerant native plants under the subtropical and semi-arid climatic conditions. The colonization of tolerant plants played a particularly important role in OC stabilization through aggregation and organo-mineral interactions, which could be attributed to the greater microbial biomass and N rich organic compounds input.

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Tables

Table 1 Selective physicochemical properties in the treatments.

Treatments*	**pH**	**EC (mS cm^{-1})^{a}**	**CEC (cmol^{+} kg^{-1})^{b}**	**WHC (%)^{c}**	**TOC:N**	**Aggregates**		
						Macroaggregate (%)	Microaggregate (%)	MWD(mm)^{f}
TD5	6.7(0.0)	3.4(0.3)$^{\alpha}$	0.79(0.27)$^{\alpha}$	19.2(1.2)$^{\alpha}$	13.7(2.4)	11.2(2.4)$^{\alpha}$	49.8(4.0)$^{\alpha}$	0.21(0.03)$^{\alpha}$
TD5+WC	6.7(0.1)	4.5(0.8)$^{\beta}$	1.66(0.41)$^{\alpha}$	21.8(0.7)$^{\beta}$	13.3(2.9)	19.2(2.4)$^{\beta}$	31.1(1.5)$^{\beta}$	0.28(0.03)$^{\beta}$
TD5+P	6.4(0.1)	5.2(0.4)$^{\beta}$	4.52(0.31)$^{\beta}$	22.1(0.4)$^{\beta}$	11.7(1.7)	19.1(2.8)$^{\beta}$	50.2(0.3)$^{\alpha}$	0.30(0.03)$^{\beta}$
TD5+WC+P	6.5(0.0)	5.6(0.7)$^{\beta}$	4.48(0.43)$^{\beta}$	21.7(0.2)$^{\beta}$	13.3(2.2)	21.1(1.1)$^{\beta}$	51.3(2.1)$^{\alpha}$	0.32(0.01)$^{\beta}$

Treatments*	Total (g kg^{-1})							Water Soluble (mg kg^{-1})				
	TOC^{d}	TN^{e}	Cu	Pb	Zn	S		C	N	S	Cu	Zn
TD5	1.5(0.0)$^{\alpha}$	0.11(0.03)$^{\alpha}$	1.1(0.1)	1.9(0.1)	4.1(0.3)	47(4)	91(6)	5.1(2.0)$^{\alpha}$	0.9(0.4)$^{\alpha}$	238(125)$^{\alpha}$	0.12(0.01)$^{\alpha}$	18.4(2.3)
TD5+WC	4.3(0.4)$^{\beta}$	0.34(0.11)$^{\beta}$	1.0(0.1)	1.9(0.2)	3.9(0.4)$^{\alpha}$	46(5)	88(4)	48.8(19.5)$^{\beta}$	3.1(0.2)$^{\beta}$	601(113)$^{\beta}$	0.33(0.01)$^{\beta}$	18.5(2.6)
TD5+P	4.7(0.2)$^{\beta}$	0.40(0.04)$^{\beta}$	1.0(0.1)	1.5(0.1)	3.5(0.6)	41(2)	83(5)	26.1(1.9)$^{\gamma}$	17.2(1.2)$^{\gamma}$	706(73)$^{\beta}$	0.45(0.06)$^{\gamma}$	17.5(0.8)
TD5+WC+P	5.5(0.0)$^{\gamma}$	0.43(0.08)$^{\beta}$	1.0(0.0)	1.6(0.1)	3.7(0.4)	38(3)	94(8)	78.3(2.4)$^{\delta}$	19.0(1.3)$^{\delta}$	710(92)$^{\beta}$	0.48(0.03)$^{\gamma}$	17.2(0.3)

*TD5: TD5 tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants. [a] Electrical conductivity. [b] Cation exchangeable capacity. [c] Water holding capacity. [d] Total organic carbon. [e] Total nitrogen. [f] Mean weight diameter. [g] Standard error; Values are means with standard error brackets (n = 3); values labelled with letters ‘α, β, γ and δ’ within the column indicate significant differences among treatments at the level of $P < 0.05$ (only labelled for selected parameters).

Table 2 OC concentrations and OC: N ratios of OC fractions in all the treatments.

Treatments*	**Macro-iPOC**		**Micro-iPOC**		**Macro-iMOC**		**Micro-iMOC**	
	OC (g kg^{-1})	OC: N	OC (g kg^{-1})	OC: N	OC (g kg^{-1})	OC: N	OC (g kg^{-1})	OC: N
TD5	3.2(0.3)^{a}	13.9(5.7)^{a}	1.8(0.1)^{a}	7.3(1.1)^{a}	2.3(0.4)^{a}	4.4(1.6)^{a}	3.7(0.4)^{a}	6.1(1.4)^{a}
TD5+WC	13.8(2.2)^{b}	33.0(6.6)^{b}	6.0(0.1)^{b}	17.7(1.8)^{b}	3.1(0.5)^{a}	4.1(0.9)^{a}	5.6(0.5)^{b}	5.3(0.2)^{a}
TD5+P	11.7(0.8)^{b}	27.2(3.4)^{b}	4.3(0.3)^{b}	16.8(3.0)^{b}	8.3(1.8)^{b}	5.9(0.7)^{a}	7.2(0.2)^{c}	6.3(1.4)^{a}
TD5+WC+P	12.5(1.0)^{b}	24.5(3.2)^{b}	4.5(0.0)^{b}	16.8(1.5)^{b}	9.5(1.4)^{b}	5.5(0.6)^{a}	7.9(0.4)^{c}	5.8(0.9)^{a}

*TD5:TD5 tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants.

Macro-iPOC, intra-macroaggegate > 53 µm particulate OC; Micro-iPOC, intra-microaggregate > 53 µm particulate OC; Macro-iMOC, intra-macroaggregate mineral associated OC < 53 µm fraction; micro-iMOC, intra-micro-aggregate mineral associated OC < 53 µm fraction. Values are means (n = 3) with standard error in brackets; values labelled with letters 'a, b, c, d' indicate significant differences among the treatments at the level of $P < 0.05$.

Table 3 Microbial biomass (MBC, MBN), microbial quotient (MBC: TOC) and net mineralization rate in all the treatments.

Treatments*	**MBC ^{a} (mg kg^{-1})**	**MBN ^{b} (mg kg^{-1})**	**MBC: TOC^{c} (%)**	**Net mineralization rate (mg mineral N kg^{-1} d^{-1})**
TD5	13.0(1.8)^{a}	0.87(0.22)^{a}	0.86(0.13)^{b}	0.018(0.003)^{a}
TD5+WC	18.3(1.3)^{ab}	1.89(0.28)^{b}	0.43(0.14)^{a}	0.030(0.009)^{ab}
TD5+P	53.8(2.4)^{c}	4.99(0.03)^{c}	1.00(0.04)^{bc}	0.045(0.011)^{b}
TD5+WC+P	76.1(7.9)^{c}	6.20(0.93)^{c}	1.37(0.14)^{c}	0.039(0.010)^{b}

*Treatments: TD5 tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants. ^{a} microbial biomass carbon; ^{b} microbial biomass nitrogen; ^{c} total organic carbon. Values are means (n = 3) with standard error in brackets; values labelled with letters 'a, b, c, d' indicate significant differences among the treatments at the level of $P < 0.05$.

Table 4 Pearson correlations among microbial biomass (MBC and MBN) and activities (Net mineralization, Dehydrogenase activity, Urease activity, Invertase activity and Neutral phosphatase activity) in all the treatments.

Microbial properties	**Net mineralization**	**Dehydrogenase activity**	**Urease activity**	**Invertase activity**	**Neutral phosphatase activity**
MBC^a	0.92**	0.77**	0.73**	0.93**	0.71**
MBN^b	0.91**	0.78**	0.68*	0.96**	0.77**

^{a} microbial biomass carbon; ^{b} microbial biomass nitrogen.

'***, ** and *' indicate significance of correlation at the levels of $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively.

Table 5 Effects of experimental factors (i.e., WC, plant) and their interactions on OC stocks in each OC fractions in the tailings.

OC fractions*	**WC**		**Plant**		**WC x Plant**	
	F	Sig.	*F*	Sig.	*F*	Sig.
TOC	65.19	0.000	202.49	0.000	52.85	0.000
Macro-iPOC	63.22	0.001	93.47	0.000	74.21	0.000
Micro-iPOC	24.37	0.002	121.95	0.000	6.95	0.030
Macro-iMOC	11.50	0.009	22.38	0.001	0.24	0.640
Micro-iMOC	0.00	0.952	310.38	0.000	0.05	0.824
MOC	0.00	0.985	6.42	0.035	2.74	0.137

*OC: organic carbon; WC: woodchips; TOC: total organic carbon; Macro-iPOC, intra-macroaggegate > 53 µm particulate OC; Micro-iPOC, intra-microaggregate > 53 µm particulate OC; Macro-iMOC, intra-macroaggregate mineral associated OC < 53 µm fraction; micro-iMOC, intra-micro-aggregate mineral associated OC < 53 µm fraction; MOC: sum of Macro-iMOC, Micro-iMOC and mineral associated OC in <53 µm fraction.

Figures captions

Fig. 1. Organic carbon (OC) fractionation procedure in the tailings.

Fig. 2 OC content as non-protected OC (Macro-iPOC), physically protected OC (Micro-iPOC) and mineral associated OC in macroaggregate (Macro-iMOC), microaggregate (Micro-iMOC) and silt+clay particles (oMOC) in all the treatments. Treatments include TD5: tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants. Values are means (n = 3) with standard error bar; the letters ‘a, b, c, d’ above each OC fractions indicate significant differences among the treatments at the level of $P < 0.05$.

Fig. 3 Activities of dehydrogenase (a), urease (b), invertase (c) and neutral phosphatase (d) in all the treatments. Treatments include TD5: tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants. Values are means (n = 3) with standard error bar; the letters ‘a, b, c, d’ above the bars indicate significant differences among the treatments at the level of $P < 0.05$.

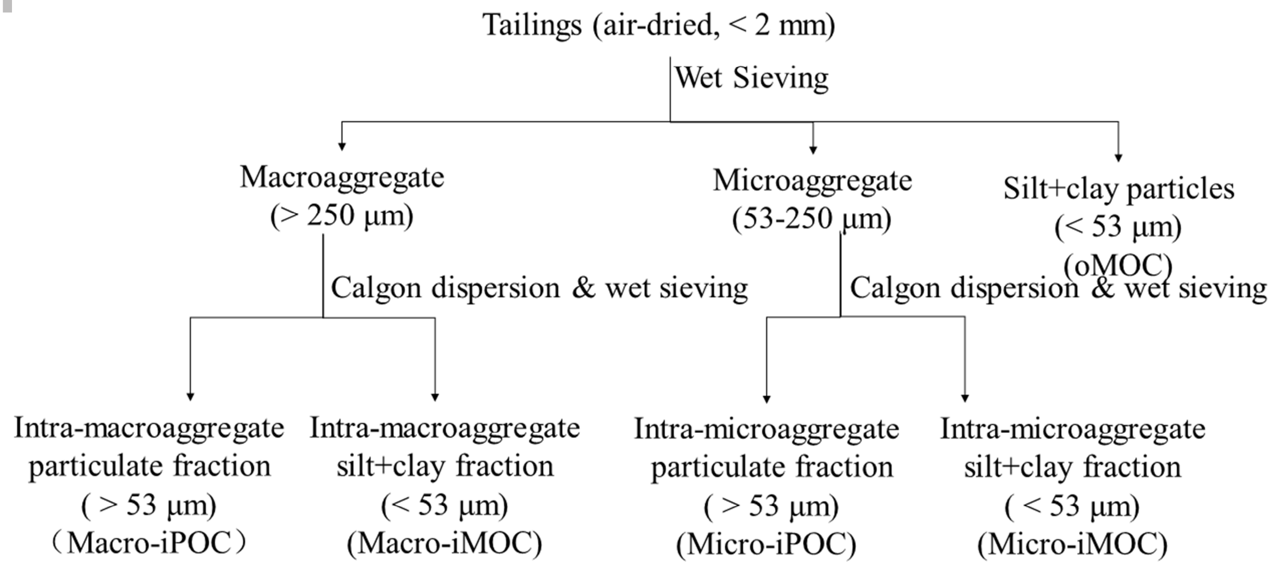

Fig. 1. Organic carbon (OC) fractionation procedure in the tailings.

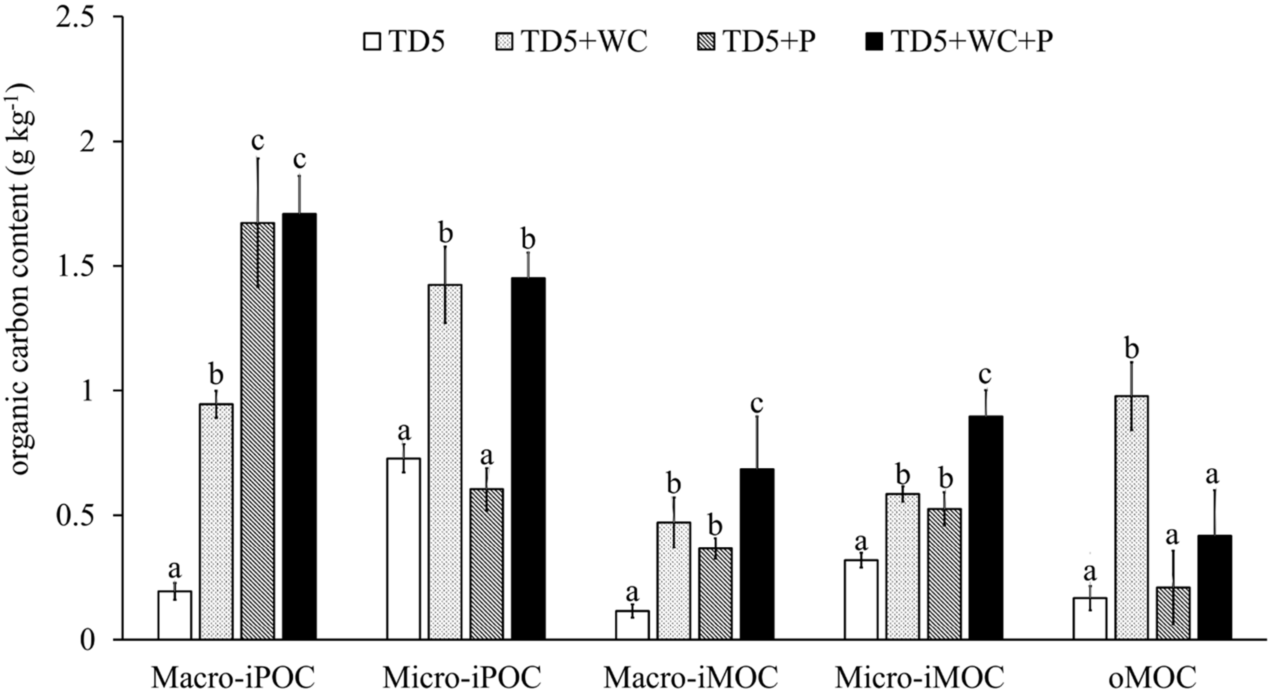

Fig. 2 OC content as non-protected OC (Macro-iPOC), physically protected OC (Micro-iPOC) and mineral associated OC in macroaggregate (Macro-iMOC), microaggregate (Micro-iMOC) and silt+clay particles (oMOC) in all the treatments. Treatments include TD5: tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants. Values are means (n = 3) with standard error bar; the letters ‘a, b, c, d’ above each OC fractions indicate significant differences among the treatments at the level of *P* < 0.05.

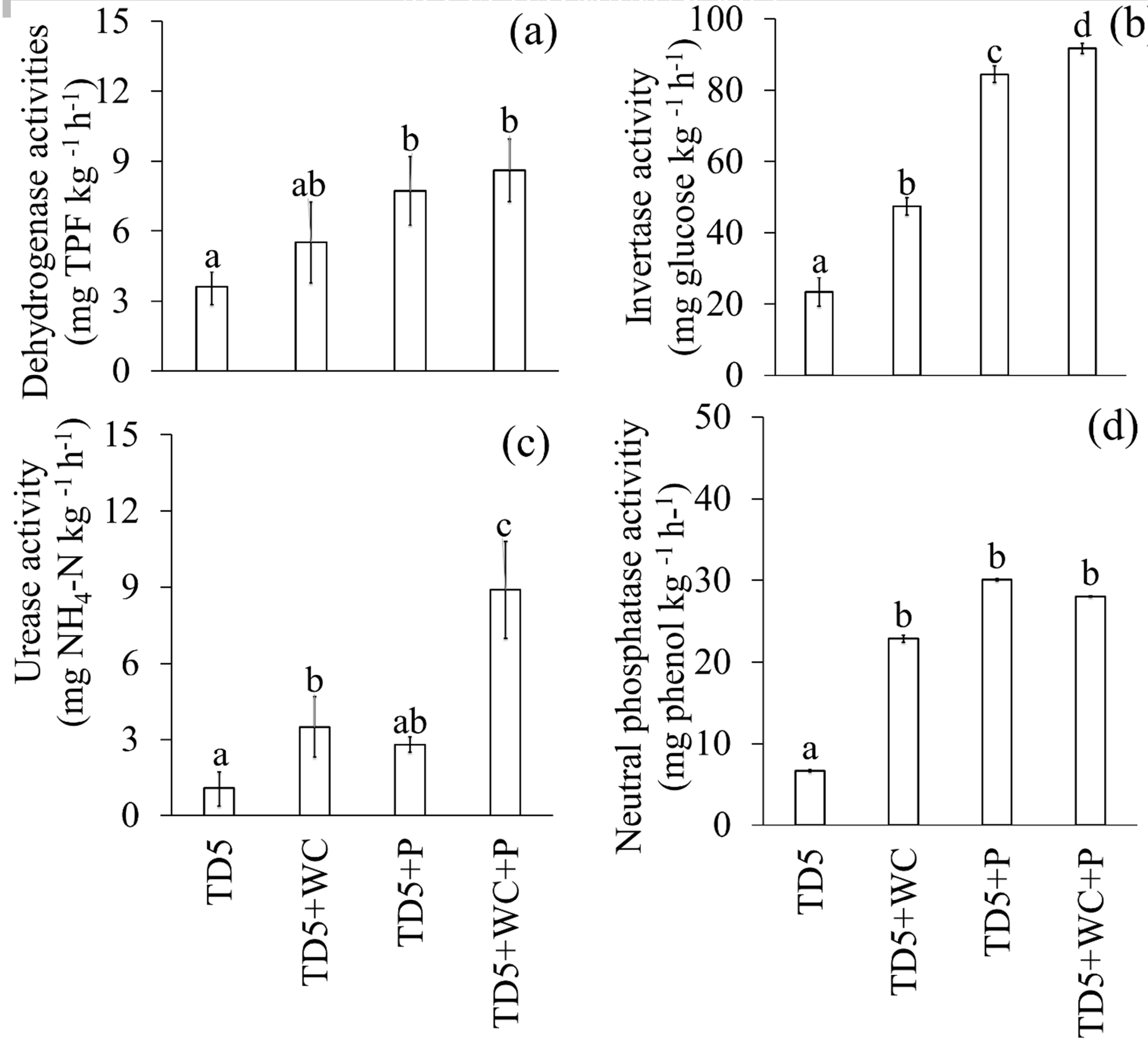

Fig. 3 Activities of dehydrogenase (a), urease (b), invertase (c) and neutral phosphatase (d) in all the treatments. Treatments include TD5: tailing, TD5+WC: TD5 amended with woodchips, TD5+P: TD5 growing plants, TD5+WC+P: TD5 amended with woodchips and growing plants. Values are means (n = 3) with standard error bar; the letters ‘a, b, c, d’ above the bars indicate significant differences among the treatments at the level of $P < 0.05$.

- Plant growth and WC input initiated soil formation in weathered Cu-Pb-Zn tailings
- The treatments increased water-stable aggregates and organic carbon stabilization
- The treatments elevated microbial biomass and key enzymatic activities in the tailings
- Combined woodchips and native plants were preferred to stimulate tailing-soil formation.