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Rehabilitation of organic carbon and microbial community structure and functions in Cu-Pb-Zn mine tailings for in situ engineering technosols — Source link

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THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Rehabilitation of Organic Carbon and Microbial Community Structure and Functions in Cu-Pb-Zn Mine Tailings for *in situ* Engineering Technosols

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Bachelor of Natural Sciences

Master of Sciences

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Abstract

Base metal mine tailings phytostabilisation has been severely hindered by the lack of growth media (soil and inert overburden), and associated hydrogeochemical instability, and phytotoxicity in the tailings. A new paradigm of *in situ* engineered pedogenesis of tailings into functional technosols has been proposed as a cost-effective and sustainable solution. This project aimed to understand critical factors and processes driving soil formation towards functional technosols in Cu-Pb-Zn tailings, which may be manipulated and stimulated by ecological engineering inputs (e.g., organic amendments, microbial inoculum, and pioneer plants).

Native soil characteristics (under native plant communities located in subtropical, semi-arid Mount Isa region) have set the direction of technosols formation and justified the plant biomass-based organic amendment option, to couple with physiological traits (slow growth rates, low water and nutrient requirements) of native plant communities dominant in the investigated region. Both Cu-Pb-Zn tailings (TD5, TD7) from Mount Isa Mines and Cu-Mo-Au tailings from Ernest Henry Mine were used in this study, representing typical hydrogeochemical conditions of tailings.

Organic carbon (OC) is recognised as an overall indicator of technosols formation in tailings. In a 2.5-year old column trial under field conditions with weathered (TD5) and fresh (TD7) Cu-Pb-Zn tailings, exogenous organic amendments (woodchips) rapidly built up OC content with 61.5-80.3 % OC physically protected in aggregates and organo-mineral complexes in the amended tailings, regardless of the mineral weathering stages. N-rich and surface charged organic compounds interacted with tailings minerals (e.g., Fe and Al (hydr-) oxides) to form organo-mineral complexes and aggregates, contributing significantly to OC stabilisation. Native plants (e.g., *Acacia chisolmii*, *Triodia pungens*) survived beyond the time of sampling in the TD5 in the field trial, but not in the TD7. Plant colonisation in TD5 further accelerated technosols formation in the amended tailings, significantly stimulating OC stabilisation, microbial biomass and functions. While the amended tailings were far from reaching the desired hydrogeochemical stability, the pioneer native plant species were proven to be critical to the colonisation of heterotrophic bacteria and associated biogeochemical processes.

Organic matter properties (e.g., labile OC, C: N ratio) induced biogeochemical changes in the tailings with different directions. In a 6-month microcosm experiment, the weathered and neutral Cu-Pb-Zn tailings were amended with plant litter (*Acacia chisolmii*) and biochar. Although little improvement was observed for microbial diversity, the plant litter significantly

increased the labile OC and N, and microbial biomass and enzymatic activities in the amended tailings. Comparatively, bacterial communities more readily recolonised than the fungi in the tailings. The abundance of heterotrophic bacteria affiliated to *Bacteroidetes* and *Proteobacteria* stimulated in the plant litter amended tailings significantly. Interestingly, biochar enhanced the dominance of autotrophic bacteria, *Thiohalobacter* sp., with suppressed rehabilitation of heterotrophic bacteria, probably related to the lack of labile OC and N in the biochar. Combined plant litter and biochar further increased the microbial diversity and functions in the amended tailings.

Topsoil underneath native plant communities rich in microbial inoculums may be used to rapidly prime microbial diversity in the amended tailings. In an 8-week microcosm experiment, the weathered and neutral Cu-Pb-Zn tailings were inoculated with native soils, which had been amended with sugarcane as the base treatment. The colonisation of heterotrophic bacteria and fungi were observed in the tailings-soil mix, strongly linkedto the microbial biomass and functions. Microbial biomass and enzymatic activities increased by 1.5-8 folds in the tailings-soil mix compared to the control, depending on soil addition rates. 25 % soil addition doubled the microbial diversity in the tailings-soil mix compared to the control. 50 % soil addition achieved a respiratory quotient, and C and N cycling processes similar to those of the native soil. Again, *Proteobacteria* and *Bacteroidetes* significantly stimulated in the tailings-soil mix. Stresses including EC (thus S) and total heavy metals (Pb, Zn) had negatively impacts on microbial community.

Biogeochemical changes were investigated in fresh Cu-Mo-Au tailings (containing low levels of reactive minerals with stable hydrogeochemistry) in response to organic amendments (i.e., sugarcane and biochar) and introduction of native grass (*Iseilema vaginiflorum*) and leguminous shrub (*Acacia chisholmii*). Microbial diversity were 2-4 folds in all the amended/revegetated tailings compared to the control. Microbial biomass and enzymatic activities in sugarcane amended and revegetated tailings significantly increased by 4-25 folds, with stimulated abundance of *Bacteroidetes* and the dominance of heterotrophic bacteria (e.g., *Algoriphagus* sp. *Sphingopyxis* sp., *Sediminibacterium* sp., *Planctomyces* sp.), enhancing plants growth. Again, biochar stimulated the dominance of autotrophic bacteria (e.g., *Thermithiobacillus* sp. *Acidiferrobacter* sp.) in the amended tailings. Furthermore, biochar contributed to Cu immobility, considerably reducing Cu uptake by plant roots from the tailings. The introduced pioneer plants effectively involved in rehabilitating microbial community structure and functions, particularly in the sugarcane amended tailings with low levels of reactive minerals and relative stable hydrogeochemistry.

In comparison, the presence of pioneer native plants seemed to be not critical to the development of microbial communities in the tailings amended with labile organic matter (e.g., sugarcane residue), unlike the Cu-Pb-Zn tailings (TD5) after long-term weathering.

In summary, engineering technosols with desired biogeochemical capacity in hydrogeochemically stable Cu-Pb-Zn tailings must be consistent with the physiological characteristics of native plant communities specific to local edaphic and climatic conditions. Technosols formation from the tailings can be initiated and accelerated by plant biomass-based organic amendments coupled with the soil inoculation and the introduction of tolerant pioneer native plants.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed, published journal papers

F You, R Dalal, L Huang. Biochemical characteristics in root zone associated with Acacia and Spinifex to sustain on infertile soil in semiarid northwest Queensland, Australia. Accepted by *Soil Research* on 18 Aug. 2015

X Li, **F You**, P Bond, L Huang (2015). Establishing microbial diversity and functions in weathered and neutral Cu–Pb–Zn tailings with native soil addition. *Geoderma*. 247-248, 108-116.

F You, R Dalal, D. Mulligan, L Huang (2015). Quantitative measurement of organic carbon in mine wastes: Methods comparison for inorganic carbon removal and organic carbon recovery. *Communications in Soil Science and Plant Analysis*. 46 (sup1), 375-389.

X Li, **F You**, L Huang, E Strounina, M Edraki (2013). Dynamics in leachate chemistry of Cu-Au tailings in response to biochar and woodchip amendments: a column leaching study. *Environmental Sciences Europe*. 25 (1):32.

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F You, R Dalal, D Mulligan, L Huang (2013). Microbial Biomass and Activities in Response to Nutrition and Toxicity Factors in Amended Base Metal Mine Tailings. ASA, CSSA, and SSSA International Annual Meetings, Tampa, Florida, USA. 3-6 November.

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Contributor	Statement of contribution
Author Fang You (Candidate) Co-first-author	Experiment set up and lab incubation (100%) Microbial activities analysis (100%) Manuscript drafting (30%)
Author Xiaofang Li	Initial the hypothesis (50 %) Physiochemical assays and bioinformatics analysis (90 %) Manuscript drafting (60%)
Author Phil Bond	Manuscript discussion and revision (5 %)
Author Longbin Huang	Initial the hypothesis (50 %) Project funding (100 %) Manuscript discussion and revision (5 %)

Note: XL and FY both contributed 50 % of the research in terms of working hours.

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Contributor	Statement of contribution
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Author Longbin Huang	Project design and manuscript preparation and revision (15 %)
Author David Mulligan	Manuscript editing (5 %)

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Contributor	Statement of contribution
Author Fang You (Candidate)	Experimental design and manuscript preparation (70 %)
Author Ram Dalal	Experimental discussions and manuscript revision (15 %)
Author Longbin Huang	Project design and manuscript preparation and revision (15 %)

Contributions by others to the thesis

My advisors, Associate Professor Longbin Huang of the University of Queensland, Centre for Mined Land Rehabilitation, Professor Ram Dalal of Department of Science, Information Technology, Innovation and the Arts, Queensland Government and Dr. Xiaofang Li of the University of Queensland, Centre for Mined Land Rehabilitation contributed to editing the thesis prior to submission.

Dr. Xiaofang Li also made significant contributions to Chapter 6 "*Establishing microbial diversity and functions in weathered and neutral Cu–Pb–Zn tailings with native soil addition*" with significant input to the interpretation of experimental data and manuscript preparation. Furthermore, he also critically revised the draft of the Chapter 5 and Chapter 7 of this thesis.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

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Keywords

technosols, OC stabilisation, microbial community, pyrosequencing, enzymes, Cu-Pb-Zn tailings, pioneer native plant, Mount Isa

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FoR code: 0605, Microbiology, 30 %

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List of Figures & Tables

List of Abbreviations used in the thesis

AMF	arbuscular micorrhizal fungi
ANOVA	analysis of variance
CCA	canonical correspondence analysis
CEC	cation exchange capacity
CF-IRMS	continuous flow isotope-ratio mass spectrometry
CFU	colony forming units
CP/MAS ^{13}C-NMR	cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance
DCB	dithionite-citrate-bicarbonate
DNA	deoxyribonucleic acid
EC	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
EHM	Ernest Henry Mine
FISH	fluorescence in situ hybridization
GC-FID	gas chromatography-flame ionization detector
GDP	gross domestic product
Gold	genomes online database
IC	inorganic carbon
ICP-OES	inductively coupled plasma optical emission spectroscopy
LOD	limit of detection
LOQ	limit of quantification
LSD	least significant differences
Macro-iPOC	organic carbon of intra-macroaggregate particulate fraction
Macro-iMOC	organic carbon of intra-macroaggregate silt+clay fraction
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
Micro-iPOC	organic carbon of intra- microaggregate particulate fraction
Micro-iMOC	organic carbon of intra-microaggregate silt+clay fraction
MIM	Mount Isa Mine
MOC	minral associated organic carbon
Mt	million tons
MV	million volts

SINA	SILVA incremental aligner
SMI	Sustainable Mineral Institute
SOM	soil organic matter
TC	total carbon
TD	Tailings dam
TD3	Tailings dam 3
TD5	Tailings dam 5
TD7	Tailings dam 7
TOC	total organic carbon
TPF	triphenyl formazan
TSF	tailings storage facility
TTC	triphenyl-tetrazolium chloride
OC	organic carbon
OM	organic matter
oMOC	organic carbon associated silt+clay particles
OTU	operational taxonomic unit
PCA	principal component analysis
PCR	polymerase chain reaction
PEAR	paired-End reAd mergeR
PGPB	plant growth promoting bacteria
PLFAs	phospholipid fatty acids
PLFA-ME	phospholipid fatty acid methyl esters
PNAST	python nearest alignment space termination tool
RDA	redundancy analysis
QC	quality control
QIIME	quantitative insights into microbial ecology
RSD	relative standard deviation
UQ	The University of Queensland
WC	woodchips
WHC	water holding capacity
WSOC	water-soluble organic carbon
WSN	water-soluble nitrogen
16S rRNA	16S ribosomal ribonucleic acid

Chapter 1 Introduction

1.1 Problem statement

Australia is a leading producer of minerals, producing at least 19 minerals (in significant amounts) from close to 400 operating miners (Geoscience Australia, 2015). The industry contributes to more than 10 % of the national gross domestic product (GDP), with the production of most minerals growing. In particular, the production of copper (Cu), black coal, lead (Pb), zinc (Zn), and iron (Fe) ores has increased markedly over the last decade (Mudd, 2010; Sutton and Dick, 1987). From the mid-1800s to 2008, cumulative Cu, Pb and Zn production (kt) in Australia reached 20473, 37945, and 48465, respectively, in which their production in Queensland accounted for 50-60 % (Mudd, 2010).

Intensive mining and processing activities generate vast volumes of tailings. Tailings are mine wastes and mineral residues left behind following extraction of the metals via various combinations of mining processing methods. Briefly, metal-rich ore is ground to a fine particle size, and then separated by flotation into metal-rich concentrates and by-products of low and uneconomic grade. These by-products, referred to as mine tailings, are usually deposited in the slurry form in purposely-constructed dams or storage facilities (EPA, 1994). In Australia, the mining industry produces 1750 million tons (Mt) of mine waste per year (Lottermoser, 2010), with an estimated 135 Mt tailings produced from Cu mines between 1899 and 2005 (Mudd, 2007).

Tailings typically contain various biotoxic and hazardous chemicals and minerals in significant quantities. These include heavy metals, metalloids, radioactive elements, acids and bases, all of which pose a long-term threat to both environmental and human health (Järup, 2003). Finely-grained tailings may contaminate the surrounding area through wind dispersion and/or water erosion. Furthermore, sulphide-rich tailings are potential sources of metals and acidity resulting from oxidation and mineral weathering (Bobos et al., 2006). High concentrations of heavy metals and acid mine drainage have not only been found in mined sites (Aykol et al., 2003), but also in local streams and waterways (Fields, 2003). Therefore, rehabilitation of tailings is a legal requirement and legitimate community expectation. Phytostabilisation (surface stabilisation with plant cover) has been advocated as a sustainable and cost effective solution across mined landscapes including those occupied by mine wastes (Mendez and Maier, 2007).

However, successful phytostabilisation for non-polluting and sustainable outcomes is challenging in tailings landscapes (Huang et al., 2012). Base metal mine (e.g., Cu, Pb, Zn) tailings present a range of physical and (geo)chemical constrains to plant growth and recruitment. They typically lack organic matter and available macronutrients (e.g., nitrogen, phosphate) (Ye et al., 2000). Fresh tailings consist of crushed rock containing large proportions of silt and fine silt, and thus lack proper physical structure for soil hydraulic functions and root penetration. Following sedimentation, tailings have a high bulk density (> 1.8×10^3 kg m^{-3}) and mechanical resistance, and lack water stable aggregates and macropores (Huang et al., 2012; Lottermoser, 2010). Furthermore, tailings are characterised by very high levels of salts, heavy metals (and/or metalloids) and potential acidity (if not sufficiently neutralised) (Dold and Fontboté, 2002). The inherent ecotoxicity from these contaminants can prevent natural colonisation of plants in tailings for many decades (Ye et al., 2000). To overcome these primary constraints and create reasonable growth conditions for pioneer plants, several amendments are usually applied before planting. Conventional practices and amendments (e.g., fertiliser, ripping, organic amendments, mulch, liming etc.) target the primary constraints in tailings related to plant establishment and growth (Ram and Masto, 2010). Most studies on tailings phytostabilisation have focused on plant growth relying on continuous inputs of amendments with little information related to long-term sustainability of the rehabilitated tailings (Mendez and Maier, 2007). In addition, conventional cover systems require mega volumes of growth media (including improved inert overburden and/or topsoil) to reconstruct root zones for target plant communities. However, at many mine sites in Australia, this option is constrained by material shortage, expensive transport cost and secondary site disturbances for sourcing those cover materials.

Recently, a new approach has been proposed based on years' of field research at Mount Isa Mines (MIM), namely, engineered pedogenesis (soil and soil horizons) of tailings into functional technosols through purposely oriented and stimulated processes to achieve non-polluting and sustainable phytostabilisation across tailings landscapes (Huang et al., 2014a; Huang et al., 2012; Li and Huang, 2015). 'Technosol' is a developed taxa by the World Reference Base for Soil Resources for soils with human interventions, such as soils in urban/industrial area followed by landfills, farming, earth movement and heavy metal contamination, and agricultural area with erosion, ripping, and/or land levelling. In this study, the use of "engineered" rather than 'anthropogenic' pedogenesis emphasises that tailings-soil are consequences of mining engineering, mineral processing and ecological engineering inputs at the initial phase. These processes initiate soil formation under the local climatic conditions with minimal continuous human inputs at the later phases.

Based on the knowledge from natural ecosystems, soil formation is the consequence of alteration and transformation of parent minerals from long-term abiotic and biotic interactions (Jenny, 1941). Decades to millennium is required to alter earth rock into parent materials for soil, with plant colonisation greatly accelerating the soil formation at the later phases (Van Breemen and Buurman, 2002). Continuous organic matter transformation (decomposition, humification, carbon and nitrogen cycles) principally from plants incorporated into the soil, greatly modifies soil physicochemical properties and biogeochemical processes. Particularly, soil microorganisms are the most active agents in stabilisation soil physical structure and facilitating the soil functions.

In contrast, tailings properties are mainly inherited from the mineralogical profile of the original ores and hosting rocks. Their geochemistry from mineral processing, are normally characterised with abundant reactive minerals (e.g., pyrite, chalcopyrite) and high specific surface area (due to their fine particle size). At best, these can be classified as parent minerals subject to rapid oxidation and dissolution when exposed to air and water. Therefore, engineered pedogenesis from geochemical unstable tailings to biogeochemically functional technosols requires purposely oriented ecological engineering to simulate the deterministic physical, chemical and biological processes that would enhance geochemistry stability, and rehabilitate biogeochemical properties and functions (such as build-up and stabilisation of organic carbon, recovery of soil-like microbial community and functions, and rehabilitation of organic matter decomposition and nutrient cycling processes to couple with target plant communities) (Huang et al., 2014; Li and Huang, 2015).

1.2 Research objectives

Understanding organic carbon dynamics and microbial community structure and functions in engineered tailings-soil (technosols) is crucial to the development of effective ecological engineering methodologies and technologies to form technosols to sustain target plant communities for tailings phytostabilisation. However, there have only been limited studies in literature systematically investigating critical factors and processes involved in *in situ* engineered technosols formation in the tailings. Biogeochemical rehabilitation is promising in the tailings with a relatively stable hydrogeochemistry, such as those have undergone rapid oxidation and weathering of most of the reactive minerals (e.g., sulphides) (Huang et al., 2014).

The present study is timely and important to the development of cost-effective and sustainable methodology for base metal mines tailings phytostabilisation within Australia and across the world. This is one important component within Mount Isa Mine Tailings Revegetation Research Project, which investigates critical factors and processes involved in the development of biogeochemical properties and functions in response to changes induced/stimulated by ecological engineering options in Cu-Pb-Zn tailings. In detail, this study will evaluate the efficacy of organic amendments, microbial inoculums and pioneer plant roles in inducing and stimulating biogeochemical changes along desired directions, such as the organic carbon stabilisation, aggregation, rehabilitation of the soil-like microbial community structure and microbial functions (such as enzymes required in nutrient cycling).The specific aims of this research include:

(1) Establish methods for characterising organic carbon pools and forms in Cu-Pb-Zn tailings with different mineral compositions and amendments.
(2) Investigate the role of organic amendments in stimulating aggregation and organic carbon stabilisation. It is hypothesized that organic carbon derived from exogenous organic matter can be stabilised via interaction with tailings minerals and aggregation, leading to gradual development of soil structure and functions in the amended tailings.
(3) Investigate the role of pioneer plants and organic amendments with contrasting labile organic carbon and N contents in the rehabilitation of microbial community structure and functions in the amended tailings. It is hypothesized that introduction of pioneer plant species and labile organic matter can stimulate the colonisation of rhizosphere microorganism and shift microbial communities from autotrophic to heterotrophic dominant species, closely linked to rehabilitation of biogeochemical processes for technosols formation.
(4) Evaluate the efficacy of topsoil inoculum in the rehabilitation of biogeochemical properties and functions in amended tailings, including the microbial diversity, microbial community composition and dominant microbial groups, and key soil enzymatic activities. It is hypothesized that native microbes in the topsoil from native vegetation sites can be introduced into the tailings amended with labile organic matter and stimulate the development of soil-like microbial communities in the tailings-soil mix; and from the above
(5) Develop integrated knowledge on effective ecological engineering inputs and associated tailings environmental factors in driving the rehabilitation of organic carbon, microbial communities and associated biogeochemical processes in relation to the formation of functional technosols.

1.3 Organisation of thesis

The thesis is divided into eight chapters.

Chapter 1 (the current chapter) presents a broad overview of project background and research objectives.

Chapter 2, *Literature Review*, includes existing knowledge in the literature, on: (1) characteristics of soil organic matter, biogeochemical processes influencing its dynamics and associated soil properties and functions; (2) biogeochemical changes induced by tailings amendments for supporting plant growth; and (3) the importance of rehabilitation of biogeochemical capacity and processes in the formation of technosols. It provides an overview of the up-to-date understanding on the topic area. A set of key research questions have been raised based on the knowledge gaps identified. These questions forms the basis for research focuses in the following chapters.

Chapter 3, *Organic carbon stabilisation in weathered and fresh Cu-Pb-Zn tailings amended with woodchips under field conditions,* details and investigates OC characteristics and stabilisation mechanisms in Cu-Pb-Zn tailings in response to exogenous organic amendments. OC fractionation and quantification methods were applied to characterise OC in the Cu-Pb-Zn tailings representing typical biogeochemical conditions in response to woodchips application in a 2.5-year field column incubation.

Chapter 4, *Plant colonisation stimulated organic carbon formation and microbial biomass and functions in weathered Cu-Pb-Zn tailings from a long-term field trial*, contains findings on the roles of introduced native plants in stimulating the colonisation and shift of microbial communities, aggregation and associated biogeochemical processes in the amended tailings. Information about underlying biogeochemical processes of OC stabilisation and preferred OC provides fundamental information to enhance OC stabilisation and associated structure and functions rehabilitation in tailings.

Chapter 5, *Biogeochemical changes induced by addition of exogenous organic matter with contrasting properties in weathered and neutral Cu-Pb-Zn tailings – a 6-month microcosm study*, presents findings from a controlled study to evaluate efficacy of organic amendments with contrasting properties applied individually or in combination on tailings amelioration in laboratory conditions. The findings explain how microbial community composition and functions responded to physicochemical properties induced by organic amendments without association of native plant species in the tailings.

Chapter 6, *Establishing microbial diversity and functions in weathered and neutral Cu–Pb–Zn tailings with native soil addition*, presents findings about the roles of topsoil from native vegetation sites in combination with organic amendments in the rehabilitation of biogeochemical properties and functions along soil addition gradients, including microbial community structure and enzymatic activities. The findings have been published in *Geoderma* under the same title.

Chapter 7, *Sugarcane and biochar differ in ameliorating Cu-Mo-Au tailings for phytostabilisation with native plant species-A greenhouse study*, presents findings from a study based on a greenhouse experiment, where neutralised Cu-Mo-Au tailings were amended with a combination of organic amendment and direct native plant colonisation. The efficacy of plant based biomass and recalcitrant biochar on tailings amelioration is not only evaluated in terms of rehabilitation of organic carbon, microbial community and functions, but it was also assessed for their linkage to typical tolerant native plants establishment and growth. This information is useful to guide efficient amendment formulation for tailings phytostabilisation with native plant species.

Chapter 8, *General discussion and conclusions and future research needs*, summarises the major findings and develops a conceptual model to encapsulate knowledge on the pathways to rehabilitate organic carbon, microbial community structure and functions in the tailings with ecological engineering inputs, leading the formation of functional technosols, which can then be integrated into reconstructed root zones to support sustainable native plant species and communities.

The three appendixes provide background information about method validation, biochemical properties of natural soil supporting the acacia-spinifex open woodland communities in a colluvial plain (a potential reference site for rehabilitation completion), and a summary of Mount Isa Mine tailings rehabilitation practices. Specifically, Appendix A compares two methods in terms of accuracy and reliability for OC quantification in mine wastes. Appendix B characterises physicochemical and biological properties in natural soils colonised by keystone native plant species (i.e., *Acacia chisolmii* and *Triodia pungens*) from a colluvial plain landscape, in order to understand plant-soil-microbe interactions to set the benchmarks and goals for engineered pedogenesis in the tailings. Lastly, Appendix C summarises current amendment practices conducted in Mount Isa Mine with general amendment strategies formulated based on previous studies.

Overall, this thesis covers a number of laboratory incubation, glasshouse and field experiments examining carbon (and nitrogen) pools and dynamics, and microbial community diversity, composition and functions in the mine tailings, which critically contribute to technosols formation in the amended tailings. The work employs many methodologies and technologies to characterise changes in the physical, chemical and biological properties and processes in the tailings treated by ecological engineering options, along the directions of technosols development. These include carbon fractionation and quantification, investigation of microbial community diversity and structure with aid of advanced molecular techniques, and microbial activities based on enzyme assays analysis (Fig. 1-1).

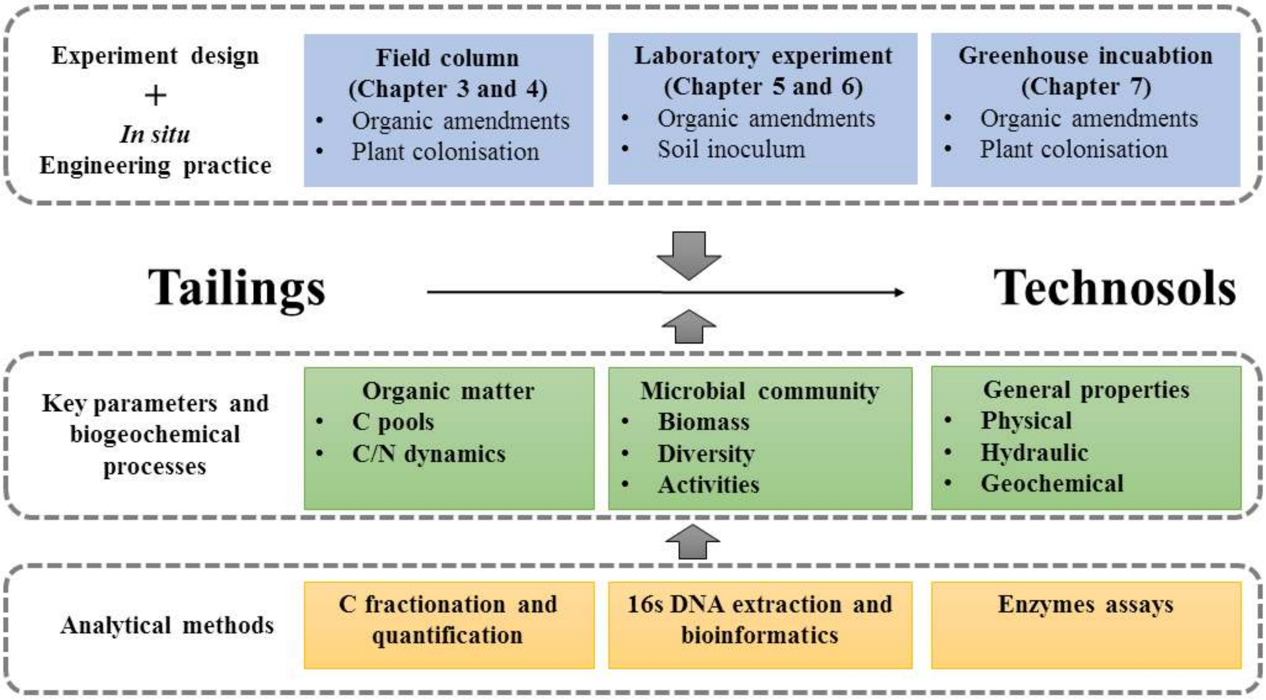

Fig. 1-1 Schematic chart of structure of this thesis

Chapter 2 Literature review

2.1 Importance of soil organic matter and microbial communities in soil biogeochemical functions

2.1.1 Soil organic matter affecting soil properties and functions

Soil organic matter (SOM) has been defined as the organic fraction of soil, including plant, animal, and microbial residues, in fresh forms and forms at all stages of decomposition, and the relatively resistant soil humus (Nelson and Sommers 1982). In natural soil, SOM is comprised mainly from inputs of plant litter, dead roots and root exudates (Tate et al., 2000), which plays a critical role in maintaining soil fertility (Torn et al., 2005) and regulating chemical environment and stabilising physical structure (Oades, 1984). SOM represents the dominant source of plant available nutrients (e.g., N, P, S) in natural ecosystems (Gardenas et al., 2011). In soils with low activity clay or low clay contents, up to 97 % of cation exchange capacity (CEC) is contributed by SOM, related to its abundant charged surface functional groups (e.g., carboxyl and phenolic-OH groups). Also, functional groups in the SOM is the foundation of organo-mineral interactions and aggregation, thus contributing to improving soil physical structure and hydraulic properties (Tisdall and Oades, 1982). Although accounting for only approximately 5 %, SOM is the critical substrate in many soil biogeochemical processes (e.g., SOM mineralisation and nutrient mobilisation, organo-mineral interactions, cation exchange and adsorption, and pollutant removal) (Rovira and Vallejo, 2002). As it is difficult to quantitatively estimate the amount of SOM present in a soil, it is normally estimated by multiplying the organic carbon (OC) concentration by a conversion factor based on the percentage of carbon (C) in organic matter alternatively. Published OC-OM conversion factors for surface soils have varied from 1.724 to 2.0 (Nelson and Sommers 1982).

Table 2-1 Turnover, composition, function and representative fractions and fractionation methods of OC pools

OC Pools	Turnover time	Percentage (%)	Composition	Functions	Representative fractions	Fractionation Methods
Labile pool	Months to years	1-5	Molecules readily removed from the soil by living organisms Readily soluble molecules	Early indicator of soil C dynamics; Energy source for microorganisms	Water soluble organic carbon (WSOC); Free OC in light fraction (<1.6 g·cm^{-3}); OC in the coarse fraction (>250 μm).	Water extraction; Density fractionation; Particle-size fractionation
Slow pool	Decades	60-85	Chemically recalcitrant but moderately decomposable materials in the form of macro-aggregate	Important source of mineralisable nutrients; Physical and chemical soil properties	OC in the aggregate (53 μm-250 μm); Mineral-associated OC soluble in hydrofluoric acid (<1.6 g·cm^{-3}).	Particle-size fractionation; HF demineralization
Stable pool	Millennia	10-40	Polymerized substrates; Microaggreates, organo-minenal complexes; Charcoal	Formation of stable aggregates; Soil structure and chemical buffering capacity	silt+clay particles associated OC (< 53 μm); Mineral-associated OC in density fractions in sodium polytungstate or other heavy liquids/ solutions (1.6-2.0 g·cm^{-3}); OC resistant to oxidation unless under high temperature and pressure (e.g., charcoal).	Particle-size fractionation; Density fractionation; Dumas high-temperature combustion (above 450 ℃)

Depending on its turnover time and decomposition rate, total OC can be categorised into labile, slow and stable OC pools (Jenkinson, 1990). As summarised in Table 2-1, the labile OC pool turns over relatively rapidly, including molecules readily consumed by soil organisms and readily soluble organic molecules (Marriott and Wander, 2006). The relatively slow pool of OC is normally represented by physically or chemically protected OC, which have slower turnover rates than the labile pool (Denef et al., 2001). The stable OC pool is mainly composed of humic polymers, intercalated OC in microaggregations, organo-mineral complexes and charcoal (John et al., 2005), which collectively contribute to chemical buffering capacity and soil physical structure and hydraulic properties (Brodowski et al., 2007).

A range of physical and chemical fractionation procedures have been developed to approximately separate various OC pools, including physical (particle size or density separation) and chemical (solubility, mobility) fractionation methods (Helfrich et al., 2007). Although these fractions are only approximate estimation of labile, slow and stable OC pools (with missing and overlay among them) rather than accurately measuring OC pools, they do contribute to our understanding about OC dynamics and associated biogeochemical processes in soils.

Metal mine tailings are normally comprised of finely ground particles (ranging from fine silt to fine sand) and consist residue minerals of high reactivity, where there are little OC contents (Lottermoser, 2010). The initial level of total organic carbon (TOC) in copper (Cu) tailings can be less than 0.1 % (Ye et al., 2002). The extremely low levels of TOC deprive the tailings from the potential to develop basic physical, chemical and biological properties and functions as 'soil' for productive and healthy plant communities. As a result, rehabilitation of SOM and OC stabilisation is critical to technosols formation, which is required for the development of geochemical stability, hydraulic properties and physical structure and nutrient cycling and supplying capacity in tailings. Although TOC in the tailings can be rapidly increased by direct input of organic amendments, the formation and distribution of various OC functional pools in tailings may be a complex process. As a result, it is necessary to understand characteristics of SOM and OC forms and distribution to evaluate effective options of ecological engineering to speed up technosols development in tailings. However, until recently there was limited information available concerning OC pools or fractions in tailings and especially those under field conditions.

2.1.2 Biogeochemical processes and SOM dynamics

SOM decomposition and coupled nutrient cycling processes

In natural ecosystems, plant available nutrients are mainly from SOM decomposition and nutrient cycling. Mineralisation of 1.5-3.5 % of the organic nitrogen (N) would provide sufficient mineral N for the growth of natural vegetation in most soils except for those with low SOM (Kemmitt et al., 2008). Also, ecophysiological requirements of plant species are closely linked to the SOM decomposition and nutrients cycling processes (Wardle et al., 2004). Specifically, a fertile and productive ecosystem is often colonised by fast growing, short-lived plants with high quality litter (high N content, low lignin) returning to soil, and rhizosphere microbial community is likely to be bacteria dominated which enable rapid decomposition rates to supply nutrients at relatively higher rates for plants. On the other hand, in an infertile and less productive ecosystem, plant species are slow growing and long-lived with low quality litter, coupled with rhizosphere microbial communities with more fungi enabling relatively slower litter decomposition and nutrient supply rates for the plants. More than 85 % of litter decomposition in natural soil is mediated by microorganisms (Zhang et al., 2008). Decomposers (e.g., bacteria, fungi) quickly utilise newly added organic materials and start the decomposition process with the aid of enzymes hydrolysing complex molecules and polymers to smaller molecules. As shown in Fig. 2-1, during decomposition, there is initially a large decrease of water soluble organic matter in litter/organic amendments, followed by a decline in cellulose and hemicellulose and an increase of lignin (Sina, 2003). Chemical composition analysis suggests that the degree of decomposition can be indicated by declines of carbohydrates, increases of relative proportion of alkyl C and carboxyl C, and the breakdown of lignin. In addition, the C: N ratio decreases as decomposition proceeds, as N retented in microbial biomass or by-products while C is respired into CO_2 (Quideau et al., 2000).

Enhancing the formation and accumulation of OC pools in tailings is a critical aspect of engineering tailings into biogeochemically functional technosols. Therefore, ascertaining SOM decomposition and nutrient cycling processes in tailings and its linkage with plant species is fundamental to the adoption of necessary and effective ecological engineering options for developing functional technosols from the tailings. This informs the necessary properties and functions that permit sustainable growth and development of target plant species and communities for tailings phytostabilisation.

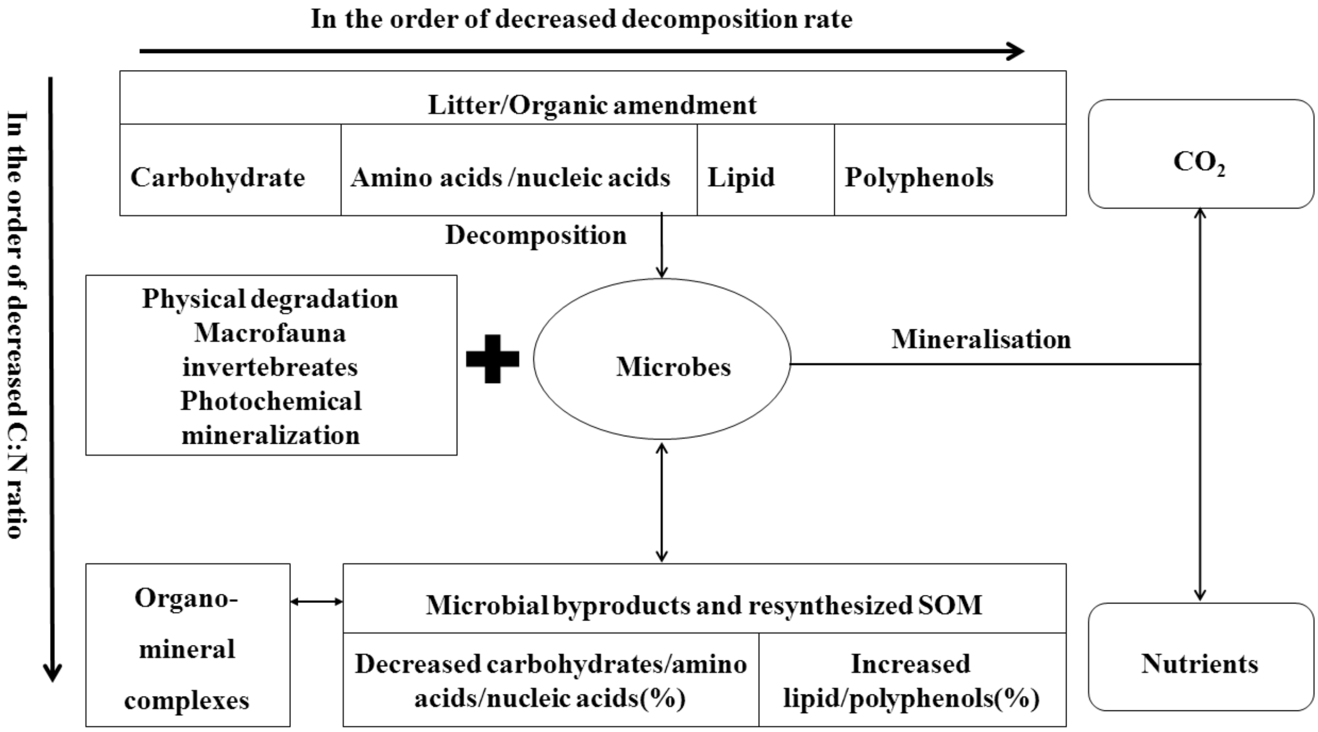

Fig. 2-1 SOM decomposition, nutrients cycling and chemical change

Mechanisms involved in SOM stabilisation, organo-mineral interactions and aggregation

The formation of stable SOM in soil is important to soil structure and functions. It is widely recognised that several biotic and abiotic processes are involved in SOM stabilisation. These include: (1) chemical recalcitrance of organic matter with complex structure retarding its degradation by microorganisms (e.g., lignin, wax) (Marschner et al., 2008); (2) interactions between organic molecules with inorganic (e.g., Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}) or other organic substances (Kleber et al., 2007); and (3) physical protection by forming barriers between microbes and enzymes to organic substance (e.g., aggregates, clay occluded OM) (Six et al., 2002).

Two typical forces between organic matter and mineral surfaces are observed, including strong force (i.e., ligand exchange, polyvalent cation bridges) and weak force (i.e., hydrophobic interaction, H-bonding and van der Waals forces) (Kogel-Knabner et al., 2008). Among them, ligand exchange and polyvalent cation bridges are the dominant mechanisms to form stable organo-mineral complexes in soil. For instance, in kaolinite and montmorillonite, contribution to absorption ability of different interactions are in the order: cation bridging (40 %)> ligand exchange (33 %)> van der Waals force (22 %) (Feng et al., 2005). The binding mechanisms depend strongly on the surface chemistry of SOM and clay minerals. Among functional groups of SOM, carboxylic C with negative charges has the highest potential to bind minerals, followed by phenolic and hydroxylic C (Kumar et al., 2007), while alkyl and aromatic C which are relatively nonpolar or hydrophobic, are little involved in organo-mineral interactions.

Table 2-2 Typical interactions between organic matter and minerals, adapted from Kögel-Knabner et al. (2008)

Interaction force	Organic matter	Minerals
Strong binding force		
Ligand exchange	OM functional groups (aliphatic or phenolic OH) Aliphatic acid (citric acid, malic acid) Amines, ring-NH, heterocyclic-N;	OH groups on Fe, Al, Mn oxides OH groups on edge sites of phyllosilicates; Allophane, imogolite.
Polyvalent cation bridges	OM functional groups (carboxyl, carbonyl, alcoholic OH-) Microbial polysaccharides with glucuronic-, galacturonic-,mannuronic-, pyruvic-, succinic-acid	Expandable layer silicate (e.g., smectite, vermiculite, illite); Electrostatic cation bridges (Fe^{3+}, Al^{3+}; Pb^{2+} Ca^{2+}, Mn^{2+}, Mg^{2+}).
Weak binding force		
Van der Waals force	Uncharged non-polar groups (aromatic, alkyl-C)	Non-expandable layer silicates, neutral microsites on smectites, quartz sand;
Hydrophobic interaction		Non-polar, uncharged surface.
H-bondings	OM functional groups (carboxyl, carbonyl, phenolic OH^-); amines, heterocyclic-N.	Minerals with oxygen surfaces (e.g., kaolinite).

In particular, organo-mineral interactions are the basis for aggregation and soil structure stability (Shang and Tiessen, 1998). Plant rootlets, fungal hyphae, cell filaments and secretions from living organisms interact with mineral components to form clumps and increase the size and stability of aggregates (Denef et al., 2001). Pores formed between and within aggregates allow gas exchange (large pores: 30-60 μm) and retention of water and nutrients (small pores: 0.2-60 μm) (Skopp, 1981).

Several studies have reported the significantly positive impacts from organic inputs on aggregation (Kong et al., 2011). Despite a much improved understanding on the organo-mineral interactions and aggregation in soil (Kaiser and Guggenberger, 2003), significant gaps remain in our current understanding about SOM stabilisation processes in the mine tailings, which is anticipated to be distinct from those in natural soils depending on tailings mineralogy and forms of extraneous organic inputs. Organic amendments may stimulate organo-mineral interactions and aggregation in tailings. Therefore, understanding mechanisms of SOM stabilisation and its linkage to aggregation is important to tailings physical structure improvement and biogeochemical functions recovery.

2.1.3 Microbial community composition and functions in tailings

SOM dynamics driven by microorganisms is indicative of biogeochemical linkages between engineered soil and plant systems, which is an useful indicator for assessing the success of tailings rehabilitation (Harris, 2003; Harris, 2009). In spite of the recognised importance of diverse and functioning microbial communities for tailings phytostabilisation, the evolution of microbial communities in the engineered technosols remains poorly understood in literature, with and without amendments.

As microorganisms rely on energy from SOM decomposition or mineral oxidisation and nutrients (e.g. N and P) to grow (Torsvik and Øvreås, 2002), forms of SOM, minerals and levels of nutrients have marked impacts on microbial community composition and associated biogeochemical processes. Other environmental variables (e.g., moisture, temperature, pH, osmotic conditions) may also significantly influence composition and functions of microbial communities in the tailings. Some optimum conditions for microbial communities in soil have been summarised in Table 2-3 (Madigan et al., 2006).

Table 2-3 Environmental factors and optimum conditions for microbial community

Environmental factor	Optimum conditions
Available soil moisture	25-85 % Water holding capacity
Oxygen	>10 % air-filled pore space for aerobic degradation
Redox potential	Eh> 50 mill volts (mV)
Nutrients	C: N: P=120: 10: 1 molar ratio
pH	6.5-8.0
Temperature	20-30 ℃
Heavy metals	≤700 ppm

Tailings represent an extremely and geochemically dynamic (rather than stable as in soil) environment with strong selection pressure on microbial colonisers. The extremity and intensity of physical and geochemical stresses are closely related to the shift of structure, composition and functions of microbial communities, due to the loss and/or gain of species/function diversity (Schimel et al., 2007). Tailings are typical of low species and functional diversity, with low levels of microbial biomass, microbial activities and energy utilisation efficiency. For instance, in Cu tailings, total cell counts are around 10^9 g^{-1} (Diaby et al., 2007), much lower than that (10^{12} g^{-1}) in typical and healthy soil (Or et al., 2007). Also, species richness is very low, with only a few species abundant in Pb-Zn tailings (Mendez et al., 2008).

Because of the lack of SOM, microbial communities in tailings mainly comprise of microorganisms that tolerant of extreme geochemical conditions and rely on chemical energy from primary and secondary minerals in the tailings (Li et al., 2014; Schimel et al., 2007). Among them, the most intensively studied microorganisms are related to S and Fe oxidation (e.g., *T. ferrooxidans*) (Fortin et al., 2000). Some recent molecular examinations of microbial communities in base metal mine tailings (Kock and Schippers, 2008) suggest that in addition to low microbial biomass and diversity, some essential groups (e.g., fungi) may be absent (Kock and Schippers, 2008; Li et al., 2014) and result in a very limited capacity to decompose complex organic compounds (e.g., lignin) (Blanchette, 1995). Furthermore, tailings are normally characterised with much lower microbial metabolic activities than soils (Chen et al., 2005). The microbial limitation in the tailings significantly lower the rate of bioweathering of unstable minerals and development of functional technosols within foreseeable timeframe (e.g., years-decades). As a result, ecological engineering options will be needed to stimulate microbial-mediated processes involved in mineral weathering and hydrogeochemical stabilisation, which forms the foundation for the development of soil-like microbial communities in the tailings-soils.

Many sulfidic tailings are biologically toxic and not hospitable for natural colonisation of soil microorganisms and plants, because of their acidic pH conditions, elevated soluble metal concentrations and acute heavy metal toxicities (Ortega-Larrocea et al., 2010). In extremely acidic tailings, all phylotypes identified are closely related to S- and Fe- oxidising bacteria with high degree of phylotype dominance (e.g., *Leptospirillus ferriphilum; Sulfobacillus, Acidimicrobium ferrooxidans*) (González-Toril et al., 2003). The relative degree of microbial diversity and community complexity increases with pH rise to slightly acidic conditions in the tailings, where microorganisms with neutrophilic growth preference are colonising (e.g., *thiosulfate*, ferrous iron and arsenite oxidisers), thus permitting the recolonisation of microbial communities resembling those in local natural soil (Mendez et al., 2008).

Sulphur/Fe-oxidising bacteria are the most intensively investigated microbes, which mediated biogeochemical processes in sulfidic tailings, such as pyrite weathering and acid mine drainage formation (Dold and Fontboté, 2001; Lindsay et al., 2009). However, the most important microbial activities in soil are decomposition and turnover of structural complex and highly diverse organic matter from plants and associated nutrient cycling (Berg, 2000; Moretto et al., 2001). Yet, to date knowledge concerning the evolution of soil-like microbial communities and associated SOM decomposition is limited, save for a few studies on the monitoring of SOM decomposition and nutrient cycling processes in tailings (Hulshof et al., 2003; Moynahan et al., 2002).

Reconstructing a soil-like habitat in tailings may be required to sustain diverse and functioning microbial communities, which underpins the development of biogeochemical functions and sustainable development of target plant community. Therefore, detailed evaluation of biogeochemical dynamics in tailings in response to ecological engineering options is required, in order to develop suitable options for stimulating recolonisation and development of soil-like microbial communities and technosols formation. In particular, detailed investigations are to be carried out to understand key environmental drivers of changes in microbial community composition and functions in the amended tailings, which forms the basis for developing efficient ecological engineering strategies and options to engineer functional technosols for tailings phytostabilisation.

Characterisation of microbial community composition and activities

Methods applied to quantify microbial community have been summarised in Table 2-4, and they comprised direct enumeration, chloroform fumigation and methods based on molecular technology (e.g., PLFA, 16S rRNA) at higher resolution. Advancement in biotechnology has allowed the development of molecular techniques using biomarkers for measurements of the whole, or selected parts of microbial communities, which has received significant development in the last decade (Chen et al., 2008; Li et al., 2014). Collectively this has enhanced knowledge of microbial communities in less known and complex soil ecosystems.

In general, methods based on molecular technology provide extensive information about taxa present in soil or tailings, but without providing the information about microbial species effectively and specifically facilitating biogeochemical processes in soil. It is common to combine microbial activities with microbial composition for microbial community characterisation in complex systems.

Depending on the biogeochemical processes concerned, various methods can be used to quantify microbial activities in soil or tailings (Table 2-5), such as microbial respiration, community levels physiological profile based on BIOLOG ® system (Cookson et al., 2007; Garland, 1997) or specific enzymatic activities (Burns, 1982).

Table 2-4 Methods applied to quantify microbial community

Index	Methods description	Advantages and limitation	Reference
Microbial biomass	Fumigation extraction organic C	Classical and reliable; Both living and dead microorganisms will be extracted;	Chotte et al., 1998
CFU	Dilution plating and culturing methods, less than 1 % of the microorganism are culturable	Easy and economic operation; Suitable for culturable microorganisms only.	Vieira and Nahas, 2005
Phospho lipid fatty acids (PLFAs) analysis	Phospholipid fatty acids have signature molecules presenting in all living cells. Specific fatty acid methyl esters are used as an accepted taxonomic discriminator for species identification.	Easily extractable molecules; Reveal the presence and abundance of particular organisms; Rely on chromatography of phospholipid component with medium resolution; Not for species identification; False signature under specific conditions.	Frostegård et al., 2011
Nucleic acid techniqu es (16S rRNA)	DNA extraction, PCR amplification, and DGGE differentiation	High resolution; Suitable for complex habitat; Detect unknown species; Multiple steps bias such as sample storage, extraction and amplification; Not suitable for large sample processing	Klindworth et al., 2012
FISH	Fixed cell, 16S or 23S rRNA is hybridized with fluorescently-labelled taxon-specific oligonucleotide probes, viewed with scanning confocal laser microscopy.	Direct identification and quantification of individual species and groups; Not suitable to the nutrient-poor soils; Familiar with sample for probe choice	Hill et al., 2000

Respiration is commonly used as an integrated indicator to measure entire metabolic processes, based on CO_2 evolution with relatively low resolution. BIOLOG ® approach combines both functional diversity and degradation rates, which is suitable for culturable microorganisms, but sometimes resulting in bias from microbial competition (Degens et al., 2001). Enzyme assay provides information at higher resolution of biological processes related to biogeochemical functions such as C, N and P mineralisation. The activities of some of the enzymes measure entire metabolic processes (e.g., dehydrogenase), whereas others (e.g., invertase, cellulose, protease, urease, phosphatase) measure specific key processes involved in nutrient cycling, such as C, N and P (Alef and Nannipieri, 1995).

Table 2-5 Methods for measurement of microbial activities

Index	Methods description	Advantages and limitation	Reference
Basal respiration	carbon dioxide (CO_2) evolution	Low resolution; Difficult to be separated from root respiration, OM decomposition;	
Community-level physiological profiles	Based on BIOLOG® system, quantify spectrophotometrically of colour change	Suitable for culturable microorganisms; Time lag bias resulting from microbial competition; Not match with real environment;	Degens et al., 2001
Enzyme activities	Substrate induced respiration, N mineralisation, nitrification; potential denitrification activity, N-fixation	Specific processes	Alef and Nannipieri, 1995

Microbial communities in tailings may be highly adapted to the changing physicochemical properties in tailings and are stimulated by rhizosphere effects of plants in the tailings subject to remediation by *in situ* engineering practices. Therefore, the associated biogeochemical processes might be greatly influenced, resulting in various levels of ecological changes (SOM content, metal and nutrient levels in its solution) in the context of soil development and formation (Fig. 2-2).

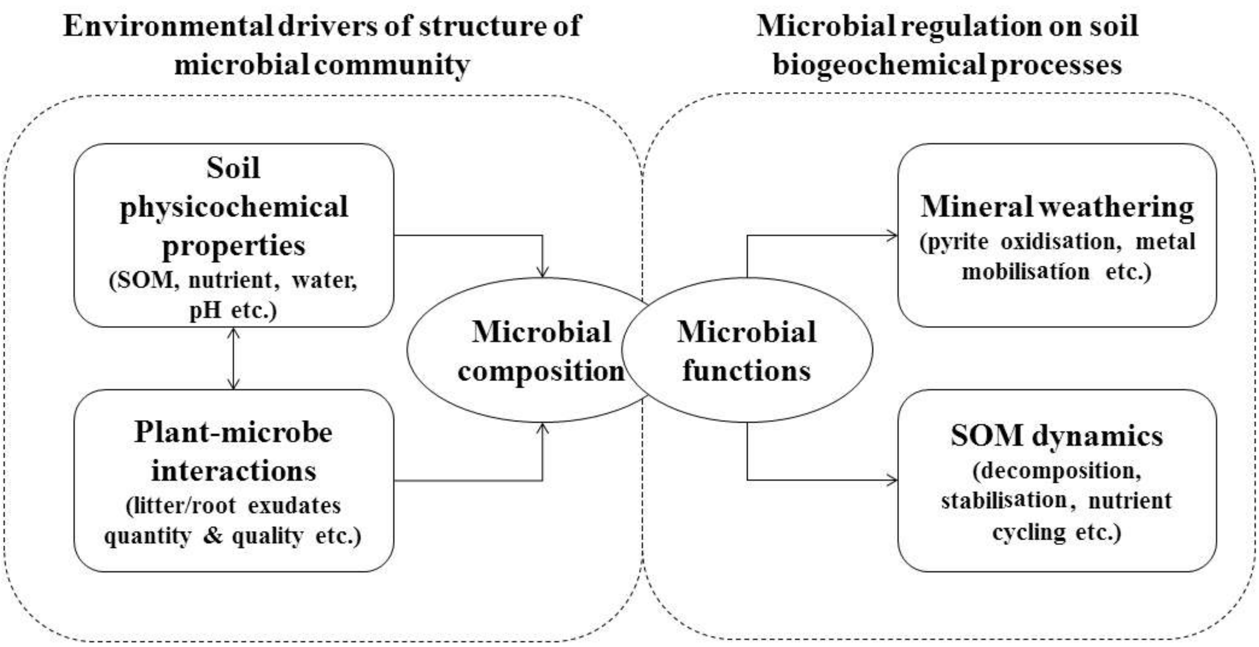

Fig. 2-2 A conceptual linkage between composition and functions of microbial community.

The above methods ranging from molecular to physiological analyses can be used to characterise microbial community composition and functions and greatly improve our understanding of the relationship among changes of microbial composition and functions and physico-chemical changes induced by ecological engineering in tailings. The expected findings are critical to the development of ecological engineering options for *in situ* engineering of technosols from the tailings.

2.2 Biogeochemical changes induced by ecological engineering practices in tailings

2.2.1 Organic amendments to build up nutrients and organic carbon

Organic amendment (OA) has commonly been used as one of the options to rehabilitate SOM and associated soil structure and functions in degraded/polluted soils as well as mine tailings (Huang et al., 2012; Ros et al., 2003; Tejada et al., 2006). Diverse microbial communities are often observed in tailings following OA (Pepper et al., 2012). The most commonly used OAs in tailings include crop residues and their compost, municipal and industrial wastes and manure, uncomposted organic materials, such as sludge, plant residues, biochar. All of them contain essential elements (e.g., C, N, P) with variable concentrations (Table 2-6), due to the differences in their origin and processing methods (Quilty and Cattle, 2011). In general, compared to fertile OAs such as compost, manure, sludge and plant residues, biochar is relatively low in nutrient quality, where low amounts of labile OC is readily available for microbial colonisers.

Table 2-6 Physicochemical properties of typical organic amendments used for tailings

Type of OAs	pH	EC (cm dm^{-1})	CEC (cmol$_+$ kg^{-1})	OC (%)	N (g kg^{-1})	P (g kg^{-1})
Compost	4.0-9.7	1.3-36	29-236.3	7.7-60.1	1.3-30.2	0.4-16.2
Manure	6.3-9.1	1.9-7.3	na	42.7-72.0	1.8-35.8	9.4-42.3
Sludge	4.8-7.8	0.27-16.0	18-33	28.1-48.4	6.8-65.0	5.2-48.6
Biochar	4.5-12.0	0.05-1.05	0.06-61.1	31-98	0.6-34.7	0.02-30.1
Plant residues	5.2-7.7	na	na	41.0-52.6	4.3-25.5	0.3-3.7

a: Data collected from Angın, 2013; Baker et al., 2011; Bolan et al., 1996; Chiu et al., 2006; Hoorens et al., 2003; Romero et al., 2005; Schwab et al., 2007; na: not available.

The major impacts of OAs on tailings properties and colonising plants have been summarised in Table 2-7. The primary benefits of these OAs in tailings are the high OC contents and plant nutrients. OA also improves nutrient retention capacity in tailings through significantly increasing CEC (Gardner et al., 2012; Shu et al., 2002). Although biochar may not be beneficial to microorganisms in terms of nutrient supply (Fellet et al., 2011), it is able to retain nutrients because of its surface charge and area properties (Laird et al., 2010).

Table 2-7 Examples of OAs applied in tailings and impacts on tailings and plants

OA types	Tailings types	Duration	Impacts on tailings	Impacts on plants	Reference
Manure, compost and biosolids.	Pb/Zn/ Cu	4 months	Increase N, P, K; Decrease extractable Pb, Zn, Cu.	Greater vegetation cover and dry weight yield.	Chiu et al., 2006
Compost, pig manure	Pb/Zn	1 year	Alter pH; Increase TOC.	Deeper root system; Greater dry weight yield	Ye et al., 2000
Domestic refuse	Pb/Zn	3 months	Decrease EC; Increase TOC and macronutrients; Decrease both total and extractable heavy metals.	Greater vegetation cover and dry weight yield.	Shu et al., 2002
Biosolids	Cu	3 years	Decrease bulk density; Increase water retention; Increase EC, CEC, TOC and microbial activity.	Increase biomass production; plant cover	Gardner et al., 2012
Pine bark	Base metal	48 hours	Increase dissolved OC; Increase water extractable metals.	Not available	Munksgaard and Lottermoser, 2010
Mushroom compost	Pb/Zn	6 months	Improve physical and chemical status; Decrease metal content.	Increase dry weight yield	Jordan et al., 2008
Paper sludge	Au	6 years	Slow decomposition.	Not available	Cousins et al., 2009
Biosolids	Cu	10 years	Not available	Noxious weed dominance	Borden and Black, 2011

Physico-chemical conditions in tailings can be greatly modified by OA. Specifically, as intrinsic densities of organic materials are much lower than minerals, they are able to hold more water (Tester, 1990). Thus, tailings receiving OA often have considerably lower bulk density and greater water holding capacity (WHC) compared the tailings without (Brown et al., 2003).

Several studies suggest that OA immobilises heavy metals through adsorption, complexation, reduction and volatilisation effects (Park et al., 2011). For instance, pig manure greatly decreased extractable concentrations of Pb, Zn and Cd in tailings (Ye et al., 1999). However, the effects of OA on metal mobility in literature are conflicting – possibly as a result of from the interaction of several environmental variables (e.g., pH, CEC). Sludge, pine bark and woodchips may even induce metal release in readily soluble form when applied to tailings (Li et al., 2013). Besides, OA may increase EC in tailings due to elevated dissolution of minerals and salts, which may further exacerbate the levels of salinity in tailings at least in the short-term, especially in semi-arid areas (Chiu et al., 2006). Therefore, comprehensive assessment is necessary for optimum utilisation of OA in tailings to (1) identify OA-induced changes in physicochemical conditions in the tailings; (2) the underlying biogeochemical processes; (3) and potential impacts on physiological requirements of target plant species.

In the context of engineered pedogenesis of mine tailings, the selection of OA should focus on the effectiveness in stimulating the weathering of reactive minerals and speeding up hydro-geochemical stabilisation as the first priority, which forms the basis for further improvement of biogeochemical capacity towards functional technosols. The perceived ecological linkages between soil and target plant subsystems must also be considered when selecting OA in relation to associated nutrient loads (such as N content), because soil fertility can influence the pattern of plant community development (Huang et al., 2012; Wardle et al., 2004).

2.2.2 Introduction of native microbial inoculum for fast development of microbial communities in the tailings-soil

Introducing native or cultured microbes through addition of soil, isolated microbes, and soil microbial extracts may speed up microbial community rehabilitation in tailings, which is also undergoing physicochemical changes induced by other amendments (e.g., OA) (van de Voorde et al., 2012). In previous studies, microbial inoculation was found to help establish diverse and functional microbial communities in tailings and improve plant growth (de-Bashan et al., 2010; Grandlic et al., 2009).

Inoculation of functional microbes such as plants growth promoting bacteria (PGPB) and mycorrhizal fungi has been found to be beneficial to root nutrient acquistion and plant tolerance of heavy metals (Alguacil et al., 2011; Ma et al., 2006). For instance, inoculating PGPB (e.g., N_2-fixing bacteria, phosphate and potassium solubilisers) enhanced plant growth in Pb-Zn tailings in arid area and alleviated metal toxicity in plants (Grandlic et al., 2009; Wu et al., 2006). Many pot and field experiments have shown the potential of arbuscular mycorrhizal fungi (AMF) in facilitating plant establishment in tailings (Ma et al., 2006). In addition, mycorrhizal colonisation of plant roots also helps reduce translocation of heavy metals to shoots by binding them to the cell walls of the fungal hyphae (Chen et al., 2007), but effectiveness of mycorrhizal colonisation varies among the introduced fungal isolates (Orłowska et al., 2005).

Microbial inoculums may differ in their tolerance to stresses present in tailings. It is likely that potential application of microbial inoculation is more efficient in slightly or moderately metal-polluted sites compared to heavily polluted areas (Wu et al., 2006). Similarly, AMF adapted to uncontaminated soil stimulate plant growth far more than those adapted to the moderately contaiminated sites (Shetty et al., 1994). Therefore, it is necessary to investigate not only the tolerance of introduced microbial species or community, but also their interactions with other amendments, which modify physicochemical properties in tailings for microbial colonisers, in order to formulate efficient amendment strategy for technosols development in tailings.

2.2.3 Rhizosphere effects of tolerant pioneer and native plants in microbial development of tailing-soil

Plant cover provides both intangible and tangible benefits in mine tailings landscapes, such as surface stabilisation, land quality improvement, and increased biodiversity in tailings landscapes. Due to the presence of many physical and biogeochemical constraints, natural colonisation of diverse native plants in tailings is largely unsuccessful if without significant physicochemical improvement, except for limited number of highly tolerant plant species (Huang et al., 2012; Shu et al., 2002). However, soil development in tailings may be stimulated by the colonisation of tolerant pioneer plants, because root turnover can provide inputs of organic matter and stimulate the colonisation of rhizosphere microbial communities, in addition to root-induced improvement of physical structure in the tailings. For example, it is reported that initially low microbial diversity in non-vegetated tailings was rapidly increased after plant establishment and succession later (Alguacil et al., 2011). Even a relatively low plant cover is sufficient to stimulate microbial community recovery (Moynahan et al., 2002). It is worth noting that the establishment of pioneer plant species is far from the rehabilitation of target plant communities consisting keystone species and expected species diversity, which might be achieved at the later stage during plant community succession.

Rhizosphere in the immediate vicinity of growing roots are known to promote microbial biomass, diversity and activities, where roots produce organic exudates, including enzymes and organic compounds (Bais et al., 2006). Some root exudates (e.g., *isofalvones*) are able to attract beneficial bacteria (e.g., *Bradyrhizobium japonicum*), working as symbiotic signals to microorganisms in nutrient-poor soil (Dakora and Phillips, 2002). For instance, legume plants routinely use flavonoid molecules in root exudates to induce transcription of nodulation genes, leading to nodule formation and N_2 fixation, particularly in infertile soil (Dakora and Phillips, 1996). Besides, root exudates stimulate the formation of mycorrhizal fungi association with roots, by inducing spore germination and/or hyphal growth in vesicular-arbuscular fungi, bringing about the overall improvement in the capacity of nutrient (particularly, P) and water acquisition (Gilbert et al., 2000). In addition, many low weight organic compounds in root exudates may chelate cations to form organo-mineral complexes, thus reducing their availability to plants and microbes (Compant et al., 2005). As a result, the introduction of tolerant native plants as pioneer plants may greatly stimulate the development of rhizosphere microbial communities, in terms of biomass, diversity and functions, which critically contribute to the development of functional technosols in the tailings.

Many metallophytes are recommended for tailings phytostabilisation, due to their tolerance of hostile habitat conditions, such as *Cynodon dactylon, Festuca rubra, Agrostis tenuis, Agrostis stolonifera, Typha latifolia and Phragmites australis* (Archer and Caldwell, 2004). However, due to the relatively high amount of metals in tailings, it is impossible to rely on hyperaccumulatiing plant species (e.g., Cd > 0.01 %, Cu, Pb > 0.1 % dry weight) (van der Ent et al., 2013) to remove such quantities metalloids and metals from sulfidic tailings of base metal mines, even assuming these plants could grow adequate biomass. Gramineous grasses and legumes are generally the favourable options for phytostabilisation purpose, due to their adaptation to nutrient deficiency and fast growing trait (Li, 2006). Native plant species well adapted to local climatic conditions are preferred to be used as pioneer plants, without residual risks of weed proliferation and associated negative impacts on native plant species (Singh et al., 2002).

Relative abundance of microorganisms and associated functions in the rhizosphere have been found to be species specific (Carrasco et al., 2010). Plants tend to actively select specific rhizosphere microorganisms to establish the habitat for themselves (Doornbos et al., 2012). Field-grown potato and wheat are associated with distinct ascomycete community in the rhizosphere (Viebahn et al., 2005). Root system of *L. spartum* presents a higher cellulose content than that of *p. miliaceum*, favouring colonisation and growth of fungi-producing extracellular cellulases (Carrasco et al., 2010). Symbiosis of AMF and hyphae with legume (*Acacia* spp.) had been observed in many native acacia species in Australia (Herrera et al., 1993), while very low or absent in some species (e.g., *Ptilotus* spp., *Triodia* spp.) (Jasper et al., 1989). Much less has been known about microbial communities in the rhizosphere of native plant species under field conditions, compared to those for crops. In the present project, key native plant species from Mount Isa/Clonccury regions of Northwest Queensland have been used to stimulate microbial colonisation and diversity in the amended tailings of Cu-Pb-Zn mines.

2.3 Rehabilitation of biogeochemical capacity and processes in tailings for engineering functional technosols

2.3.1 Biogeochemical processes critical to technosol development

In base metal mine tailings, the weathering and transformation of minerals are considered to be indicators of pedogenesis, particularly those abundant in reactive primary minerals (e.g., pyrites) (Uzarowicz and Skiba, 2011). In addition to geochemical stabilisation resulted from mineral weathering in the tailings, soil-like structure and functions are to be developed through coupled physical, chemical and biological processes to stimulate technosols formation in tailings (Remenant et al., 2009). Therefore, a comprehensive evaluation of pedogenesis in tailings require clear characterisation of mineralogical, hydrogeochemical and biogeochemical processes, in response to various ecological engineering inputs (Huang et al., 2014).

Soil formation in natural ecosystems is typically an extreme slow process that occurs of lengthy periods of time (thousands and even millions of years) (Jenny, 1941). Natural soil formation from bedrocks undergoes intertwined physical, chemical and biological processes under the influence of pedogenetic factors (i.e., climate, biological activity, topography and time) (Jenny, 1941). However, technosols are a new reference soil group (as noted in World Reference Base for Soil Resources), and they contain a large range of materials of natural and/or anthropic origin, such as mine tailings (Uzarowicz and Skiba, 2011).

When comparing the tailings with natural soil in terms of mineralogy, fertility and microbial community, the most significant differences are elevated metals of lithogenic origin, low in clay minerals and organic matter and stressed microbial community dominated by extremophiles in the base metal mine tailings (except for red mud) (Li and Huang, 2015; Lottermoser, 2010). Tailings are normally deficient in SOM due to the absence of plant cover or low productivity of the plants. SOM levels in tailings tend to increase with the progress of vegetation establishment and development (Lorenz and Lal, 2007; Ussiri et al., 2006). However, the efficiency of OC stabilisation in the tailings seemed to be low. For example, in Cu tailings rehabilitated more than 20 years with a dominated grass vegetation, the levels of TOC in tailings were only 18 % of those in soil samples from the reference site (Huang et al., 2011).

Accumulation of organic matter is often reported as a primary pedogenetic process occurring in technosols formation (Hernández-Soriano et al., 2013). Different processes such as solute (e.g., sulphates, carbonates) movement, aggregation involving minerals and organic materials, changes in structure and hydrodynamics (Hartmann et al., 2010) have also been shown to occur during technosols formation. Ecological engineering inputs and direct/indirect interactions with natural climatic or environmental factors are expected to accelerate the processes of physical, chemical and biological changes and influence technosol formation in the tailings.

Although advanced extraction technologies has extracted majority of metals for profit purpose, the concentrations of heavy metals and metalloids (e.g., As, Cu, Mn, Pb, Cd, Zn) in tailings remain very high, which largely exceed the ecological investigation levels (Li and Huang, 2015). There has been increasing evidence that phytostabilisation of base metal mine tailings require more knowledge about how to develop functional soil and root zones to support target plant communities, rather than simply rely on the unrealistic potential of hyperaccumulating plant species (Huang et al., 2012; Li and Huang, 2015; Monserie et al., 2009). The pedogenesis on coal mining sites have been studied with pioneer plant cover, the function of soil fauna and organic matter accumulation (Hafeez et al., 2012; Novo et al., 2013). Yet, few studies have been conducted to understand the mineral transformation and translocation in the early stage of pedogenesis (Huot et al., 2014). As a result of the research undertaken for this literature review, it is apparent that the success of phytostabilisation of tailings landscapes depends on the development of hydrogeochemically stable and biogeochemically functional tailing-soils (technosols) by utilising effective ecological engineering options, and this is closely linked with the tolerance capacity and growth requirements for the candidate plant species. Therefore, the scientific merits and environmental and economic benefits have justified the present project to investigate critical factors and processes involved in technosol formation from sulfidic tailings, in response to suitable ecological engineering options.

2.3.2 Research questions

The present project aims to develop a suit of effective ecological engineering options (including organic amendment, microbial inoculation and introduction of pioneer native plant species), based on the comprehensive evaluation the efficacy and underlying mechanisms involved in the amelioration of tailings' biogeochemical properties and biogeochemical processes. The physicochemical and biochemical properties in the rhizosphere soils underneath key native plant species (i.e., *Acacia chisolmii* and *Triodia pungens*) in natural sites colonised by target native plant community have been characterised to provide the benchmark and target for engineered pedogenesis in the tailings. Detailed research questions to be investigated may include:

(1) What are the characteristics of OC pools and its dynamics in tailings?

(2) What are the characteristics the microbial community (diversity, composition and biogeochemical processes) in response to amendment options (e.g., organic amendment, microbial inoculum and plant introduction)? What are the environmental drivers?

(3) What are the relationships of microbial community and functions in tailings and how it is linked to the pioneer plant survival and growth?

Specifically, a series of laboratory, glasshouse and field trials have been designed and carried out:

(1) In Chapter 3, *Organic carbon stabilisation in weathered and fresh Cu-Pb-Zn tailings amended with woodchips under field conditions* aims to investigate OC pools and forms in typical Cu-Pb-Zn tailings with different mineral composition with the purpose to explore possible mechanisms of OC stabilisation in tailings, in response to exogenous organic amendment in field conditions

(2) Chapter 4, *Plant colonisation stimulated organic carbon formation and microbial biomass and functions in weathered Cu-Pb-Zn tailings from a long-term field trial* aims to characterise OC pools and associated microbial biomass and functions in tailings from long-term field trial to evaluate organic amendments and plants roles in technosols formation in tailings;

(3) Chapter 5, *Biogeochemical changes induced by addition of exogenous organic matter with contrasting properties in weathered and neutral Cu-Pb-Zn tailings – a 6-month microcosm study* aims to evaluate how typical organic amendments in driving the shift of microbial community structure and functions in tailings under controlled laboratory conditions;

(4) Chapter 6, *Establishing microbial diversity and functions in weathered and neutral Cu–Pb–Zn tailings with native soil addition* aims to investigate the dosage effects of soil inoculum on rehabilitation microbial community diversity and functions in tailings with the purpose to identify important physicochemical factors driving the shift of microbial communities;

(5) Chapter 7, *Sugarcane and biochar differ in ameliorating Cu-Mo-Au tailings for phytostabilisation with native plant species-A greenhouse study* aims to investigate the efficacy of organic amendments on rehabilitation of microbial communities interacting with native plants based on greenhouse study, which provide basic information to guide selection of candidate pioneers plants for sustainable tailings phytostabilisation.

From the findings against these objectives, a conceptual model has been synthesized to illustrate the processes stimulating the development of technosols in the Cu-Pb-Zn tailings and provide a basis for further research progress.

Chapter 3 Organic carbon stabilisation in weathered and fresh Cu-Pb-Zn tailings amended with woodchips under field conditions

3.1 Introduction

Organic carbon (OC) stabilisation is one of the key processes involved in soil formation (Quideau et al., 1998). OC is an essential constituent in the soil matrix, playing a crucial role in the biological (provision of substrate and nutrients for microbes and plants) (Alguacil et al., 2011), chemical (buffering and pH changes; chelation of metals) (Park et al., 2011) and physical (aggregation and stabilisation of soil structure) properties (Abiven et al., 2009) and functions in soil. The degree of OC stabilisation in tailings could be an overall indicator of engineered soil formation and technosols development (Bendfeldt et al., 2001; Shukla et al., 2004). In natural soils, mechanisms contributing to OC stabilisation include: (1) the chemical recalcitrance of complex organic matter, retarding its degradation by microorganisms (e.g., lignin, wax etc.) (Marschner et al., 2008); (2) interaction between organic and inorganic substances (e.g., Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}) or other organic substances with charged surface (Kleber et al., 2007); and (3) physical protection by forming barriers among microbes, enzymes and organic substance (e.g., aggregates, clay occluded OC) (von Lützow et al., 2008). Over the last decade, characterising OC distribution and chemistry through physically fractionation methods has contributed significantly to our understanding about the mechanisms of OC stabilised in soil (Six et al., 2002; von Lützow et al., 2007), but with little published information about OC fractions in base metal mine tailings.

Endogenous accumulation of OC in tailings is very slow due to the harsh physicochemical conditions limiting natural colonisation of plants. Therefore, the addition of exogenous organic matter (e.g., plant residue, biosolids, biochar, manure etc.) is one of the common practices to provide organic carbon, and also improve the growth conditions for plants (Fellet et al., 2011; Huang et al., 2011a). However, to what extent that OC could be stabilised in tailings from organic amendments is still unclear.

It is widely accepted that microbial communities colonising the tailings are low in biomass, diversity and activities compared to natural soils (Huang et al., 2011b; Li et al., 2014; Pepper et al., 2012). Therefore, we expect that the turnover of organic matter by biological processes in tailings is fairly slow compared to natural soil. Direct interactions between tailings minerals and organic compounds may be more important to OC stabilisation in the tailings in the initial phase of soil formation.

Furthermore, tailings vary significantly in physical and biogeochemical properties, due to ore mineralogy, processing technology and stages of mineral weathering (Lottermoser, 2010). For example, Mount Isa Mine (MIM) tailings from Tailings dam 5 (TD5), were decommissioned about 40-year ago. This dam, had received mixed streams of Cu and Pb-Zn tailings, with higher ratio of Cu to Pb-Zn tailings. Tailings from Tailings dam 7 (TD7) had been recently deposited less than 2-year from mixed streams of Cu and Pb-Zn tailings, with much lower ratio of the former to the latter. Due to changes of ore composition and mineral processing techniques, tailings in TD7 composed of minerals with a finer particle size and higher sulphide (e.g., pyrite) content compared to TD5 (Gao and Young, 2002). Changes in tailings composition occur during mineral weathering in terms of secondary mineral precipitates, sulphide mineral oxidation, primary mineral dissolution and secondary mineral formation (Lottermoser, 2010; Wakelin et al., 2012). Forsyth (2014) suggested that the rates of sulphide depletion ranged from 5 to 17 mM pyrite per year with consumption of 24 to 39 mM $CaMg(CO_3)_2$ per year in TD5 during the period of deposition. Mineralogy of these two tailings is summarised in Table 2-1. Overall, fewer secondary minerals were observed in TD7 compared to TD5 (Forsyth, 2014).

Therefore, we hypothesize OC will be stabilised in tailings via organo-mineral interactions and aggregation, and their accumulation rates and associated mechanisms are markedly different between TD5 and TD7 tailings, which may be influenced by their particle size, mineralogy and geochemistry. The present study aimed to characterise: (1) the distribution of OC in different physical fractions of TD5 and TD7 amended with woodchips after 2.5-year field trial; and (2) the major forms of OC in the tailings, with the purpose to explore possible mechanisms of OC stabilisation in tailings, in response to exogenous organic amendment. The information about physical and chemical forms of OC in tailings could help us formulate effective amendment strategies to enhance OC stabilisation and the formation of technosols for successful phytostabilisation.

Table 3-1 Major minerals in TD5 and TD7, reproduced from Forsyth (2014)

Minerals (> 2 % by weight)	Formulae	TD5	TD7
Quartz	SiO_2	√	√
Amphibole	SiO_4	√	√
Plagioclase feldspar	$NaAlSi_3O_8$-$CaAl_2Si_2O_8$	√	√
Talc	$Mg_3Si_4O_{10}(OH)_2$		√
Chlorite	$Mg_6Si_4O_{10}(OH)_8$	√	√
Muscovite	$KAl_2(Si_3Al)O_{10})(OH)_2$	√	√
Microcline	$KAlSi_3O_8$	√	
Pyrite	FeS_2	√	√
Ankerite	$Ca(Fe^{2+}(CO_3)_2)$	√	
Ankerite ± sphalerite	$Ca(Fe^{2+}(CO_3)_2)$ ± ZnS		√
Dolomite	$CaMg(CO_3)_2$	√	√
Calcite	$CaCO_3$	√	
Gypsum	$CaSO_4(H_2O)_2$		√
Goethite	α-$Fe^{3+}O(OH)$	√	√
Most likely trace apatite and secondary phosphates	$Ca_{10}(PO_4)_6(OH)_2$	√	√

3.2 Materials and methods

3.2.1 Field column incubation

The Cu-Pb-Zn tailings were collected from the top 1 m of the MIM Tailings TD5 and TD7 in late 2009. The tailings were excavated in bulk, air-dried, crushed and mixed thoroughly on site before use. A column (1 x 1 x 1 m bulk container) trial was established at the end of 2009, located at the edge of the TD5. The TD5 and TD7 tailings were thoroughly mixed with woodchips (WC) (namely TD5+WC, TD7+WC) (C: N ratio of 98) at the rate of

20 % (v/v), and incubated in the columns under field conditions. Woodchips applied in the field column were from road tree pruning, with particle size ranging from 5-10 cm. Both the control (tailings without WC, namely TD5, TD7) and the WC amended tailings were replicated in three columns, which were randomly laid out and blocked according to the tailings type. Treatments included TD5, TD5+WC, TD7 and TD7+WC (Fig. 3-1).

The climate of the incubation site is defined as subtropical and semi-arid, with generally warm to hot temperatures (17-32 °C), an annual pan evaporation of 2800 mm, and an average rainfall of 465 mm (Bureau of Meteorology Australian Government, 2015). Rainfall is highly variable between the wet season (during November to February) and dry season.

The tailings and the amended tailings from each column 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 column. All the samples were dried at 40 °C and sieved less than 2 mm for physicochemical analysis and OC fractionation.

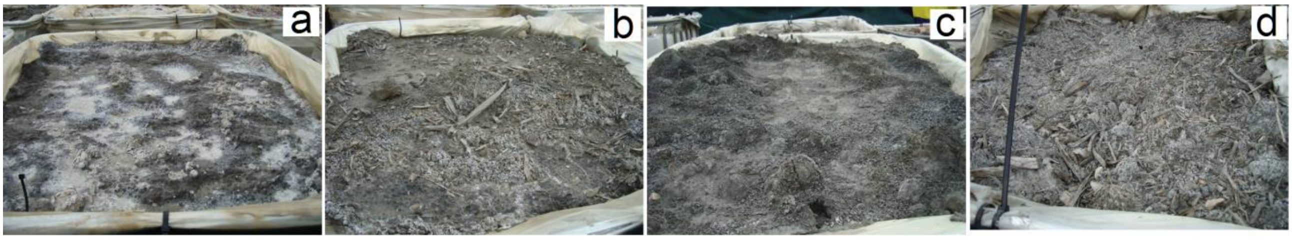

Fig. 3-1 Cu-Pb-Zn tailings (TD5) field incubated tailings under semi-field conditions at TD5 east of Mount Isa Mines, northwest Queensland, Australia. The treatments were TD5 Control(a), TD5+WC (b), TD7 (c), and TD7+WC (d) (Source: Longbin Huang, The University of Queensland).

3.2.2 Physicochemical analysis

The pH and electrical conductivity (EC) (1: 5 tailings: water) in the water-extracts of the samples were measured 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 (Pleysier and Juo, 1980). Water holding capacity (WHC) was measured using the method as described by Wang et al. (2003). Total elemental concentrations were determined by means of inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian) after aqua-regia digestion (Li et al., 2013). 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 of 90 ± 10%.

Water-soluble organic carbon (WSOC) was extracted by shaking samples with deionised water at a ratio of 1: 2 (w/v) on an end-over-end shaker at 20 °C for 1 h (Tao and Lin, 2000). The suspension was then centrifuged at 4000 rpm for 10 min and passed through 0.45 µm pore filter before WSOC determined using the dichromate digestion method (Bremner and Jenkinson, 1960). Concentrations of water-soluble elements were analysed with ICP-OES after shaking 1 g samples in 50 ml deionised water for 1 h (Dold, 2003).

Microbial biomass (MBC) in the samples was determined using the chloroform fumigation and extraction method. Briefly, aliquots of fresh samples were fumigated with chloroform vapour in darkness for 24 h. Both fumigated and unfumigated samples were extracted with 0.25 M potassium sulphate and filtrated through Whatman® No. 42 filter paper. Soluble OC in the extracts was determined using the dichromate digestion method (Bremner and Jenkinson, 1960). 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).

3.2.3 Analysis of iron and aluminium (hydr-) oxides

Dithionite-citrate-bicarbonate (DCB) extractable Fe and Al (Fe_d, Al_d) were determined in the tailings samples for crystalline Fe oxides, Al substituted in crystalline Fe oxides and amorphous Fe and Al. Acid-oxalate extractable Fe and Al (Fe_o, Al_o) were determined following the method as described by Rayment and Lyons (2011), mainly as estimates of amorphous and poorly crystalline minerals (e.g., ferrihydrite and imogolite). The difference between DCB and acid-oxalate extractable Fe represents crystalline Fe (Fe_{d-o}). Fe and Al concentrations in the extracts were measured using ICP-OES.

3.2.4 OC fractionation and analysis

Tailings was fractionated following the procedure as shown in Fig. 3-2. Aggregates and further fractionation was undertaken using the modified method of Six et al. (2002). In brief, 150 g of air-dried samples were submerged in deionised 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. The sieving was carried out manually by moving the sieves up and down 3 cm at the rate of 50-time in 2 minutes. Floating material was collected with a syringe for weight and OC measurement. As weights of this fraction for each sample was negligible compared to total weight (accounting for 0-0.5 %), they were not taken into consideration for mass and OC recovery calculation in the present study. 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 Eq. 3-1, 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.

$$MWD = \sum_{i=1}^{n} x_i \, w_i \quad \text{(Eq. 3-1)}$$

Following initial separation, a 15 g sub-sample of macroaggregate (> 250 μm) and microaggregate (53-250 μm) fractions were dispersed in 0.5 % sodium hexametaphosphate using a mechanical end-over-end shaker for 15 h at the speed of 30 rpm at room temperature (22 ± 1 °C). The dispersed macroaggregate and microaggregate fractions were further separated by passing the fractions through 53 μm sieve. 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; and 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).

The OC and N concentrations in the bulk tailings and each fractions were determined by dry-combustion with a LECO CNS-2000 analyser (LECO Corporation, MI, USA) after acid-removal of inorganic carbon (You et al., 2014. Refer to Appendix A). There was a mean of 99.7 % mass recovery in aggregate dispersion and fractionation procedure. Recovery of OC ranged from 85.5 to 110.6 % (mean, 96.6 %) in macroaggregate dispersion and fractionation, and ranged from 84.8 to 111.1 % (mean, 96.9 %) in microaggregate dispersion and fractionation, respectively.

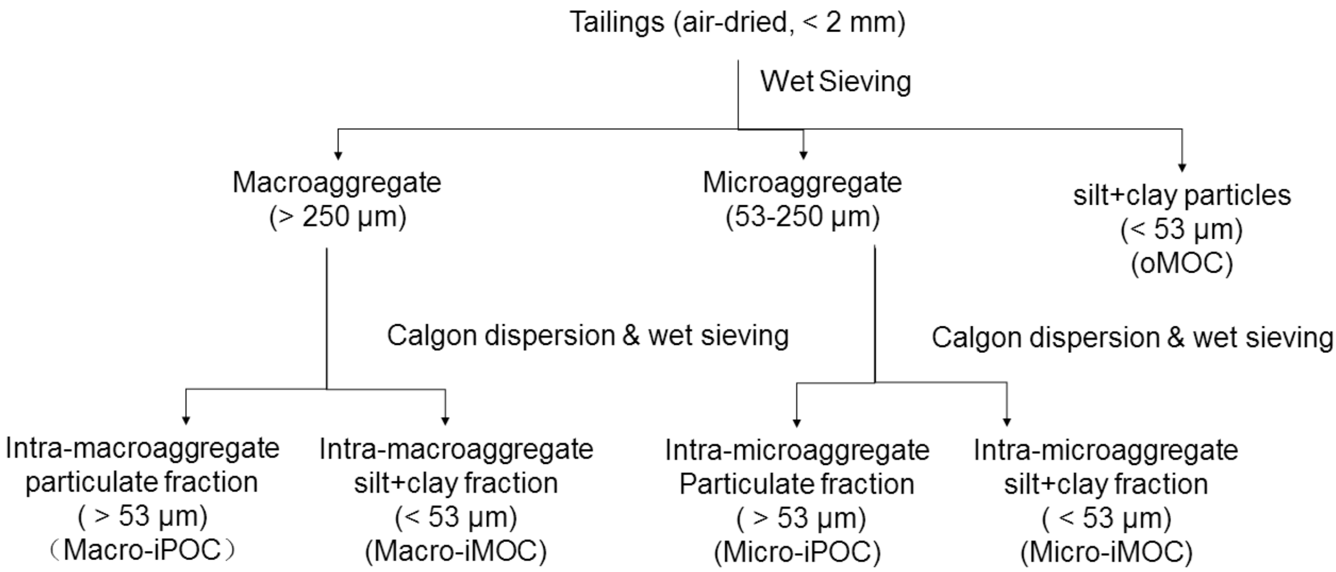

Fig. 3-2 Organic carbon (OC) fractionation procedure in the tailings

3.2.5 Statistical methods

Primary data processing was performed using Microsoft® Excel. One-way analysis of variance (ANOVA) was carried out for evaluating the significant differences among the treatments after normality check. Two-way ANOVA was performed to test significant differences between the types of tailings and effects of woodchips. Means were compared using the least significant differences (LSD) test at P = 0.05. Pearson linear correlations among physicochemical properties, mineral composition, and OC fractions were also conducted. All statistical analyses were conducted using the SPSS software package (SPSS Statistics 20.0, Chicago, IL, USA).

3.3 Results

3.3.1 General physicochemical properties in the tailings

General physicochemical properties of the tailings in all the treatments were summarised in Table 3-2. Both TD5 and TD7 were finely-textured, with 11.2-13.3 % particles in macroaggregate and 49.8-54.8 % particles were in microaggregate. They were slightly acid to neutral (6.7-6.9) with extremely low levels of TOC, TN, CEC, WHC and WSOC, especially in TD5. Microbial communities in these two tailings were with low MBC, less than 16 mg kg^{-1}, accounting for 0.5 % of TOC in both TD5 and TD7. Both were sulphidic saline tailings, containing high levels of salts, and the levels of EC were greater than 3 mS cm^{-1}. The salinity problem was even more severe in TD7 with the EC as high as 5.4 mS cm^{-1}. They were also with high levels of total Cu, Pb and Zn, which were above the ecological investigation limits (Mendez and Maier, 2008), especially in TD7.

Application of WC brought about significant changes of some physicochemical properties in the WC amended TD5 and the WC amended TD7 after 2.5-year field incubation. TOC significantly increased from 1.5 to 4.3 g kg^{-1} in the WC amended TD5 compared to TD5. Similarly, TOC increased from 3.9 to 10.5 g kg^{-1} in the WC amended TD7 compared to TD7. Moreover, significant improvements of TN ($P < 0.01$), WHC ($P < 0.001$), CEC ($P < 0.01$), WSOC ($P < 0.001$), and a greater abundance of macroaggregate ($P < 0.01$) and MWD ($P < 0.01$) were observed in the WC amended TD5 compared to TD5. MBC in the WC amended TD5 also slightly increased. However, this increase was not statistically significant. Similarly, all the properties mentioned above were improved significantly in the WC amended TD7 compared to TD7.

Table 3-2 Selective physicochemical properties in TD5 and TD7 with woodchips application

Treatments		pH	EC ($mS\ cm^{-1}$)[a]	CEC ($cmol_+\ kg^{-1}$)[b]	WHC (%)[c]	MBC ($mg\ kg^{-1}$)[d]	C:N	Total ($g\ kg^{-1}$)							Water Soluble ($mg\ kg^{-1}$)				Aggregates		
								TOC[e]	TN[f]	Cu	Pb	Zn	S	Fe	C	S	Cu	Zn	Macro aggregate(%)	Micro aggregate(%)	MWD(mm)[g]
TD5	Mean	6.69^{α}	3.4^{α}	0.79^{α}	19.2^{α}	13.0^{α}	13.7	1.5^{α}	0.11^{α}	1.1^{α}	1.9^{α}	4.1^{α}	47^{α}	91	5.1^{α}	238^{α}	0.12^{α}	18.4^{α}	11.2^{α}	49.8^{α}	0.21^{α}
	S.D.[h]	0.04	0.3	0.27	1.2	1.8	2.4	0.0	0.02	0.1	0.1	0.3	4	6	2.0	125	0.01	2.3	2.4	4.0	0.03
TD5 +WC	Mean	6.66^{α}	$4.5^{\alpha\beta}$	1.66^{α}	21.9^{β}	$18.3^{\alpha\beta}$	13.3	4.3^{β}	0.34^{β}	1.0^{α}	1.9^{α}	3.9^{α}	46^{α}	88	48.8^{β}	601^{β}	0.33^{β}	18.5^{α}	19.2^{β}	31.1^{β}	0.28^{β}
	S.D.	0.08	0.8	0.41	0.7	6.4	2.9	0.4	0.11	0.1	0.2	0.4	5	4	19.5	113	0.01	2.6	2.4	1.5	0.03
TD7	Mean	6.86^{β}	5.4^{β}	1.61^{α}	29.1^{γ}	15.9^{α}	12.5	3.9^{β}	0.31^{β}	1.6^{β}	3.8^{β}	11.8^{β}	75^{β}	105	$18.0^{\alpha\beta}$	1016^{γ}	0.16^{α}	20.5^{α}	13.3^{α}	54.8^{γ}	$0.24^{\alpha\beta}$
	S.D.	0.04	0.1	0.42	1.0	6.4	0.9	0.4	0.05	0.2	0.4	1.6	9	9	3.1	146	0.02	2.5^{α}	1.5	1.1	0.01
TD7 +WC	Mean	6.52^{γ}	8.7^{γ}	3.85^{β}	32.8^{δ}	24.8^{β}	18.8	10.5^{γ}	0.57^{γ}	1.5^{β}	3.1^{β}	13.6^{β}	77^{β}	101	83.4^{γ}	2321^{δ}	0.37^{β}	85.2^{β}	29.6^{γ}	29.4^{β}	0.39^{γ}
	S.D	0.02	1.2	1.17	1.6	5.9	1.4	1.2	0.10	0.1	1.0	1.1	3	5	12.7	514	0.04	12.0	5.2	2.4	0.05

[a] Electrical conductivity. [b] Cation exchange capacity. [c] Water holding capacity. [d] Microbial biomass carbon. [e] Total organic carbon. [f] Total nitrogen. [g] Mean weight diameter. [h] Standard deviation. Values are means (n = 3); values labelled with letters 'α, β, γ and δ' within the column indicate significant differences among the treatments at the level of $P < 0.05$.

However, the WC application had some negative impacts on tailings physicochemical properties. For example, EC was significantly increased from 3.4 to 4.5 mS cm^{-1} in the WC amended TD5 compared to TD5. In addition, water-soluble S and Cu significantly increased from 238 to 601 mg kg^{-1} ($P < 0.01$) and 0.12 to 0.33 mg kg^{-1} ($P < 0.01$) respectively in the WC amended TD5 compared to TD5. In the WC amended TD7, pH decreased from 6.86 to 6.52 compared to TD7. In addition, the WC amended TD7 became more saline with EC increased to 8.7 mS cm^{-1}. Significant amounts of water-soluble S, Cu and Zn were released in the WC amended TD7 compared to TD7. Specifically, water-soluble S and Cu almost doubled in the WC amended TD7 compared to TD7. Water-soluble Zn in the WC amended TD7 was as high as 85.2 mg kg^{-1}, 3-fold greater compared to the level in TD7.

3.3.2 Chemical forms of iron (Fe) and aluminium (Al) (hydr-) oxides

There were several types of Fe and Al containing minerals in both TD5 and TD7 tailings (Table 3-1), which present in different forms (Table 3-3). Both TD5 and TD7 tailings contained similar amounts of crystalline Fe (Fe_{d-o}) and amorphous Fe (Fe_o). The amorphous Fe (Fe_o) in the tailings raged from 4.5 to 5.2 g kg^{-1}, without significant differences among all the treatments. Both amorphous Fe and crystalline Fe were abundant in TD5 and TD7, with Fe_o/Fe_d ratio ranging from 0.43 to 0.46. The WC treatment did not change the relative distribution of amorphous and crystalline Fe in the WC amended TD5, while in the WC amended TD7, there was significantly increased amount of crystalline Fe, and Fe_o/Fe_d decreased from 0.48 to 0.27 ($P < 0.01$) (Table 3-3).

Overall, Al contents in both Al_o and Al_d were significantly greater in TD7 compared to TD5. The majority of Al in TD5 was in the form of amorphous Al (Al_o) (0.25 g kg^{-1}), with Al_o/Al_d ratios around 1. Levels of Al_d increased following WC application with the Al_o/Al_d ratios decreased from 0.71 in the TD7 to 0.53 in WC amended TD7, suggesting the increased levels of crystalline Al in latter treatment compared to the former.

Table 3-3 Iron and aluminium (hydr-) oxides in TD5 and TD7 with woodchips application

Treatments	Fe_o (g kg^{-1})	Fe_d (g kg^{-1})	Fe_{d-o} (g kg^{-1})	Fe_o/Fe_d	Al_o (g kg^{-1})	Al_d (g kg^{-1})	Al_o/Al_d
TD5	4.5(0.7) a	10.0(0.9) a	5.5(1.5) a	0.46(0.10) a	0.25(0.05) a	0.26(0.01) a	1.00(0.22) a
TD5+WC	5.2(0.5) a	12.0(0.5) a	6.9(0.5) a	0.43(0.03) a	0.25(0.00) a	0.23(0.01) a	1.06(0.08) a
TD7	4.7(0.3) a	9.8(0.6) a	5.1(0.3) a	0.48(0.01) a	0.45(0.03) b	0.64(0.01) b	0.71(0.04) b
TD7+WC	5.0(0.2) a	18.3(2.5) b	13.3(2.3) b	0.27(0.03) b	0.43(0.05) b	0.81(0.06) c	0.53(0.07) b

Values are means (n = 3) with standard deviation in brackets; values labelled with letters 'a, b, c, d' within the column indicate significant differences among the treatments at the level of $P < 0.05$.

3.3.3 OC concentrations and C: N ratios in the tailings fractions

The concentrations of OC and C: N ratios in each OC fractions were shown in Table 3-4. Background OC was detected in the unamended TD5 and TD7 tailings. In TD5 samples, OC concentrations were generally low in all of the fractions, ranging from 1.8 to 3.7 g kg^{-1}. The C: N ratios in these fractions were variable. Specifically, in TD5, the C: N ratio of the particulate fraction associated with macroaggregates (Macro-iPOC) was the highest (13.9). C: N ratios were lower in mineral associated fractions (4.4-6.1), including both Macro-iMOC and Micro-iMOC fractions in TD5. In the TD7 tailings, the OC concentrations in all the fractions were also low, ranging from 2.9 to 7.0 g kg^{-1}; the OC fraction with the greatest C: N (14.6) was also found in Macro-iPOC fraction. Comparatively, the C: N ratios (9.2-10.9) in TD7 fractions of Macro-iMOC and Micro-iMOC were greater than those in the same fractions in TD5 (4.4-6.1) ($P < 0.01$).

In the WC amended TD5, OC concentrations in all the fractions significantly increased. For example, the OC concentration in Macro-iPOC in TD5+WC was 4-fold (12.1 g kg^{-1}) compared to TD5 ($P < 0.001$). C: N ratios of this fraction in TD5+WC (33.0) were also significantly higher than those in TD5 (13.9) ($P < 0.05$). Similar findings were observed for Micro-iPOC fraction between TD5+WC and TD5. In the mineral associated fractions, OC concentrations in both Macro-iMOC and Micro-iMOC slightly increased in TD5+WC (3.1-5.6 g kg^{-1}) compared to those in TD5 (2.3-3.7 g kg^{-1}). C: N ratios in these two fractions were not different between the treatments of TD5 and TD5+WC.

Table 3-4 OC concentrations and C: N ratios in the tailings fractions in TD5 and TD7 with woodchips application.

Treatments	Macro-iPOC		Micro-iPOC		Macro-iMOC		Micro-iMOC	
	OC (g kg^{-1})	C: N	OC (g kg^{-1})	C: N	OC (g kg^{-1})	C: N	OC (g kg^{-1})	C: N
TD5	3.2(0.3)^{a}	13.9(5.7)^{a}	1.8(0.1)^{a}	7.3(1.1)^{a}	2.3(0.3)^{a}	4.4(1.6)^{a}	3.7(0.4)^{a}	6.1(1.4)^{a}
TD5+WC	12.1(0.7)^{b}	33.0(6.6)^{b}	6.0(0.1)^{b}	17.7(1.8)^{b}	3.1(0.1)^{a}	4.1(0.9)^{a}	5.6(0.5)^{b}	5.3(0.2)^{a}
TD7	3.6(0.3)^{a}	14.6(1.1)^{a}	2.9(0.2)^{a}	7.8(0.6)^{a}	7.0(0.9)^{b}	10.9(2.4)^{b}	6.0(0.2)^{b}	9.2(1.2)^{b}
TD7+WC	37.3(10.3)^{c}	40.1(11.4)^{b}	6.0(0.4)^{b}	10.6(2.0)^{c}	9.4(0.9)^{c}	9.9(0.9)^{b}	10.0(0.5)^{c}	10.4(1.1)^{b}

Values are means (n = 3) with standard deviation in brackets; values labelled with letters 'a, b, c, d' within the column indicate significant differences among the treatments at the level of $P < 0.05$.

The WC application also significantly increased OC concentrations in all the fractions in TD7+WC compared to TD7. The Macro-iPOC fraction in the TD7+WC contained the highest OC concentration (37.3 g kg^{-1}) and C: N ratio (40.1), significantly greater compared to respective fraction in TD7 ($P < 0.001$). The OC concentration in Micro-iPOC fraction in TD7+WC doubled compared to the respective fraction in TD7. The C: N ratio in this fraction was also significantly greater in TD7+WC compared to TD7 ($P < 0.05$) Furthermore, OC concentrations in Macro-iMOC and Micro-iMOC in the TD7+WC increased significantly (9.4-

10.0 g kg^{-1}) compared to the TD7 (6.0-7.0 g kg^{-1}), but with no effect on C: N ratios in these two fractions.

In both TD5+WC and TD7+WC, C: N ratios were highest in unprotected OC (Macro-iPOC) (33.5-40.1), followed by physically protected OC (Micro-iPOC) (10.6-17.7) and mineral associated OC (Macro-iMOC and Micro- iMOC) (4.1-10.9).

3.3.4 OC distribution in the tailings fractions

The OC content in each of the OC fractions was calculated based on OC concentrations and mass aggregate distribution (%) of each fraction (Fig. 3-3). The pattern of OC distributed in each fraction and the contributing mechanisms to OC stabilisation in these tailings (TD5 and TD7) differed from each other.

Generally, OC contents in the unprotected fraction of Macro-iPOC (0.18-0.19 g kg^{-1}) were extremely low in both TD5 and TD7, accounting for 4.7-12.8 % of TOC in these tailings (Fig. 3-3). The OC stored in this fraction was significantly increased by the addition of WC to as much as 0.85 and 4.11 g kg^{-1} in the TD5+WC and TD7+WC, respectively, accounting for 19.6 % and 38.5 % of TOC in these two tailings.

The physically protected OC (Micro-iPOC) (0.60-0.73 g kg^{-1}) was low in TD5 and TD7, accounting for 47.8 % and 15.8 % of TOC in these tailings, respectively. In TD5+WC and TD7+WC, the physically protected OC (Micro-iPOC) increased to 1.42 and 1.45 g kg^{-1}, accounting for 33.6 % and 13.9 % of the TOC, respectively. Physical protected OC was a major form of OC stabilised in both TD5 and TD5+WC.

The mineral associated OC (MOC), sum of Macro-iMOC, Micro-iMOC and oMOC, in TD5 was as low as 0.11-0.32 g kg^{-1} in each of the fractions and a combined total of 0.60 g kg^{-1}. Addition of WC significantly increased the OC in TD5+WC in form of organo-mineral complexes, which appeared in Macro-iMOC (0.38 g kg^{-1}) and Micro-iMOC (0.98 g kg^{-1}), particularly in the latter. Overall, MOC is a dominant form of the TOC (45.3 %) in TD5+WC, compared to the physically protected OC (33.6 %).

The MOC in TD7 was considerably higher at 0.53-1.90 g kg^{-1} in each of the fractions (a combined total of 3.00 g kg^{-1}). The addition of WC in TD7 significantly increased OC content in the fractions of Macro-iMOC (1.70 g kg^{-1}), Micro-iMOC (0.90 g kg^{-1}) and oMOC (2.21 g kg^{-1}) ($P < 0.001$). Total MOC increased to 4.82 g kg^{-1} in TD7+WC, accounting for 45.9 % of the TOC in this treatment.

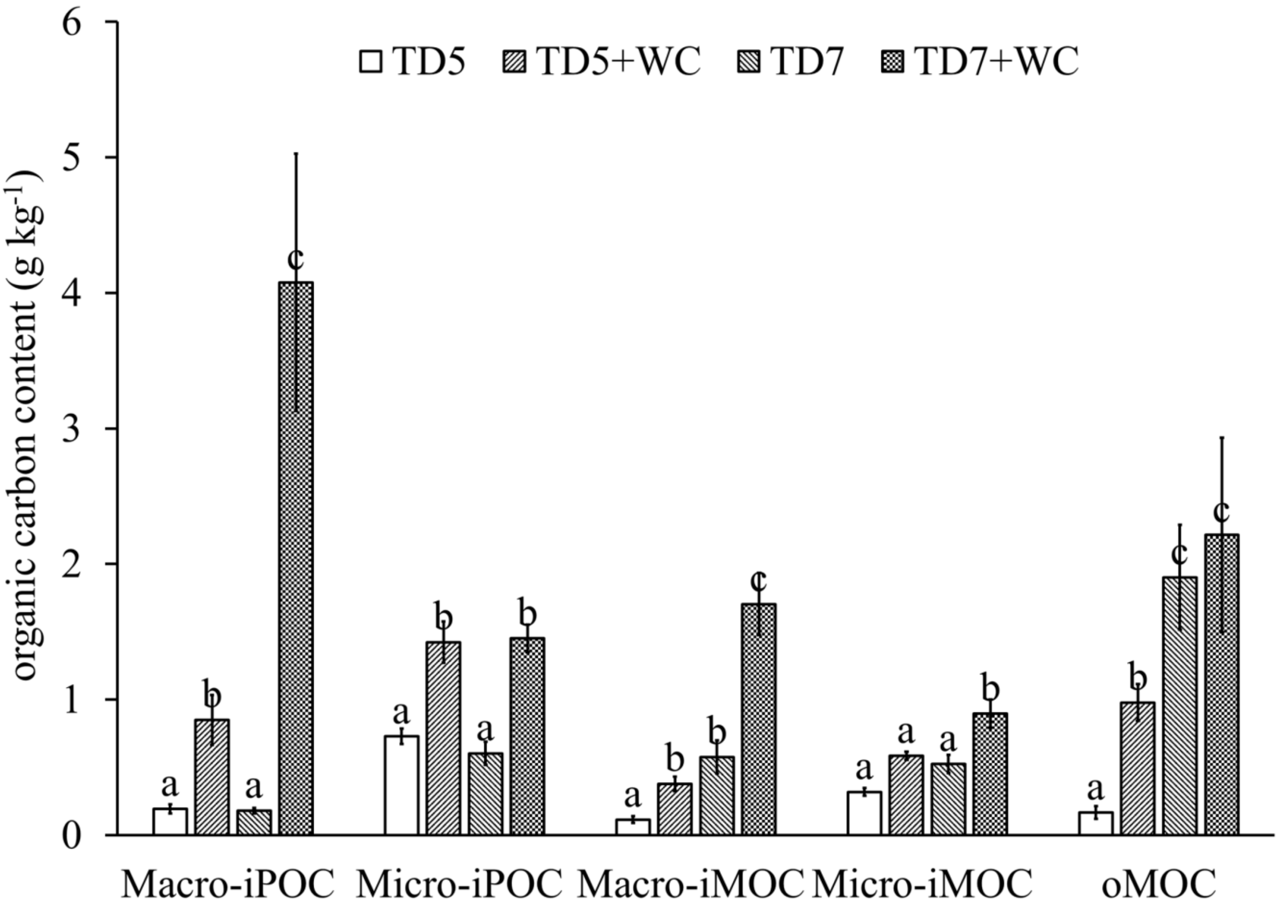

Fig. 3-3 OC content as non-protected OC (Macro-iPOC), physically protected OC (Micro-iPOC) and mineral associated OC in macroaggregate (Macro-iMOC), microaggregate (Micro-iMOC) and silt+clay particles (oMOC) in TD5 and TD7 with woodchips application. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above each OC fractions indicate significant differences among the treatments at the level of $P < 0.05$.

As shown in Fig. 3-4, distribution of OC in different fractions indicated that majority of the OC (61.5-95.3 %) was either physically protected in microaggregate or mineral associated in all the tailings. Application of WC in both TD5 and TD7 enhanced OC in a non-protected form (Macro-iPOC), which could be further decomposed. Physically protected OC in microaggregate (Micro-iPOC) was the most important component (47.8 %) of TOC in TD5 compared to the mineral associated OC (39.4 %). The proportion of MOC was as high as 45.3-77.3 % of TOC in TD5+WC, TD7 and TD7+WC. Overall, physically protected OC had greater contribution in TD5 and TD5+WC compared to the respective fraction in TD7 and TD7+WC.

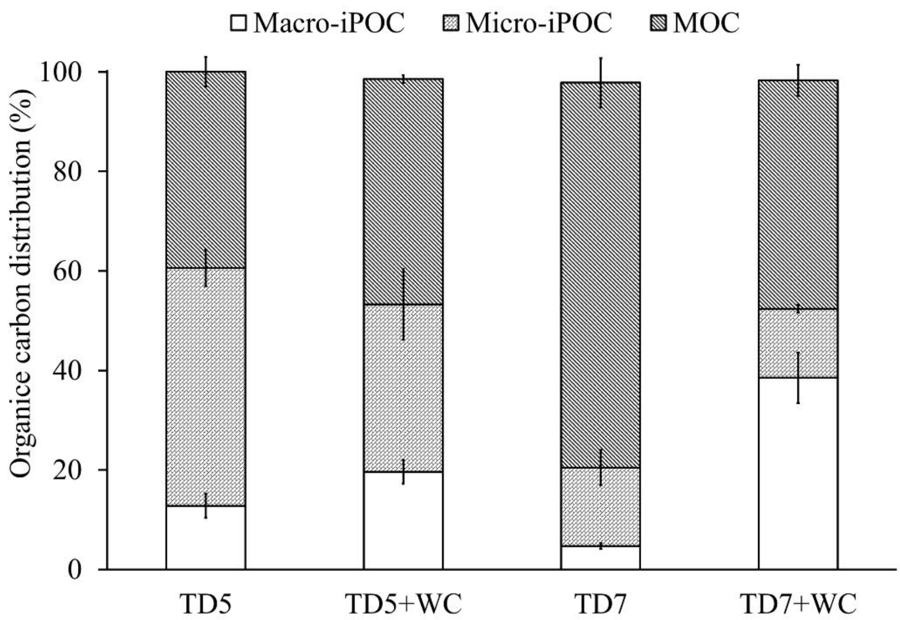

Fig. 3-4 Distribution (%) of Macro-iPOC, Micro-iPOC and MOC (sum of Macro-iMOC, Micro-iMOC and oMOC) in TD5 and TD7 with woodchips application. Values are means (n = 3); error bars indicate standard deviations.

We also found closely positive relationship between the amounts of OC stabilised in the tailings and contents of Fe and Al (hydr-) oxides in the tailings (Table 3-5). Specifically, the crystalline Fe, and both crystalline and amorphous Al had significantly positive correlations with MOC in the tailings ($P < 0.01$).

Table 3-5 Correlations among OC fractions and Fe and Al (hydr-) oxides in TD5 and TD7 with woodchips application.

OC fractions	Al_o	Fe_o	Al_d	Fe_{d-o}
TOC	0.51	0.31	0.76**	0.86**
Micro-iPOC	-0.12	0.45	0.10	0.65*
MOC[a]	0.75**	0.25	0.89**	0.72**

Values followed with '** and *' indicate significance of correlations at the levels of $P < 0.01$ and $P < 0.05$ respectively.

3.4 Discussion

The importance of TOC as an overall indicator of soil quality has been widely recognised (Anderson, 1977; Bendfeldt et al., 2001). Increasing TOC in tailings is also a critical factor in the process of soil development toward biogeochemically functional technosols supporting sustainable plant communities (Li and Huang, 2015). TOC (1.5-3.9 g kg^{-1}) in the TD5 and TD7 tailings without organic amendment was close to the levels of surface (0-10 cm) desert soils (0.5-3.5 g kg^{-1}) (Charley and West, 1975). As well as many other limiting physicochemical factors, the low levels of TOC in the tailings make it difficult for colonisation of microorganisms and thus limiting the biogeochemical processes to sustain productive and healthy plant communities (Harris, 2009). The present study showed that the addition of exogenous organic matter (e.g., woodchips of local native trees) rapidly and significantly increased the TOC in the Cu-Pb-Zn tailings. The rate of stabilised OC (physically protected and mineral associated) increase in the tailings in the present study appeared to be faster (0.5-1.5 g kg^{-1} y^{-1}) than those formed under naturally rehabilitated vegetation cover in glacial till mine spoil in a semi-arid climate and those stabilised at an early stage of ecosystem development (Anderson, 1977; Crews et al., 2001) and the re-established forest (Richter et al., 1999) (< 0.1 g kg^{-1} y^{-1}). Consistent with previous studies (Bendfeldt et al., 2001; Shukla et al., 2004), in the present study, significant positive relationships were observed between TOC and nutrient availability (TN, $R^2 = 0.93$, $P < 0.01$, n = 12), aggregation (MWD, $R^2 = 0.88$, $P < 0.01$, n = 12), water relations (WHC, $R^2 = 0.82$, $P < 0.01$,, n = 12), chemical buffering capacity (CEC, $R^2 = 0.90$, $P < 0.01$, n = 12) and microbial properties (MBC, $R^2 = 0.64$, $P < 0.05$, n = 12) (Table 3-2). Thus, compared to unamended tailings, the WC amended tailings, including both TD5+WC and TD7+WC, with greater TOC (4.5-10.5 g kg^{-1}) are likely to be less deficient in N and with greater water and nutrient retention capacity. The percentage of macroaggregate in both TD5 and TD7 (< 15 %) were significantly increased to (19.2-29.6 %) with WC application, getting close to those found in natural soils (> 30 %) (Bird et al., 2002). More macroaggregates and pores in these amended tailings will allow improved water and gas movement (Tisdall and Oades, 1982).

Stimulating aggregation by organic amendments, especially microaggregation, is expected to contribute to OC stabilisation in the tailings. Based on the hierarchy aggregation model (Tisdall and Oades, 1982), microaggregation of mineral particles largely depends on binding agents, such as amorphous Fe and Al oxy/hydroxides, and organic molecules probably derived from resistant fragments of roots, hyphae, and bacterial cells. Macroaggregation of mineral particles largely depends on plant roots and hyphae, mainly comprised of undecomposed OC (Caesar-TonThat et al., 2008). The physically protected OC was one major form of OC stabilised in TD5 and TD5+WC, accounting for 33.6-47.8 % of TOC. Relative contribution of this fraction is almost 10-fold of those reported in natural soils, but OC content (1.5 g kg^{-1}) in this fraction is much lower than those found in natural soils (von Lützow et al., 2006; Wiesmeier et al., 2012). It is widely recognised that both plant and microbes in tailings are stressed by the unfavourable physicochemical conditions (Santibáñez et al., 2008). The binding agents to aggregate tailings particles may be more related to the presence of secondary minerals such as Al/Fe-oxyhydroxides, and less to organic binding compounds produced by roots and microbes. In addition, increased acids and free cations from mineral weathering in the tailings may result in the dissociation of aggregates (Kögel-Knabner et al., 2008). Therefore, the introduction of pioneer plant species may be required to further enhance aggregation and OC stabilisation processes in the tailings, through improved abundance and activities of associated rhizosphere microbial communities and self-mulching of organic compounds with charged surface from plant litter and root exudates.

Isotope studies showed that the organo-mineral complexes are the dominant form of stable OC below the A horizon in many soil types (Six et al., 2000), which were mainly associated with polymeric Fe and Al hydroxide through sorption, entrapment or complexation (Mikutta et al., 2006; von Lützow et al., 2006). The relative distribution of the OC among various physical fractions appeared different between the weathered (TD5) and fresh (TD7) tailings, due to their differences in mineralogy and geochemistry. The distribution of OC in various fractions in the WC amended tailings may be the combined effects of organic matter decomposition, aggregation, and organo-mineral interactions. Generally, WC application increased the formation of stabilised OC more in the TD7 (fresh) than TD5 (weathered), especially in the mineral associated form (Table 3-4). In the present study, positive relationships were found among amorphous Al (Al_o) and crystalline Fe (Fe_{d-o}) and MOC, suggesting that Fe, Al (hydr-) oxides in the tailings are important in the formation of organo-mineral complexes and associated OC stabilisation (Denef et al., 2002; Duiker et al., 2003). In soils, crystalline Fe and Al oxides are prevalent (Richards et al., 2009), contributing to most of the OC in mineral associated fractions. In the tailings, we also found crystalline Fe and Al oxides also significantly contributed to MOC. The increasing crystalline Fe and Al in the WC amended tailings (Table 3-3), particularly in TD7+WC, might play more important roles in OC stabilisation in latter phase. In addition to Fe and Al (hydr-) oxides, the difference of levels and distribution of MOC between TD5 and TD7 may also have been influenced by the Ca/Mg minerals (Jastrow et al., 2007), reactive surface ratio (CEC, clay content), pH conditions (Carrasco et al., 2009) or abundance of metal ions (e.g., Cu^{2+}, Zn^{2+}) (Manceau and Matynia, 2010), which need further invesitgation.

We found significant decreases of C: N ratios in both physically protected OC and organo-mineral complexes compared to those non-protected form (Table 3-3). The findings are consistent with previous observations (Plaza et al., 2013). This might result from the preferential enrichment of N-containing compounds (e.g., those of protein, microbial by-product etc.) (Wang and Lee, 1993) bound to tailings particles. N-rich organic materials (e.g., crop residues, compost, leguminous plant biomass etc.) may be used partially or wholly (depending on their local availability) to enhance efficacy of OC stabilisation in tailings. Organic materials with relative high N and density of functional anion groups may be advantageous to stimulate organo-mineral interactions in tailings, such as sugarcane residue and compost (Yuan et al., 2014).

In addition to N-level in the organic amendment used in the tailings, the low microbial biomass may have limited the decomposition of exogenous organic matter and thus the formation of stabilised OC associated with aggregates. The relative effectiveness of different organic materials in stimulating soil development of the tailings, in relation to their effects on microbial community abundance, composition and functions, will be investigated later in this thesis (see Chapter 5 and 7).

As sulphidic Cu-Pb-Zn tailings, both TD5 and TD7 contain abundant sulphide minerals, mainly in the form of pyrites (Forsyth, 2014). The content of pyrite in TD5 (1-2 % w/w) is much lower than that of TD7 (12-15 %), as the former is much older (nearly 40-year old since deposition) compared to the latter (< 2 years) (Forsyth, 2014). Oxidation and co-dissolution of reactive minerals (e.g., galena, sphaleriete, chalcopyrite etc.) are common phenomenon in sulphidic tailings, resulting in acidification and/or release of soluble salts (Aguilar et al., 2004). In the present study, WC application increased the levels of soluble salts (EC, water-soluble S) and heavy metals (water-soluble Cu and Zn) in the WC amended TD5 and TD7. Salts may move to the surface of tailings via water evaporation capillary rise although due to the woodchips addition improved the structure, macropore formation and thus the water infiltration water conductivity. In addition, the considerable increase of salts and heavy metals suggested further mineral weathering and pyrite oxidisation were enhanced by the WC treatment, especially in the fresh tailings (e.g., TD7). The enhanced geochemical reactions by organic amendments may be exploited to further enhance the weathering of tailings minerals and thus consolidate the degree of hydrogeochemical stability in the early phase of soil formation, leading to an improved chance of biogeochemical rehabilitation in the later phase.

3.5 Conclusions

In summary, organic amendment (woodchips) rapidly increased the amount of stabilised OC in neutral Cu-Pb-Zn tailings regardless their weathering stages, which is critical to the technosols formation and the associated rehabilitation of physical structure and biogeochemical processes in the long term. The majority of OC (61.5-80.3 %) was either physically protected through aggregation or in the form of organo-mineral complexes in the amended tailings. Physically protected OC was a dominant form of OC in TD5 and mineral associated OC was the dominant form of OC in TD7 and WC amended tailings (45.3-77.3 %). The Fe and Al (hydr-) oxides in tailings contributed significantly to OC stabilised in mineral associated fractions. In addition, OC stabilised in both tailings was enriched with N. Organic amendments with high N and density of surface functional groups (such as sugarcane mulch, compost, legume biomass, etc.) may be advantageous in enhancing OC stabilisation and aggregation in tailings, compared to organic amendment with very high C: N ratios (such as woodchips of native trees). This could be investigated in future research.

Chapter 4 Plant colonisation stimulated organic carbon stabilisation and microbial biomass and functions in neutral and weathered Cu-Pb-Zn tailings from a long-term field trial

4.1 Introduction

The success of phytostabilisation of sulphidic tailings depends on the development of plant-tolerable root zone conditions in the tailings profiles, specifically a high degree of hydrogeochemical stability and biogeochemical functionality (Huang et al., 2012; Li and Huang, 2015). In natural and cultivated soils, soil organic matter is regarded as an overall indicator of soil quality (Lal, 2009). As an essential constituent in the soil matrix, soil organic matter plays crucial roles in biological (provision of substrate and nutrients for microbes and plants) (Alguacil et al., 2011a), chemical (buffering and pH changes; chelation of metals) (Park et al., 2011) and physical (aggregation and stabilization of soil structure) (Abiven et al., 2009) properties in soils. Tailings, in contrast, are dominated by residue minerals and contain little organic matter in the profile (Lottermoser, 2010). Therefore, stimulating OC stabilisation is expected to rehabilitate the soil physical structure and biogeochemical functions (e.g., nutrients cycling) to support plants colonised in tailings (Huang et al., 2011).

Without inputs, weathered tailings lack a proper physical structure, have only stressed microbial community and functions. They are also low in macronutrients (e.g., N, P, K) and high in soluble salts, and metals and metalloids (Mendez and Maier, 2007; Ye et al., 2002) for direct revegetation. Addition of exogenous organic matter (e.g., plant residue, biosolids, biochar, manure etc.) is a common practice to stimulate soil formation and improve the growth conditions for plants (Fellet et al., 2011; Munksgaard and Lottermoser, 2010). Our earlier findings suggest that, if plants were not introduced, OC was physically protected by organo-mineral interactions and aggregation in both weathered and fresh tailings (Chapter 3) due to the electrostatic interactions between functional groups of organic matter and tailings mineral particles (Yuan et al., 2014; Zech et al., 1997). However, little is known whether the introduction of tolerant native plant species could stimulate OC stabilisation and improve the physical structure, and microbial community and biogeochemical functions in the weathered and neutral Cu-Pb-Zn tailings.

Previous studies also found that the introduction of tolerant native plant species as pioneer plants stimulated the development of heterotrophic bacterial communities (Li et al., 2014), because plant roots can produce exudates and stimulate bacterial colonisation in the rhizosphere (Bais et al., 2006). Alternation of OC in tailings with plant colonisation is assumed to be different from those without plant cover. The recolonisation of rhizosphere bacterial communities may in turn stimulate biogeochemical processes and OC associated with tailings mineral particles through aggregation and organo-mineral interactions (Harris, 2009; Six et al., 2006).

The present study aims to provide a snap-shot analysis of the distribution of OC, microbial biomass and functions in weathered and neutral Cu-Pb-Zn mine tailings from a long-term (2.5-year) field trial under subtropical and semi-arid climatic conditions at Mount Isa, northwest Queensland, Australia. Native plant species were introduced into the tailings treatments with/without organic amendment to test the hypothesis that pioneer plants could stimulate microbial community development with associated functions recovery, and increase the OC stabilisation in aggregates and organo-mineral complexes.

4.2 Materials and methods

4.2.1 Experimental design and sampling

Background information regarding the location and climatic conditions of experimental site was described previously (Li et al., 2014) and Chapter 3. The weathered and neutral Cu-Pb-Zn tailings from Tailings dam 5 (TD5) of Mount Isa Mine (MIM) were excavated in bulk, air-dried, crushed and mixed thoroughly on site before use. An appropriate volumes of the tailings was mixed with 20 % (v/v) woodchips (WC). The properties of WC were described in Chapter 3.2.1. Control (tailings only, TD5) and WC amended tailings (TD5+WC) were loaded into modified industry bulk containers (1 x 1 x 1 m dimension) *in situ* in November, 2009. Native plants (P) (*Triodia pungens, Acacia chisolmii, Ptilotus exaltatus*) were introduced into 3 containers of each treatment, compared to 3 replicates of each treatment without plants introduction, forming 4 treatments in total: TD5, TD5+WC, TD5+P, and TD5+WC+P (Fig. 4-1). During incubation, no fertiliser was applied and regular irrigation watering (about once per week) was performed provided by drip-irrigation in the initial phase, with increasing intervals between watering later.

Tailings and the amended/revegetated tailings from each column 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 column. The fresh samples were sealed in plastic bags in the field and stored at 4 °C for transporting to the laboratory for microbial analysis within 1 week after collection. For physicochemical analyses, subsamples were dried at 40 °C and sieved less than 2 mm prior to use.

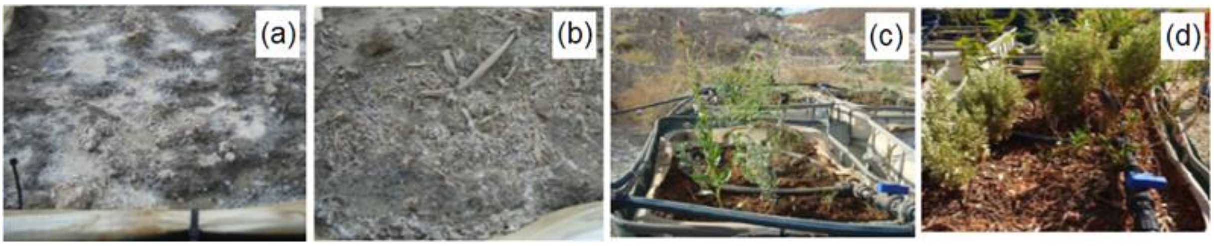

Fig. 4-1 View of field incubated tailings with treatments of TD5 (a), TD5+WC (b), TD5+P (c), and TD5+WC+P (d) at the time of sampling, in Mount Isa, northwest Queensland, Australia (Source: Longbin Huang, the University of Queensland).

4.2.2 Physicochemical analysis

Selected physicochemical properties were measured as described in Chapter 3.2.2. Total N in the water extract was determined colorimetrically with the salicylic acid method (Cataldo et al., 1975) following potassium persulphate digestion (Raveh and Avnimelech, 1979).

4.2.3 Aggregates separation and OC fractionation

Aggregates separation and OC fractionation were conducted as described in Chapter 3.2.4. 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 contents in each fraction were calculated based on OC concentrations and mass distribution (%) of each fraction.

4.2.4 Microbial biomass, organic matter mineralisation and enzyme assays

Microbial biomass was extracted and microbial biomass carbon (MBC) was measured as describe in Chapter 3.2.2. Microbial biomass nitrogen (MBN) was calculated as the difference of ninhydrin nitrogen (Inubushi et al., 1991) between fumigated and unfumigated samples with a conversion factor K_{EN} as 0.54 (Joergensen, 1996). Microbial quotient was calculated as the ratio between MBC and TOC.

The nitrogen mineralisation 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 potassium chloride (2M) for the analysis of mineral nitrogen, the sum of ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) in the extract. The net mineralisation rate was calculated from the difference of mineral N in the extracts of each incubated sample between day 28 and day 0 (Eq. 4-1). Concentrations of NH_4-N in 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).

$$\text{Net mineralisation rate} = \frac{Mineral\ N\ (dn) - Mineral\ N\ (d0)}{n} \quad \text{Eq. 4-1}$$

Where n is the days for incubation, and d_n indicate the mineral N at n^{th} day. D_0 indicate mineral N at day 0.

Activities of 4 soil enzymes (dehydrogenase, invertase, urease, and neutral phosphatase) were measured in this study with fresh samples. Dehydrogenase activity was measured using the incubation method followed the method of Serra-Wittling et al., (1995). In brief, 2 g of samples was incubated with 2 ml of 0.5% 2,3,5-triphenyl-tetrazolium chloride (TTC) and 2 ml of Tris–HCl buffer (0.5 M, pH 7.6) for 2 h at 30 °C in the dark. Sample sterilised by autoclaving are used as a paired blank. Immediately after incubation, the triphenyl formazan (TPF) formed was extracted with 100 ml of methanol by shaking vigorously for 1 min. TPF was measured spectrophotometrically at 485 nm using methanol as the blank. Dehydrogenase activity was calculated as the difference between unsterilised and sterilised samples and expressed as μg TPF g^{-1} h^{-1}. Invertase activity was determined using sucrose as the substrate (Frankeberger and Johanson, 1983). In brief, 0.4 ml of toluene was added to 2 g tailings sample and allowed to stand for 15 min. Then, 2 ml of 10 % sucrose and 2 ml acetic acid buffer (0.2 M, pH 5.5) were added. The mixture was incubated for 24 h at 37 °C and made up to 100 ml with deionised water. After filtration, reducing sugars in 1 ml of filtrate were measured using a molybdenum-blue method (Gusakov et al., 2011). Invertase activity was based on the difference in reducing sugar concentrations between the substrate induced samples and a blank control and calculated as μg of reducing sugar g^{-1} h^{-1}. Urease activity was determined with urea as substrate for incubation (McGarity and Myers, 1967). In brief, 0.4 ml of toluene was added to a 2 g sample and allowed to stand for 15 min. The samples were then mixed with 2 ml of 10 % urea solution and 2 ml citric acid buffer solution (1 M; pH 6.7). After incubation at 37 °C for 24 h, the culture solution was made up to 100 ml with deionised water and immediately filtered. The resulting ammonium product in the filtrate was measured colorimetrically using the indophenol-blue method (Ivančič and Degobbis, 1984). Urease activity was calculated from the difference between the produced ammonium and the initial ammonium content of the control. Neutral phosphatase activity was analysed by the disodium phenyl phosphate method (Shen et al., 2006). Briefly, 0.4 ml toluene was added to 2 g of sample and allowed to stand for 15 min. The sample was then mixed with 2 ml of 0.5 % (w/v) disodium phenyl phosphate and 2 ml of citric acid buffer (0.2 M, pH 7.0) and incubated for 24 h at 37 °C. The culture solution was then made up to 100 ml with 38 °C distilled water and filtered. 1 ml filtrate was diluted to 5 ml with distilled water. After the addition of 4 ml of borate buffer (0.05 M, pH 10), 0.5 ml of 2 % 4-amino antipyrine and 0.5 ml of 8 % potassium ferricyanide, absorbance at 510 nm of the solution was measured spectrophotometrically. Neutral phosphatase activity was calculated as the difference of phenol formed between the substrate-induced sample and the sample free control.

4.2.5 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 significant different effects of organic amendments and plant colonisation. 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).

4.3 Results

4.3.1 Selective physicochemical properties of the tailings

The analyses of physicochemical properties of the tailings across treatments (Table 4-1) showed TD5 tailings were characterised by the lowest levels of TOC, N, WHC and CEC. Also, the water-soluble organic carbon (WSOC) and water-soluble nitrogen (WSN) in TD5 were the lowest among all treatments, measuring 5.1 and 0.9 mg kg^{-1} respectively. Tailings in TD5 contained high concentrations of soluble salts (EC) and S. Cu, Pb and Zn were abundant in tailings at the levels far higher than those of ecological investigation limits. Tailings in TD5 were acid to neutral with pH of 6.69. The water-soluble heavy metals (such as Cu and Zn) were 1000-fold lower than their total levels. Aggregate distribution in TD5 was dominated by microaggregate fraction (49.8 %) with the lowest abundant of macroaggregate (11.2 %) and smallest MWD (0.21 mm) among all the treatments.

Table 4-1 Selective physicochemical properties in TD5 with woodchips application and plant colonisation.

Treatments		pH	EC (mS cm^{-1})[a]	CEC (cmol$_+$ kg^{-1})[b]	WHC (%)[c]	C:N	Total (g kg^{-1})							Water Soluble (mg kg^{-1})					Aggregates		
							TOC[d]	TN[e]	Cu	Pb	Zn	S	Fe	C	N	S	Cu	Zn	Macroaggregate (%)	Microaggregate (%)	MWD(mm)[f]
TD5	Mean	6.69	3.4$^{\alpha}$	0.79$^{\alpha}$	19.2$^{\alpha}$	13.7	1.5$^{\alpha}$	0.11$^{\alpha}$	1.1	1.9	4.1	47	91	5.1$^{\alpha}$	0.9$^{\alpha}$	238$^{\alpha}$	0.12$^{\alpha}$	18.4	11.2$^{\alpha}$	49.8$^{\alpha}$	0.21$^{\alpha}$
	S.D.[g]	0.04	0.3	0.27	1.2	2.4	0.0	0.03	0.1	0.1	0.3	4	6	2.0	0.4	125	0.01	2.3	2.4	4.0	0.03
TD5+WC	Mean	6.66	4.5$^{\beta}$	1.66$^{\alpha}$	21.8$^{\beta}$	13.3	4.3$^{\beta}$	0.34$^{\beta}$	1.0	1.9	3.9$^{\alpha}$	46	88	48.8$^{\beta}$	3.1$^{\beta}$	601$^{\beta}$	0.33$^{\beta}$	18.5	19.2$^{\beta}$	31.1$^{\beta}$	0.28$^{\beta}$
	S.D.	0.08	0.8	0.41	0.7	2.9	0.4	0.11	0.1	0.2	0.4	5	4	19.5	0.2	113	0.01	2.6	2.4	1.5	0.03
TD5+P	Mean	6.41	5.2$^{\beta}$	4.52$^{\beta}$	22.1$^{\beta}$	11.7	4.7$^{\beta}$	0.40$^{\beta}$	1.0	1.5	3.5	41	83	26.1$^{\gamma}$	17.2$^{\gamma}$	706$^{\beta}$	0.45$^{\gamma}$	17.5	19.1$^{\beta}$	50.2$^{\alpha}$	0.30$^{\beta}$
	S.D.	0.06	0.4	0.31	0.4	1.7	0.2	0.04	0.1	0.1	0.6	2	5	1.9	1.2	73	0.06	0.8	2.8	0.3	0.03
TD5+WC+P	Mean	6.50	5.6$^{\beta}$	4.48$^{\beta}$	21.7$^{\beta}$	13.3	5.5$^{\gamma}$	0.43$^{\beta}$	1.0	1.6	3.7	38	94	78.3$^{\delta}$	19.0$^{\delta}$	710$^{\beta}$	0.48$^{\gamma}$	17.2	21.1$^{\beta}$	51.3$^{\alpha}$	0.32$^{\beta}$
	S.D.	0.03	0.7	0.43	0.2	2.2	0.0	0.08	0.0	0.1	0.4	3	8	2.4	1.3	92	0.03	0.3	1.1	2.1	0.01

[a] Electrical conductivity. [b] Cation exchangeable capacity. [c] Water holding capacity. [d] Total organic carbon. [e] Total nitrogen. [f] Mean weight diameter. [g] Standard deviation; Values are means (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)

The application of WC and plant colonisation brought about significant changs of physicochemical properties in the tailings (Table 4-1). With WC application and plant colonisation, WHC in all the amended tailings increased significantly ($P < 0.01$), and there were significant increase in macroaggregate (19.2-21.1 %) in all the amended and/or revegetated tailings with significantly increased MWD ranging from 0.28 to 0.32 mm ($P < 0.05$). OC levels and nutrients conditions improved considerably in these amended tailings. Specifically, TOC, TN, CEC increased by 2-6 folds in all the amended tailings ($P < 0.05$) compared to TD5, with the greatest level found in the treatment of TD5+WC+P.

Around 1-2 % of TOC in these amended tailings were in the water-soluble form (WSOC), which also significantly increased ($P < 0.05$), particularly in the treatment of TD5+WC+P. Moreover, WSN increased from 0.9 to 3.1 mg kg^{-1} in TD5+WC compared to TD5. Particularly, in treatments with plants, including TD5+P and TD5+WC+P, WSN increased by 5-fold compared to those in TD5+WC, which were 17.2 and 19.0 mg kg^{-1} respectively.

Furthermore, WC application and plant colonisation shifted the geochemistry in the amended tailings. Tailings in all the treatments were acid to neutral with pH ranging from 6.41 to 6.69. A slight decrease of pH was found in all the amended tailings but was not statistically different from the control. In contrast, EC in the amended tailings ranged from 4.5 to 5.6 mS cm^{-1}, significantly greater than the level in TD5 (3.4 mS cm^{-1}) ($P < 0.001$). In addition, the levels of water-soluble S and Cu in the amended tailings were 2-3 folds compared to those in TD5.

4.3.2 OC concentrations, C: N ratios and content in the tailings fractions

In general, 98-99 % TOC in the tailings was in the insoluble form, a vast majority (64-87 %) of which was physically protected in microaggregate or in organo-mineral complexes in the tailings. Similar to TOC, OC concentrations and OC contents in all the fractions were lowest in TD5. WC and plants colonised in tailings increased OC concentrations and OC contents in all the fractions significantly (P < 0.05), except the OC concentration of Macro-iMOC fraction in TD5+WC (Table 4-2).

In bulk tailings, C: N ratios ranged from 11.7 to 13.7 with no significant differences among the treatments (Table 4-1); this ratio did vary greatly among different OC fractions (Table 4-2). Specifically, we observed that in all the amended tailings, C: N ratios were highest in the Macro-iPOC (24.5-33.0) fraction, followed by Micro-iPOC (16.8-17.7) and MOC (4.1-6.3) (e.g., Macro-iMOC, Micro-iMOC). Compared to respective fractions in TD5.

Table 4-2 OC concentrations and C: N ratios of OC fractions in TD5 with woodchips application and plant colonisation

Treatments	Macro-iPOC		Micro-iPOC		Macro-iMOC		Micro-iMOC	
	OC ($g\ kg^{-1}$)	C: N	OC ($g\ kg^{-1}$)	C: N	OC ($g\ kg^{-1}$)	C: N	OC ($g\ kg^{-1}$)	C: 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

Values are means (n = 3) with standard deviation in bracket; values labelled with letters 'a, b, c, d' indicate significant differences among the treatments at the level of $P < 0.05$.

Changes of C: N ratios with WC application and plants colonisation also varied among fractions and treatments (Table 4-2). Specifically, C: N ratios in fraction of Macro-iPOC significantly increased from 13.9 in TD5 to 24.5-33.0 ($P < 0.05$) in the amended tailings. The ratios in physically protected OC also increased from 7.3 in TD5 to 16.8-17.7 (P < 0.05) in the amended tailings. In contrast, C: N ratios in Macro-iMOC and Micro-iMOC between TD5 and amended tailings were not statistically significantly different.

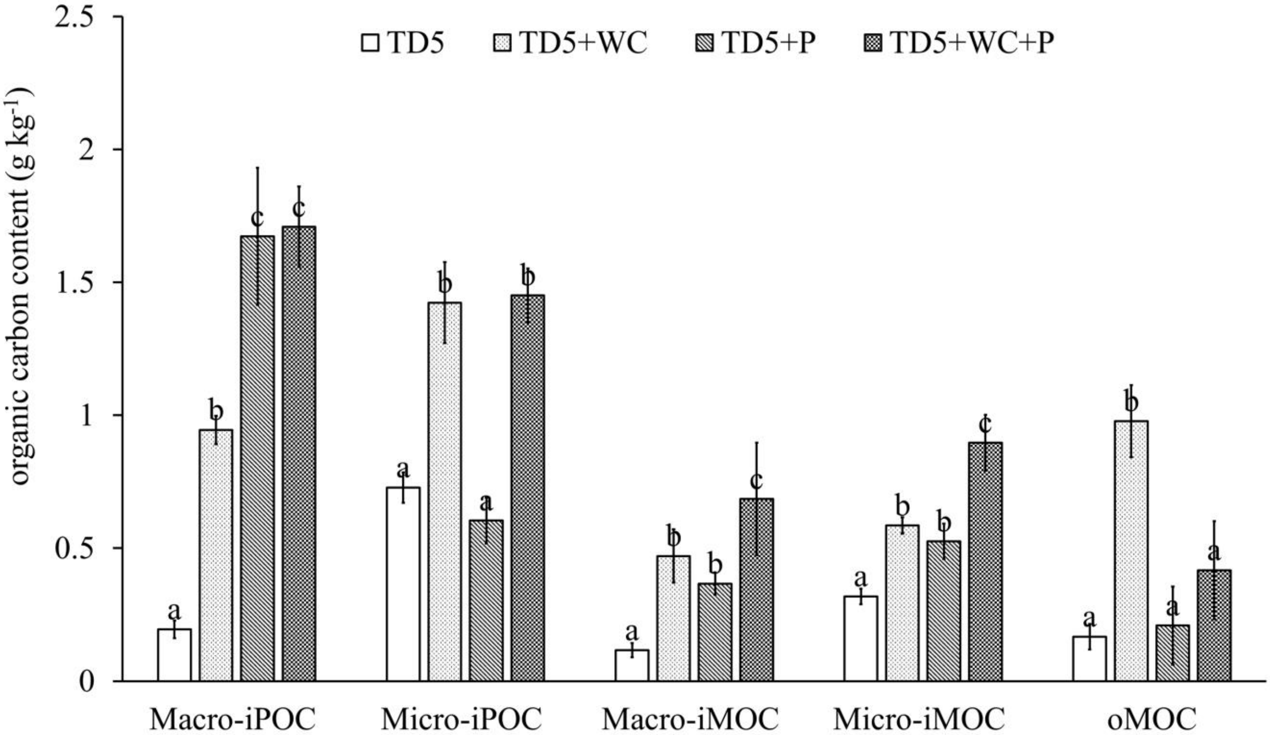

Fig. 4-2 OC content as non-protected OC (Macro-iPOC), physically protected OC (Micro-iPOC) and mineral associated OC in macroaggregate (Macro-iMOC), microaggregate (Micro-iMOC) and silt+clay particles (oMOC) in TD5 with woodchips application and plant colonisation. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above each OC fractions indicate significant differences among the treatments at the level of $P < 0.05$.

4.3.3 Microbial community and functions among treatments

Microbial biomass (MBC, MBN), microbial quotient (MBC: TOC), and net mineralisation rate were measured for all the treatments. As shown in Table 4-3, MBC only accounted for a small portion (0.5-1.4 %) of TOC in all the treatments. Consistent with TOC, it is not surprising that microbial biomass (MBC and MBN) were lowest in TD5. Microbial biomass (MBC and MBN) changed significantly with application of WC and plant colonisation, increasing in all the amended tailings, particularly in the TD5+WC+P treatment.

A similar trend of net N mineralisation rates was observed among these treatments (Table 4-3). In particular, compared to treatment of TD5+WC, tailings in both treatments of

TD5+P and TD5+WC+P were characterised by greater biomass, microbial quotient (MBC: TOC) and net N mineralisation rate (Table 4-3).

Table 4-3 Microbial biomass (MBC, MBN), microbial quotient (MBC: TOC) and net N mineralisation rate in TD5 with woodchips application and plant colonisation.

Treatments	MBC ($mg\ kg^{-1}$)	MBN ($mg\ kg^{-1}$)	MBC: TOC (%)	Net N mineralisation 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}

Values are means (n = 3) with standard deviation in brackets; values labelled with letters 'a, b, c, d' indicate significant differences among the treatments at the level of $P < 0.05$.

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. 4-3). Microbial biomass was positively linked to their activities in the tailings (Table 4-4), which increased in all the amended tailings, especially in the treatment of TD5+WC+P.

The activities of dehydrogenase, invertase and neutral phosphatase were consistently higher in the treatment of TD5+P and TD5+WC+P regardless of WC treatment. In contrast, the activity of urease activity was less effectively rehabilitated in TD5+P tailings and only significantly increased in TD5+WC and TD5+WC+P. Moreover, neutral phosphatase activity showed no significant differences among the amended treatments, TD5+WC, TD5+P, and TD5+WC+P.

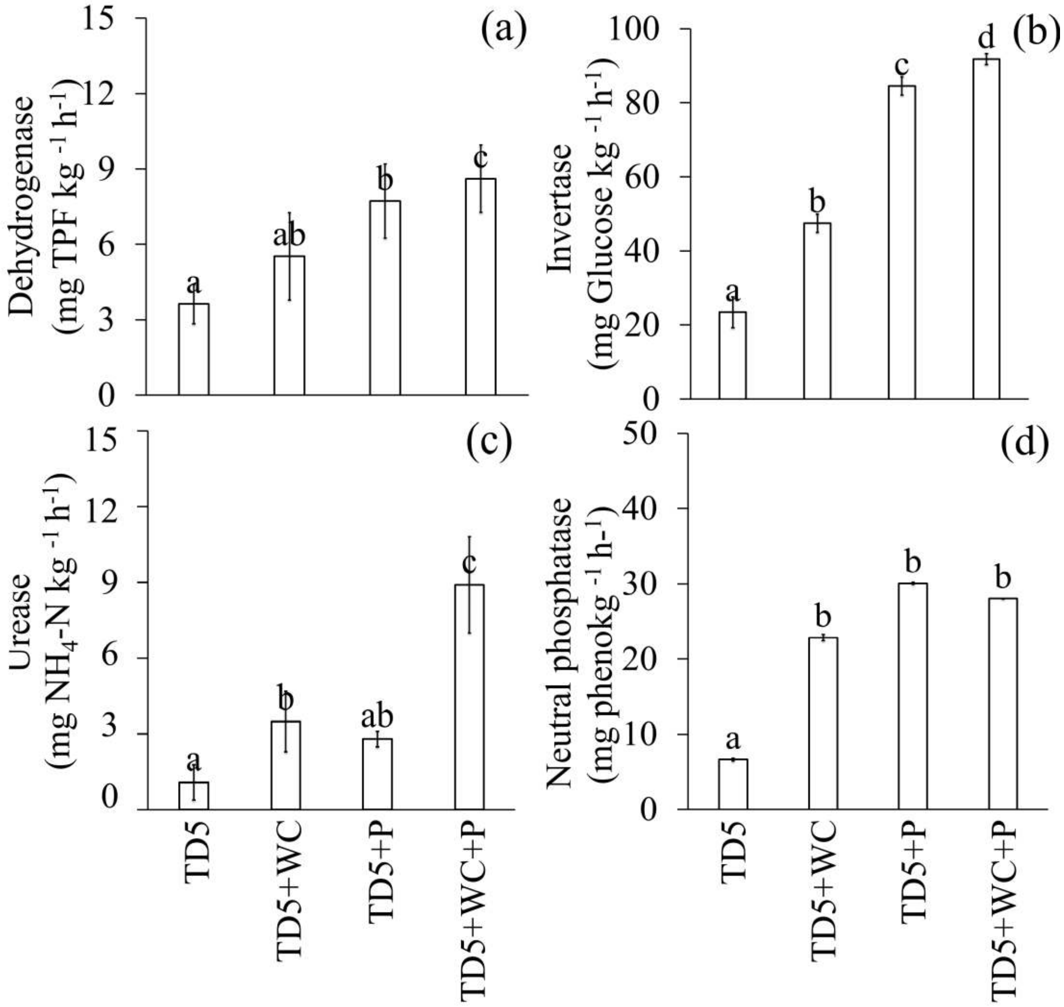

Fig. 4-3 Activities of dehydrogenase (a), urease (b), invertase (c) and neutral phosphatase (d) in TD5 with woodchips application and plant colonisation. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above the bars indicate significant differences among the treatments at the level of $P < 0.05$.

Table 4-4 Correlations among microbial biomass and activities in TD5 with woodchips application and plant colonisation.

Microbial properties	Net mineralisation rate	Dehydrogenase activity	Urease activity	Invertase activity	Neutral phosphatase activity
MBC	0.92**	0.77**	0.73**	0.93**	0.71**
MBN	0.91**	0.78**	0.68*	0.96**	0.77**

Values followed with '** and *' indicate significance of correlation at the levels of $P < 0.01$ and $P < 0.05$ respectively.

4.4 Discussion

The present findings have shown that OC in the weathered and neutral Cu-Pb-Zn tailings increased through the addition of organic matter (woodchips), which were further significantly enhanced by the colonisation of native plants under field conditions. In addition, microbial community and functions were rehabilitated in the amended tailings, particularly in those with a combined application of organic amendment and plant colonisation.

4.4.1 OC stabilisation in the tailings in response to woodchips application and plants colonisation

Both WC and plants colonised in tailings are important sources of OC which would be decomposed by microbial colonisers and stabilised in tailings. TOC in TD5 is extreme low (1.5 mg kg^{-1}). Only 13 % TOC in TD5 were in the unprotected form with C: N ratio of 13.9, close to that (13.7) of bulk tailings. Organic matter in TD5 may be at late stage of decomposition with majority of them comprised with recalcitrant organic compounds (Couˆteaux et al., 1995). In all the amended tailings, unprotected OC contained 22-36 % of TOC with the greatest C: N ratio ranging from 24.5-33.0 (Table 4-2). These OC might be furthered decomposed and stabilised in the amended tailings (Bruun et al., 2010).

It is widely accepted that recalcitrance, spatial inaccessibility and organo-mineral interactions are major mechanisms contributing to OC stabilisation (von Lützow et al., 2008). We observed that majority of OC (64-77%) in the amended tailings were physically protected or in the form of organo-mineral complexes. In the TD5 treatment, physically protected OC were dominant than mineral associated OC in the tailings, presenting as high as 47.8 % of TOC. MOC became dominant in tailings with WC application and plant colonisation, accounting for 45-77 % in the amended tailings. In the present study, OC stabilised (physically protected and mineral associated) via WC application and plant colonisation found to be time efficient compared to early soil ecosystem development (Anderson, 1977; Crews et al., 2001).

Physically protected OC is closely linked to the aggregation processes in soil. In the present study, stimulated aggregation in the amended tailings not only contributed to the stabilisation of OC in tailings (Table 4-5), it is also fundamentally important to improve the tailings' physical structure and hydraulic properties (Table 4-1) (Six et al., 2000). Stimulating aggregation and OC stabilisation strongly depends on the presence of binding agents (e.g., fragments of roots, hyphae, polysaccharides, bacterial cells and colonies) (Tisdall and Oades, 1982). In the examined tailings, WC application stimulated the macroaggregate development, with greater MWD compared to TD5. In the plant colonised tailings, both macroaggregate and microaggregate development stimulated, particularly in the tailings with combined WC and plant colonisation (Table 4-1), which might be attributed to aid of plants roots and a greater microbial biomass (Table 4-3).

Moreover, plants are also particularly important in stimulating OC stabilised with tailings mineral particles (Table 4-5). OC concentrations were significantly greater in these mineral associated fractions (Macro-iMOC and Micro-iMOC) with plant colonisation (TD5+P and TD5+WC+P) compared to tailings without (TD5 and TD5+WC). Capacity of OC associated with tailings minerals depends strongly on the surface chemistry of organic matter and minerals. For example, carboxylic C with negative charge has the largest potential to bind to minerals, followed by phenolic and hydroxylic C (Kumar et al., 2007), while alkyl and aromatic C, which are relatively nonpolar or hydrophobic involve little in organo-mineral interactions. 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) (Vieublé Gonod et al., 2006) are likely to strongly bind with minerals (e.g., Fe, Al, Mn oxides, edge sites of phyllosilicates, allophane, imogolite, smectite, vermiculite, illite) to form resistance organo-mineral complexes in the soil (Feng et al., 2005; Kögel-Knabner et al., 2008). The different OC stabilised with tailings mineral particles among treatments could be at least partially attributed to the different forms of OC entering into tailings and the biogeochemical modification of organic compounds. Interestingly, levels of WSN were particularly higher in treatments with plant colonisation than those in TD5 and TD5+WC. Natural vegetation improving N supply is not only important to plant growth but also to microorganism development. Variations among treatments suggested that limited N is available in WC, while colonisation of plants aid the fertility improvement in the root zone tailings, which may be consequences of root exudates and/or litter return from established plants. In this case, N_2 fixation in roots of the planted leguminous plants (*Acacia* spp.) might have occurred and contributed to the N supply in the rhizosphere. The enriched soluble N-containing compounds might also contribute to OC stabilisation in tailings with plants colonisation (Plaza et al., 2013).

Table 4-5 Two-way ANOVA of woodchips and plant colonisation impacts on 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
oMOC	0.00	0.985	6.42	0.035	2.74	0.137

WC low in N (C: N ratio of 98) and WSN might be decomposed slowly in the examined tailings (Aber and Melillo, 1982), constrained by the small size and tolerant dominant bacterial community (e.g., *rubrobacter*) (Li et al., 2014). In contrast, in tailings with plant colonisation, root exudates, the mixture of enormous range of small molecular weight compounds (e.g., carbohydrates, carboxylic acids and amino acids) (Lynch and Whipps, 1991), might enter into tailings. Due to their negative charges, these substances may be sorbed to the mineral phase through cation binding (Jones and Brassington, 1998). Meanwhile, organic matter derived from roots of plant colonised in tailings could be incorporated into microbial biomass, stimulating the decomposition and element cycling processes in the improved tailings (Fig. 4-3). As decomposition proceeds, N is retained in the microbial products while C is respired (Quideau et al., 2000). Therefore, these N enriched organic compounds might readily interact with tailings mineral particles, contributing to OC stabilised in the form of organo-mineral complexes.

4.4.2 Rehabilitating microbial community and functions in the tailings

These snap-shot analyses provided initial evidence on the importance of plants and microbes in the formation of OC fractions associated with microaggregates and organo-mineral complexes, and thus technosols formation in tailings. The importance of a diverse and functioning microbial community to support a plant community has been well documented in the literature (Harris, 2009, Bais et al., 2006). Furthermore, existence of plant is crucial to shape microbial structure and driving biogeochemical processes in the tailings. These plants also likely benefit from microbe-root interactions to gain competitive advantages in stressed environments. For example, some plant growth promoting bacteria and fungi benefit plants in terms of tolerance capacity to nutrients deficiency and ecotoxicity in tailings (Christopher et al., 2008; Compant et al., 2005). Previous studies have indicated initially low microbial diversity in non-vegetated tailings rapidly increases after plant establishment and later succession (Alguacil et al., 2011b; 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). The presence of surviving plants is likely to shift the microbial community in tailings away from the autotrophic dominant structure (Li et al., 2014). We also observed the importance of introduced pioneer native plants in rehabilitation of microbial biomass and functions in amended tailings, which is essential to technosols development for tailings phytostabilisation.

In the present study, OC, microbial biomass and biogeochemical processes (net mineralisation rate, enzymatic activities) had been improved to some degree through WC application and plant colonisation. The weathered and neutral Cu-Pb-Zn tailings were depleted in reactive minerals (e.g., pyrite) and had a relatively stable geochemistry (Forsyth, 2014). Microbial communities present in this type of tailings are likely to be more diverse than those in acid Cu-Pb-Zn tailings (Mendez et al., 2008). The enhanced OC in the amended tailings provides energy and nutrients for microbial colonisers, greatly stimulating development of heterotrophic bacteria and contributing to the decomposition and element cycling processes in tailings, especially in tailings with combination of WC and plant colonisation.

In general, the efficacy of WC and plant colonisation in regards to OC stabilisation and microbial structure in the examined tailings is still limited, compared to those in natural soils, probably due to the short history and low rate of OC turnover. The greatest TOC in TD5+WC+P was only 5 g kg^{-1} (Table 4-1), which is 10-50 % of those in natural soil under similar climatic conditions (Bird et al., 2002; Kihara et al., 2012; Mendham et al., 2002, also refer to Appendix B). Additionally, bacterial communities in all the amended tailings were dominated by tolerant species and the fungal community is hardly detected in these tailings (Li et al., 2014), much less diverse than microbial communities that colonise in natural soils (Berg and Smalla, 2009; Burke et al., 1998). The relatively low diversity of the microbial community and absence of eukaryote in the tailings concerned may have resulted from other stresses such as elevated salinity, high concentrations of heavy metals, extreme temperature changes, and low rainfall in the field (Madigan et al., 2006).

Nevertheless, the incorporation of WC and plant colonisation greatly improved the nutrients, WHC, CEC and aggregation in the amended tailings, resulting in much-improved microbial biomass and enzymatic activities, compared to the control. In addition, we found that further mineral weathering was induced in this relatively weathered Cu-Pb-Zn tailings, resulting in a further increase of EC, levels of soluble S and metals (Cu, Zn) (Table 4-1). This is consistent with our previous findings that there were abundant *Thiobacillus*, an autotrophic sulphur oxidiser in the amended tailings (Li et al., 2014). The induced mineral weathering associated with organic amendments in tailings has been reported in previous studies (García et al., 2007; Hayes et al., 2012; Li et al., 2013). It is likely that during the process of mineral weathering, stresses from acids, soluble salts or trace metals might be aggravated, constrained both microbial community and plant colonisation in these tailings. From the present study, stabilisation of OC could be achieved through the application of WC and plant colonisation. However, whether the microbial community in tailings could be rehabilitated with the structure and function close to those in soils supporting desired native tolerant plant species is yet to be determined. It is still a fundamental question relevant to sustainable tailings phytostabilisation.

4.5 Conclusions

Organic amendment (WC) and introduction of pioneer plants effectively increased the nutrients availability and improved physical structure and chemical buffering capacity, with induced mineral weathering processes in the weathered and neutral Cu-Pb-Zn tailings. Combined treatments of organic amendment (WC) and introduction of pioneer plants rapidly built up OC and effectively rehabilitated microbial biomass and functions in the amended/revegetated tailings. The majority of OC (64-77 %) in the amended/revegetated tailings was physically protected in microaggregate and organo-mineral complexes. Plants played a particularly important role in OC stabilisation through aggregation and organo-mineral interactions with stimulated microbial biomass and N rich organic compounds inputs. Furthermore, plants colonisation also contributed significantly to rehabilitate microbial structure and functions in the amended/revegetated tailings. Microbial community composition and functions in the tailings may have been altered by changes of physicochemical properties induced by organic amendments and plant colonisation, which will be investigated in the later chapters.

Chapter 5 Biogeochemical changes induced by exogenous organic amendments with contrasting properties in weathered and neutral Cu-Pb-Zn tailings – a 6-month microcosm study

5.1 Introduction

Organic carbon (OC) stabilisation is one of the critical benefits of organic amendments, which plays essential roles in the physical structure and biogeochemical functions of soils (Ros et al. 2003; Tejada et al. 2006). An important impact from organic amendment in tailings is the improved colonisation of diverse microbial communities facilitating the biogeochemical processes in tailings (Pepper et al., 2012). Our previous findings from a field trial demonstrated that the addition of exogenous organic matter (woodchips of native trees) in the neutral Cu-Pb-Zn tailings enhanced the OC stabilisation and physical structure (Chapter 3 and 4) (also refer to Huang, Baumgartl, et al. 2011). With the hosting effects of pioneer native plants in the weathered and neutral Cu-Pb-Zn tailings, microbial community structure in the amended/revegetated tailings began to shift from the autotrophic to heterotrophic bacterial communities with increased biomass, even when only organic matter with high C: N ratios (woodchips with C: N of 98) added (Li et al., 2014) and also refer to Chapter 3 and 4.

Organic amendments not only provide OC and nutrients (Chèneby et al., 2010), they also have marked impacts on the physical stability, hydraulic properties, mineral weathering and heavy metal mobility in the amended tailings (Li et al., 2013; Steiner et al., 2008). Various organic amendments have been applied for tailings amelioration, including compost, manure, biosolids, plant residues and biochar (Chiu et al., 2006; Gardner et al., 2012). However, nutrient-rich organic amendments such as manure and biosolids may not be suitable for the local native plant communities, which are slow growing and have low nutrient requirements (Huang et al., 2012) and also refer to Appendix B. Our previous results have demonstrated the benefits of native plant-biomass with relatively high C: N ratio (woodchips) in improving biogeochemical properties and functions in the tailings (refer to Chapter 3 and

4). Alternative sources of biomass from native acacia species are also readily available in semi-arid regions. As leguminous shrub, the acacia biomass contains relatively a high N content and a large proportion of labile carbon and may further improve the rate and intensity of biogeochemical changes in tailings towards the desired state of functional technosols (Huang et al., 2014). It is critical to investigate how microbial community composition and functions in tailings are driven by the changed physicochemical properties induced by different organic amendments. However, little published information is available regarding detailed changes in microbial community composition and associated biogeochemical processes in Cu-Pb-Zn tailings, in relation to organic amendments with contrasting properties (e.g., acacia biomass, recalcitrant biochar).

The present study evaluated biogeochemical changes induced by the addition of acacia biomass and recalcitrant biochar within the context of engineered pedogenesis in the tailings, including microbial community composition and functions, and associated physicochemical properties in the tailings, to formulate efficient amendment strategies to accelerate technosols formation. The specific objectives were to: (1) investigate the effects of acacia biomass and biochar on the diversity, composition, and functions of microbial communities in the amended tailings, in relation to the induced physicochemical changes; and (2) identify important physicochemical factors driving the shift of microbial communities in response to the different organic amendments. We hypothesized that microbial communities might substantially shift because of the changed physicochemical properties in response to amendments of acacia biomass with large proportions of labile organic carbon and nitrogen compared to the biochar.

5.2 Materials and methods

5.2.1 Experimental design

The weathered and neutral Cu-Pb-Zn tailings applied in this study were taken from Mount Isa Mine (MIM), TD5 from the top 50 cm with 5 replicates mixed as bulk samples in May, 2013. The tailings are neutral with an average pH of 7.1 ± 0.1, abundant in quartz, dolomite, pyrites, gypsum and kaolinite and contain 6.0 ± 0.2 % Fe, 4.9 ± 0.1 % Ca, 3.3 ± 0.0 % S, 0.13 ± 0.03 % Cu, 0.18 ± 0.02 % Pb, and 0.29 ± 0.01 % Zn.

Acacia biomass (AC) (mainly shoots) of dominant native leguminous species (woody shrub) was collected in bulk, from natural vegetation located at George Fischer colluvium plain, at about 26 km north of the MIM tailing impoundments. The biomass was oven dried at 65 °C. Briefly, AC was of near neutral pH (6.8 in water) and contained 40.2 % total organic carbon (TOC) and 1.05 % total N with a C: N ratio of 38. Biochar (BC) was produced from pine wood heated to a temperature of 650 °C. It was neutral to slightly alkaline in pH (7.8) and contained about 79.0 % TOC and 0.19 % TN with a C: N ratio of 416. Both AC and BC were ground to pass through 1 mm sieve and to achieve uniform mixing with the tailings. Both were sterilised before they were added to the tailings. We recognise that organic amendment in tailings would be more feasible in the form of mulch rather than powder in future operations under field conditions.

200 g air-dried tailings were thoroughly mixed with designated proportions (w/w basis) of the sterilised AC and BC using an end-over-end shaker for 15 h. There were 4 treatments with 3 replications in each treatment, including the control (tailings only), tailings mixed with 5 % AC (5%AC); tailings mixed with 5 % BC (5%BC), and tailings mixed with combined 5 % AC and 5 % BC (5%AC+5%BC). The mixtures were adjusted to 15 % water content and aerobically incubated for 6 months from May 2013 in the dark at 25 °C. All the containers were covered with plastic film perforated with several pinholes to ensure gas exchange while avoiding rapid water loss. Sterilised deionized water was used to adjust moisture contents in the treatments every 2-3 days to compensate for water loss through evaporation throughout the incubation period. At the end of incubation, the samples were collected and divided into 2 portions in each replicate: one portion was stored at 4 °C for Mineral N and basal respiration analyses within 48 h and for microbial biomass determination, enzyme assays and DNA extraction within 4 weeks, the other portion was air-dried for physicochemical analyses.

5.2.2 Physicochemical analysis

Methods to determine physicochemical properties of the samples can be found in Chapter 3.2.2, including pH, EC, CEC, WHC, TOC, TN, WSOC and ICP-OES analysis of elements after aqua-regia digestion. Mineral N was sum of ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were extracted and determined as described in Chapter 4.2.4.

5.2.3 Microbial biomass, respiration and enzyme assays

Microbial biomass and enzyme assays were measured as described in Chapter 4.2.4. Microbial basal respiration rate in fresh samples was measured at the end of the incubation period. Samples were placed in a closed chamber attached with infrared gas carbon dioxide (CO_2) analyser (Q-Box SR1LP soil respiration package, Oregon, Canada). Gas accumulated in the chamber was collected 2 s^{-1} for 10 minutes for the analysis of CO_2 concentrations.

5.2.4 DNA extraction, pyrosequencing and data analysis

DNA extraction in the samples was performed with a PowerSoil® (Deoxyribonucleic acid) DNA Isolation Kit (MO BIO LACoratories, Inc.) following cell enrichment using sucrose density gradient centrifugation (Li et al., 2014). DNA concentrations and quality were determined with a Nanodrop spectrometer (Thermo Scientific, US). Quality DNA was selected and submitted to the Australian Centre for Ecogenomics, The University of Queensland for pyrosequencing with paired-end Illumina MiSeq platform (Caporaso et al., 2012). Universal fusion primers 926F (5′-AAACTYAAAKGAATTGACGG-3′) and 1392wR (5’-ACGGGCGGTGWGTRC-3’) were applied, which cover most bacteria, archaea and eukaryotes (Cayford et al., 2012). Paired-ends reads were assembled by aligning the 3’ ends of forward and reverse reads with Paired-End reAd mergeR (PEAR) (PEAR 0.9.4) with a minimum output length of 200 bp after quality trimming at Q_{20}. Assembled sequences along with the corresponding quality values were processed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010). Primers were trimmed using Seqtk (Li, 2012). Sequences with 97 % similarity were classified as an operational taxonomic unit (OTU) and were sorted by abundance followed by UCLUST clustering employed USEARCH (Edgar, 2010). Full length duplicate sequences were removed and singletons were discarded. Chimera were filtered using the Genomes on Line Database (Gold) as a reference (Liolios et al., 2008). UCLUST was applied to assign taxonomy to the representative OTUs, which were aligned by Python Nearest Alignment Space Termination tool (PYNAST) (Caporaso et al., 2010). The rarefaction curve and the non-normalised OTUs table, with the abundance of different OTUs and their taxonomic assignments for each sample were generated in QIIME.

Mean number of OTUs and Shannon diversity values based on the non-normalised OTUs table were calculated in R (package 'vegan'). Nomaliser (Imelfort and Dennis, 2011) was used to find a centroid normalised OTUs table. A heatmap (version 2.15.1; package 'heatmap2') was created in R (Kolde, 2012). The most abundant OTUs sequences and reference sequences from GenBank were aligned using SILVA incremental aligner (SINA) (Pruesse et al., 2012) and a neighbour-joining phylogenetic tree was generated using MEGA 6 (Tamura et al., 2013) based on the alignment.

5.2.5 Statistical methods

Primary data processing was performed using Microsoft® Excel. One-way analysis of variance (ANOVA) was carried out for significant test among treatments. Means were compared using the least significant differences (LSD) test at P = 0.05. Pearson linear correlations among physicochemical properties and microbial properties in tailings were also analysed. All statistical analyses were conducted using the SPSS software package (SPSS Statistics 20.0, Chicago, IL, USA).

Canonical correspondence analyses (CCA) were carried out using CANOCO software for Windows 4.5 (Biometris-Plant Research international, Wageningen, The Netherlands). CCA were performed for correlations of microbial types and measured environmental variables.at the phyla level for microbial species and elected environmental parameters (e.g., pH, EC, Cu, S).

5.3 Results

5.3.1 Physicochemical changes detected in the tailings

As shown in Table 5-1, the control in the present study contained low levels of TOC, N and P, limited water-soluble OC (WSOC) and nutrients (mineral N). The tailings were saline with an EC of 6.3 mS cm^{-1}. There were elevated levels of heavy metals (i.e., Cu, Pb and Zn). At neutral pH, the soluble heavy metals (e.g., WS Cu, WS Zn) were low, accounting for less than 0.1 % of the total heavy metals.

Physiochemical conditions in the amended tailings were altered by the AC and BC amendments, with different magnitude of changes. The pH conditions in all the treatments remained neutral to alkaline, regardless of the organic amendments. The AC and BC treatments (individually or in combination) significantly increased the WHC in the amended tailings, especially in the 5%AC+5%BC treatment. Levels of nutrients in the amended tailings significantly increased in the AC-treatments, including the 5%AC and 5%AC+5%BC treatments. For example, levels of TOC, TN, WSOC and mineral N increased in these two treatments more than 10-fold, compared to the control. Furthermore, the CEC was significantly elevated in the 5%AC and 5%AC+5%BC treatments. In contrast, BC had only limited impacts on nutrient availability and CEC in the amended tailings. The levels of TN, WSOC, mineral N and CEC in 5%BC treatment did not differ significantly from those in the control.

Table 5-1 Selective physicochemical properties in the control and amended tailings with acacia biomass and biochar application.

Treatments		pH	EC (mS cm^{-1})[a]	CEC ($cmol_{+}$ kg^{-1})[b]	WHC (%)[c]	Total (g kg^{-1}) TOC[d]	N[e]	P	Cu	Pb	Zn	Water/salt extractable (mg kg^{-1}) WSOC[j]	Mineral N	WS Cu[g]	WS Zn[h]
Control	Mean	7.11	6.3$^{\alpha}$	3.4$^{\alpha}$	29.9$^{\alpha}$	2.5$^{\alpha}$	0.15$^{\alpha}$	0.19	2.1	2.2	4.8	2.8$^{\alpha}$	1.8$^{\alpha}$	0.12$^{\alpha}$	0.06$^{\alpha}$
	S.D.[i]	0.08	0.3	0.2	2.2	0.6	0.01	0.02	0.1	0.2	0.3	0.2	0.6	0.01	0.02
5% AC	Mean	7.14	6.4$^{\beta}$	6.9$^{\beta}$	38.1$^{\beta}$	22.7$^{\beta}$	0.45$^{\beta}$	0.19	1.7	1.9	4.1	63.1$^{\beta}$	15.4$^{\beta}$	0.37$^{\beta}$	0.76$^{\beta}$
	S.D.	0.04	0.2	0.4	3.6	1.5	0.04	0.04	0.1	0.1	0.4	5.6	2.0	0.06	0.21
5%BC	Mean	7.29	5.9$^{\beta}$	3.2$^{\alpha}$	40.2$^{\beta}$	42.0$^{\gamma}$	0.20$^{\alpha}$	0.22	1.7	1.9	4.3	2.4$^{\alpha}$	1.1$^{\alpha}$	0.13$^{\alpha}$	0.03$^{\alpha}$
	S.D.	0.11	0.1	0.1	3.0	1.6	0.03	0.05	0.1	0.2	0.4	1.0	0.4	0.01	0.01
5%AC+5%BC	Mean	7.31	5.2$^{\beta}$	6.2$^{\beta}$	54.3$^{\gamma}$	53.7$^{\delta}$	0.42$^{\beta}$	0.16	1.7	1.9	4.0	48.4$^{\gamma}$	15.4$^{\beta}$	0.22$^{\gamma}$	0.35$^{\gamma}$
	S.D.	0.06	0.3	0.4	3.8	2.6	0.05	0.01	0.1	0.2	0.2	1.8	3.2	0.01	0.07

[a] Electrical conductivity. [b] Cation exchangeable capacity. [c] Water holding capacity. [d] Total organic carbon. [e] Total nitrogen. [f] water-soluble organic carbon. [g] water-soluble Cu. [h] water-soluble Zn. [i] Standard deviation. Values followed with letters ‘α, β, γ and δ’ within the column indicate significant differences among the treatments at the level of $P < 0.05$ (only labelled for selected parameters).

Although EC in the 5%AC+5% BC treatment significantly decreased (P < 0.05) (perhaps, due to dilution effect), high levels of salinity remained in all the treatments, ranging from 5.2 to 6.4 mS cm^{-1}. In the 5%BC treatment, the difference in WS Cu and WS Zn did not differ significantly from the control, while both WS Cu and WS Zn significantly increased in the 5%AC and 5%AC+5%BC treatments (P < 0.05). In particular, the highest levels of WS Cu and WS Zn were found in the 5%AC treatment.

5.3.2 Microbial biomass, respiration and profiling of microbial diversity

Lowest levels of MBC and basal respiration were observed in the control. There were no significant differences in MBC and basal respiration between the control and 5%BC treatments (P < 0.05). Among the treatments, AC addition significantly stimulated microbial biomass and respiration in the amended tailings (Fig. 5-1a and Fig. 5-1b).

Specifically, levels of MBC increased from 5.1 mg kg^{-1} in the control to 84.7 mg kg^{-1} and 99.0 mg kg^{-1} in 5%AC and 5%AC+5%BC treatments respectively. The basal respiration rates in AC-amended tailings (5%AC and 5%AC+5%BC treatments) were 10-fold compared to the control. Using all the samples, MBC and basal respiration were positively correlated with each other (R^2 = 0.98, P < 0.01, n = 12).

The pyrosequencing analysis of DNA extracted from the tailings samples uncovered a total of 116,619 good quality reads (Fig. 5-2), which were classified into 172 $OTUs_{0.97}$. In general, microbial diversity in the tailings was lowered by AC and BC application, but not the species richness (Fig. 5-1c and Fig. 5-1b). Specifically, no significant differences of the microbial communities in species richness and diversity were found between the control and the 5%AC+5%BC treatment. Both species richness and diversity of the microbial communities in the 5%AC treatment were lower than those in the control (P < 0.05). However, species richness significantly increased in 5%BC treatment compared to the control (P < 0.05), although there was lower species diversity in this treatment.

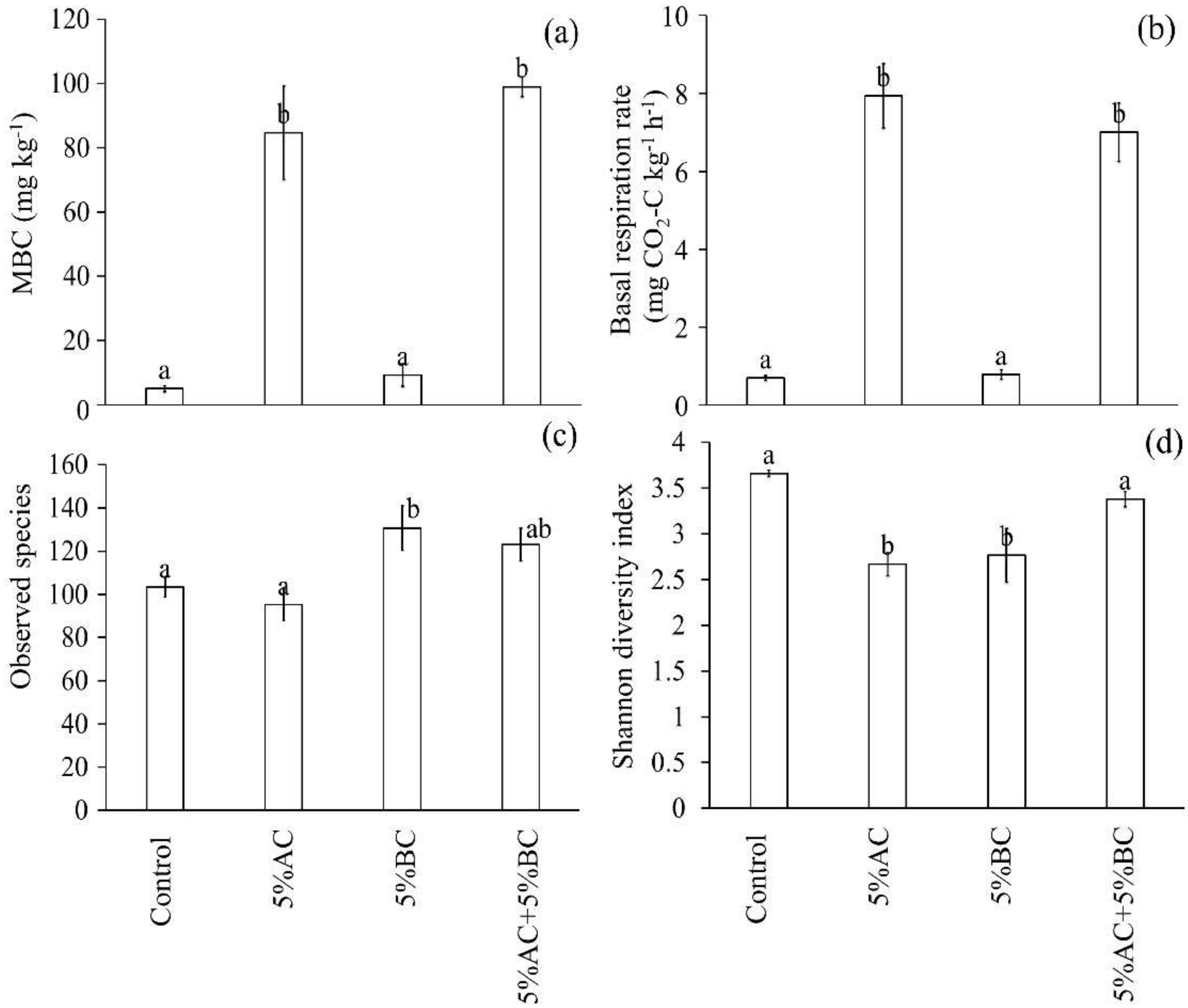

Fig. 5-1 Microbial biomass carbon (MBC) (a), basal respiration rate (b), observed species (c) and Shannon diversity (d) in the tailings and the amended tailings with acacia biomass and biochar application. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above the bars indicate significant differences among the treatments at the level of $P < 0.05$.

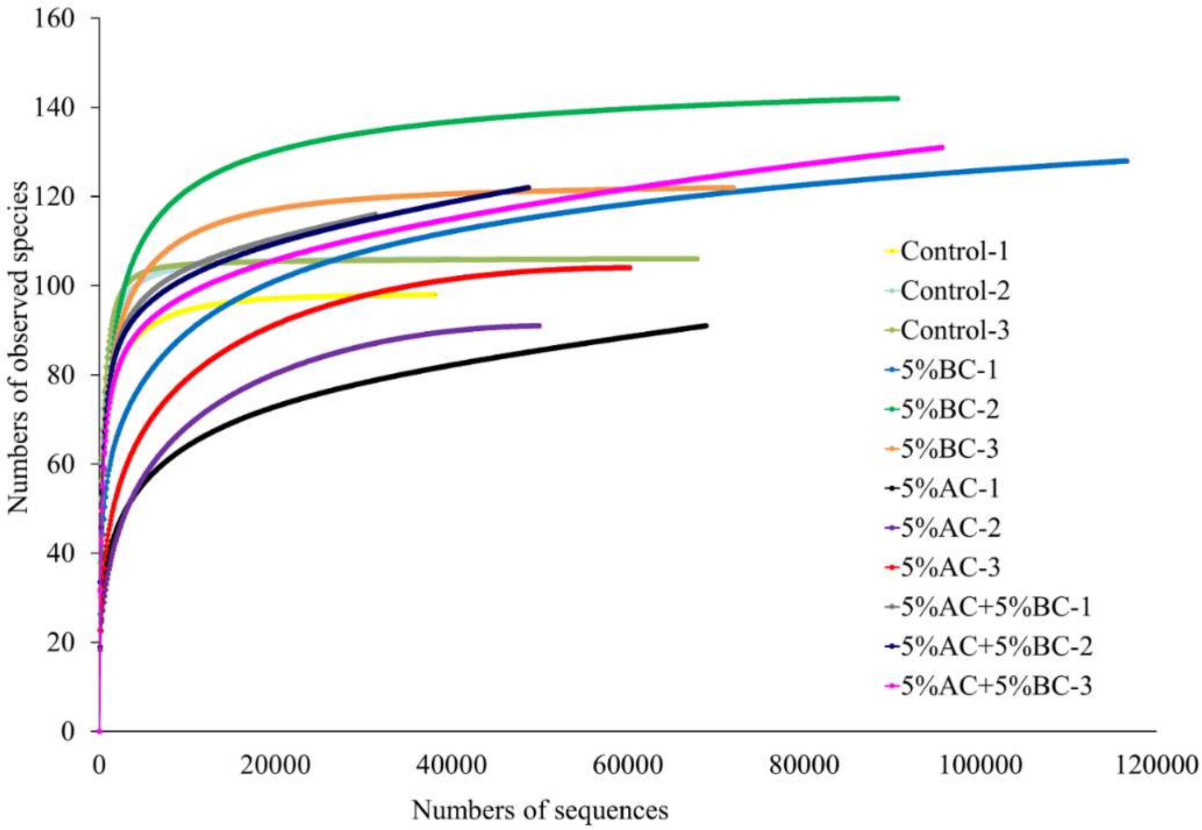

Fig. 5-2 Rarefaction curves in the control and the amended tailings with acacia biomass and biochar application.

5.3.3 Microbial composition and variation across amendment treatments

Most importantly, the AC and BC treatments altered microbial community composition and dominant microbial groups. In general, microbial communities in all treatments were dominated by prokaryotes with negligible amount of eukaryotic microbes (only one OTU affiliated to eukaryote microbes with less than 0.2 % in all samples).

The major OTUs and their affiliations were shown in Fig. 5-3. The most abundant archaeal sequences were affiliated with *Crenarchaeota*, sharing 99 % of similarity with *Nitrososphaera* sp.. Bacterial OTUs dominated all the microbial assemblages and those within the phylum *Proteobacteria* and *Bacteroidetes* were most abundant. Abundant bacterial OTUs (> 10 % in at least one sample) could be classified into 3 groups, Group 1, best hit with *Sphingobacterium* sp., *Brevundimonas* sp., *Ochrobactrum* sp., *Delftia* sp. and

Pseudomonas sp.; Group 2, affiliated to *Methylophaga* sp. and *Limnobacter* sp.; and Group 3, including *Legionella* sp. and *Thiohalobacter* sp..

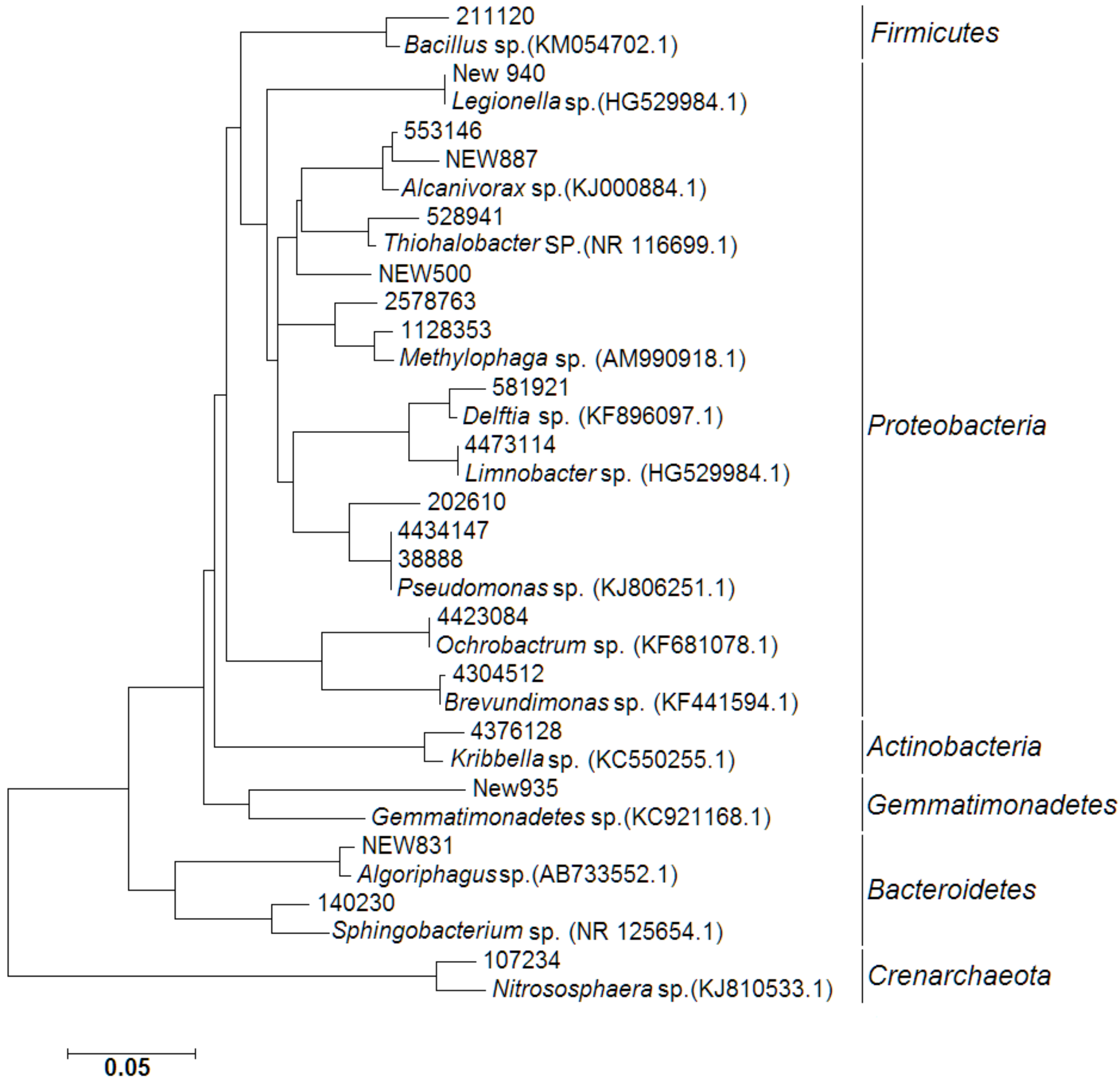

Fig. 5-3 A neighbour-joining phylogenetic tree of 16S rRNA gene sequences recovered from in the control and the amended tailings with acacia biomass and biochar application. Only representative OTUs were shown in this tree; the taxonomic groups are delineated on the right; scale bar stands for 0.05 changes per site.

Specifically, significant differences in microbial community composition were detected among treatments at both phylum and genus levels (Fig. 5-4 and Fig. 5-5). As indicated by the cluster analysis, the microbial communities in the 5%AC and 5%AC+5%BC treatments were clearly different from those in the control and the 5%BC (Fig. 5-5).

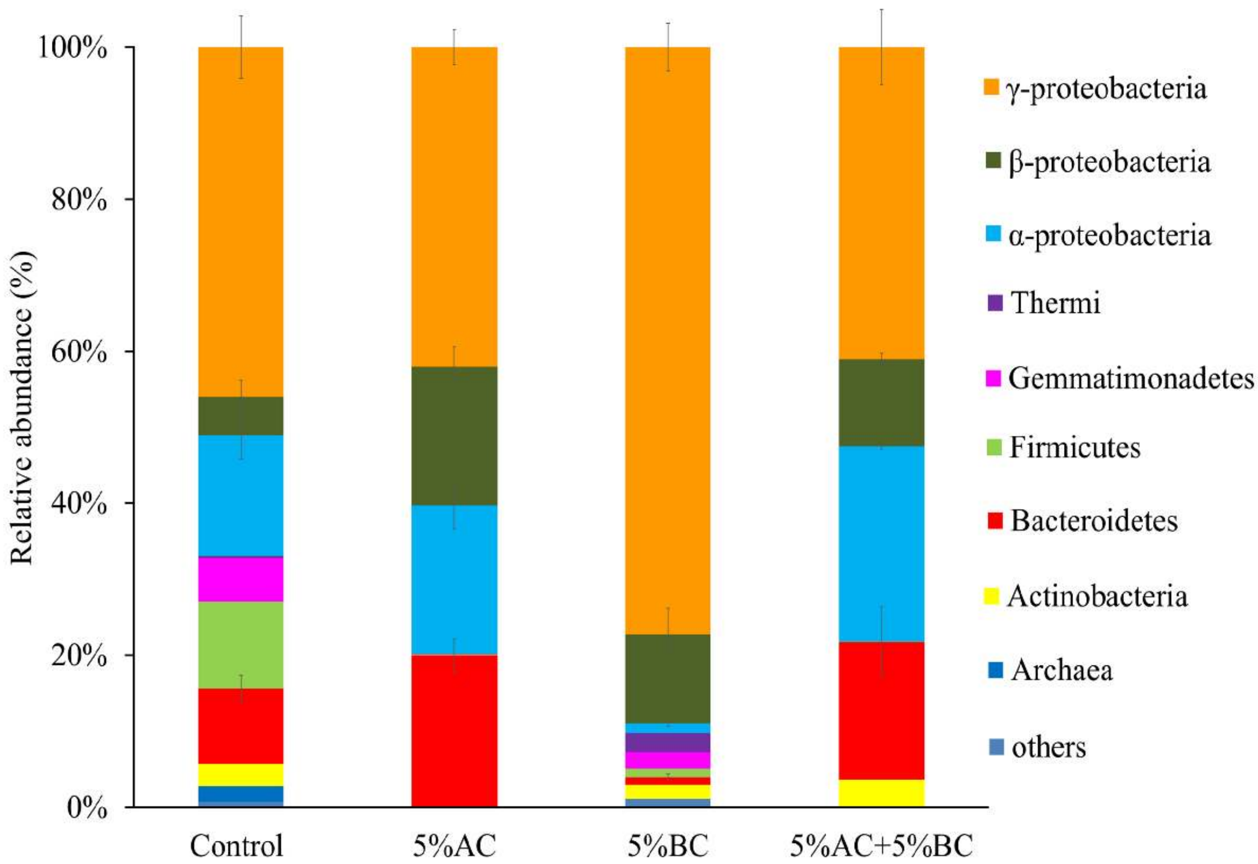

Fig. 5-4 Relative abundance of different phylum and classes of *Proteobacteria* in the control and the amended tailings with acacia biomass and biochar application. The bacterial phyla with a frequency less than 1 % are shown as 'others'; values are means (n = 3); error bars indicate the standard deviation; error bars were drawn for the most abundant phylum and classes of *Proteobacteria*.

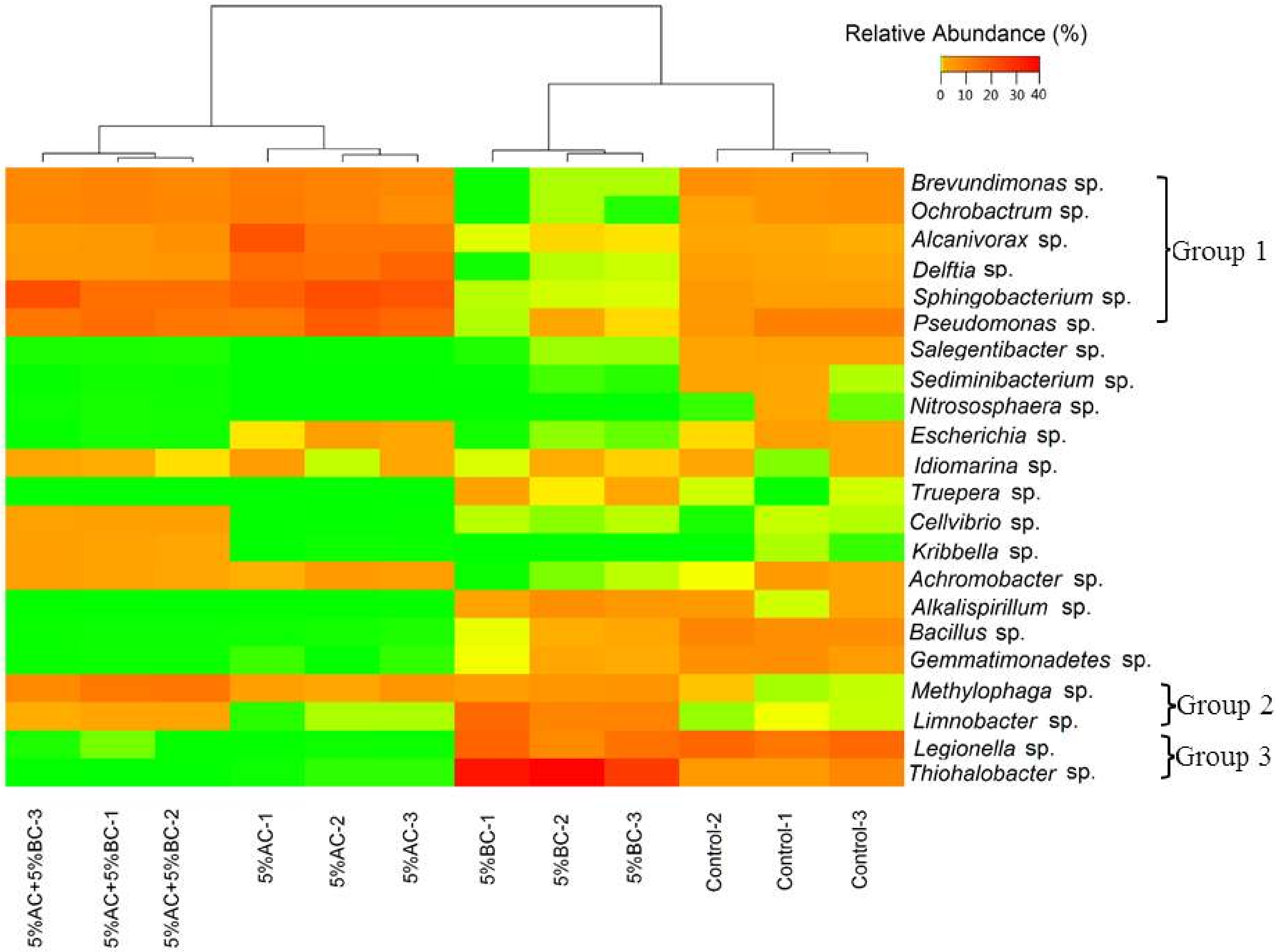

Fig. 5-5 Microbial community composition detected at the genus level in the control and the amended tailings with acacia biomass and biochar application. The abundance of genera is indicated and cluster analysis of the community composition between the samples is shown. Only the OTUs with a frequency of greater than 1 % were shown in the figure.

The most prominent group was *γ-Proteobacteria* in both the control and the 5%BC treatments. The most abundant genus of *γ-Proteobacteria* sequence in the control was affiliated to *Legionella* sp. (14.8 %) in Group 3. The abundance of this genus significantly decreased in the 5%BC treatment ($P < 0.05$). In contrast, the abundance of species affiliated to Groups 2 and Group 3 significantly increased in 5%BC treatment, including *Thiohalobacter* sp. (33.5 %), *Limbobacter* sp. (11.4 %) and *Methylophaga* sp. (4.8 %) (P <

0.05). Meanwhile, genera in Group 1 (e.g., *Brevundimonas* sp., *Ocharobactrum* sp., *Pseudomonas* sp.) were significantly depressed ($P < 0.05$) in the 5%BC treatment compared to the control.

The shift of microbial community composition in the 5%AC and 5%AC+5%BC treatments was similar. The abundance of *β-Proteobacteria* and *Bacteroidetes* in these 2 treatments was much higher compared to the control ($P < 0.05$). Specifically, bacteria in Groups 1 were greatly stimulated in these AC-amended tailings. For example, *Brevundimonas* sp. and *Pseudomonas* sp. increased by 40 % and 50 % respectively in both 5%AC and 5%AC+ 5%BC treatments compared to the control. Furthermore, the abundance of *Ochrobactrum* sp. and *Sphingobacterium* sp. doubled in both 5%AC and 5%AC+5%BC treatments compared to the control. Meanwhile, the abundance of bacteria in Group 3 (e.g., *Legionella* sp. and *Thiohalobacter* sp.) significantly decreased in both 5%AC and 5%AC+5%BC treatments ($P < 0.05$). In contrast, the abundance of *Limbobacter sp.* belonging to Group 2 greatly increased in the 5%AC+5%BC treatment, but not the 5%AC treatment.

5.3.4 Enzymatic activities across amendment treatments

Across the treatments, dehydrogenase ($R^2 = 0.90$, $P < 0.01$, n=12), invertase ($R^2 = 0.93$, $P < 0.01$, n=12), urease ($R^2 = 0.77$, $P < 0.01$, n=12) and neutral phosphatase ($R^2 = 0.90$, $P < 0.01$, n=12) activities were positively correlated with MBC in the present study. Similar to MBC, the lowest enzymatic activities were found in the control and the 5%BC treatment, which were not statistically different from each other.

All the 4 enzymes were significantly increased in the 5%AC and 5%AC+5%BC treatments (Fig. 5-6). Compared to the control, the levels of dehydrogenase, invertase and urease activities in the 5%AC treatment were elevated to 3-4 folds, while neutral phosphatase activities were around 10-fold greater. Moreover, no differences were found between the 5%AC and 5%AC+5%BC treatments for dehydrogenase and neutral phosphatase activities, while invertase and urease activities in the 5%AC+5%BC treatment were much greater than those in the 5%AC treatment (P < 0.05).

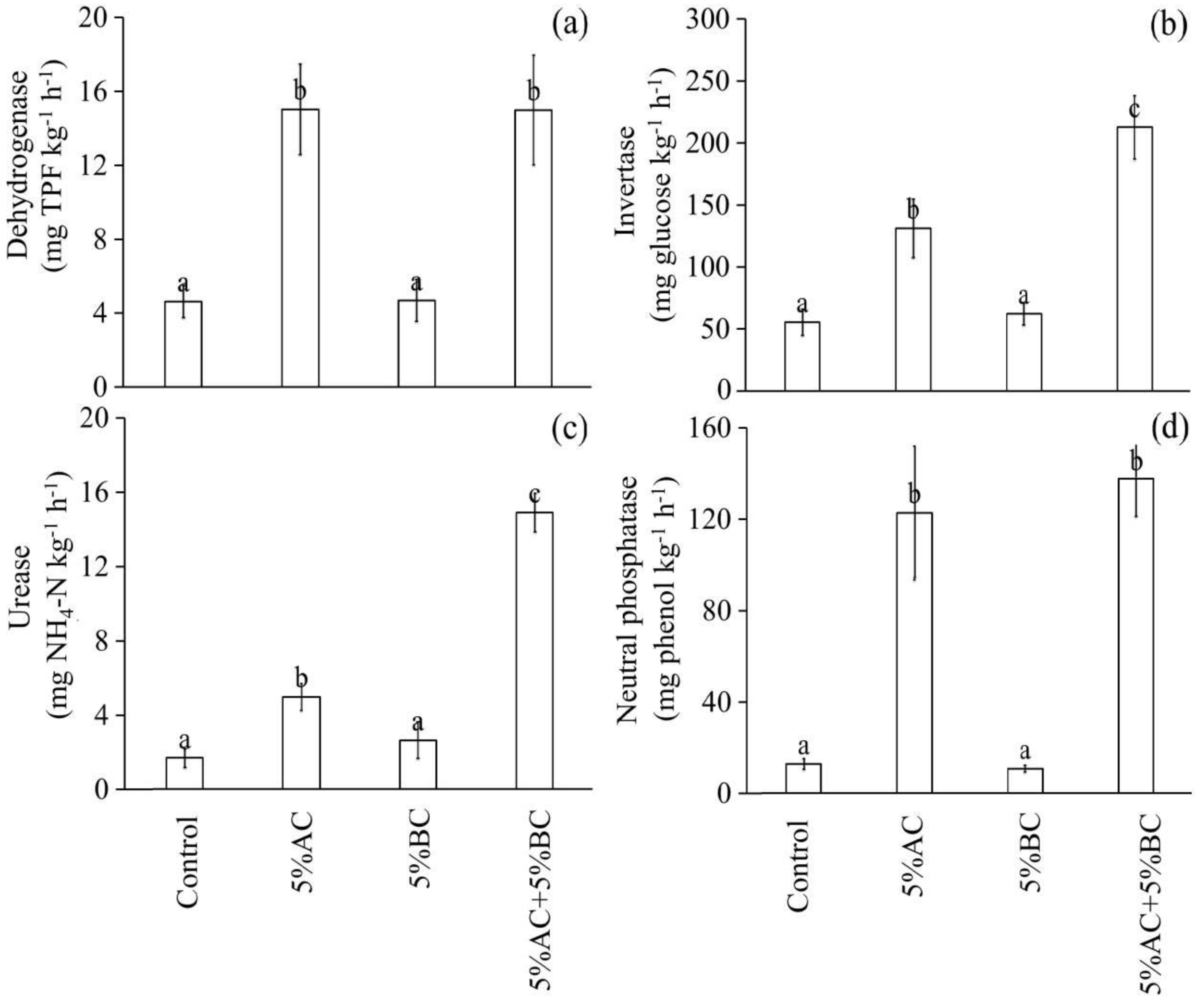

Fig. 5-6 Activities of dehydrogenase (a), invertase (b), urease (c) and neutral phosphatase (d) in the control and the amended tailings with acacia biomass and biochar application. Values are means (n = 3); error bars indicate standard deviations; the letters ‘a, b, c, d’ above the bars indicate significant differences among the treatments at the level of P < 0.05.

5.4 Discussion

5.4.1 Biogeochemical changes induced by different organic amendments

Through purposeful ecological engineering inputs (e.g., plant biomass-based organic amendments), it is possible to shift microbial community composition and dominant microbial groups towards those functioning species driving the biogeochemical processes, critical to the initiation of technosols development in the tailings (Huang et al., 2014). The present results demonstrated that organic amendments of contrasting properties resulted in different biogeochemical changes in the amended tailings, based on observed physicochemical properties, microbial community composition and functions. These changes are critical to the rehabilitation of biogeochemical processes and the technosols formation in the weathered and neutral Cu-Pb-Zn tailings.

Although the 6-month incubation did not significantly enhance the microbial diversity in the amended tailings, the microbial community composition and the relative abundance of phylum and genera were significantly altered by the organic amendments which were associated with changes in physicochemical properties (particularly those amended with AC). For example, in the weathered and neutral Cu-Pb-Zn tailings (control) without any amendments, the most abundant genus *Legionella* sp. is an aerobic chemoorganotrophic gram-negative bacilli which proliferates in neutral pH conditions (Hao et al., 2010). This specie was greatly depressed in all the amended tailings, especially in the AC amended tailings. Interestingly, in the tailings amended with BC, abundant microorganisms were mainly autotrophic and tolerant bacteria, reflecting the selective pressure by environmental stresses in the Cu-Pb-Zn tailings. For example, the most abundant microorganism *Thiohalobacter* sp., in the BC amended tailings, closely linked to sulphur- (S-) oxidising processes, is salt-tolerant *γ-Proteobacteria* (Sorokin et al., 2010). The stimulated *Limnobacter* sp. and *Methylophaga* sp. in BC amended tailings were also metal and salt tolerant (Lu et al., 2011; Villeneuve et al., 2013). Also, the relative abundance of species potentially beneficial to plant growth (e.g., *Brevundimonas* sp., *Pseudomonas* sp.) (Bakker and Schippers, 1987; Park et al., 2008) were depressed in BC amended tailings. We assume that the shift of the microbial community in the BC amended tailings might accelerate sulphide oxidisation and geochemical reactions, leading to faster transition from geochemically unstable tailings into the state of high hydrogeochemical stability (Kock and Schippers, 2008). This is particularly important in tailings containing a relative high percentage pyrites (Li et al., 2015). The hypothetical effects of BC in enhancing the abundance and functions of autotrophs and associated sulphide oxidation should be tested in fresh tailings, in relation to accelerating weathering of reactive minerals and the transition into a hydrogeochemically stable state, which is the foundation of engineered pedogenesis (Li et al., 2015).

Overall, biochar in the form of recalcitrant OC may not be suitable as a primary amendment option in terms of stimulating the colonisation of soil-like microbial communities in the tailings. The AC biomass contained much higher proportion of labile OC and higher levels of N than the BC used here (Table 5-1), and favoured the shift of dominant microbial groups from autotrophic extremophiles towards heterotrophic bacteria in the evolving microbial communities, in response to direct and indirect changes of physicochemical conditions in the tailings. Microbial community composition in 5%AC and 5%AC+5%BC treatments were similar to each other, both shifting towards microbial communities resembling those in local natural soils. Both the potential heterotrophic and plant growth promoting bacteria in Group 1 (e.g., *Ochrobactrum* sp., *Sphingobacterium* sp., *Brevundimonas* sp., and *Pseudomonas* sp.) were greatly stimulated in these AC-amended tailings (P < 0.05) (Fig. 5-6). These bacteria in Group 1 are potentially important in degradation of complex organic compounds (e.g., phenol, cellulosic fibres) (Kılıç, 2009; Lednická et al., 2000), improving plant survival and growth via enhanced tolerance resistance (Sharma et al., 2003) and nutrient acquisition (Mehnaz et al., 2007; Vessey, 2003). Therefore, AC appeared to be more effective in rehabilitating soil-like microbial communities and functions in tailings, thus stimulating the soil formation process of highly weathered tailings to biogeochemically functional technosols. It is worthwhile to point outthat the effects observed in a relatively short-term laboratory incubation experiment might be difficult to relate to those in the tailings under field conditions, but the findings at least suggest that it is important to apply suitable types of organic amendments for the purpose of engineering technosols in the tailings and stable carbon (e.g., biochar) may not be effective in the rehabilitation of soil-like microbial communities and biogeochemical functions in the tailings. Further field studies involved in temperature and hydraulic movement are required for to verify the present findings in the tailings landscapes under local climatic conditions.

The main impacts of organic amendments on the microbial community composition were on the bacterial communities, which recovered more readily than the fungal communities in the amended tailings. Although fungi are only a minor part of the microbial communities in soils (20 %), they play important roles in soil functions and associated plant communities, affecting, for example, plant diversity and productivity (Ingram et al., 2005; Van Der Heijden et al., 2008). As one of the significant groups, fungi is ubiquitous and diverse in soil ecosystems of low productivity (Griffiths and Philippot, 2012). Fungi is also detected in extreme environments (e.g., acid mine drainage, tailings and solar salterns) (Baker et al., 2004; Cantrell et al., 2006; Huang et al., 2011), tolerant to stresses caused by acidity and elevated levels of heave metals and salinity. However, fungi was almost absent in all the treatments in the present study, which should be further investigated in future experiments. Supplement amendment strategies (fungi inoculation, introducing tolerant native plants) may require for the development of fungal communities in the tailings.

5.4.2 Physicochemical drivers of microbial composition and functions

The results showed that AC and BC contained contrasting forms of organic compounds (labile carbon, carbohydrates) and nutrients contents (e.g., N and P) (Table 5-1). The application of AC and BC resulted in an array of change in physicochemical properties (e.g., pH, EC, water soluble heavy metals) in the microcosm system (incubated tailings), which collectively drove the shift of microbial community composition and functions along different directions.

Unlike natural soils, where there is usually a positive relationship between TOC and MBC (Brejda et al., 2000), increased TOC by organic amendments in the amended tailings was less related to the levels of MBC in this study. The high amounts of labile forms of OC and N (WSOC and mineral N) were more closely related to the build-up of microbial biomass. The solubility of OC is closely linked to corresponding chemical composition of organic amendments. For example, lignin is not a significant source of soluble OC compared to other organic compounds (e.g., carbohydrate, protein) (Engelhaupt and Bianchi, 2001). Organic amendments with relatively high content of lignified organic compounds (e.g., biochar used in the present project) may inhibit the production of labile OC and consequently the biogeochemical processes (Fellet et al., 2011; Jones et al., 2012). The labile OC and nutrients from AC can be utilised by heterotrophic microorganisms in the tailings, which in turn, stimulate organic matter decomposition and nutrients cycling processes.

Species richness and diversity of the microbial community were found to be more related to pH and EC conditions in the tailings. Neutral pH conditions favour the majority of microorganisms (Ussiri and Lal, 2005); extreme pH conditions (e.g., acidic or alkaline) may result in the loss of microbial diversity by favouring those microbial groups tolerant of these extreme pH conditions (Rothschild and Mancinelli, 2001). Previous studies have investigated the impacts of a range of pH conditions, from extreme acidic (pH: 2-3) to alkaline (pH: 7-8), on microbial communities in mine tailings. Results indicated that increasing the pH was beneficial to the development of diverse microbial communities in tailings as acidic pH conditions usually elevated the solubility of heavy metals and associated ecotoxicity (Kock and Schippers, 2008; Londry and Sherriff, 2005; Mendez et al., 2008). Although the tailings used in the present study are neutralised, the small change in pH (7.1-7.3) induced by the organic amendments may have caused marked impacts on microbial community composition, albeit relatively weaker than other physicochemical conditions (such as the levels of soluble OC and N). The level of EC is negatively correlated to microbial diversity in the tailings in the present study (Table 5-2), which is consistent with previous studies where loss of microbial diversity was found along gradients of increasing salinity (Casamayor et al., 2002). High concentration of salts results in hyper-osmotic stresses to microbial colonisers (Muhammad et al., 2006). In the present study, neither AC nor BC were effective in alleviating salinity problem in the tailings, where the lowest EC in the 5%AC+5%BC treatment was 4-fold greater compared to saline soil (1.5 mS cm^{-1}). Water-soluble heavy metals seemed to have negative impacts on the microbial diversity (Table 5-2).

Table 5-2 Correlations among tailings physicochemical properties and microbial biomass, respiration and diversity in the control and the amended tailings with acacia biomass and biochar application.

Physicochemical properties	MBC	Basal respiration	Observed species	Shannon diversity
pH	0.195	0.049	0.658*	0.011
EC	-0.346	-0.171	-0.638*	-0.227
WHC	0.706*	0.567	0.486	-0.031
CEC	0.944**	0.982**	-0.352	-0.253
TOC	0.498	0.361	0.679*	-0.273
TN	0.962**	0.987**	-0.249	-0.355
WS Cu	0.736**	0.853**	-0.497	-0.487
WS Zn	0.798**	0.900**	-0.535	-0.439
WSOC	0.949**	0.989**	-0.381	-0.318
Mineral N	0.947**	0.963**	-0.279	-0.176

Data followed with '** and *' indicate significance of correlation at the levels of $P < 0.01$ and $P < 0.05$ respectively.

Several studies have shown the capacity of organic amendments for metal immobilisation via absorption, complexation, reduction and volatilization (Park et al., 2011). In particular, metal complexation by dissolved OC is an important factor in the mobilisation of metals like Cu^{2+} (Bolan et al., 2011). However, in this study, levels of water-soluble heavy metals significantly increased in the AC amended tailings (5%AC and 5%AC+5%BC treatments) although there are considerable increases of WSOC. This may be the reason that dominant metal ions (e.g., Cd^{2+}, Pb^{2+}, and Zn^{2+}) concerned in the present study have low affinity for soluble organic molecules in the pore water. The differences in the efficacy of AC and BC in heavy metals immobilisation may result from the differences in the (1) initial pH conditions of organic amendments (BC is slightly alkaline than AC); (2) increase levels of labile OC in AC amened tailings favouring the development of heterotrophic bacteria, which may stimulate mineral weathering and dissolution processes (Ribeta et al., 1995).

Shift of microbial community composition in the tailings is closely associated with the physicochemical variables induced by AC and BC application in the present study. The presence of dominant bacteria (e.g., *Sphingobacterium* sp., *Brevundimonas* sp. and *Ochrobactrum* sp.) was positively driven by the improved nutrient availability (e.g., WSOC, mineral N, TN) in the 5%AC and 5%AC+5%BC treatments and to some extent, negatively driven by the levels of total heavy metals. In the neutral Cu-Pb-Zn tailings, the soluble heavy metals (i.e., WS Cu, WS Zn) may not be the major limiting factors to the dominance of heterotrophic bacteria and functions of resultant microbial communities, as demonstrated by the observed microbial biomass, respiration rates and enzymatic activities.

The changes of pH and WHC in the BC amended tailings favoured colonisation of *Thiohalobacter* sp., *Methylophaga* sp. and *Limnobacter* sp. in the 5%BC treatment. The dominant physicochemical factors driving these changes are the levels of nutrients (TOC, TN, WSOC, and mineral N) and total heavy metals (e.g., Cu, Pb and Zn) in the tailings. The impact of salinity (EC) on the changes in composition of the microbial communities in the tailings at the genus level is weaker than the other environmental variables (Fig. 5-7).

Current understanding concerning fungal community diversity and functions in extreme environments is limited (compared to bacterial community). Although a number of studies have demonstrated the impacts of environmental factors and plant traits on fungal composition and diversity, the conclusion on dominant site factors remains conflicted in the literature (Guo and Gong, 2014; Sikes et al., 2014). The low fungal diversity and abundance in the present tailings might be a result of the co-occurrence of multiple stresses (e.g., salts, heavy metals) (Casamayor et al., 2002; Nordgren et al., 1983). Since the universal primers 926F and 1392R are16S rRNA-gene group specific primers targeting bacterial and archaea, they don't completely cover the 18S rRNA gene in eukarya (Engelbrekston et al., 2010) with SILVA database for eukaryotic amplicons (Pruesse et al., 2007). As a result, the quantification of fungal community in the present study is significantly underestimated and require further sequencing using a primer targeting fungi.

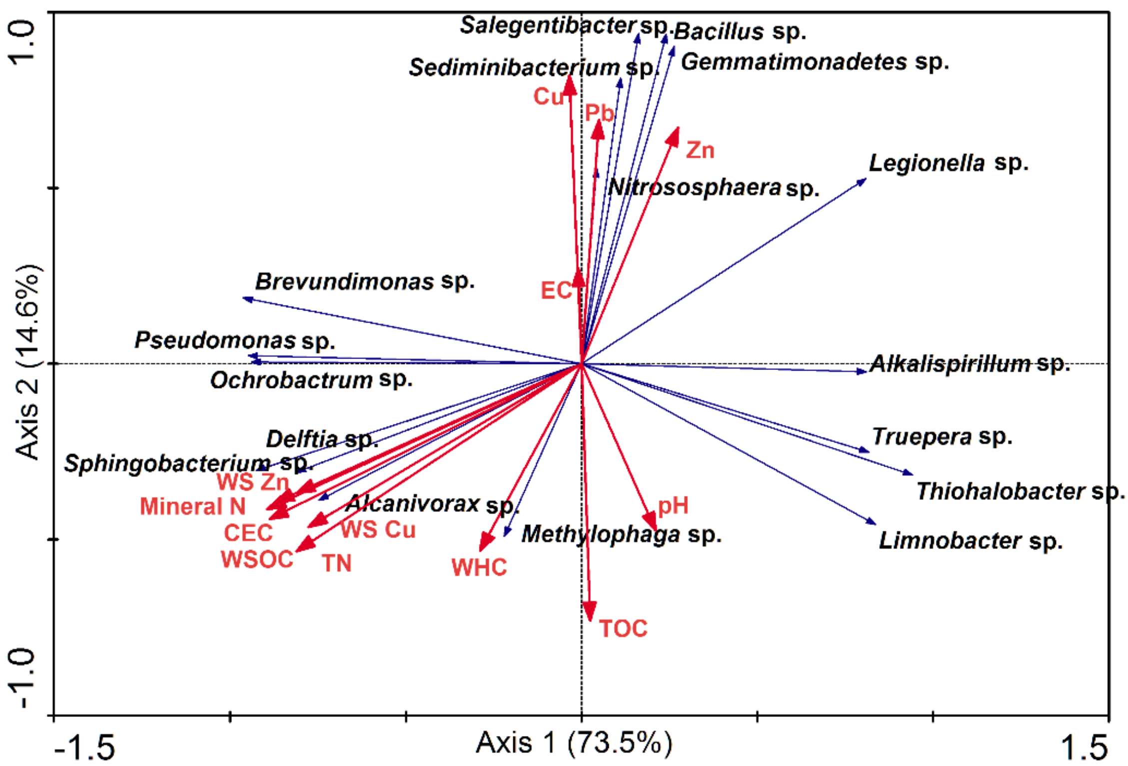

Fig. 5-7 Canonical correspondence analyses (CCA) ordination biplot of relative genera abundance of microbial community and dominant site factors in the control and the amended tailings with acacia biomass and biochar application. Genera less than 1 % are excluded in this analysis

Microbial inoculums might be a fast-track solution to rehabilitate a diverse and abundant fungal community in tailings. Furthermore, as increase of abundance and diversity of fungal community is found positively related to plant productivity and diversity, regardless of stresses levels (Guo and Gong, 2014; van Ryckegem and Verbeken, 2005), it is possible to rehabilitate fungal community in the tailings with the introduction of pioneer plant species (due to plant-hosting effects). The role of pioneer native plant species can further galvanise the positive effects of AC amendment on the recovery of soil-like microbial communities via intensive root-microbe interactions in the weathered and neutral tailings. Where acute toxicity has been alleviated after extensive weathering and neutralisation, pioneer native plants could survive in the tailings, due to the high tolerance of salinity and low nutrient availability in the semi-arid environment. A follow-up trial with native plant species was conducted to test this hypothesis (Chapter 7).

5.5 Conclusions

In the weathered and neutral Cu-Pb-Zn tailings, microbial community composition will shift along different directions by applying organic amendments with contrasting chemical/biochemical forms (mainly the degree of OC solubility and nutrient contents). Among the physicochemical factors examined, the levels of N, total and soluble OC, and total and water-soluble heavy metals induced the strongest changes in diversity and relative abundance of different groups of microbial communities in the tailings. Acacia biomass with relative greater levels of lability of organic matter and total N brought about significant increases of labile OC and nutrients, and favoured the development of potential heterotrophic bacteria in the neutralised Cu-Pb-Zn tailings. The enhanced heterotrophic bacteria in the AC amended tailings could in turn facilitate the organic matter decomposition and nutrient cycling processes as indicated by the greatly elevated enzymatic activities. These biogeochemical processes are critical to the rehabilitation of soil functions and the development of the amended tailings towards functional technosols. In contrast, inert organic amendment such as biochar with little soluble OC and nutrients, is far less effective to rehabilitate heterotrophic microbial community and associated biogeochemical processes in the amended tailings. However, the positive effects of BC on the relative abundance of S-oxidising bacteria suggest that it may be possible to accelerate microbial mediated oxidisation of sulphide minerals and thus hydrogeochemical stabilisation in sulphidic tailings. A combination of acacia biomass and biochar appeared to rehabilitate microbial communities in the tailings with better efficiency, in terms of the diversity of microbial community and microbial functions.

Chapter 6 Establishing microbial diversity and functions in weathered and neutral Cu–Pb–Zn tailings with native soil addition

6.1 Introduction

Successful phytostabilisation of tailing landscapes is often hindered by the lack of adequate volume of growth medium (e.g., soil), leading to the necessity of ecologically engineering technosols by amending tailings for direct revegetation (Li and Huang, 2015; Li et al., 2013; Scalenghe and Ferraris, 2009; Uzarowiczand Skiba, 2011). Our previous studies show that organic amendments with high proportion of labile organic carbon (OC) and nutrients could stimulate microbial communities and functions in tailings (Chapter 3, 4 and 5), while the reactive geochemistry of base metal mine tailings may prevent a rapid pedogenesis and thus development of microbial diversity in the amended tailings (Li and Huang, 2015; Li et al., 2014; Li et al., 2013), also refer to Chapter 5. Within this context, we proposed a new amendment strategy of natural soil addition, in addition to incorporating plant-biomass organic amendment, for accelerating the development of soil biogeochemical functions in weathered and neutral Cu-Pb-Zn tailings.

Base metal mine tailings are abundant in reactive minerals, among which pyrites dominate and the presence of these will suppress the development of microbial consortia with biological functions similar to that of a functional soil (Li and Huang, 2015). It is found that only tolerant and/or lithotrophic microbial phylotypes are sustained in Cu-Pb–Zn tailings (Li et al., 2014; Wakelin et al., 2012; Zhang et al., 2007b). The rehabilitation of native microbial communities in the amended tailings is a critical step in the process of technosols formation (Li and Huang, 2015). Natural soils from local plant communities are rich in microbes tolerant of *in situ* edaphic and climatic conditions. Addition of local soil containing native microbial communities may not only ameliorate the tailings physicochemical conditions, but also accelerate the shift toward soil-like microbial communities.

In previous studies, plant biomass-based organic amendments were shown to be essential for the development of microbial communities in the tailings from the mine site of this study (Li et al., 2014) and also refer to Chapter 4 and 5. However, amending tailings with organic matter alone is seldom adequate for establishing microbial diversity and functions resembling those in local soil (see Chapter 4, 5 and Appendix B). Therefore, the present study aims to investigate the effects of adding local top soil collected from native vegetation habitat on the establishment of microbial diversity and functions in the neutral and weathered Cu-Pb-Zn tailings from Mount Isa mines, north Queensland, in addition to plant-biomass organic matter. Specifically, we aimed to examine (1) whether microbial functions (e.g., microbial biomass, basal respiration and enzymatic activities) and diversity could be established in the Cu–Pb–Zn tailings with inputs of natural soil in combination with sugarcane; and (2) how the microbial community composition, examined using in-depth sequencing with universal primers, changes with the soil addition regime. The expected findings would contribute to the identification of ecological engineering options to accelerate technosols formation in the tailings for supporting native plant communities.

6.2 Methods and materials

6.2.1 General

The Cu-Pb-Zn tailings were sampled in May in 2013 from Mount Isa Mine (MIM) Tailings dam 5 (TD5) as described in Chapter 4.2.1. Local climatic conditions were described in Chapter 3.2.1. Natural vegetation at Mount Isa is dominated by *Eucalyptus*, *Spinifex* and *Acacia* species (Appendix C). The tailings area was still devoid of natural plant colonisation, despite their weathered state (Li et al., 2014). The soil used for tailings amendment was sampled from a natural vegetation land adjacent to the TD5.

The soil is a typical highly-weathered and mineralised soil with fragmented structure, alkaline in pH (8.3), low content of organic C (< 0.1 %), low electrical conductivity (EC, 0.8 mS cm^{-1}) and high abundances of iron oxides (mainly goethite and hematite) (Li and Huang, 2015; Li et al., 2014). Both tailings and soil were oven dried at 40 °C, sieved through 2 mm and mixed thoroughly before use.

Sugarcane was used as a tailing amendment option in this study, in combination with natural soil. The sugarcane (debris, including leaves and stem) was purchased from Bunnings Warehouse (Brisbane, Australia; http://www.bunnings.com.au). It contained large amounts of carbohydrates (86 %), and had the properties of: EC 2.4 mS cm^{-1}, pH 6.5 and CEC 42.7 $cmol_+$ kg^{-1}. The sugarcane was dried at 65 °C, ground and sieved through 1 mm before use.

6.2.2 Tailings treatments

The tailings were mixed with the natural soil at the rate of (% w/w): 12.5, 25 and 50, with a total of 400 g of the tailings-soil mixtures. The tailings and soil only were used as positive and negative controls, for the purpose of comparison. All treatments (including the controls) were replicated 3 times in the pots. In all the treatments, 5 % (w/w) sterilised sugarcane debris was added as a basal amendment, based on our previous tests. The treatments were designated as tailings, 12.5% soil, 25% soil, 50% soil and soil, respectively.

All the treatments were incubated in plastic containers in a temperature controlled incubator in the dark at 25 °C for 8 weeks. Water loss was adjusted with sterilised deionised water every 2 days, to maintain about 15 % water content (based on weight changes). At the end of incubation, tailings-soil mix samples were subsampled at 4 °C for the analysis of microbial biomass, enzyme assays and DNA extraction. A separate set of subsamples were dried at 40 °C for physicochemical analyses.

6.2.3 Physicochemical analysis

All the selected physicochemical analysis were measured as described in Chapter 3.2.2.

6.2.4 Microbial biomass, respiration and enzyme assays

Microbial biomass and enzyme assays were measured as described in section 4.2.4. Basal respiration was measured as described in Chapter 4.2.3.

6.2.5 DNA extraction, pyrosequencing and analysis

DNA was extracted as described in Chapter 5.2.4. Sequence data was analysed through the ACE Pyrosequencing Pipeline (Imelfort and Dennis, 2011). The sequence reads were sorted according to the barcode in QIIME (Caporaso et al., 2010), trimmed to 250 bp length and de-noised using ACACIA (Bragg et al., 2012). Sequences with 97 % similarity were classified as an operational taxonomic unit (OTU) using CD-HIT-OTU (Wu et al., 2011) and then aligned by PYNAST (Caporaso et al., 2010). All sequences were assigned through the GreenGenes database (2011 Fed release) to the taxonomy with BlastTaxonAssigner in QIIME. The OUTs table, rarefaction curve, the heatmap and neighbour-joining phylogenetic tree were conducted as described in Chapter 5.2.4.

6.2.6 Statistic methods

Primary data processing was performed using Microsoft® Excel. All significant tests were done using SigmaPlot 12.5 (Systat Software Inc., London, UK). Principal component (PCA) and canonical correspondence analyses (CCA) were performed using R with 'vegan' package (Oksanen et al., 2007). PCA and CCA were performed at the class level for microbial species and for selected environmental variables (e.g., Pb, Zn, S) to detect the importance of microbial community composition and tailings treatments, and for correlations of microbial community composition and measured environmental variables.

6.3 Results

6.3.1 Physicochemical changes in the tailings in response to treatments

Table 6-1 Selective physicochemical properties of the tailings-soil mix treatments

Treatments		pH	EC (mS cm^{-1})[a]	CEC (cmol$_+$ kg^{-1})[b]	WHC (%)[c]	TOC[d]	TN[e]	WSOC[f]	Total concentration (g kg^{-1})						Water soluble concentration (mg kg^{-1})				
						g kg^{-1}			S	Ca	Al	Fe	Pb	Zn	S	Ca	N	Pb	Zn
Tailings	Mean	7.70	2.51$^{\alpha}$	3.5$^{\alpha}$	46.6$^{\alpha}$	25.5$^{\alpha}$	0.44$^{\alpha}$	0.30$^{\alpha}$	32.6	75.4	4.8	78.6	2.78	6.23$^{\alpha}$	4181	2722	66	0.05	0.35$^{\alpha}$
	S.D.[g]	0.00	0.27	1.0	1.4	2.0	0.04	0.03	3.3	6.7	0.9	11.3	0.46	1.01	135	18	11	0.01	0.01
12.5% soil	Mean	7.57	2.68$^{\alpha}$	6.5$^{\beta}$	50.9$^{\alpha}$	26.5$^{\alpha}$	0.46$^{\alpha}$	0.24$^{\alpha\beta}$	33.8	75.2	6.9	74.3	2.70	5.21$^{\beta}$	4085	2715	34	0.02	0.27$^{\beta}$
	S.D.	0.06	0.16	0.4	3.2	2.8	0.03	0.05	1.1	4.4	0.5	2.1	0.63	0.28	37	21	2.	0.01	0.02
25% soil	Mean	7.53	2.68$^{\alpha}$	7.3$^{\beta}$	49.3$^{\alpha}$	24.3$^{\alpha}$	0.46$^{\alpha}$	0.18$^{\beta\gamma}$	31.3	72.0	9.0	71.8	2.24	5.08$^{\beta}$	4048	2733	26	0.02	0.26$^{\beta}$
	S.D.	0.06	0.05	0.5	2.6	0.5	0.02	0.03	5.1	11.7	1.63	10.9	0.32	0.69	34	7	2	0.01	0.02
50% soil	Mean	7.63	2.63$^{\alpha}$	10.4$^{\gamma}$	50.2$^{\alpha}$	23.4$^{\alpha}$	0.45$^{\alpha}$	0.16$^{\gamma}$	22.4	62.7	14.6	69.1	1.34	3.35$^{\gamma}$	3927	2748	21	0.03	0.27$^{\beta}$
	S.D.	0.06	0.04	0.2	3.8	0.6	0.05	0.05	4.2	6.6	3.1	12.2	0.21	0.05	48	21	2	0.02	0.06
Soil	Mean	7.73	0.83$^{\beta}$	9.9$^{\gamma}$	49.7$^{\alpha}$	25.3$^{\alpha}$	0.52$^{\beta}$	0.18$^{\gamma}$	1.4	30.1	22.2	43.8	0.02	0.14$^{\delta}$	840	528	12	0.02	0.03$^{\gamma}$
	S.D.	0.06	0.05	0.7	5.6	2.7	0.04	0.05	0.2	9.8	1.8	6.9	0.00	0.02	35	27	1	0.01	0.01

[a] Electrical conductivity. [b] Cation exchange capacity. [c] Water holding capacity. [d] Total organic carbon. [e] Total nitrogen. [f] Water soluble organic carbon. [g] Standard deviation. Values followed with letters 'α, β, γ and δ' indicate significant differences among the treatments at the level of $P < 0.05$ (only labelled for selected parameters).

After the 8-week incubation, the tailings-soil mix treatments had similar pH, TOC, TN and WHC. The soil had much lower EC and water-soluble Zn levels than the tailing treatments. In contrast, the tailings' EC and water-soluble Zn levels were high in all the tailings-containing treatments, regardless of the level of soil addition rates (Table 6-1).

In the tailings-soil mix, MBC (Fig. 6-1a) and Shannon diversity (Fig. 6-1d) increased linearly with increasing soil addition rates, while WSOC and soil respiration showed a reverse trend. A significantly negative correlation between MBC and WSOC (Table 6-1) was detected across the treatments ($P = 0.010$). The microbial respiratory quotient differed significantly among the 5 treatments ($P < 0.001$), with tailings being the highest and the 50% soil and soil treatments not statistically different.

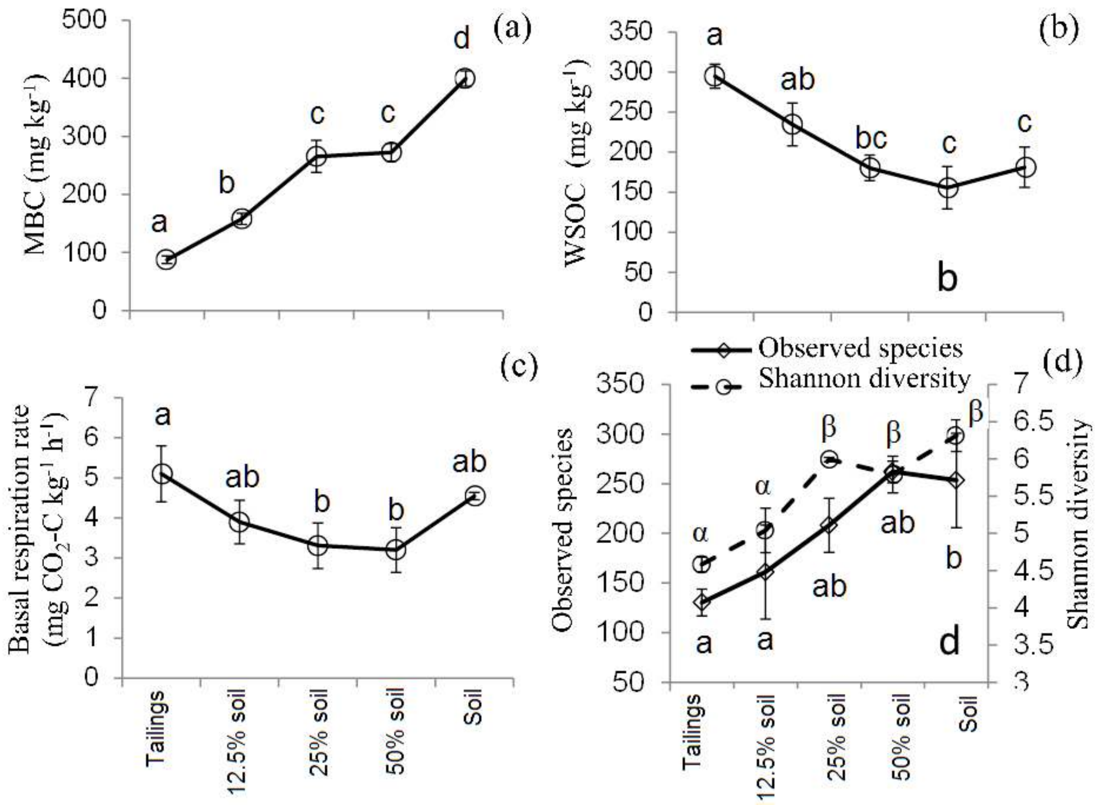

Fig. 6-1 MBC (a), WSOC (b), basal respiration rate (c) and microbial community diversity indexes (d) in the tailings-soil mix treatments. Values are means (n = 3); error bars indicate standard deviations; letters 'a, b, c and d' above indicate significant differences among the treatments at the level of $P < 0.05$; letters 'α, β, γ and δ' above indicate significant differences of Shannon diversity among the treatments at the level of $P < 0.05$.

The measured activities of the selected enzymes including dehydrogenase, invertase and urease increased with increasing soil addition rates, with the 50% soil treatment and soil being significantly ($P < 0.05$) higher than the other treatments (Fig. 6-2). The increased levels of these enzymatic activities in the treatments coincided with decreased levels of WSOC and water-soluble N (Table 6-1). Neutral phosphatase activities were not significantly different among the 4 tailings-containing treatments, but was significantly lower ($P < 0.002$) in the soil only treatment.

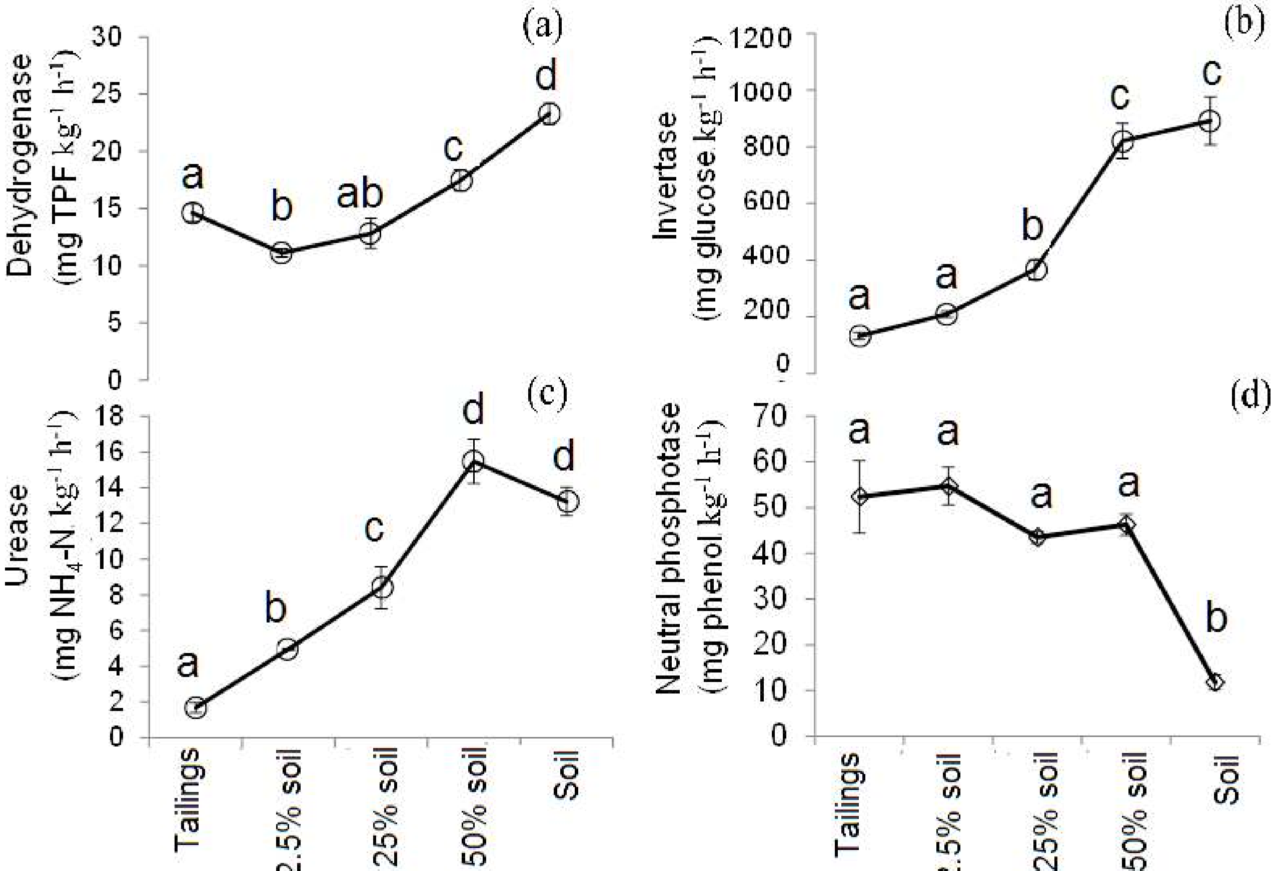

Fig. 6-2 Activities of dehydrogenase (a), invertase (b), urease (c), and neutral phosphatase (d), in the tailings-soil mix treatments. Values are means (n = 3); error bars indicate standard deviations; letters ‘a, b, c and d’ above indicate significant differences among the treatments at the level of $P < 0.05$.

6.3.2 Microbial community composition and variation across the treatments

Pyrosequencing of the small subunit rRNA genes was performed to explore the detailed microbial diversity within different treatments of tailings-soil mix. It was apparent that the depth of sequencing well covered the microbial diversity therein (Fig. 6-3). A total of 71,351 good quality reads were obtained by pyrosequencing in the present study. These sequences were classified into 1200 $OTUs_{0.97}$. Major OTUs and their affiliations were shown in Fig. 6-4. The 1200 $OTUs_{0.97}$ were affiliated with 54 classes of 2 phyla of bacteria, 5 classes within 2 archaeal phyla, and 4 phyla of fungi. There were 17 non-fungi eukaryotic OTUs and 3 unknown OTUs.

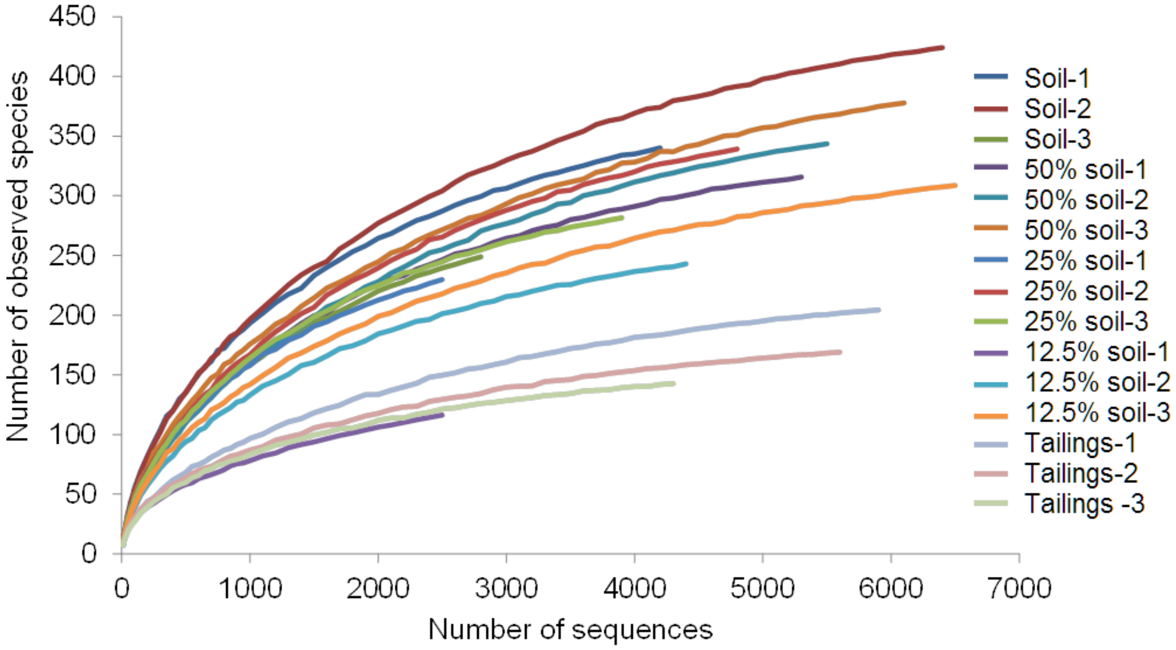

Fig. 6-3 Rarefaction curve of OTUs recovered from the tailings-soil mix treatments.

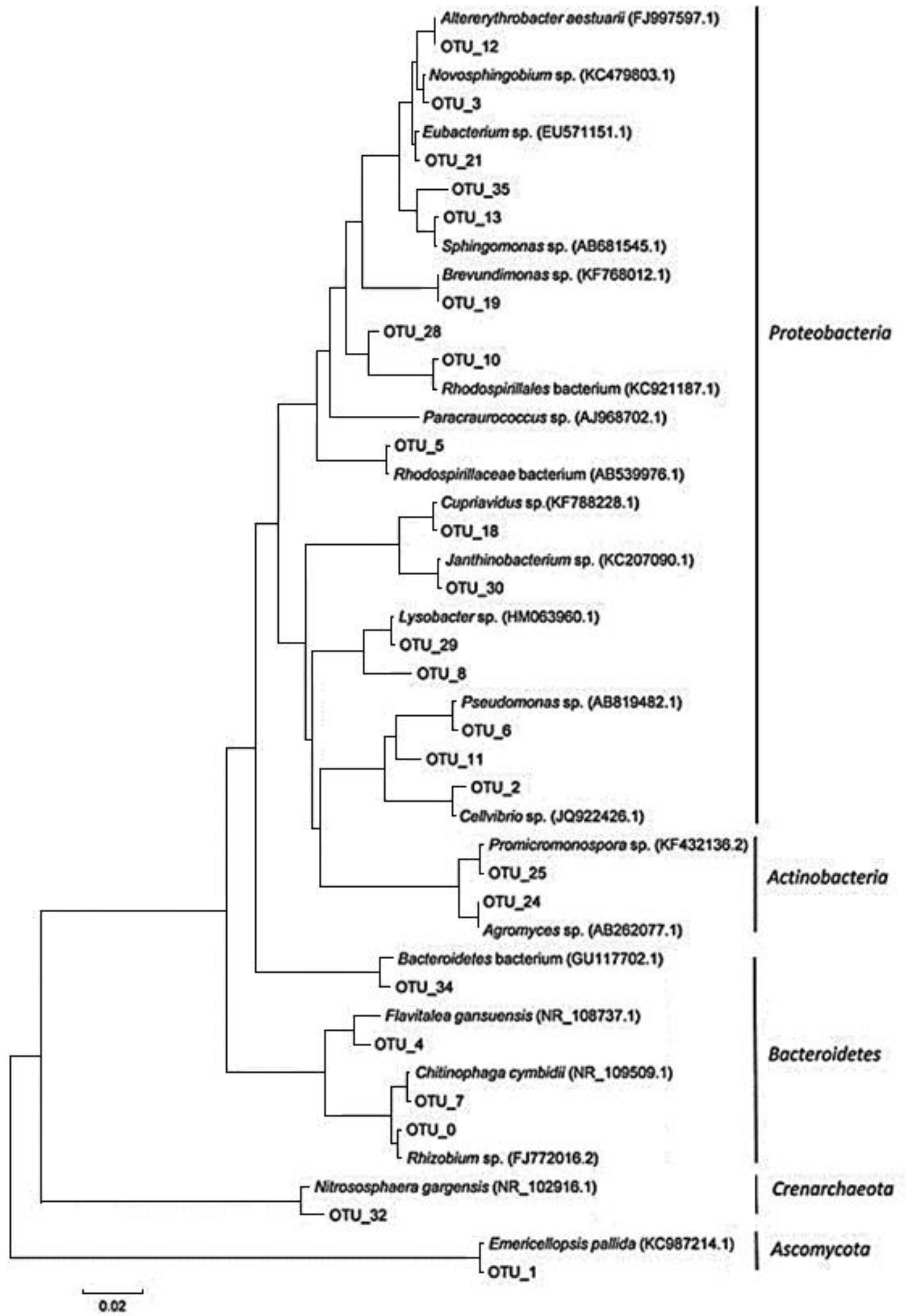

Fig. 6-4 A neighbour-joining phylogenetic tree of 16S rRNA gene sequences recovered from the tailings-soil mix treatments. Only representative OTUs were shown in this tree; the taxonomic groups are delineated on the right; scale bar stands for 0.02 changes per site.

The observed species of the microbial communities in the tailings-soil mix treatments ranged from 130 ± 23 (tailings) to 262 ± 19 (50% soil), and the Shannon index ranged from 4.58 ± 0.17 (tailings) to 6.31 ± 0.37 (soil). The observed species was not significantly different among the treatments ($P < 0.07$), despite the 50% soil treatment being significantly higher than the tailings ($P < 0.01$). In contrast, the Shannon diversity was significantly different ($P < 0.001$) among the treatments, with higher microbial diversity in the soil and 50% soil treatments than those in the other 3 treatments.

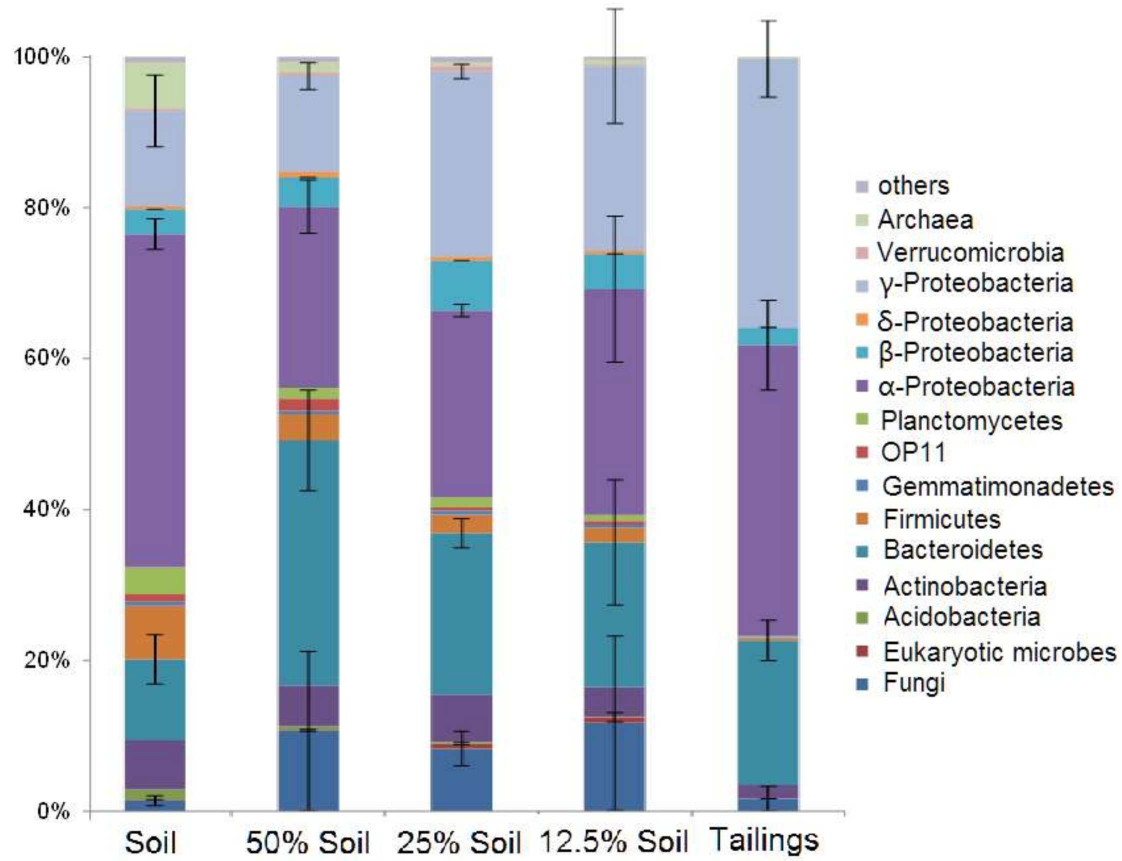

Fig. 6-5 Pyrosequencing-based microbial community composition (bacterial phyla, archaea, fungi and non-fungal eukaryotic microbes) of the tailings-soil mix treatments.The bacterial phyla with a frequency less than 1 % are shown as ‘others’; values are means (n = 3); error bars indicate standard deviations included for the top 4 abundant groups (*α-Proteobacteria*, *γ-Proteobacteria*, fungi and *Bacteroidetes*).

Bacterial OTUs dominated all the microbial assemblages in tailings-soil mix treatments and those within the phyla of *Proteobacteria* and *Bacteroidetes* were most abundant (Fig. 6-5). Dominant bacterial OTUs (> 1 % in all samples) were closest to *Rhizobium* sp., *Novosphingobium* sp., *Agromyces* sp., *Pseudomonas* sp. and *Lysobacter* sp. Some other abundant bacterial OTUs (> 10 % in at least 1 sample), which were detected in all the treatments but not all samples, were affiliated with *Cellvibrio* sp., *Flavitalea* sp., *Altererythrobacter* sp. and *Sphingomonas* sp. (Fig. 6-6). The most abundant archaeal OTU was assigned to *Nitrososphaera* sp. In comparison of all the treatments, significant differences were found for abundances of *Planctomycetes* ($P < 0.001$) and *Firmicutes* ($P = 0.002$), both of which increased with soil percentage.

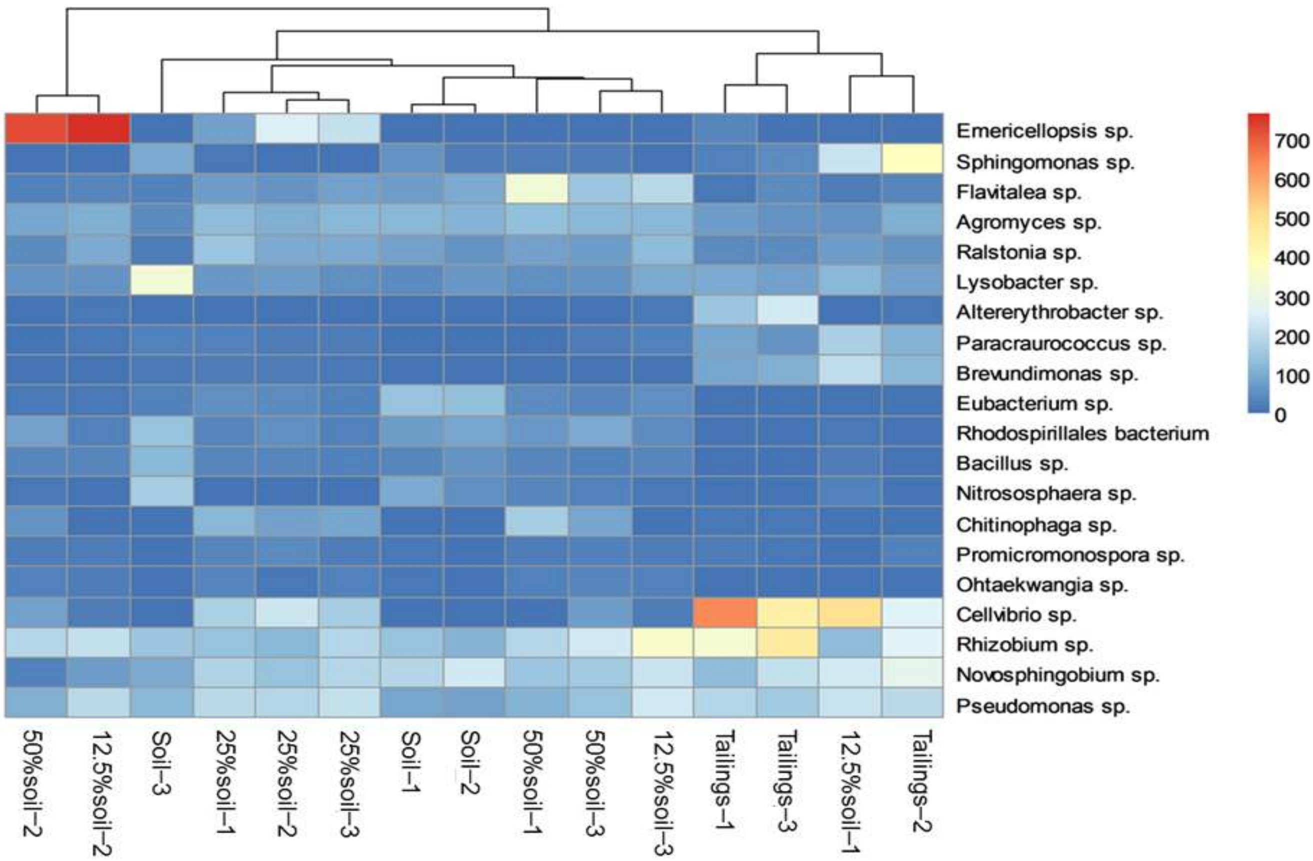

Fig. 6-6 Microbial community composition detected at the genus level in the tailings-soil mix treatments. The abundance of genera is indicated and cluster analysis of the community composition between the samples is shown. Only genera with a frequency of greater than 1 % are shown in the figure.

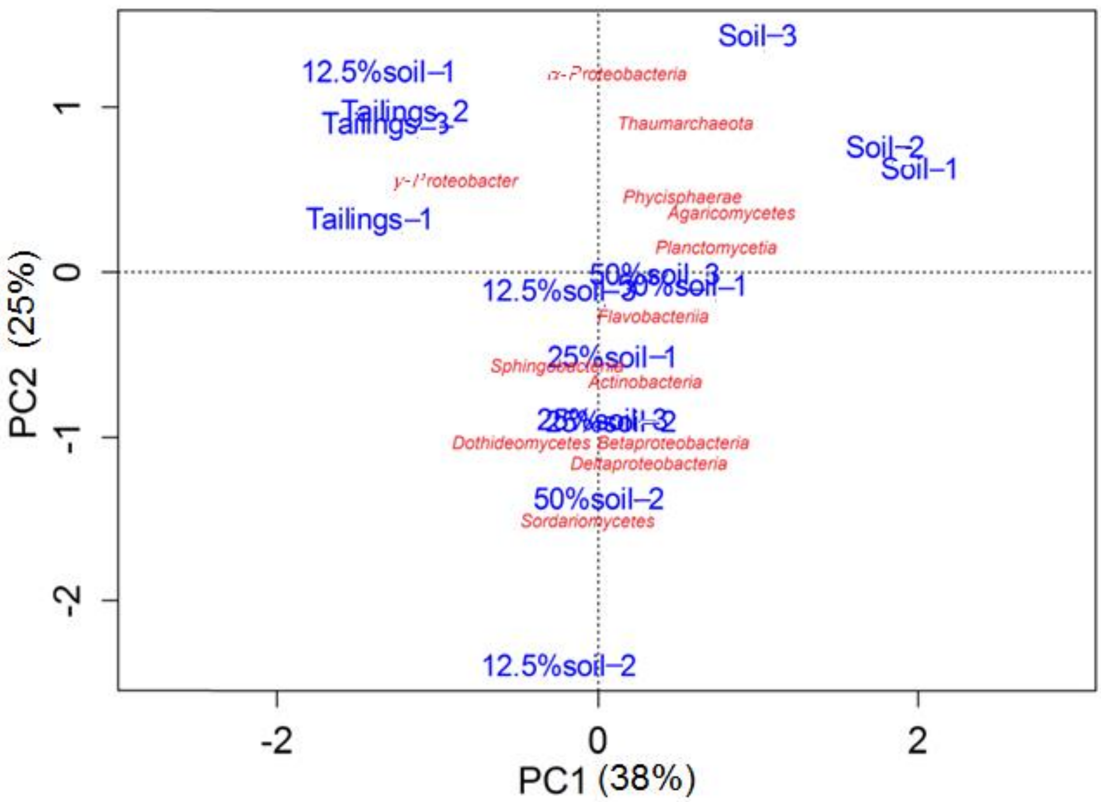

Fig. 6-7 Principal component analysis (PCA) of the microbial community composition detected in the tailings-soil mix treatments. Correlations with classes of microorganisms (in red) are made; the PCA axes differentiate the tailing samples according to their microbial community composition

Fungi was detected in all the treatments, though their abundance was low in the soil and tailings only, but their abundance was around 10 % in the tailings-soil mix treatments (Fig. 6-5). The major fungal phylotypes (> 1 % in at least one sample) were affiliated with the phyla of *Ascomycota* and *Basidiomycota*, and were closest to *Emericellopsis* sp., *Stachybotrys* sp., *Pleosporales* sp. and *Sebacina* sp. The most abundant of these, *Emericellopsis* sp., is potentially alkalitolerant in the tailing treatment (Fig. 6-6).

The CCA analysis indicated a strong association between fungi (*Agaricomycetes*) and microbial biomass and the activities of enzymes related to C and N cycling (Fig. 6-8). Non-fungi eukaryotes, mainly affiliated with the phyla of *Nematoda* and *Protozoa*, were detected in all soil-containing treatments but not in the tailings only. The most abundant *Nematoda* was closest to *Aphelenchus* sp., a typical mycophagous nematode.

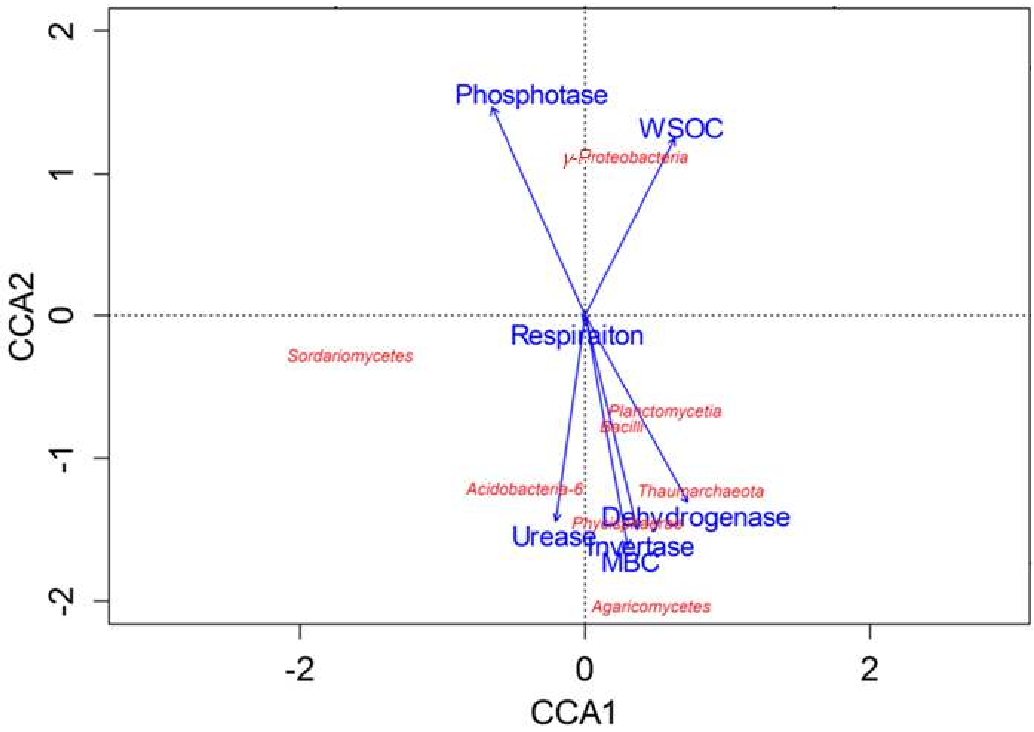

Fig. 6-8 Canonical correspondence analysis (CCA) of class abundance vs. enzymatic activities, microbial biomass carbon, respiration and water soluble organic carbon in the tailings-soil mix treatments. Abbreviations are as indicated in Table 6-1.

6.3.3 Microbial community structure vs. environmental variables

To explore whether the amendment strategy had driven the shift of microbial community structure and what environmental variables contributed to the shift, multivariate analyses were performed on the data assemblages of microbial community composition and environmental variables. At higher taxonomic levels, the microbial community structure in the tailings was clearly separated from those of the soil only, and the tailings-soil mix treatments which formed another cluster (Fig. 6-6 and Fig. 6-8).

Tailings were abundant in *γ-Proteobacteria*, mainly *Cellvibrio* sp., *Pseudomonas* sp. and *Lysobacter* sp., while soils were abundant in *α-Proteobacteria*, mainly *Novosphingobium* sp., *Eubacterium* sp. and *Rhodospirillales* sp.. A CCA analysis further indicated that the dominance of *γ-Proteobacteria* in tailings was strongly associated with the stressors, including EC (thus S), total Zn and total Pb (Fig. 6-9).

Further, it is worth noting that large differences in abundance were observed for fungi and protists at the genus level among replicates across the treatments. These genera included *Emericellopsis* sp., *Pleosporales* sp., *Acremonium* sp., and *Aphelenchus* sp.. The CCA indicated that community differences were associated with the physiochemical properties of the different treatments. For example, CEC and N may be factors important for the *Agaricomycetes* (Fig. 6-9).

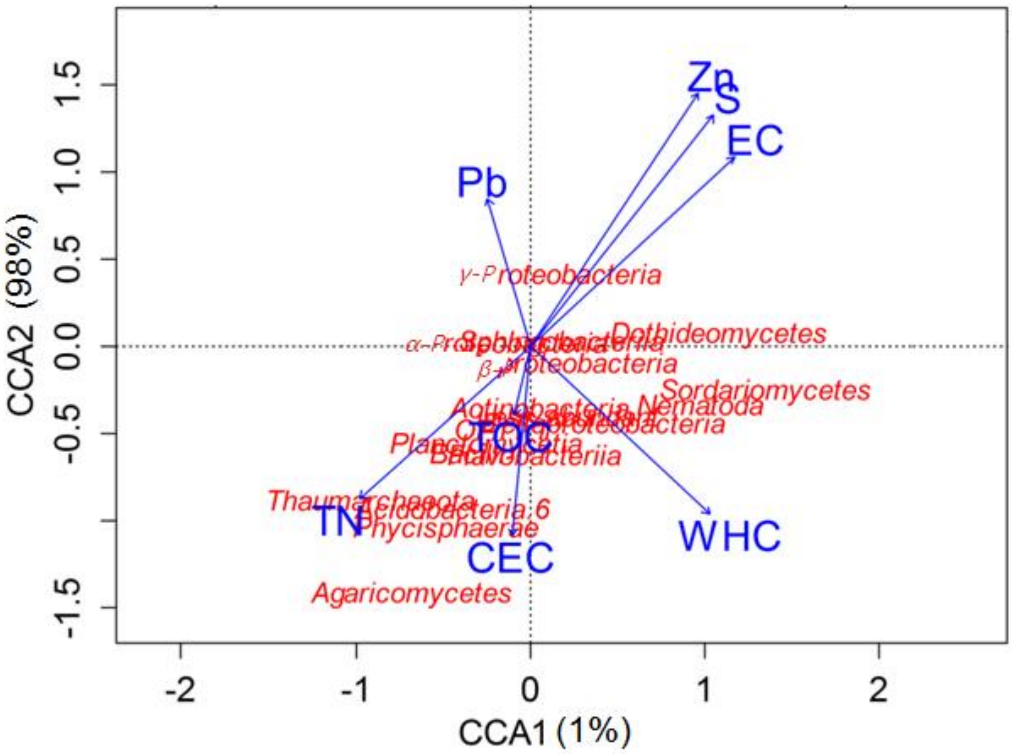

Fig. 6-9 CCA of class abundance vs. environmental variables in the tailings-soil mix treatments. Abundance less than 1 % are excluded from this analysis.

6.4 Discussion

From the present results, incorporation of local soil from native vegetation into the weathered and neutral Cu-Pb-Zn tailings significantly changed the composition and structure of microbial communities in the tailings-soil mix. The addition of up to 50 % local soil increased the microbial diversity and enzymatic activities for C and N turnover, with a respiratory quotient similar to that of the native soil. The phyla *Proteobacteria*, *Ascomycota* and *Bacteroidetes* dominated in all the treatments, but the microbial community composition was clearly differentiated among the treatments of tailings only, the tailings-soil mix and the soil only. In the mixtures of tailings and soil, opportunistic development of fungi and protists was found. The development of fungi in the treatments was also strongly associated with the microbial biomass and selected enzymatic activities.

6.4.1 Colonisation of native soil microbial community in the tailings

Consistent with previous studies, the microbial assemblages in all the tailings-containing treatments were dominated by prokaryotes (bacteria and archaea); however, non-negligible amounts of eukaryotic microbes were also detected in this study. Prokaryote diversity has been examined in various tailing environments in the past decade (Baker and Banfield, 2003; Bond et al., 2000a; Bond et al., 2000b) and eukaryotes have been detected in some studies (Baker et al., 2004; Baker et al., 2009). To our knowledge, this is the first report to detect the presence of prokaryotic and eukaryotic microbes in tailings using in-depth sequencing. Possibly the importance of eukaryote microorganisms in tailings biogeochemistry has been overlooked in past studies.

Most of the dominant species across all the microbial assemblages were potential heterotrophic decomposers which would be required for litter decomposition in soil (Mergaert et al., 2003; Sohn et al., 2004; Zgurskaya et al., 1992), or nitrogen-fixers (Sawada et al., 2003; Spang et al., 2012). The abundances of *Planctomycetes* (P < 0.001) and *Firmicutes* (P = 0.002) in the tailings-soil mix increased with increasing soil addition. Both the *Planctomycetes* and *Firmicutes* are important microbial phyla in natural bulk and rhizosphere soils (Fierer et al., 2007) but are generally much less abundant in Pb-Zn tailings (Li et al., 2014; Zhang et al., 2007a; Zhang et al., 2007b). These phyla may be an indicator of the ameliorating effects of the soil amendment and the rehabilitation of soil-like biological capacity in the engineered technosols.

There is the possibility that the findings of the pyrosequencing are influenced by DNA that is extracellular or from dead cells. It may be that extracellular DNA from dead bacteria can be a significant proportion of the sample DNA. However, it is reported that the turnover of soil DNA can be considerable, for example > 90 % of extracellular microbial DNA may be degraded within 4 days (Herdina et al., 2004). So we consider the high abundance of these bacterial types detected by DNA sequencing to indicate the presence of these microorganisms in the tailings-soil mix samples after the 8-week incubation. It would be useful to validate and quantify these findings in future work by designing specific probes and using fluorescence in situ hybridisation.

The measured activities of the selected enzyme dehydrogenase, invertase and urease increased with increasing soil amendments. These three enzymes are involved in C and/or N turnover in soil (Das and Varma, 2011). Due to the added sugarcane, the starting levels of soluble organic carbon and N were similar in all the treatments. It is logical to deduce that the increased levels of these enzyme activities in the treatments coincided with decreased levels of WSOC and water soluble N. It was surprising to have observed that neutral phosphatase activities was not significantly different among the four tailings-containing treatments in our present experiment, as the tailings may contain stressors that suppress phosphatase activity (Nannipieri et al., 2011). A possibility here is that the tailing microbial communities were adapted to the stresses and the phosphatase activity of these organisms was somehow induced by the addition of the sugarcane debris. Organic amendment has been found to induce phosphatase activity in other contaminated soils (Renella et al., 2005). It would also be useful to examine the dynamics of these changes by analysis of multiple points as the samples develop over time. This would confirm whether the observed enzymatic activities were the results of the active colonisation of the soil microbes or just the residual activities (Smith and Parsons, 1985).

It is known that microorganisms can have major impacts on geochemical processes in mine tailings (Diaby et al., 2007). Changes in microbial communities, in response to remediation measures, also have functional implication for tailings' soil biological capacity for plant establishment (Li et al., 2014). The observed species of microbial communities in tailings and soil as well as the mix treatments was fairly low in comparison to fertile natural or arable soils (Li et al., 2014). The alpha diversity in the tailings may be primarily limited by toxicity factors in the tailings, even if microbial community composition can be changed by the soil amendment. It was apparent that further chemical weathering of minerals had occurred in the amended treatments within the 8-week period to maintain soluble ion levels similar to that detected in the tailing treatments. Such rapid weathering is not common in normal soils, but has been found in many sulphidic coal and base metal mine tailings (Dold and Fontboté, 2001; Park et al., 2013), particularly when organic matter is added in the tailings (Li et al., 2014; Li et al., 2013).

The stimulation of the fungi colonisation in the tailings-soil mix is rather unexpected, since fungi abundance was low in the soil and tailings only treatments. In the mixed treatments of the present experiment, the abundance of fungi accounted for about 10 %, indicating their potential importance in the establishment of microbial communities in the engineered tailings-soils. The major fungal phylotypes (> 1 % in at least one sample) were typical heterotrophic decomposers in soil (Basiewicz et al., 2012; Haugland et al., 2001; Zhang et al., 2009; Zuccaro et al., 2004). Among these fungi, the most abundant, *Emericellopsis* sp., is potentially alkalitolerant, a trait possibly aiding its survival in the unfavourable geochemical conditions of the tailing treatments. From an engineering viewpoint, the colonisation of fungi in the amended tailings is of particular importance in soil with low organic carbon and nutrients, in addition to the development of prokaryotic microbial communities. In agriculture, high soil fungal: bacterial ratios are well-recognized as indicative for more sustainable soil ecosystems with low fertility background (De Vries et al., 2006). Fungi play special roles in recycling and retaining nutrients (Averill et al., 2014) and improving soil physical structure (Ritz and Young, 2004), and soil ecosystem tends to be dominated by fungi in late terrestrial succession (Crawford et al., 2012). A CCA analysis indicated a strong association between fungi (*Agaricomycetes*) and microbial biomass and the activities of enzymes related to C and N cycling (Fig. 6-7). Therefore, the colonisation of fungi may be not only an indicator of amelioration of the tailings environment but also a driving factor in tailing pedogenesis after amendment.

The survival of non-fungi eukaryotes in all soil-containing treatments is also interesting. The occurrence of the most abundant *Nematoda*, *Aphelenchus* sp. may be highly connected to the development of fungi (Walker, 1984), as supported by our findings that there was a strong correlation between the total abundance of fungi and soil protists ($R^2 = 0.60$, $P < 0.05$, n = 15) in all the samples. Although the protists were low in abundance, the survival of the protists in the tailings-soils mix implies the possibility of reconstructing a food-web similar to natural soil in the tailings by the current amendment strategy.

6.4.2 Environmental drivers in the establishment of microbial community

The additions of local soil from native vegetation habitat not only provide the inocula of soil microbes, but also altered the physicochemical conditions in the tailings through dilution, addition and interactions between the soil and tailing factors. At the end of the 8-week of incubation tests, the microbial communities were the result of selection of soil microbes caused by physicochemical conditions induced by soil addition. The microbial community structure differences may be a reflection of the different magnitudes of metal stresses of the tailing environment, while ample carbon sources were provided for microorganisms in the tailings and the soil treatment. Metal stresses can induce changes of soil microbial communities in not only the community structure (Deng et al., 2009) but also the abundance of specific functional groups (Li et al., 2009; Li et al., 2012). In a study of Pb–Zn tailings from the *Aravaipa Valley*, *Graham County*, *Arizona γ-Proteobacteria* (in percentage) were detected an order of magnitude higher in comparison to that in the reference soil where *α-Proteobacteria* were much more abundant (Mendez et al., 2008). Moreels et al. (2008) also found that microbial communities sensitive to metals can evolve toward *γ-Proteobacteria*-enriched communities under metal stress. These results imply that *γ-Proteobacteria* may be more tolerant of environmental stresses than other classes. Indeed, it was found in the present study that the dominance of *γ-Proteobacteria* in tailings was strongly associated with the stressors, including EC (thus S), total Zn and total Pb.

Despite the apparent difference in patterns of microbial community structure between tailings and soil, the microbial community structure of their mix was not a simple result of superimposition. Rather, opportunistic development of fungi and nematodes was found in these treatments. This could be related to the complex dynamics of nutrient levels and environmental stresses created by the additions of sugarcane and soil materials. Further, it is worth noting that large differences in abundance were observed for fungi and protists at the genus level among replicates in all treatments. CCA indicated that community differences can be associated with the physiochemical nature of the different treatments. Stochastic assembly processes can also contribute to microbial community variation, as found in soils and other habitats after disturbance (Ferrenberg et al., 2013; Zhou et al., 2014). However, the occurrence of community clusters based on the tailing treatments (Fig. 6-7) implies that stochastic processes were not the dominating influence on the community patterns detected here. The dynamic of tailing microbial community structure merits a further investigation in long-term and under field conditions.

6.5 Conclusions

In weathered and neutral Cu-Pb-Zn tailings, the native soil inoculation approach may be used to fast-track the establishment of native microbial communities and initiate the rehabilitation of biogeochemical processes in the technosol for establishing native plant species well adapted to the local soil conditions, although field trials are required to investigate the persistence across seasons and years.

Chapter 7 Biogeochemical changes induced by organic matter of contrasting properties and native plant colonisation in fresh and neutral Cu-Mo-Au tailings of low phytotoxicity

7.1 Introduction

Functional technosol development in base metal mine tailings consists of two critical phases – (1) weathering of reactive minerals and hydrogeochemical stabilisation and (2) development of biogeochemical structure and functional processes (Huang et al., 2014a; Huang et al., 2012). Direct revegetation of base metal mine tailings, even in the significantly amended tailings, without successful soil formation *in situ* has had little success in establishing a healthy plant cover and plant communities of high diversity and sustainability (Chen et al., 2007; Chiu et al., 2006; Song et al., 2004). Technosols with adequate biogeochemical capacity and functions is anticipated to develop from sulphidic tailings only after many decades of weathering of sulphides and pedological development under natural conditions (Uzarowicz and Skiba, 2011).

As microbial community and activities are crucial for ecosystem functions and plant productivity (Harris, 2009), rehabilitation of functional microbial communities is an essential part of technosols formation from the amended tailings (Li and Huang, 2015; Mendez and Maier, 2008). The development of microbial communities and their functions in amended tailings has become an critical indicator of technosol formation in tailings, subjected to various ecological engineering inputs. It is widely recognised that plant species and matrix properties together shape the structure and functions of microbial communities (Berg and Smalla, 2009). As a result, the introduction of tolerant pioneer plant species should be considered when investigating ecological engineering options to stimulate the technosols formation in tailings.

Our previous studies using the weathered and neutral Cu-Pb-Zn tailings have demonstrated that it is highly possible to accelerate the process from tailings to biogeochemically functional technosols with purposely designated ecological engineering inputs, such as the incorporation of organic amendments and introduction of pioneer plant species (Huang et al., 2011; Li et al., 2015; also refer to Chapter 3, 4, and 5). However, based on field trials and laboratory pre-tests, the initiation and stimulation of biogeochemical changes towards functional technosols is far less efficient in the fresh tailings which contain abundant reactive minerals and are undergoing dynamic hydrogeochemical actions of reactive minerals (e.g., pyrites and the equivalent) (refer to TD7 in Chapter 3). The weathered Cu-Pb-Zn tailings from TD5 tested in the laboratory incubation (refer to Chapter 5 and 6) are too saline for the examined plant species even after 20 years of weathering under field conditions.

Many fresh tailings from Cu- and Cu-Mo-Au mines contain low amounts of reactive minerals and exhibit relatively stable geochemical conditions (Forsyth, 2014; Huang et al., 2011). For example, the EHM tailings (tailings from Ernest Henry Mine from processing chalcopyrite ores) in the resent study are much less hydrogeochemically reactive than those from Mount Isa Mines (TD5 and TD7), due to the mineralogical composition of the former (Siliezar et al., 2011). It is possible to initiate technosols formation in this type of fresh tailings by directly focusing on biogeochemical structure and function rehabilitation (Huang et al., 2014b). Additionally, a field trial has already shown that native grass species can be successfully established in this fresh Cu-Mo-Au tailings amended with organic amendment (hay) under subtropical and semi-arid climatic conditions; the native grass also flowered, seeded and self-recruited in the field trials (Huang et al., 2011). Therefore, the Cu-Mo-Au EHM tailings may be used to support the conceptual proposition that biogeochemical rehabilitation phase in the engineered pedogenesis is critically dependent on the hydrogeochemical stabilisation in the tailings. The primary objective of this study was to investigate the responses of microbial community structure and functions in fresh Cu-Mo-Au tailings amended with organic matter containing high proportions of labile organic carbon and the colonisation of native plant species under greenhouse conditions compared to those amended with recalcitrant biochar.

The adverse conditions in tailings (e.g., nutrients depletion, low water holding capacity, heavy metal toxicity, salinity) favour the development of tolerant microbial communities (Li et al., 2014; Li et al., 2015; Schimel et al., 2007). Microbial diversity, biomass and associated biogeochemical activities are therefore extremely low compared to natural soil (Kock and Schippers, 2008; Li et al., 2014). Organic amendments may rehabilitate diverse and functioning microbial communities in tailings that are beneficial to plant establishment and growth (Pepper et al., 2012). Furthermore, exogenous organic amendments (e.g., plant residue, biochar, compost, manure etc.) may rapidly build up organic carbon pools, contributing to microbial biomass and functions rehabilitation (Rosario et al., 2007) (also see Chapter 3, 4 and 5). In particular, the introduced pioneer plant roots would assist the colonisation of beneficial rhizosphere microbes that would benefit plants in nutrient acquisition and tolerance resistance in the stressed tailings environment (Fellet et al., 2014; Santibáñez et al., 2008; Solís-Domínguez et al., 2011). Besides, the effects on the colonisation of rhizosphere microbial communities may differ between leguminous and gramineous plant species.

This study compared the efficacy of two typical organic amendments and the colonisation of two native plant species on biogeochemical properties and processes (such as microbial community structure and functions), applied in fresh and neutral Cu-Mo-Au tailings with a relatively stable geochemistry and low contents of reactive minerals (e.g., sulphides). The native species *Iseilema vaginiflorum* (grass) and *Acacia chisholmii* (leguminous shrub) were tested in this study. Both are drought and nutrient efficient tolerant species typically distributed in the region of Cloncurry and Mount Isa, northwest Queensland, Australia (Hunter and Melville, 1994). A field survey shows these two species are capable of colonising infertile land (Diagne et al., 2013), with high tolerance to salinity (Marcar et al., 1991) and elevated levels of heavy metals (Justin et al., 2011). The specific objectives of this study were to: (1) characterise the growth and element uptake by native grass *Iseilema vaginiflorum* (IV) and leguminous shrub *Acacia chisholmii* (AC) in the tailings; (2) investigate the efficacy of organic amendments on rehabilitation of microbial communities interacting with native plants. Information from this study is foundation to formulate effective organic amendment strategies for technosols formation in relatively stable and neutral base metal mine tailings rehabilitated with tolerant native plant species.

7.2 Materials and methods

7.2.1 Ernest Henry Mine and sample collection

The tailings used in this study came from processing ores containing iron oxides (23 % magnetite), Cu (1 %) and Au (0.5 g t^{-1}) (Siliezar et al., 2011) at Ernest Henry Mine (EHM), Cloncurry, northwest Queensland, Australia (refer to Appendix C). The tailings were collected from tailings impoundment areas in June 2012. The tailings are slightly alkaline with an average pH of 7.7, EC of 1.5 mS cm^{-1} and contain 3 % S, 0.3 % Fe, 0.1 % Mn and 0.01 % Cu.

Bulk tailings were transported to the laboratory and dried at 40 °C in an aerated oven until a constant weight was reached. Prior to incubation, the tailings were sieved through 2 mm stainless mesh steel and homogenised. Organic amendments, sugarcane as fresh plant biomass and biochar as recalcitrant carbon, were used in this study. Properties of sugarcane (SC) were described in Chapter 5.2.1. Biochar (BC) properties were described in Chapter 4.2.1. Both were ground to less than 1 mm and sterilised prior to incubation.

7.2.2 Experiment design and greenhouse incubation

The seeds of *Iseilema vaginiflorum* (IV) and *Acacia chisholmii* (AC) were collected from the native plant in the sampling area of the tailings. *Iseilema vaginiflorum* seedlings were prepared by germinating in an incubator and healthy seedlings grown in greenhouse culture for 3-week before transplantation into the amended tailings. 3 seedlings were selected for each pot in each treatment to permit rapid saturation of the root mass in the pot. *Acacia chisolmii* seeds were sown and cultured for 12-week prior to transplanting into the amended treatments. Due to the relatively larger root system per plant, only one healthy AC seedling transplanted in each pot (replicate) of the treatments. Seedlings with similar growth conditions were selected for the experiment. At transplanting, all seedlings were carefully washed with tap water to remove soil particles and quickly rinsed 3 times with deionised water.

The treatments included: control, 10%SC+IV, 10%BC+IV, 10%SC+AC and 10%BC+AC, with 3 replicates in each treatment. Tailings and 10 % (w/w) of SC or BC were thoroughly mixed and subsequently placed in pots (12 cm in diameter and 10 cm in height) with a piece of 0.1 mm moisture mat underneath to retain the fine tailings particles. All pots and mats were sterilised before use. The rate of amendment application was selected based on our preliminary experiments under laboratory and field conditions (Li et al., 2014; Li et al., 2015). Preliminary experiments showed that neither of these plant species could survive in the tailings without organic amendments due to high mechanical compaction and anoxia conditions.

The plants were cultured under greenhouse conditions with an atmospheric temperature of 20-29 °C and 55 % relative humidity. Deionised water was added daily to counter water loss through evaporation; soil moisture in the pots was maintained to 50-60 % of the maximum water holding capacity. The pots were arranged in a randomised block design and incubated for 8 weeks (i.e., early May to the end of June, 2013).

At harvest, plants shoots were cut off at the base of each plant with a stainless steel blade. Roots were then gently separated from the tailings to remove any attached particles by hand. For all the revegetated treatments, tailings attached to the plant roots were purposely collected as bulk rhizosphere tailings, which were stored at 4 °C for DNA extraction and subsequent microbial community analyses. Bulk tailings were sampled and stored at 4 °C for microbial biomass and enzyme assays analyses.

For plant analyses, both shoots and roots were rinsed with deionised water 3 times, followed with 0.01M hydrochloride acid and millipore water and dried at 65 °C until a constant weight. They were ball milled and stored in a desiccator prior to chemical analysis.

7.2.3 Plant analysis

Total N concentrations in plant tissues were determined by dry-combustion with a LECO CNS-2000 analyser (LECO Corporation, MI, USA). Plant samples (0.05-0.10 g) were digested with concentrated nitric acid using an open-vessel microwave (Milestone Start D) (Huang et al., 2004). Total elements were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian) .A standard reference plant material (ASPAC 61, Canola leaf, Australian Soil and Plant Analysis Council) was used to test the accuracy of the measurement with the recoveries ranging from 90 ± 10 %.

7.2.4 Physicochemical analysis

All the selected physicochemical analyses were measured as described in Chapter 3.2.2

7.2.5 Microbial biomass, basal respiration and enzyme assays

Microbial biomass and enzyme assays were determined as described as Chapter 4.2.4. Basal respiration measurements were conducted as described in Chapter 5.2.3.

7.2.6 DNA extraction, pyrosequencing and analysis

DNA extraction, pyrosequencing and analyses were performed as decribed Chapter 5.2.4

7.2.7 Statistical methods

Primary data processing was performed using Microsoft® Excel. One-way analysis of variance (ANOVA) was carried out for significant tests among treatments. Two-way ANOVA was carried out for significant tests of differences in microbial communities between organic amendments and plant species. Means were compared using the least significant differences (LSD) test at P = 0.05. Pearson linear correlations among plants properties, biogeochemical properties and microbial properties in tailings were also analysed. All statistical analyses were conducted using the SPSS software package (SPSS Statistics 20.0, Chicago, IL, USA).

Principal component (PCA) and canonical correspondence analyses (CCA) were carried out using CANOCO software for Windows 4.5 (Biometris-Plant Research international, Wageningen, The Netherlands). PCA and CCA were performed at the class level for microbial species and for selected environmental variables (e.g., pH, EC, Cu, S) to detect the distribution of microbial communities in the tailing treatments, and for correlations of microbial composition and measured environmental variables.

7.3 Results

7.3.1 Physicochemical properties in the tailings

Basic physicochemical properties of the tailings are summarised in Table 7-1. The results showed that the tailings were neutral to alkaline and slightly saline. The tailings contained low levels of TOC and nutrients (e.g., TN), but elevated levels of sulphur (S) and iron (Fe) bearing minerals. In addition, the levels of heavy metals (e.g., Mn, Cu and As) were in the toxic range for agriculture purpose (Mendez and Maier, 2008).

The organic treatments significantly modified the physicochemical properties of the amended tailings, compared to the control. Both SC and BC increased WHC by 2-3 folds in the amended tailings, compared to the control. The SC amendment significantly increased levels of TOC ($P < 0.001$), TN ($P < 0.01$) and CEC ($P < 0.01$) compared to the control. The addition of BC in the tailings brought about significant increase of TOC ($P < 0.001$), but not TN and CEC compared to the control.

Organic amendment and plant colonisation did not change total concentrations of most elements (e.g., S, Ca, Mn, Cu and As). However, the levels of water-soluble Cu and As in the tailings were significantly increased by the SC treatments ($P < 0.01$) and water-soluble Mn and Cu decreased by the BC treatments significantly ($P < 0.01$) compared to the control.

Table 7-1 Selective physicochemical properties in the sugarcane and biochar amended tailings with native plants

Treatments		pH	EC (mS cm^{-1})[a]	CEC (cmol+ kg^{-1})[b]	WHC (%)[c]	TOC[d]	TN[e]	Total concentration (g kg^{-1})					Water soluble concentration (mg kg^{-1})				
						g kg^{-1}		S	Ca	Mn	Cu	As	S	Ca	Mn	Cu	As
Control	Mean	7.7	1.4$^{\alpha\beta}$	1.2$^{\alpha}$	14.0$^{\alpha}$	2.7$^{\alpha}$	0.27$^{\alpha}$	32.0	46.6	3.2	1.25	0.10	181	234	0.25$^{\alpha}$	0.07$^{\alpha}$	0.004
	S.D.[f]	0.07	0.1	0.3	3.3	1.5	0.10	1.2	4.3	0.5	0.20	0.02	48	66	0.08	0.03	0.005
10%SC+IV	Mean	7.6	1.6$^{\beta}$	3.3$^{\beta}$	41.2$^{\beta}$	38.2$^{\beta}$	0.48$^{\beta}$	24.1	38.8	2.7	1.19	0.06	119	151	0.32$^{\alpha}$	0.14$^{\beta}$	0.01
	S.D.	0.02	0.2	0.8	3.8	3.3	0.02	3.3	1.7	0.1	0.01	0.03	24	36	0.09	0.03	0.003
10%BC+IV	Mean	7.7	1.2$^{\alpha}$	1.4$^{\alpha}$	36.9$^{\beta}$	70.2γ	0.29$^{\alpha}$	32.3	46.0	3.3	1.19	0.11	159	192	0.15$^{\beta}$	0.01$^{\alpha}$	0.015
	S.D.	0.04	0.2	0.3	1.3	1.3	0.07	4.8	2.9	0.3	0.03	0.03	53	64	0.04	0.00	0.003
10%SC+AC	Mean	7.6	1.4$^{\beta}$	3.7$^{\beta}$	41.6$^{\beta}$	38.2$^{\beta}$	0.49$^{\beta}$	26.2	41.9	3.3	1.37	0.09	164	221	0.33$^{\alpha}$	0.30$^{\gamma}$	0.018
	S.D.	0.03	0.1	0.9	2.7	1.3	0.05	4.0	5.0	0.9	0.44	0.03	18	72	0.11	0.10	0.003
10%BC+AC	Mean	7.6	1.1$^{\alpha}$	1.6$^{\alpha}$	39.3$^{\beta}$	68.6γ	0.30$^{\alpha}$	28.7	42.0	2.7	1.13	0.09	178	208	0.14$^{\beta}$	0.01$^{\alpha}$	0.014
	S.D.	0.05	0.0	0.2	2.1	2.6	0.01	2.6	2.1	0.1	0.05	0.03	27	34	0.05	0.00	0.007

[a] Electrical conductivity. [b] Cation exchangeable capacity. [c] Water holding capacity. [d] Total organic carbon. [e] Total nitrogen. [f] Standard deviation. Values are means (n = 3); values labelled with letters 'α, β, γ and δ' within the column indicate significant differences among the treatments at the level of $P < 0.05$ (only labelled for selected parameters).

7.3.2 Plants responses in the amended tailings

All the plants transplanted in the tailings survived during the course of the greenhouse experiment. Overall, both IV and AC grew well in the amended tailings (Fig. 7-1).

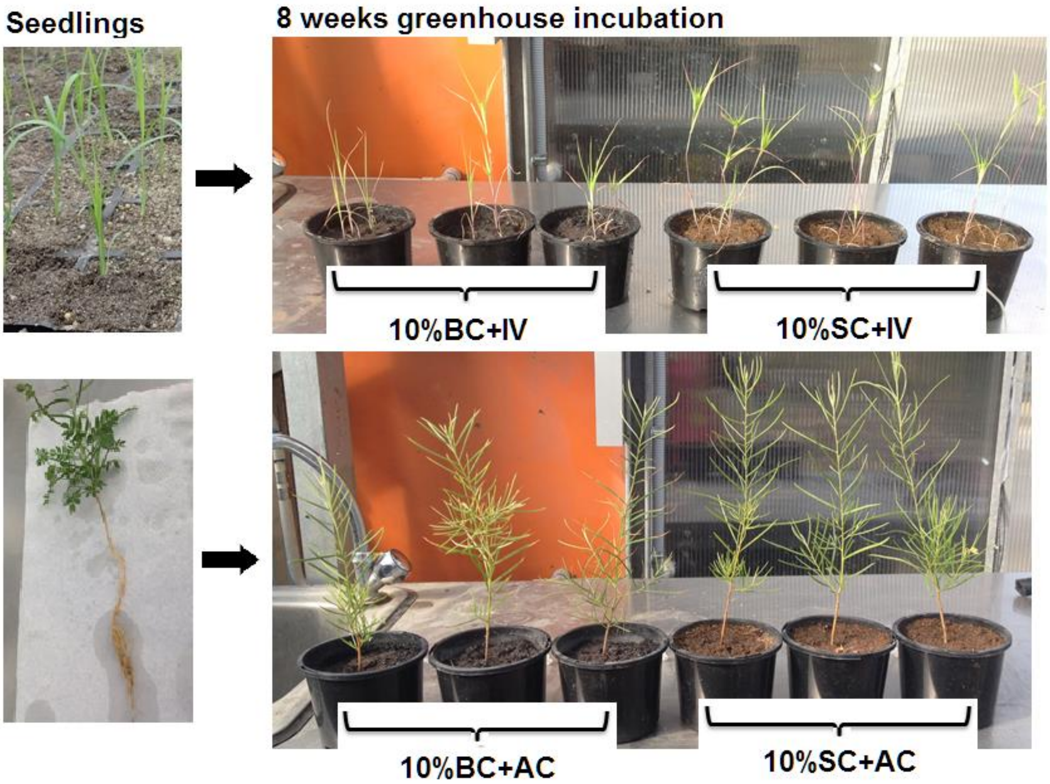

Fig. 7-1 Growth pattern of transplanted mature plant seedlings in the sugarcane and biochar amended tailings with native plants after 8-week greenhouse incubation.

These 2 native plant species had different growth responses to the SC and BC treatments. As shown in Fig. 7-2, plants grown in the SC amended tailings showed an overall greater biomass compared to those grown in the BC treatments. Both shoot and root biomass of AC grown in the SC amended tailings were significantly greater than the AC grown in the BC amended ($P < 0.05$).

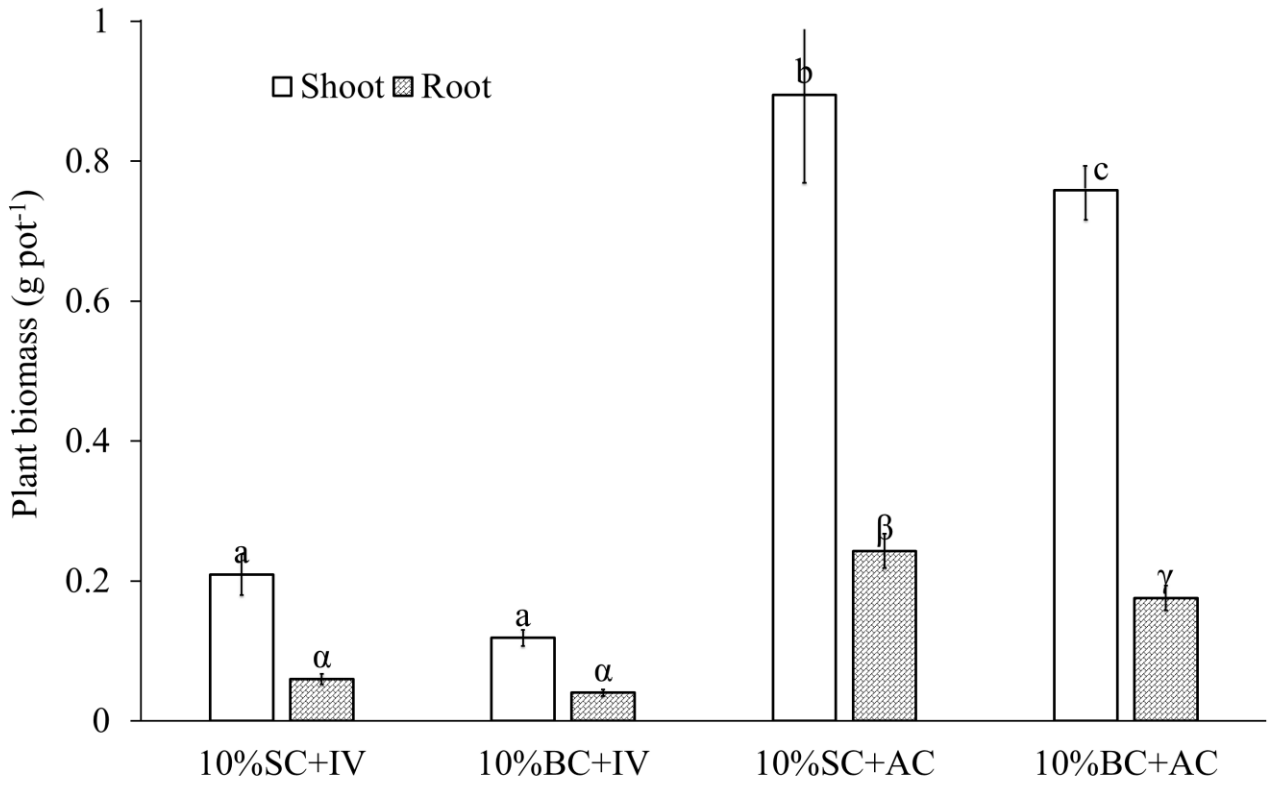

Fig. 7-2 Plant biomass of shoot and roots in the sugarcane and biochar amended tailings with native plants. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above indicate significant differences of plant shoots among the treatments at the level of $P < 0.05$; the letters 'α, β, γ and δ' above indicate significant differences of plant roots among the treatments at the level of $P < 0.05$.

The main effects of the SC and BC treatments seemed to be on Cu concentrations in the plant roots. Specially, Cu concentrations in the roots of IV in the BC amended tailings (127-199 mg kg^{-1}) were much lower than those in the SC amended tailings (207-328 mg kg^{-1}) ($P < 0.05$) (Fig. 6-3d). The same pattern was observed for Cu concentrations in the AC roots, which significantly decreased in the BC amended tailings (259-371 mg kg^{-1}) compared to those grown in the SC amended tailings (451-651 mg kg^{-1}) ($P < 0.05$).

Moreover, there were significant differences between IV and AC in element concentrations in the plant shoots. For example, N concentrations (Fig. 6-3a) in the AC shoots (13.1-19.1 g kg^{-1}) were significantly greater than those in the IV shoots (22.6-25.6 g kg^{-1}) ($P < 0.01$), while the Cu concentrations in the AC shoots (3-5 mg kg^{-1}) were lower than those in the IV shoots (8-17 mg kg^{-1}) ($P < 0.05$) (Fig. 6-3d).

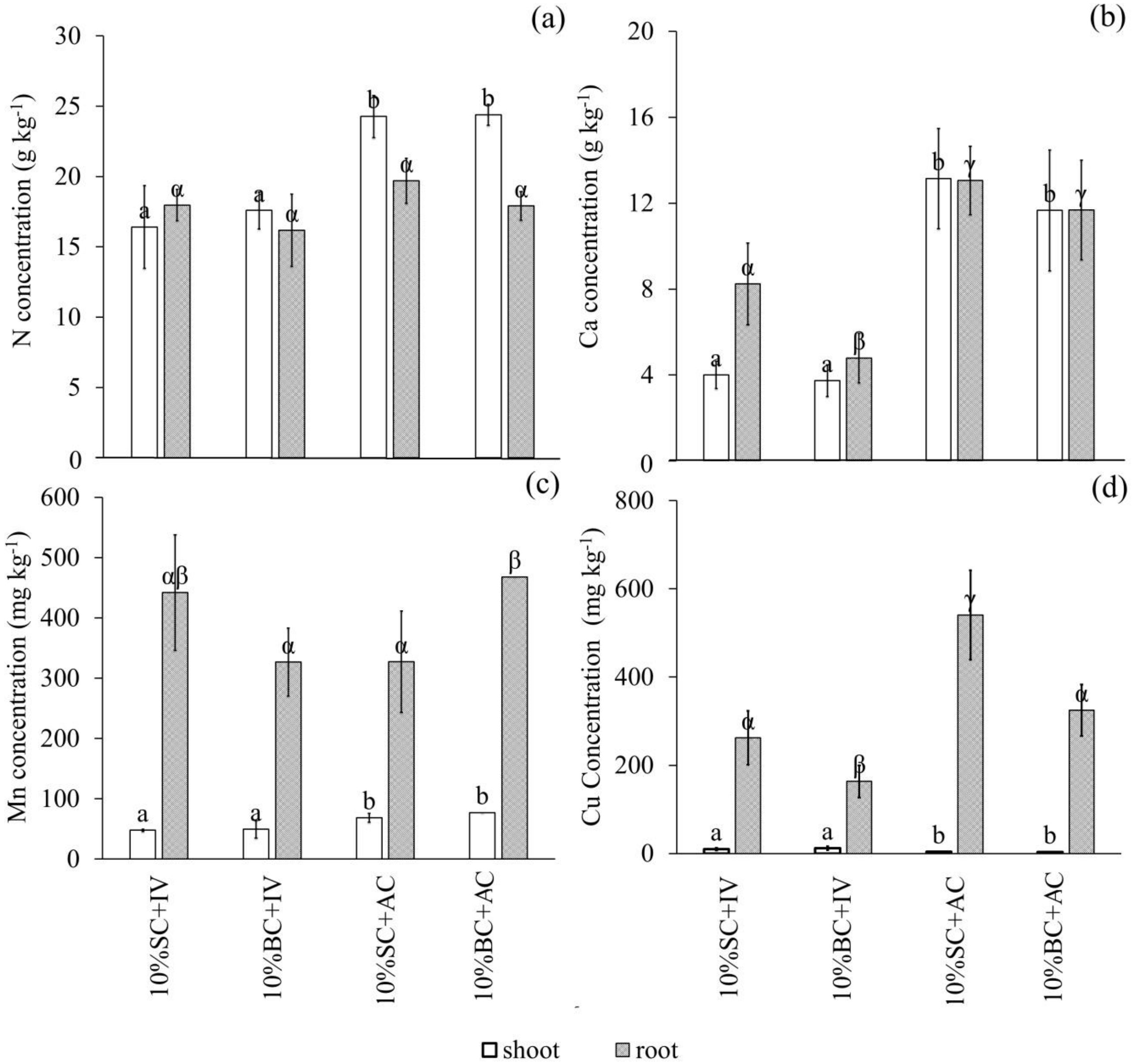

Fig. 7-3 Concentrations of N (a), Ca (b), Mn (c) and Cu (d) in shoot and root of *Iseilema vaginiflorum* and *Acacia Chisholmii* in the sugarcane and biochar amended tailings with native plants. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above indicate significant differences of plant shoots among the treatments at the level of $P < 0.05$; the letters 'α, β, γ and δ' above indicate significant differences of plant roots among the treatments at the level of $P < 0.05$.

7.3.3 Microbial functions in the tailings

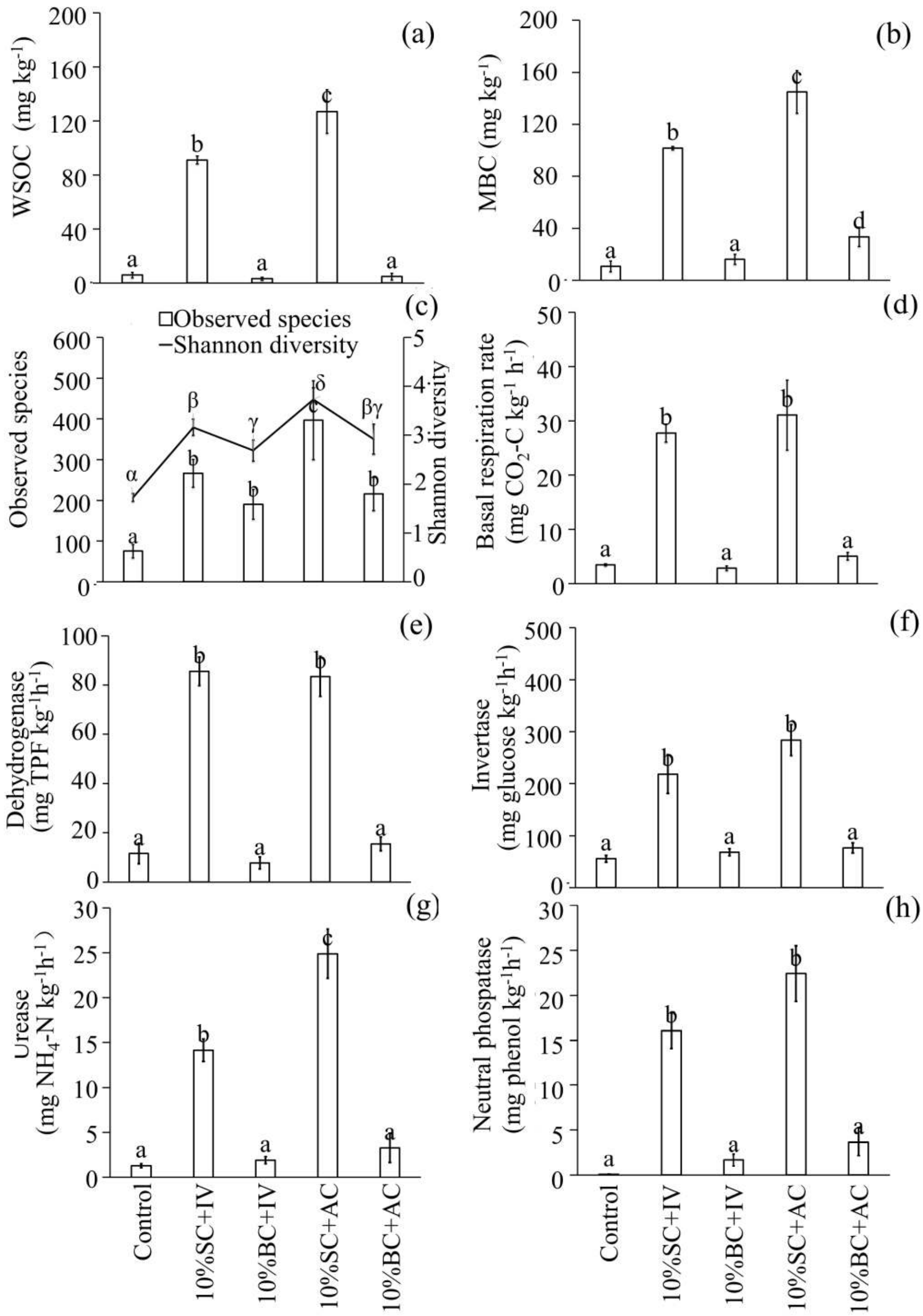

Fig. 7-4 WSOC (a), MBC (b), microbial community diversity indexes (c), basal respiration (d), dehydrogenase (e), invertase (f), urease (g) and neutral phosphatase (h) activities in the control and the sugarcane and biochar amended tailings with native plants. Values are means (n = 3); error bars indicate standard deviations; the letters 'a, b, c, d' above indicate significant differences among the treatments at the level of $P < 0.05$; the letters 'α, β, γ and δ' above indicate significant differences of Shannon diversity among the treatments at the level of $P < 0.05$.

Organic amendments with contrasting properties significantly shifted microbial communities and functions in the amended tailings. As shown in Fig. 7-4a, SC amendment significantly increased the levels of labile OC (WSOC) (P < 0.05), especially in the 10%SC+AC treatment. In the BC-amended tailings, there was limited WSOC regardless of plant species transplanted. Across the treatments, there was a significant positive relationship between WSOC and MBC (R^2 = 0.83, P < 0.01, n = 15). Consistent with the trend of WSOC in all treatments, microbial biomass (Fig. 7-4b) significantly increased in the SC amended tailings, with the highest in the 10%SC+AC treatment.

Microbial colonisers are closely linked to the biogeochemical processes in tailings. Among all samples, MBC positively related to basal respiration (R^2 = 0.97, P < 0.01, n = 15), dehydrogenase (R^2 = 0.92, P < 0.01, n = 15), invertase (R^2 = 0.98, P < 0.01, n = 15), urease (R^2 = 0.96, P < 0.01, n = 15) and neutral phosphatase (R^2 = 0.94, P < 0.01, n = 15) activities. Therefore, basal respiration and the 4 enzymatic activities significantly stimulated in SC amended tailings compared to the control. Moreover, the greatest levels of basal respiration and the enzymatic activities were observed in the 10%SC+AC treatment, especially the urease activities. In contrast, these biogeochemical processes were still at low levels in both 10%BC+IV and 10%BC+AC treatments regardless of introduction of pioneer plants, which were not different from those in the control (Fig. 7-4).

7.3.4 Microbial community composition in the tailings

Pyrosequencing of 16s rRNA genes was performed to explore the composition of microbial communities within different treatments. A total of 77,055 good quality reads were obtained by pyrosequencing, which were classified into 950 $OTUs_{0.97}$ (Fig. 7-5). These 950 $OTUs_{0.97}$ were affiliated to 11 phyla of eukaryotic microorganisms, 3 classes within 2 archaeal phyla and 63 classes within 24 bacterial phyla. Microbial communities in all the tailings were dominated by prokaryotic microorganisms. Both species richness and Shannon diversity of the microbial communities significantly increased in all the amended tailings compared to the control (P < 0.05), with the most diverse microbial communities in the 10%SC+AC treatment (Fig. 7-4c).

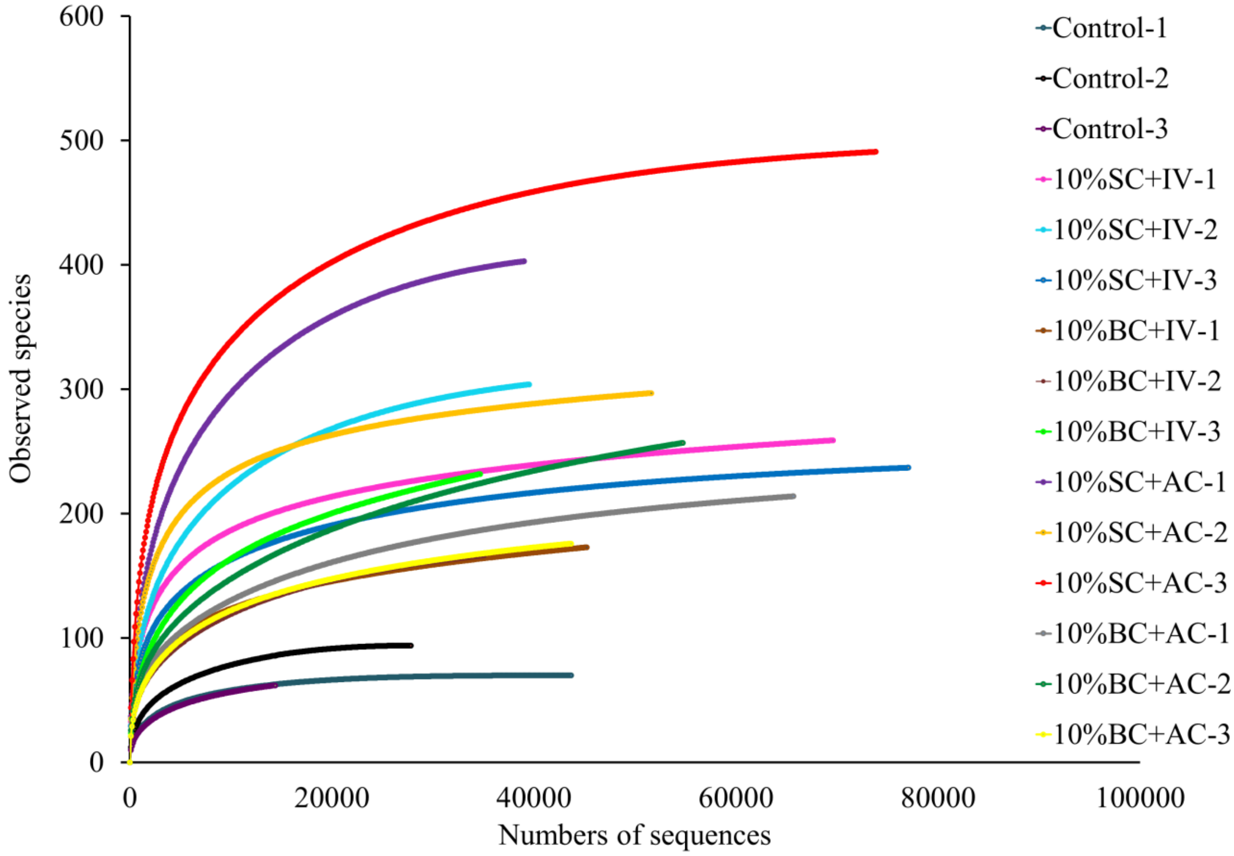

Fig. 7-5 Rarefaction curve of OTUs and microbial diversity in the control and the sugarcane and biochar amended tailings with native plants.

The depth of sequencing well captured the microbial diversity present (Fig. 7-5). Bacterial OTUs dominated all the microbial assemblages in all the treatments. Eukaryotic microorganisms were detected in all the treatments but at low levels of abundance (< 1 %). There were 14 OTUs affiliated to fungi and 37 OTUs to other eukaryotic microbes. Specifically, the most abundant fungal OTUs were within the phylum of *Dikarya*, closest to *Lentinula* sp. Other eukaryotic microorganisms were mainly affiliated to the phylum of *Alveolata*, *Viridiplantae* and *Rhizaria*, closest to *Colpodella* sp., *Albizia* sp. and *Spongomonas* sp., respectively (Fig. 7-6). OTUs numbers (14) and relative abundance (< 0.5 %) affiliated to archaea, mainly affiliated to *Crenarchaeota*, were low in all the treatments without statistical differences (Fig. 7-7).

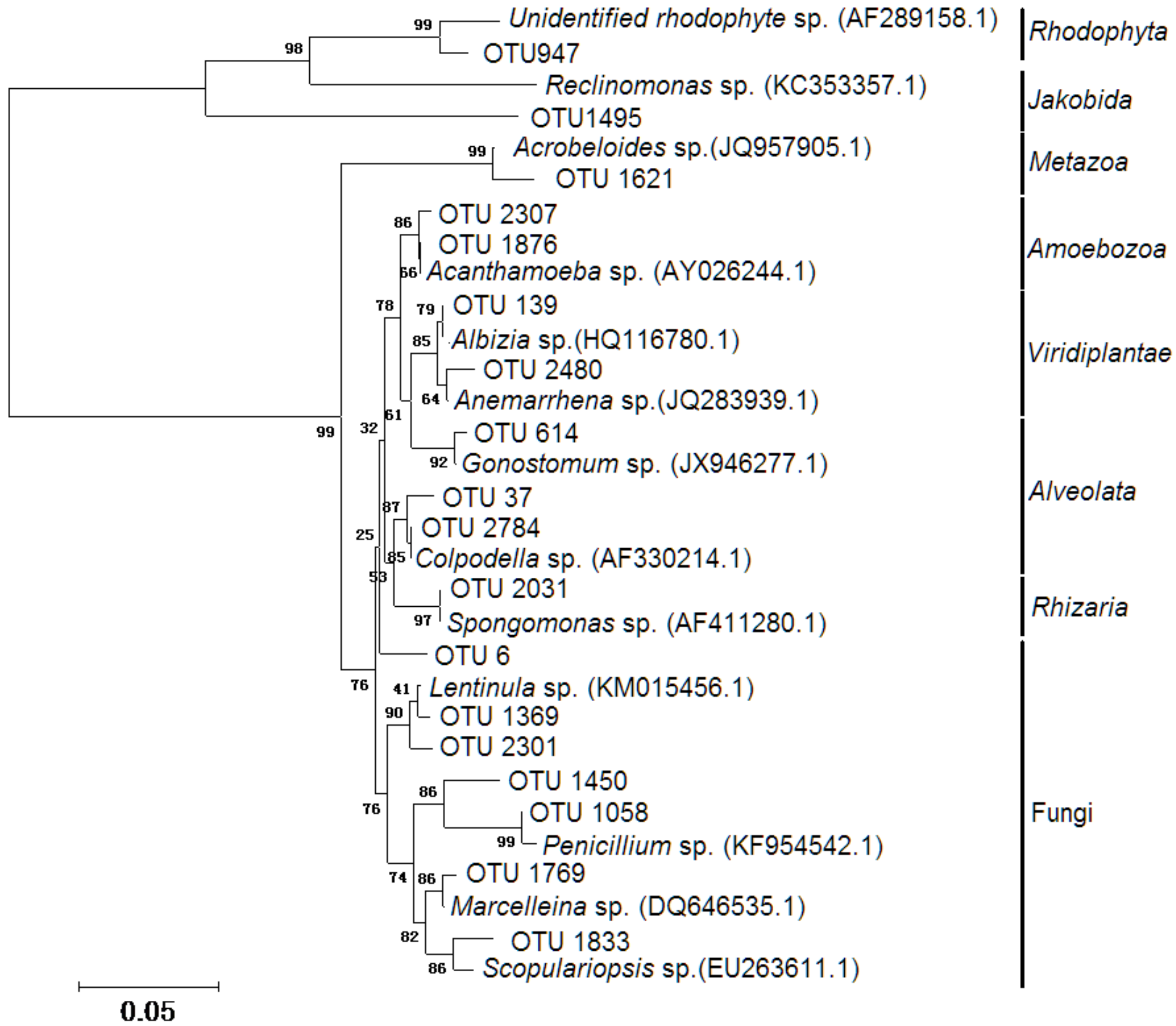

Fig. 7-6 A neighbour-joining phylogenetic tree of 16S rRNA gene sequences detected for eukaryotic microorganisms in the control and the sugarcane and biochar amended tailings with native plants. Only representative OTUs were shown in this tree; the taxonomic groups are delineated on the right; scale bar stands for 0.05 changes per site.

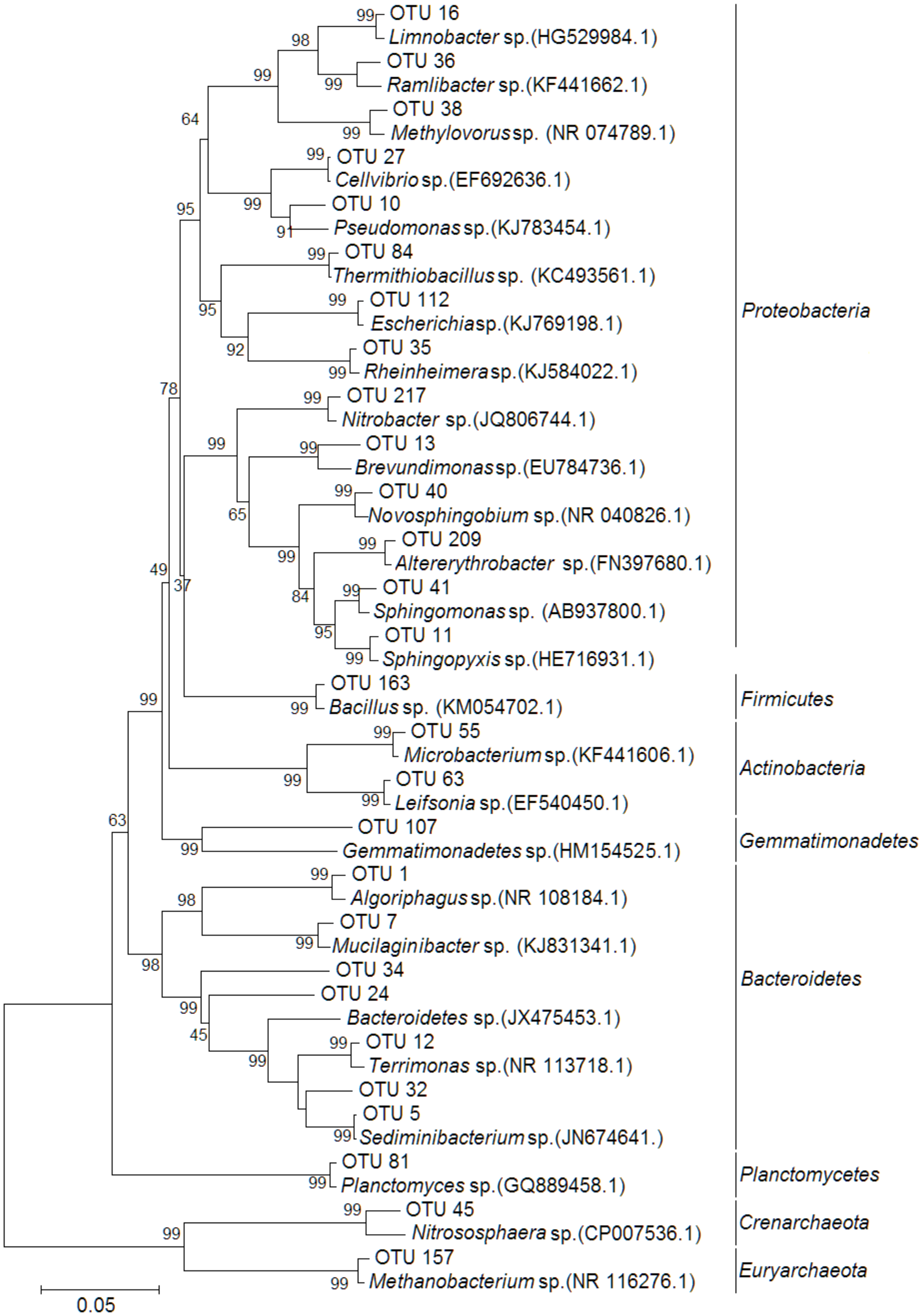

Fig. 7-7 A neighbour-joining phylogenetic tree of 16S rRNA gene sequences detected for archaeal and bacterial communities in the control and the sugarcane and biochar amended tailings with native plants. Only representative OTUs were shown in this tree; the taxonomic groups are delineated on the right; scale bar stands for 0.05 changes per site.

The organic amendments and introduction of native plant species significantly induced the changes of bacterial community composition in the tailings along different directions, compared to those in the control (Fig. 7-9 and Fig. 7-10). Specifically, microbial communities in the SC amended tailings formed one group, the BC amended tailings formed another group. Both differed from microbial communities in the control.

Comparatively, microbial communities in the control were dominated by *Proteocbacteria* and *Firmicutes* (Fig. 7-8). The most abundant bacterial OTUs in tailings was closet to *Bacillus* sp. (34.0 %), belonging to *Firmicutes*. Furthermore, *Brevundimonas* sp. (28.0 %) belonging to *α-Proteobacteria* was also found to be abundant in the control. The abundance of these dominant microorganisms in the control (i.e., *Bacillus* sp. and *Brevundimonas* sp.) significantly decreased ($P < 0.05$) in all the SC and BC amended tailings.

In the BC amended tailings, the abundance of *Actinobacteira* and *β-Proteobacteria* were significantly increased ($P < 0.05$). The most abundant bacterial OTUs in these tailings were shifted to *Thermithiobaillus* sp., belonging to *γ-Proteobacteria*. It accounted for 16.8-25.8 % of microbial communities in the BC amended tailings. Other abundant bacteria significantly increased in the BC amended tailings compared to the control, included OTUs affiliated to *Leifsonia* sp. (11.6-17.8 %), *Ramlibacter* sp. (7.4-8.3 %), *Sphingopyxis* sp. (5.9-6.0 %), *Sphingomonas* sp. (3.6-7.7 %), *Microcella* sp. (3.8-4.1 %), *Limnobacter* sp. (1.9-3.4 %) and *Nitrobacter* sp. (1.8-1.9 %). The abundance of genera of *Sphingopyxis* sp. and *Sphingomonas* sp. also increased significantly in the BC amended tailings compared to the control.

In the SC amended tailings, the abundance of *Bacteroidetes* were significantly increased ($P < 0.05$) (Fig. 7-8). Specifically, the most abundant bacterial OTUs shifted to *Algoriphagus* sp. accounting for 19.4-20.2 % of microbial communities in the SC amended tailings. The abundance of genera of *Sphingopyxis* sp. and *Sphingomonas* sp. significantly increased in the SC amended tailings ($P < 0.05$). Furthermore, in the SC amended tailings, the abundance of dominant bacterial OTUs affiliated to *Sediminibacterium* sp. (5.9-9.1 %), *Pseudoxanthomonas* sp. (3.6-4.2 %), *Terrimonas* sp. (1.7-9.3 %), *Bacteroidetes* sp. (1.9-6.5 %), *Microbacterium* sp. (1.6-3.5 %), *Planctomyces* sp. (1.0-2.6 %) and *Opitutus* sp.(1.0-3.2 %) significantly increased, compared to the control ($P < 0.05$), which were not abundant in both the control and the BC amended tailings.

Two-way ANOVA showed different impacts of organic amendments and plant species on microbial community composition. The two plant species (IV and AC) had different effects on microbial community composition in the tailings. Specifically, there was a significantly greater abundance of Fungi (e.g., *Lentinula* sp.) ($P < 0.05$), *Cyanobacteria* ($P < 0.001$) and

Gemmatimonadetes ($P < 0.05$) in 10%SC+AC compared to the 10%SC+IV. Microorganisms belonging to phylum *Bacteroidetes* ($P < 0.05$) and *Verrucomicrobia* ($P < 0.01$) were less abundant in 10%SC+AC than 10%SC+IV. Nevertheless, the effects of SC amendment on microbial community composition appeared dominant over those of plant species (Fig. 7-10).

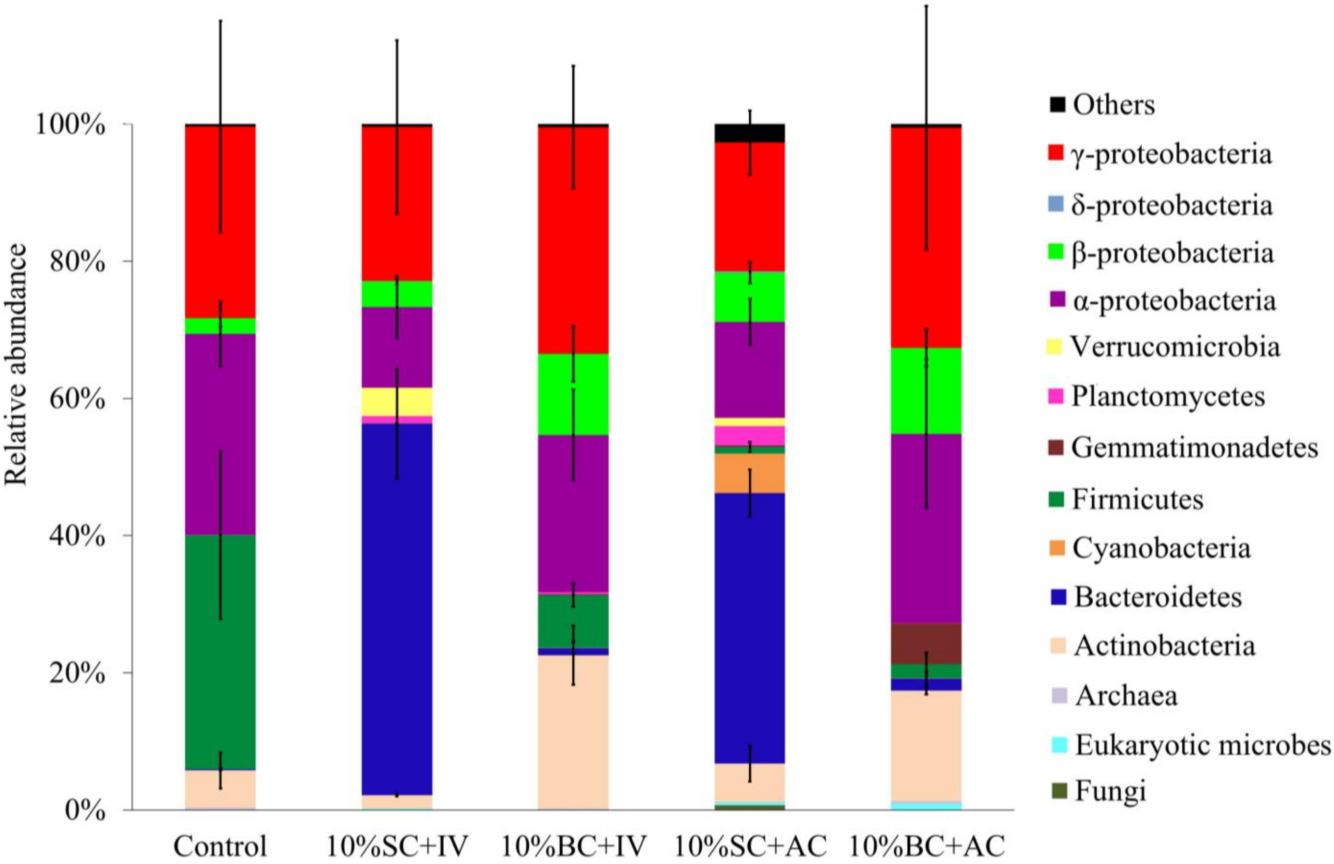

Fig. 7-8 Microbial community composition in the control and the sugarcane and biochar amended tailings with native plants. The bacterial phyla with a frequency less than 1 % are shown as 'others'; values are means (n = 3); error bars indicate standard deviations, drawn only for abundant phylum or classes of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria.*

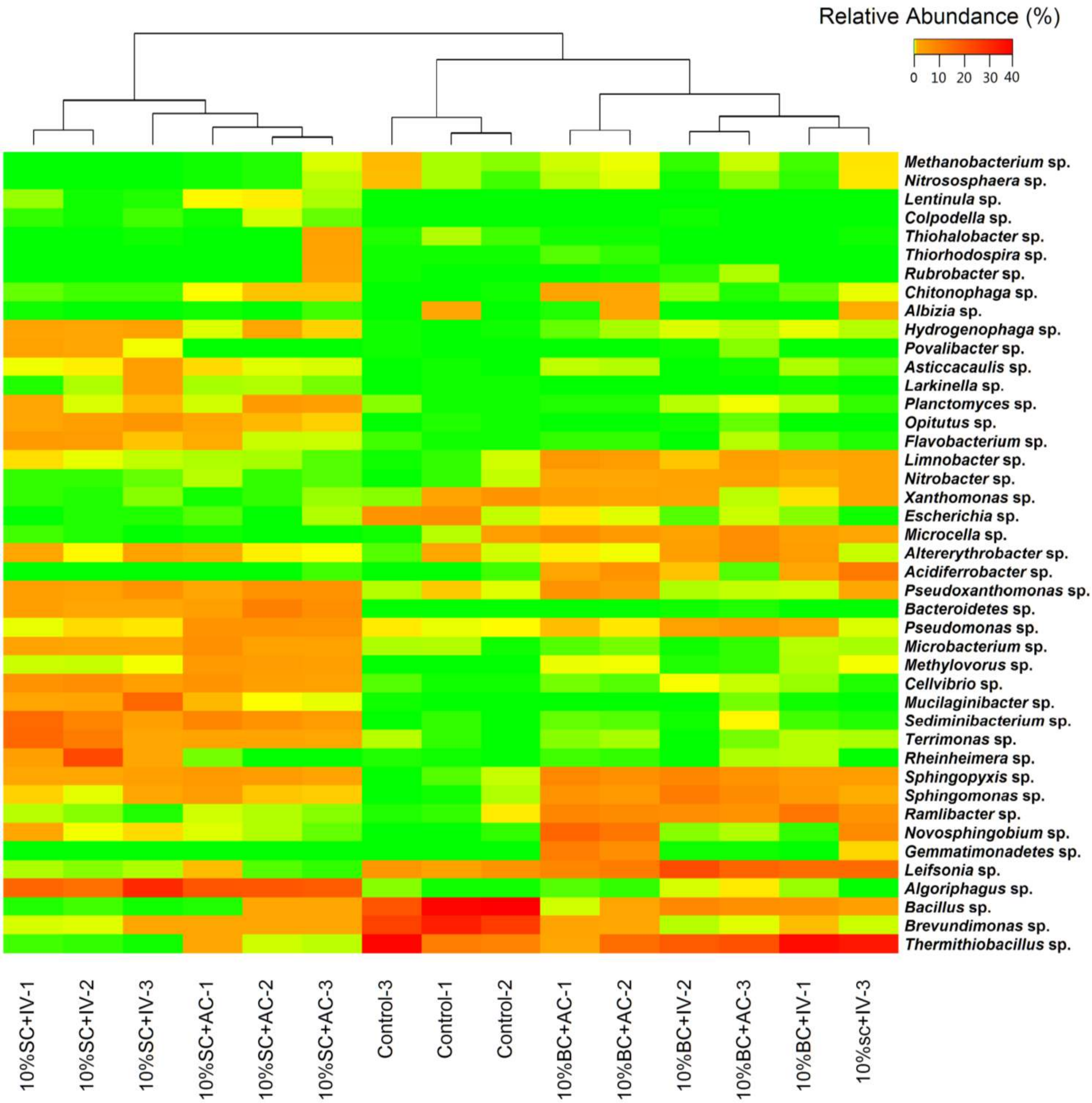

Fig. 7-9 Microbial community composition detected at the genus level in the control and the sugarcane and biochar amended tailings with native plants. The abundance of genera is indicated and cluster analysis of the community composition between the samples is shown. Only the OTUs with a frequency of greater than 1 % were shown in the figure.

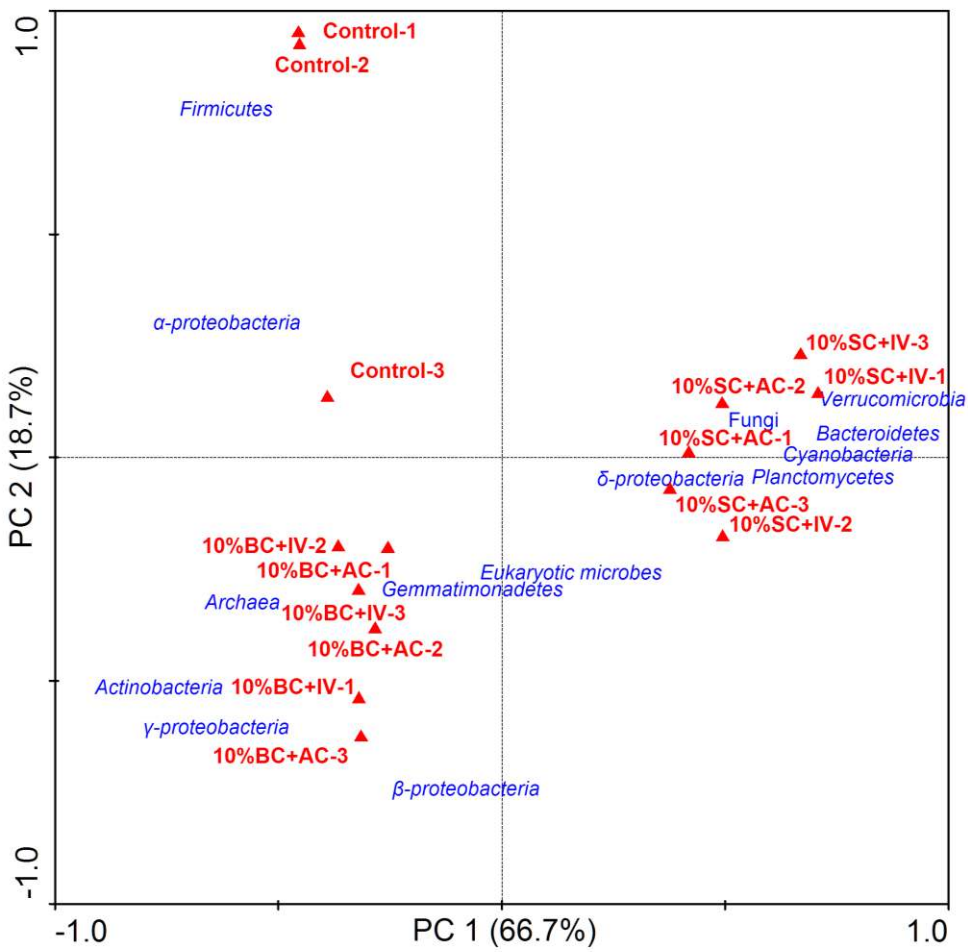

Fig. 7-10 Principal component analysis (PCA) of the microbial community composition detected in the control and the sugarcane and biochar amended tailings with native plants. Correlations of phylum and classes of *Proteobacteria* of microbial community (in blue) are made. The PCA axes differentiate the tailing samples according to their microbial community composition.

7.4 Discussion

The present findings showed that both SC and BC amendments in the Cu-Mo-Au tailings induced substantial changes of microbial community composition and enzymatic activities along different directions. The shift of the microbial community structure in the amended tailings can be explained largely by the environmental variables induced by the amendments and plant colonisation.

7.4.1 Modifications of physicochemical properties in the tailings

Organic amendments and plant colonisation had significant impacts on the physicochemical properties in the amended/revegetated tailings. With the addition of SC and BC followed by plant colonisation, we detected increased organic matter content (TOC) and WHC in all the tailings; this was observable, because organic matter had a lower bulk density and capable of retaining more water in comparison to minerals (Tester, 1990). The inputs of exogenous organic matter are particularly important to improve the physical structure and hydraulic properties in the tailings. In particular, interactions between organics and tailings mineral particles stimulate the aggregation processes in the amended tailings (Lenka et al., 2012) (also see Chapter 3 and 4). The development of various sizes of aggregates and pores are crucial allowing for water/nutrients retention and movement (Monreal and Kodama, 1997). In addition, organo-mineral interactions and aggregation processes may be limited in BC amended tailings because of low intensity of negative charges at the surfaces of high temperature biochar (Jiang, 2014; Yuan et al., 2014). The improvement of the physical structure and hydraulic properties in BC amended tailings are more likely attributed to BC-induced reduction of bulk density, which is expected to persist in tailings for a long term (Fellet et al., 2011).

The availability of energy and nutrients (TOC, TN and WSOC) in the amended tailings were significantly improved by the application of SC and introduction of these native plants. Comparatively, the 10%SC+AC treatment had higher levels of WSOC than the 10%SC+IV treatment, suggesting that AC roots might have also contributed more to the WSOC than IV. Soluble OC is an important fraction representing the most mobile and readily available form to microbes in soil, driving element cycling processes in soil ecosystems (Marschner and Kalbitz, 2003). Moreover, the soluble OC can also adsorbed onto clay minerals, contributing to OC stabilisation without further access to microbes and decomposition (Neff et al., 2000). Therefore, WSOC fraction is closely linked with several biogeochemical processes and soil formation in the amended tailings. SC application also induced increased levels of water-soluble metals, but within the tolerance capacity of these two native plant species. These increased levels of water-soluble heavy metals may be caused by rhizosphere acidification and/or further mineral weathering processes in the amended tailings (Li et al., 2013; Marabottini et al., 2013). In contrast, although BC did not increase labile OC and nutrients in the amended tailings, significantly lower levels of water-soluble Mn and Cu were observed in the BC amended tailings, compared to the control. The biochar used here had a metal adsorption capacity (Jiang, 2014), which may ameliorate the tailings by lowering heavy metal phytotoxicity to colonised microbes and plants (Fellet et al., 2014), in addition to other improvements.

7.4.2 Efficacy of rehabilitation of microbial diversity in the tailings

Clearly, tailings without improvement represent a harsh environment for both microbes and plants based on our pilot experiment and long-term field trials. We observed and measured low levels of biomass, microbial diversity and enzymatic activities in the tailings without amendments (the control). The colonising microorganisms in the tailings were highly bacteria dominant, with negligible fungi and other eukaryotes. Many strains of the dominant species *Bacillus* sp. and *Brevundimonas* sp. in these tailings were tolerant to heavy metals (Wakelin et al., 2010) and irradiation (Dartnell et al., 2010).

Substantial changes of microbial community composition were observed in all the amended/revegetated treatments. Specifically, the phylum of *Actinobacteria* and the class, *β-Proteobacteria* stimulated in the BC amended tailings compared to the control. At the genus level, *Thermithiobacillus* sp. and *Acidiferrobacter* sp. were abundant in the BC amended tailings, which were mainly chemolithoautotrophic bacteria catalysing S- and Fe-oxidising processes (Kelly and Wood, 2000). We assume that these microorganisms could facilitate mineral weathering processes, especially in tailings with abundant pyrites (Li and Huang, 2015).Consistent with the findings in Chapter 5, the interesting observation of stimulated autotrophic S- and Fe- oxidising bacteria in the BC amended tailings should be investigated further in relation to the stimulation of mineral weathering and further consolidation of hydrogeochemical stabilisation in the sulphidic tailings.

In comparison, microbial communities in the SC amended tailings were dominated by heterotrophic bacteria (e.g., *Sphingopyxis* sp., *Pseudoxanthomonas* sp., *Gemmatimondetes* sp., and *Sediminibacterium* sp.). These species are mostly found in soil and contribute to organic matter decomposition and nutrient cycling processes (Cayford et al., 2012; Yamatsu et al., 2006). As a result, organic matter with high contents of labile OC (such as sugarcane residues) is preferable for priming and stimulating rehabilitation of biogeochemical properties and functions in the tailings along the direction of functional technosols formation.

Fungi play an important role in phytostabilisation of base metal mine tailings due to its unique roles in infertile and arid terrestrial ecosystems, through improving nutrient cycling and supply and soil structure (Ma et al., 2006; Solís-Domínguez et al., 2011). In the present study, the most abundant fungi, *Lentinula* sp. was found in the amended/revegetated tailings, which could facilitate lignin degradation in the N limiting environment (Boyle, 1998). There was also a positive correlation between plant biomass (sum of shoot and roots) and eukaryotic microorganisms ($R^2 = 0.66$, $P < 0.05$, n = 12) in this study. However, both diversity and abundance of eukaryotic microorganisms (0.05-1 %) in the present study were less than 10 % of those microbial communities in natural forest soils (Bailly et al., 2007) and native soils under plant cover in Mount Isa area (refer to Appendix B). Since the universal primers 926F and 1392R are 16S rRNA gene group specific primers targeting bacterial and archaea, they do not allow completely covering the 18S rRNA gene in eukarya (Engelbrekston et al., 2010) with SILVA database for eukaryotic amplicons (Pruesse et al., 2007). The quantification of fungal community is underestimated and require further investigation using fungi-targeting primers. A factor limiting the abundance of fungi in the amended tailings may be the lack of fungal inoculum. Inoculation of topsoil from native vegetation sites (Chapter 6) may provide large amounts of fungal inoculum for rapid rehabilitation of diverse and functional fungal communities in the amended tailings (Li et al., 2015; Orłowska et al., 2005).

Different microbial community composition in the amended tailings was closely linked to amendment induced changes of physicochemical factors (Fig. 7-11). Changes in physicochemical properties (e.g., pH, EC, total heavy metals) in the amended/revegetated tailings collectively drove the shift of microbial community composition and functions towards different trajectories. The abundance of dominant phyla in the control, *Firmicutes,* and the class, *α-Proteobacteria,* was positively related to heavy metal contents (e.g., Cu, As) in the tailings. Seemingly, forms of organic compounds and nutrient contents in SC and BC represented contrasting cases in the gradient of organic matter quality, in terms of nutrient (e.g., N and P) and labile OC (e.g., carbohydrates) contents. The labile OC and nutrients from AC could be utilised by the heterotrophic microorganisms in tailings, which in turn have stimulated the organic matter decomposition and nutrient cycling processes. Consistent with previous studies (Chapter 5 and 6), the abundance of *Bacteroidetes* again increased in the SC amended tailings compared to the control, which were positively related to nutrient conditions (e.g., WSOC, TN). The lack of labile OC and nutrients in the BC amended tailings constrained the development of organic matter decomposers. Increased abundance of phylum *Actinobacteria*, and the class, *β-Proteobacteria*, were mainly ascribed by the changed TOC in the BC amended tailings.

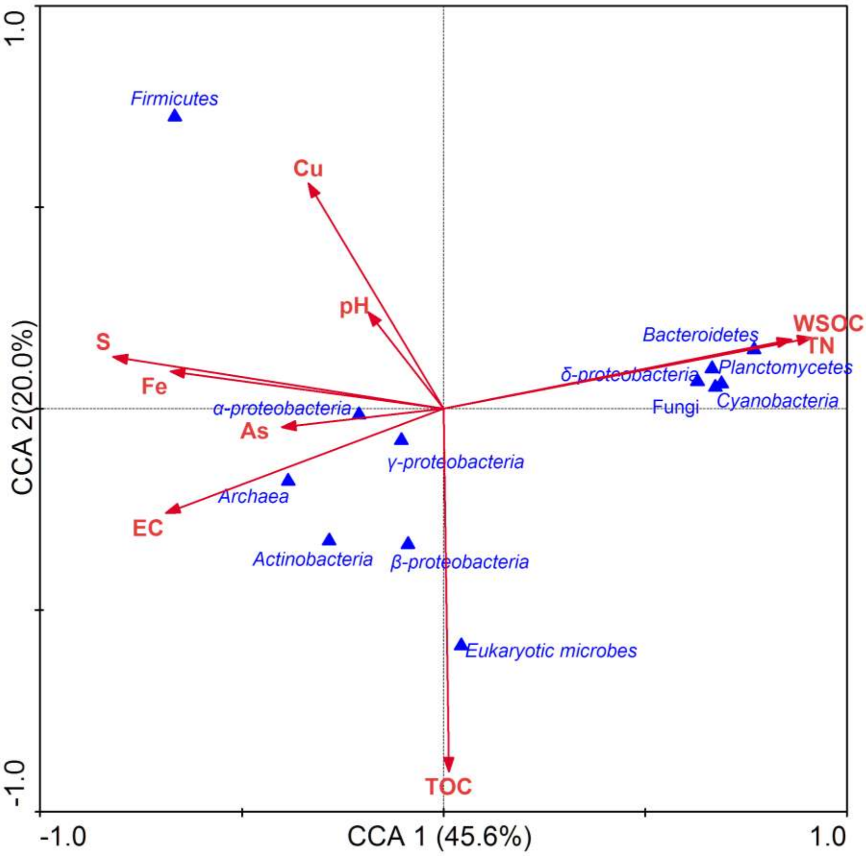

Fig. 7-11 Canonical correspondence analysis (CCA) of class abundance vs. environmental variables in the 15 samples including the control and the sugarcane and biochar amended tailings with native plants.

7.4.3 Growth and element uptake of pioneer native plants

Organic amendments such as SC and BC applied in the present study are pre-requisites to overcome the primary constraints in the tailings for establishment and growth of the introduced pioneer plants. In addition to nutrition, the addition of organic amendments improved the physical structure and hydraulic properties in the amended tailings, benefiting plant root penetration and growth (Ye et al., 2002). Furthermore, the success of these two native plants in the amended tailings implied that they are tolerant of nutrient deficiency (e.g., N), salinity and heavy metals. Particularly, compared to the native grass species, *Iseilema vaginiflorum* (IV), *Acacia chisolmii* (AC) had a much higher growth rate after transplanting, and with relatively higher biomass and N concentrations in their shoots. This is possibly because AC is a leguminous specie more tolerant of the rooting environment and is able to obtain a better N supply via N_2-fixation (Meeks, 1998). Therefore, *Acacia chisolmii* is a good candidate pioneer specie in colonising newly improved tailings and stimulate biogeochemical rehabilitation toward functional technosols.

The SC and BC amendments also resulted in different impacts on heavy metal mobility and plant uptake in this study. Particularly, the levels of water-soluble Cu increased in the tailings receiving SC, but decreased significantly in the BC amended tailings compared to those in the control. The levels of water-soluble Cu in the tailings were closely correlated with the Cu uptake by the plants, particularly in the plant roots (R^2 = 0.66, P < 0.05, n = 12). Impacts of BC on Cu immobilisation may be attributed to its relative high surface pH and the presence of some functional groups interact with metal ions (Uchimiya et al., 2011). The BC used was pyrolysed at a high temperature (650 °C) with most carboxylic and phenolic groups remove (Jiang, 2014). As biochar pyrolysed at low-moderate temperature (< 400 °C) would have much higher capacity of metal adsorption (Keiluweit et al., 2010), this type of biochar may be explored for maximising the effects of BC adsorption of heavy metals in pore water and thus lowering the severity of heavy metal toxicity in plants.

The levels of Cu in the shoot (3-12 mg kg^{-1}) and root (163-541 mg kg^{-1}) tissues of the grass and acacia species were comparable with those of plant species grown in Cu tailings reported in the literature (Chen et al., 2007; Chiu et al., 2006; Song et al., 2004) (Table 7-2). In general, Cu uptake by these two plant species was as low as 0.2 mg pot^{-1}, representing less than 0.01 % of the total Cu in the tailings. These two native plant species from Cloncurry-Mount Isa region were tolerant of the levels of total Cu in the tailings, with Cu levels in the shoot below the toxicity threshold reported for crop species (20-30 mg kg^{-1}) (Mendez and Maier, 2008). As a result, both native plant species would be a good pioneer species for providing quick plant cover, while improving the rhizosphere conditions and stimulating technosols formation from the fresh and neutral tailings containing relatively low contents of reactive minerals and stable hydrogeochemistry.

Table 7-2 Cu concentrations in plant tissues grown in Cu tailings in the literature.

Plant species	Cu concentrations in plant tissues (mg kg^{-1})		Reference
	Shoot	Root	
P. vittata,	40-160	700-800	Chen et al. 2007
T. repens	20-60	350-400	Chen et al. 2007
C. drummondii	20-120	180-200	Chen et al. 2007
L. perenne.	20-30	420-500	Chen et al. 2007
V. zizanioides	9-20	124-384	Chiu et al. 2006
P. australis	14-39	211-539	Chiu et al. 2006
S. vulgaris	3-262	33-2882	Song et al. 2004
E. splendens	4-215	120-6450	Song et al. 2004

7.5 Conclusions

Application of sugarcane and biochar induced the physicochemical properties in the tailings and caused substantial changes of microbial community composition and functions in different directions, compared to the control. Amendments with labile OC (such as sugarcane) coupled with the introduction of pioneer plant species significantly stimulated the development of microbial communities with a greater diversity and increased dominance of heterotrophic bacterial groups, with much improved microbial functions in the amended tailings. This is in contrast to the effects of biochar amendment, which enhanced the microbial diversity while with dominance of chemolithoautotrophic S- and Fe- oxidising bacteria, might further consolidating hydrogeochemical stabilisation in the sulphidic tailings. Pioneer plant species of *Iseilema vaginiflorum* and *Acacia chisolmii* contributed to the biogeochemical rehabilitation of examined tailings to some extent less important to organic amendments. Overall, combined use of labile organic amendment and pioneer plant species would be an effective approach to initiate and accelerate the biogeochemical rehabilitation in the fresh and neutral Cu-Mo-Au tailings with low levels of reactive minerals (such as sulphides) and a high degree of hydrogeochemical stability.

Chapter 8 General discussion, conclusions and future research needs

8.1 General discussion

The vast volumes of tailings from base metal (e.g., Cu, Pb, Zn) mining industry present serious threats to environmental and human health. They consist primarily of the ground-up gangue with its inherited mineralogy profile causing long-term ecotoxicity (Lottermoser, 2010). Stabilising mine tailings with native plant communities (phytostabilisation) helps reduce pollutant transportation through wind and/or water pathways. Furthermore, metals may be sequestered, precipitated or taken up by and stored in plants (Mendez and Maier, 2007). A lot of researches have focused on techniques for immediate vegetation establishment, concentrating on plant species selection for the extreme biogeochemical conditions in tailings. However, very few studies have addressed the fundamental requirements for a natural recruitment of self-sustaining plant community. Until now, plant establishment and life cycle have largely failed in the hostile tailings environments (Hayes et al., 2009). Even when tolerant plant species were established successfully, diversity was limited and the low functional ecosystem would remain unchanged for decades or centuries (Shu et al., 2002). As soil is defined as a natural medium for the growth of land plants, many authors have argued that a shift from plants establishment to soil development should be the focus for tailings phytostabilisation (Bradshaw, 1997; Scalenghe and Ferraris, 2009).

Natural soil formation is the consequences of alteration and transformation of novel parent minerals following long-term abiotic and biotic interactions. They are normally assemblages of unsolidated minerals and organic matter on the Earth's surface experiencing major developmental phases (e.g., bedrock weathering, organic matter accumulation, leaching, clay movement) and becoming relatively stable (Jenny, 1941). Soil formation in tailings commences as finely ground novel parent minerals with a relative high proportion of reactive minerals (e.g., pyrite, chalcopyrite). These are continuously oxidised when being exposed to biotic (autotrophic bacteria) and abiotic water and oxygen (Li and

Huang, 2015). Therefore, engineered pedogenesis from geochemical unstable tailings to technosols require rapid simulation of deterministic physical, geochemical and biological processes that would enhance hydrogeochemical stability, organic matter accumulation, and rehabilitation of microbial community structure and biogeochemical functions, with much improved physicochemical conditions and much reduced ecotoxicity (Uzarowicz and Skiba, 2011). A conceptual pathway of pedogenesis from geochemically unstable tailings to technosols of primary structure and biogeochemical functions, may undergo 3 stages of transition before plants can tolerate and proliferate in the medium (Fig. 8-1).

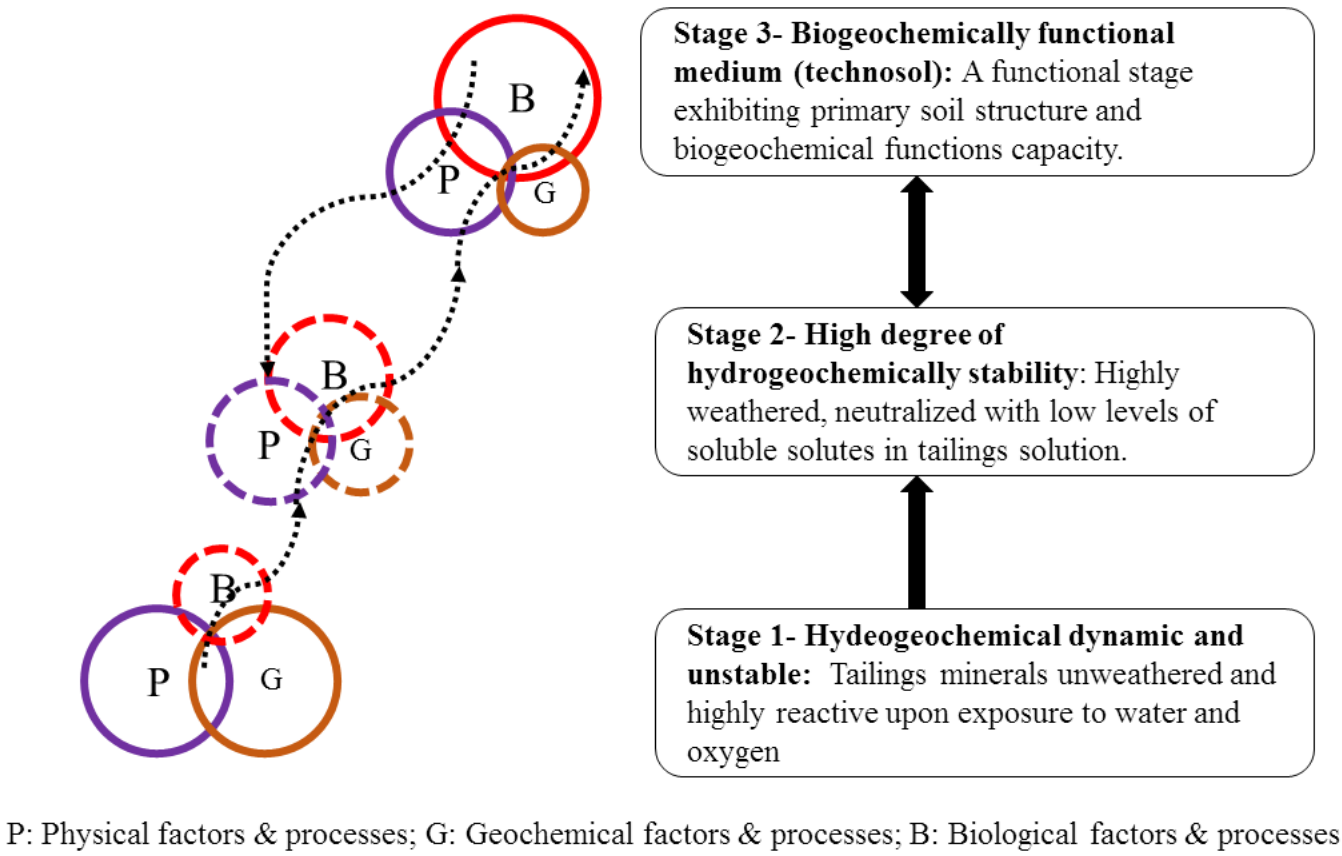

Fig. 8-1 A conceptual pathway of pedogenesis from reactive tailings to technosols of primary structure and biogeochemical functions. Rings with dash line suggest less understood factors and processes in each stage; ring sizes suggest the relative importance of the factors and processes in the corresponding stage, reproduced from Huang et al. (2014).

The present study is the first comprehensive evaluation of technosols formation in Cu-Pb-Zn tailings in response to ecological engineering techniques. The key processes involved in engineered pedogenesis in the tailings include: (1) enhance mineral weathering towards a high degree of geochemical stability without rapid acidification, mineral dissolution and acute phytotoxicity; (2) physical structure and hydraulic properties improvement either from aggregation or directly incorporated materials with lower density and greater capacity to retain water than ground minerals; (3) recolonisation and proliferation of diverse microbial communities driving biogeochemical processes and functions, consistent with physiological requirements (e.g., nutrient and growth requirements) of target native plant species.

While recognising that significant technosols formation from tailings can only occur after extensive weathering of sulphidic minerals and other reactive minerals in the tailings, this study focused on the 2nd phase of rehabilitating biogeochemical properties and functions by means of effective ecological engineering inputs in neutral tailings with relatively stable hydrogeochemistry (Fig. 8-1). The present findings have provided the basis for an integrative ecological engineering strategy consisting of suitable organic amendments, topsoil inoculum and/or pioneer plant introduction, which stimulate biogeochemical changes along the desired directions towards functional technosols from Cu-Pb-Zn tailings.

Soils from native plant communities consisting of *Acacia chisolmii* and Spinifex grasses (*Triodia* spp.) (Appendix B) were characterised by relative low levels of soil organic carbon (6.03-14.17 g kg^{-1}), microbial biomass (104.3-219.2 mg kg^{-1}) and enzymatic activities, yet they could sustain the growth and recruitment of these native plant species, which are dominant in the infertile and dry landscapes of northwest Queensland (Appendix C). This native soil information sets the direction of technosols formation in the tailings and justify the choice of plant biomass-based organic amendments (e.g., woodchips, acacia biomass and sugarcane) (rather than N and P rich amendments, such as biosolids, manure or fertiliser), because of unique physiological traits of these native plant species (slow growth rates, low water and nutrient requirements and sensitive to high levels of nutrients supply).

Inputs of exogenous organic matter such as woodchips from local trees actively stimulated aggregation with improved physical structure and hydraulic properties in the tailings and elevated organic carbon (OC) in both weathered and fresh Cu-Pb-Zn tailings. Plant community colonisation further contributed to OC stabilisation in the vegetated tailings (Chapter 4). Majority of OC (61.5-80.3 %) with N enriched was stabilised in the physically protected microaggregates and organo-mineral complexes in the amended/revegetated tailings (Chapter 3 and 4). In particular, compared to woodchips, contribution of labile OC and N from plant roots colonising the tailings stimulated microbial biomass and functions in the revegetated tailings significantly.

Based on these findings, a conceptual diagram has been proposed to illustrate (Fig. 8-2) critical factors and processes in relation to OC stabilisation in tailings regulated by ecological engineering inputs. The level of OC is widely recognised as an overall indicator of soil quality (Bendfeldt et al., 2001) that drive the associated changes of biogeochemical properties and functions in the tailings, and the formation of resultant technosols. N containing and negative charged organic compounds from exogenous organic matter and/or plants before or after modification by microbial colonisers are main source of OC to be stabilised in tailings, contributing significantly to physical structure (organo-mineral interactions, aggregation) and biogeochemical functions (decomposition and element cycling) recovery. As microbial community to be with low biomass, diversity and activities adapted to the stressed habitat conditions in the tailings (Li et al., 2014; Mendez et al., 2008) with colonised plant community with low productivity and diversity in the tailings (Ye et al., 2000, 2002; Archer and Caldwell, 2004), input of N rich and negative charged organic compounds (e.g., microbial biomass, microbial by-products, plant root exudates etc.) is low to anticipate in organo-mineral interactions and aggregation processes in the tailings (Plaza et al., 2013). The instinct properties of tailings directly inherited from ore mineralogy, mineral processing and deposition period (Lottermoser, 2010) influenced the capacity and efficiency of OC stabilisation by ecological engineering inputs. Tailings type determined the binding mineral agents and surface area where organo-mineral interactions occur (Yuan et al., 2014).

It is possible to stimulate OC stabilisation via introduction of pioneer plants and direct application of stable OC or plant-biomass based organic amendments with relative relatively greater N content and lower C: N ratio.

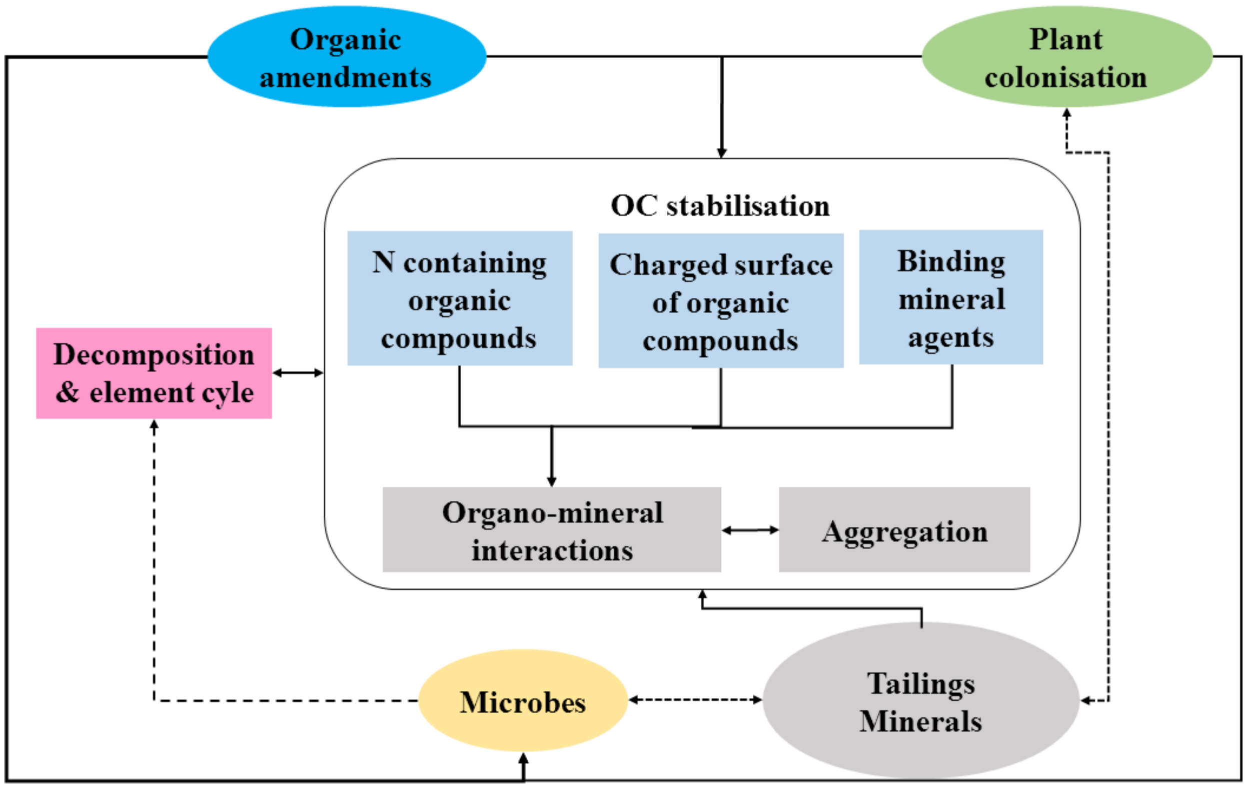

Fig. 8-2 A Conceptual diagram that OC stabilisation in tailings in response to organic amendments and/or introduction of pioneer plants (dash line suggested processes to be investigated).

Functional microbial communities of increased diversity in the amended tailings can be successfully rehabilitated with the addition of plant-biomass based organic amendments and/or soil with/without introducing pioneer plants. Organic amendments with contrasting properties (labile OC content, N contents) induce biogeochemical changes in the amended tailings along different directions. Microbial community composition and relative dominance of autotrophic and heterotrophic bacteria were also substantially shifted by the organic amendments, inoculation of topsoil, and introduction of pioneer plants in response to the induced changes in the physicochemical properties in the amended tailings (e.g., WSOC, available N, EC, total and water-soluble heavy metals, aggregates, WHC etc.) (Fig. 8-3).

Generally, both plant biomass forms (e.g., sugarcane and acacia biomass) and recalcitrant biochar application contribute to physical structure stability with more water retained, when incorporating exogenous materials into the neutral Cu-Pb-Zn (Chapter 5 and 6) and Cu-Mo-Au tailings (Chapter 7).

Mineral weathering occurred continuously in the tailings abundant in primary mineral (Forsyth, 2014; Li et al., 2013). In the present study, both plant biomass and recalcitrant biochar application seemed to have potential to accelerate mineral weathering processes and hydrogeochemical stabilisation in the amended tailings. The positive impacts of plant biomass on mineral weathering and dissolution were observed in all the amended tailings with significantly increased soluble salts and heavy metals. This might be due to the overall stimulated microbial biomass and activities as indicated by elevated enzymatic activities in response to available OC and nutrients. Some heterotrophic bacteria can also catalyse S-oxidation in sulphidic tailings (Ňancucheo and Johnson, 2011). Recalcitrant biochar stimulated the abundance of S- and Fe oxidising bacteria in the amended tailings (Chapter 5 and 7), although associated functional consequences were not yet confirmed.

Plant biomass based amendments in the amended tailings increased the labile OC and nutrients which favoured the development of potential heterotrophic bacteria (Chapter 5, 6 and 7). When nutrients were not the limiting factor, microbial diversity in tailings was more related to the levels of EC and total and soluble heavy metals (Chapter 5 and 6). Plant colonisation further increased microbial community diversity and heterotrophic bacteria in the revegetated Cu-Mo-Au tailings (Chapter 7) via root-microbe interactions (Lilia et al., 2013). As organic matter decomposition and nutrients cycling processes were mainly driving by heterotrophic microorganisms, these biogeochemical processes greatly increased in these amended/revegetated tailings, which is critical to the rehabilitation the tailings towards functional technosols for tailings phytostabilisation. Furthermore, nutrient cycling from organic matter decomposition in the amended tailings are also important to sustain the growth of the introduced pioneer tolerant native plant species (Kemmitt et al., 2008; also refer to Chapter 7).

In contrast, the stable C, biochar with little labile OC and nutrients is less effective in rehabilitating heterotrophic bacterial communities and associated biogeochemical processes. On the other hand, its application increased the relative abundance of S- and Fe- oxidising bacteria in the amended tailings(Chapter 5 and 7). This suggests that biochar application may accelerate microbial mediated oxidation of sulphide minerals in fresh tailings, containing high levels of sulphidic minerals, thus accelerating the weathering process and hydrogeochemical stabilisation. The exact mechanisms involved in BC stimulated abundance of S- and Fe- oxidising bacteria are yet to be understood.

In general, the bacterial community is more readily rehabilitated than the fungal community in the amended/revegetated tailings (Chapter 5 and 7). Inoculation of topsoil from native plant communities may fast-track the colonisation and recovery of the fungal community by providing inoculum. Addition of up to 50 % local soil increased the microbial diversity and enzyme activities for C and N turnover with a respiratory quotient similar to that of the local soil. In addition, fungi and protists can be stimulated in tailings-soil mix with the addition of plant biomass based amendment. Combining the addition of plant biomass with native soil inoculation may be used to fast-track the establishment of native microbial communities and initiate the biogeochemical functional technosols formation in tailings (Chapter 6).

Induced change of physicochemical properties in amended tailings following organic amendments enhanced the survival and growth rates of native plants. The examined native plant species *Iseilema vaginiflorum* and *Acacia chisolmii* could grow in neutral Cu-Mo-Au tailings (low in sulphide minerals and relatively stable in hydrogeochemistry) recieving organic amendments, especially in those amended with plant biomass. Overall, both native plant species showed promising potential for Cu tailings phytostabilisation with preference to accumulate Cu in plant roots rather than shoots (Chapter 7).

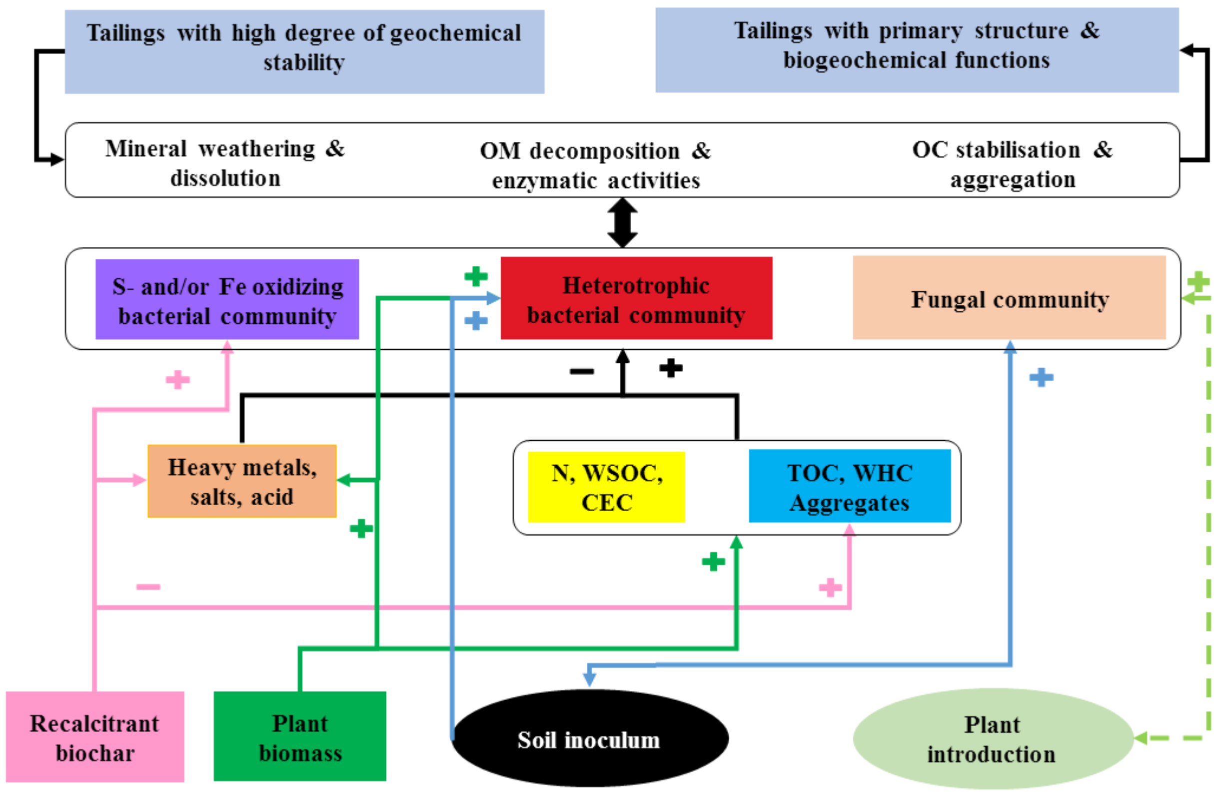

Fig. 8-3 A conceptual diagram illustrating the key biogeochemical processes and driving environmental factors affecting technosol formation in the tailings. '+' / '-' indicate amendments/key environmental variables that have positive / negative impacts (dash line suggested relationship to be investigated).

8.2 Conclusions

Based on the findings reported in this study, we can conclude the following:

(1) Plant biomass based organic amendments rapidly increased the amount of stabilised OC in Cu-Pb-Zn tailings with majority of OC either physically protected through aggregation or in organo-mineral complexes in the amended tailings. OC stabilised in the tailings was enriched with N; Fe and Al (hydr-) oxides in the tailings contributed significantly to OC stabilised in the mineral associated fractions. Plant colonisation stimulated microbial community and functions with greater N, further enhanced OC

stabilisation through aggregation and organo-mineral interactions in the revegetated tailings.

(2) In the neutral weathered Cu-Pb-Zn tailings or fresh Cu-Mo-Au tailings, microbial community composition can be shifted along different directions by organic amendments with different availability of labile OC and nutrient contents. Plant biomass based organic amendment with relatively greater levels of labile OC and nutrients favoured the development of potential heterotrophic bacteria in the amended tailings, and in turn, facilitated organic matter decomposition and nutrient cycling processes in contrast to recalcitrant biochar.

(3) Microbial communities in the tailings-soil mix with plant biomass based organic amendment were substantially changed with opportunistic development of fungi strongly associated with the microbial biomass and selected enzymatic activities. A combination of plant biomass based organic amendment and native soil inoculation could be used to fast-track the establishment of native microbial communities and initiate biogeochemical processes to rehabilitate amended tailings towards functional technosols.

(4) Organic amendments effectively improved the physicochemical properties in the fresh and neutral Cu-Mo-Au tailings with low contents of sulphide minerals, facilitating tolerant native plant species survival and growth. Microbial community composition in the amended/revegetated tailings substantially shifted with greater diversity of heterotrophic bacteria and fungi compared to the control. Plant biomass amended tailings were abundant with potential heterotrophic bacteria with stimulated organic matter decomposition and nutrient cycling sustaining native plant growth with greater biomass compared to the recalcitrant biochar. The positive impact of biochar on heavy metal immobilisation in the amended tailings may be beneficial in terms of reduced heavy metal concentrations in the pore water and thus lower plant uptake of heavy metals.

8.3 Future research needs

For the future research needs, the following recommendations are made:

(1) Simulating biological weathering processes in sulphidic tailings for rapid stabilisation of hydrogeochemistry

This study demonstrated that high-temperature biochar had the potential to stimulate the abundance of Fe- and S-oxidising bacteria in the amended tailings. Further investigation should determine if physiological functions of these autotrophic bacteria could be intensified to accelerate the weathering of reactive minerals (e.g., pyrites) in tailings.

(2) Relationship between changes of microbial community composition and biogeochemical functions

Further studies are required to relate the microbial community changes to physiological functions of enhanced microbes, in relation to biogeochemical processes, such as *in situ* litter decomposition and nutrient cycling. The information will enrich the model of engineered pedogenesis in base metal mine tailings and long-term direction of technosols development under local climatic conditions, in response to purposely designated ecological engineering options in the short-term.

Chapter 9 Reference

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Appendix A Quantitative measurement of organic carbon in mine wastes: Methods comparison for inorganic carbon removal and organic carbon recovery

A.1 Introduction

Quantitative measurement of organic carbon (OC) is often required for site characterisation and ecological assessment of plant communities revegetated in mined land, which may contain mine wastes (such as tailings) rich in primary and secondary minerals of inorganic carbon (IC) such as carbonates (calcite, dolomites, etc.), chlorides and metals (ferrous Fe and Mn oxides) (Guilbert and Park, 1986). Tailings are residue wastes from processing ores [e.g., rock ores for metals gold (Au), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), uranium (U)] and industrial minerals (e.g., bauxite, coal), which contain unstable primary and secondary minerals rich in metals and metalloids [e.g., aluminium (Al), arsenic (As), Au, chromium (Cr), Cu, Ni, Pb, Zn, and U], salts, and unwanted gangue minerals (e.g., silicates, carbonates, oxides/hydroxides, sulphides, etc.) (Lottermoser, 2010). Although conventional wet-oxidation OC measurement techniques such as those of Walkley and Black (1934) and Heanes (1984) are not affected by the presence of carbonates, other minerals [such as chlorides, ferrous iron (Fe) and manganese (Mn) oxides] interfere with the wet-oxidation process and colour development (Nelson and Sommers, 1982). Elevated amounts of calcite and dolomite are common carbonate minerals present in mine tailings and show similar effects to carbonate soils on OC determination (Guilbert and Park, 1986). Additionally, OC concentrations in mine wastes is very low initially but it can be increased by organic amendment and plant litter inputs from established vegetation. For example, OC content in mine wastes increased from 3 to 50 g kg^{-1} when mined land was rehabilitated by vegetation for a number of years (Rodríguez et al., 2009).

Dry combustion methods, in which carbon dioxide (CO_2) evolved from combustion of both organic matter and carbonate is measured by gas chromatography or infrared analysis, provide a direct measurement of total C (Tiessen et al., 1981). Methods based on the dry-combustion technique for determination of OC in carbonate-rich soils must consider either

(1) IC subtraction, in which OC is estimated as the difference between total C measured directly by dry combustion and inorganic C measured as CO_2 evolution upon acid treatment of a replicate sample such as in volumetric calcimeter; or (2) direct OC quantification by dry combustion in the same sample after IC removal using acid pretreatment.

In the first method, one of duplicate subsamples of a mine waste is treated by hydrochloric acid (HCl) of appropriate concentration in a sealed container to hydrolyse carbonate minerals and release IC as CO_2, which is subsequently quantified by gravimetric (Sherrod et al., 2002), titrimetric (Bundy and Bremner, 1972), volumetric (Wagner et al., 1998), spectrophotometric, or gas chromatographic techniques. The other replicate subsample is directly analysed for total C content by dry-combustion method. However, this method is not recommended for highly carbonate-rich soil samples (e.g., > 59 %) with low OC levels (Schmidt et al., 2012), due to the large errors brought about by two separate analyses. In the direct OC analysis method, a sample is at first thoroughly pretreated with acids, such as dilute HCl (Connin et al., 1997), a mixture of dilute sulphuric acid (H_2SO_4) and ferroferric oxide (Fe_3O_4) (Nelson and Sommers, 1982), hydrofluoric acid (HF) (Rumpel et al., 2006), sulphurous acid (H_2SO_3) (Schmidt et al., 2012), metaphosphoric acid (HPO_3) (Midwood and Boutton, 1998), or HCl vapour (Harris et al., 2001), to hydrolyse carbonate minerals into CO_2 without the need for precise IC quantification, prior to dry-combustion analysis of the C remaining in the same sample. The risks of acid pretreatment (acid wash or fumigation) are the incomplete reaction with (or dissolution of) carbonates, a rapid hydrolysis of acid-susceptible organic molecules (e.g., small proteins and water soluble carbohydrates), or underestimation of volatile OC compounds (Hewitt, 1998). Currently, detailed evaluation of these two methods has not yet been done for quantification of OC in mine wastes such as base metal mine tailings.

OC is an integral indicator of organic matter decomposition and soil quality, which is useful for assessing the development of a soil-like matrix composed of mine wastes and amending materials and the changes of revegetated land after mining. High levels of IC and metal elements and/or very low levels of OC are commonly present in mine wastes, hindering the direct adoption of routine soil OC analytical methods. As a result, it is necessary to develop or modify OC quantification method(s) in mine wastes and mined land under revegetation. The primary aims of the present study are to evaluate two different methods of OC quantification in mine wastes based on dry combustion technique: (1) IC subtraction and (2) direct OC determination after acid pretreatment, in terms of recovery and precision. The performance of these two methods for analysis of OC in mine wastes is evaluated in three aspects: (1) the recovery rate of inorganic and organic materials added to mine waste samples; (2) the lower and upper limits of IC/OC quantification; and (3) a comparison between the two methods in the analyses of OC in base metal mine tailings.

A.2 Materials and methods

A.2.1 Carbonate and organic materials

A range of typical carbonate and organic materials were quantitatively added into mine waste samples to test their recoveries using both methods (Table A-1).

Table A-1 Total carbon (TC) content in carbonate and organic materials

Carbonate material	Formula	C content ($g\ kg^{-1}$)	Organic material	Formula	C content ($g\ kg^{-1}$)
Calcium carbonate	$CaCO_3$	116.6	Sucrose	$C_{12}H_{22}O_{11}$	420.7
Magnesium carbonate basic	$MgCO_3$	121.2	EDTA	$C_{10}H_{16}N_2O_8$	411.2
Calcite[a]	$CaCO_3$	85.5	Cellulose	$(C_6H_{10}O_5)_n$	403.0
Magnesite [a]	$MgCO_3$	133.4	Litter	na	398.8
Dolomite [a]	$(CaMg)(CO_3)_2$	100.2	Charcoal	C	947.4

a: major chemical composition in carbonate minerals; na: not available. Values are means (n = 3).

The carbonate compounds and minerals used to spike the samples were calcium carbonate ($CaCO_3$, Sigma-Aldrich, DE), magnesium carbonate ($MgCO_3$, Sigma, AU), and carbonate mineral powder (calcite, magnesite, dolomite) (general grade, Pottery Supplies, Brisbane, AU). Organic C materials used to spike the samples were sucrose ($C_{12}H_{22}O_{11}$, Sigma chemicals, AU), ethylenediaminetetraacetic acid (EDTA) ($C_{10}H_{16}N_2O_8$, Sigma-Aldrich, AU), cellulose microcrystalline (Alfa Aesar, UK), litter (general grade, acacia leaf and twig litter, finely ground < 0.5 mm, AU), and charcoal (C, Sigma-Aldrich, USA). The C contents in these carbonate and organic materials were determined by the dry-combustion method for total C determination (LECO Corporation, St Joseph, MI) (Table A-1).

A.2.2 Properties of mine wastes

7 samples of base metal mine tailings were collected from a field revegetation trial established in 2010 at a tailings storage facility. There are 3 types of base metal mine tailings, which are highly weathered Cu-Pb-Zn tailings, freshly deposited Cu-Pb-Zn tailings and Cu-Mo-Au tailings. The general properties of tailings samples are summarised in Table A-2. All the samples were collected from top 10 cm layer in each treatment. The samples were dried at 40 °C in an air-drafted oven until constant weights. Subsamples representative of the bulk samples were further ground to pass through a 0.5 mm sieve for OC analyses by the two methods described below.

A.2.3 Dry combustion for total carbon quantification

A CNS-2000 automated analyser (Leco Corporation, St Joseph, MI) was used to determine C content in the samples by dry combustion. Aliquotes (0.2–0.3 g) of standard materials or tailings samples were accurately weighed to a preweighted tin foil sheet. Samples and standards were combusted on a TRUSPEC instrument at 1350 °C and evolved CO_2 was measured using an infrared detector cell. The EDTA calibrations, blanks, and ASPAC quality control (QC) check samples were included for quality control. Each sample was analysed in triplicate.

Table A-2 Properties of mine waste samples collected from field trials located in Mount Isa, northwestern Queensland, Australia

Tailings Sample No	Mine	Tailings type	Treatment	Vegetation	pH[a]	Mineralogy
1	Cu-Pb-Zn	Fresh	Pure tailings	No vegetation	6.9	Quartz: 20-60 % Dolomite/ calcite/ankerite: 18-25 % Pyrite: 6-35 % Chlorite and feldspar: 10-5 % Other < 5 %
2	Cu-Pb-Zn	Fresh	Amended with plant biomass	No vegetation	6.5	
3	Cu-Pb-Zn	Highly weathered	Pure tailings	No vegetation	6.7	
4	Cu-Pb-Zn	Highly weathered	Amended with plant biomass	No vegetation	6.7	
5	Cu-Pb-Zn	Highly weathered	Amended with plant biomass	vegetation cover	6.5	
6	Cu-Mo-Au	Fresh	Pure tailings	No vegetation	7.4	
7	Cu-Mo-Au	Fresh	Pure tailings	vegetation cover	7.2	

a: In1:5 sample/water extract followed pH electrode (TPS 900-P).

A.2.4 IC quantification for IC subtraction method

For the IC subtraction method, TC was done by the dry combustion method. Recovery test was performed for TC quantification using the dry-combustion technique according to ISO 19446 (ISO, 1995).

The IC (carbonate standard materials) recovery test was conducted for IC quantification procedure. In this test, an appropriate amount of standard IC (calcium carbonate, $CaCO_3$) and OC (sucrose, $C_6H_{12}O_6$) were spiked into 1.0000 g ± 0.0010 g tailings sample 1 to test the IC recovery at the range of 0-70 % $CaCO_3$ equivalent (e.g., 0-80 g IC kg^{-1}). The amounts of standard materials added were $CaCO_3$ at the levels of 0.0200 ± 0.0010, 0.0400 ± 0.0010, 0.0800 ± 0.0010, 0.1600 ± 0.0010, 0.3200 ± 0.0010, 0.6400 ± 0.0010 g. In addition, 5 types of carbonate minerals ($CaCO_3$, $MgCO_3$, dolomite, magnesite, calcite) were spiked into 1.0000 g tailings sample 1 separately at the rate of 0.1600 ± 0.0010 g to test the recoveries of various IC standard materials. To test the interference from OC in the IC quantification procedure, 0.010 ± 0.001 g sucrose was spiked in all the mixtures as well. Tailing sample 1 (1.0000 ± 0.0001 g) without inorganic and organic materials was used as the blank.

The IC content was determined in triplicates of the samples using methods modified from Bundy and Bremner (1972). Samples were treated with an acidic reagent to hydrolyse carbonates into CO_2, which was absorbed in a base solution and estimated by the titrimetric method. Briefly, mixtures of tailings and standard materials described in previous paragraphs were accurately weighed into 120 ml polythene containers. A 20 ml beaker containing 10 ml of 2 M sodium hydroxide (NaOH) was affixed to the side of the upper section of each container. This enabled the CO_2 absorption up to 120 g kg^{-1} IC in mixture within the range designated for carbonate content. Both the sample container and the beaker containing the base solution were placed in an air tight 1 L glass jar and tightly stoppered. An aliquot of 30 ml 2 M HCl was injected into the sample container through the needle-puncture stopper using a 50 ml hypodermic syringe. After adding the acid solution, the whole jars were gently swirled for 1-2 minutes to mix the content and allowed to stand at room temperature (20-25 °C) for 48 h. After this, the NaOH solution was transferred into a 150 ml Erlenmeyer flask and made up to 50 ml with CO_2-free deionized water. 3 droplets of phenolphthalein indicator solution were added into the base solution, which was titrated with 0.5 M HCl until the pink colour became colourless. The volume of acid used for each sample solution was recorded and IC content (as dissolved CO_2) was calculated according to a standard curve prepared from CO_2 evolved from known amounts of $CaCO_3$ using the same procedure. IC contents of each treatment were calculated as the difference between samples and blank. IC added into tailings were calculated based on the C concentration (Table A-1) and weights of added carbonate minerals. The IC recovery of all carbonate minerals were calculated as follows:

$$\text{IC recovery (\%)} = \frac{\text{IC treatment (mg) - IC blank (mg)}}{\text{IC standard concentration (mg g}^{-1}\text{)} \times \text{IC standard weight (g)}} \times 100\ \% \qquad \text{Eq A-1}$$

The 7 types of tailing samples were analysed for TC and IC content with the same method, and the difference between TC and IC concentration was the TOC of tailings samples.

A.2.5 Acid pretreatment for direct OC quantification by dry combustion

For direct OC quantification with acid pretreatment method, and to test the OC (organic standard materials) recovery, appropriate amount of organic C (sucrose, $C_6H_{12}O_6$) and standard IC (calcium carbonate, $CaCO_3$) were spiked into 1.0000 g ± 0.0010 g tailings sample 1 and used to test the OC recovery within the range of 0-7 % organic C (e.g., 0-70 g OC kg^{-1}). The amounts of standard materials added were sucrose at the levels of 0.0050 ± 0.0005, 0.0100 ± 0.0005, 0.0200 ± 0.0005, 0.0400 ± 0.0005, 0.0800 ± 0.0005, and 0.1600 ± 0.0005 g. In addition, 5 types of organic minerals (sucrose, EDTA, cellulose, litter, and charcoal) were spiked into 1.0000 ± 0.0010 g tailings (sample 1) separately at the rate of 0.0400 ± 0.0005 g to test the recoveries of various OC standard materials. To test the interference from IC in the OC quantification procedure, 0.1000 ± 0.0010 g dolomite was spiked in all the mixtures as well. Tailing sample 1 (1.0000 ± 0.0001 g) without inorganic and organic materials was used as the blank. Moreover, mixture of sucrose (0.0400 ± 0.0005 g) and dolomite (0.1000 ± 0.0010 g) was included in each batch to test the recovery of OC without any tailings samples.

The procedure to have these spiked samples treated is as follows: As preliminary experiments demonstrated that H_2SO_3 was not effective and sufficient to remove all carbonates in the tailings samples, probably due to the low penetration ability into mineral matrix (data not shown), the acid pretreatment used to treat the samples containing carbonates in this study was HCl. Any surplus or residual HCl following pretreatment was removed by hot plate and vacuum drying processes in the fume hood to minimise the corrosive risk to the total C analyser (LECO Corporation, St. Joseph, MI). Briefly, 1 ml of 5 M HCl solution was evenly added to the mixtures in a 25 ml preweighed glass beaker. The sample beaker was placed on a hot plate at 60 °C to enhance moisture evaporation. An additional 5 M HCl solution was added into the beaker until no effervescence was observed. The acid pretreated samples were finally dried on the hot plate (60 °C) overnight (15 h). The dried samples were further gently vacuumed by increasing pressure up to 85 kPa and holding at this pressure for 2 h. At the end of this period, the mass change for each sample after the acid pretreatment was recorded for mass conversion in the final calculation of OC content per unit of original sample weight. The pretreated samples were then finely ground in a mortar and pestle and stored in a desiccator before being subsampled for OC analysis by dry combustion in a total C analyser (LECO Corporation, St Joseph, MI). The OC contents of each sample were calculated as the difference between samples and blank. The OC added into tailings were calculated based on the OC concentration (Table A-1) and weights of added organic materials. The OC recovery of all organic minerals was calculated as follows:

$$\text{OC recovery (\%)} = \frac{\text{OC treatment (mg)} - \text{OCblank (mg)}}{\text{OC standard concentration (mg g}^{-1}) \times \text{OC standard weight (g)}} \times 100\ \%$$

Eq. A-2

The 7 types of tailings were measured with dry combustion for direct OC quantification using the same method.

A.2.6 Acid pretreatment methods comparison

In the acid pretreatment, in addition to spiked standard recoveries of IC subtraction and direct OC quantification methods, limit of detection (LOD) and limit of quantification (LOQ) were calculated to identify the lower limits of C contents, below which analytical precision becomes unacceptable. Relative standard deviation (RSD) was calculated to assess the analytical precision. Moreover, both methods were applied in OC content quantification in 7 tailings samples to investigate their correlations and differences.

Limits of detection (LOD): the lowest quantity of a substance that can be distinguished from the absence or zero. The value of LOD is given by the following equation:

$$LOD = X_{bl} + kSD_{bl} \quad \text{Eq. A-3}$$

where X_{bl} is the mean of the n blank measures, SD_{bl} is the standard deviation of the n blank measures, and k is a numerical factor chosen according to the confidence level desired, which is commonly set as 3 for LOD (Mocak, 1997). For calculation of LOD, blank is the sample without any tailings/standard materials.

Limits of quantification (LOQ) are the limits at which we can confidently measure the differences between two different values, which are generally taken as a concentration equal to 10 standard deviation of the blank, therefore, 3.3 times of LOD (Eq. A-4) (Currie 1999):

$$LOQ = 3.3\ LOD \quad \text{Eq. A-4}$$

Relative standard deviation (RSD) is the absolute value of the coefficient of variation. Values with RSD less than 10 % have been suggested as acceptable in terms of analytical precision (Green, 1996). The calculation is based on all the samples used in this study, including spiked samples, standard materials and tailings samples.

$$RSD\ (\%) = SD/\ Mean \times 100\% \quad \text{Eq. A-5}$$

A.2.7 Data analysis

The relationship between the IC/OC spiked (based on weight of standard materials) and the IC/OC measured for both methods were tested with a simple linear regression model. The same simple linear regression model was also used for OC values measured with both methods for the 7 tailing samples. In addition, means comparison (T-test) was used to test whether the OC values measured by the two methods were significantly different ($P < 0.05$). All statistical analysis were conducted using the SPSS software package (SPSS Statistics 20.0, Chicago, IL, USA).

A.3 Results

A.3.1 Inorganic carbon quantification recovery

The IC subtraction method achieved a satisfactory recovery of added IC in the form of carbonates and various minerals spiked in the tailings samples (Table A-3). The volume and strength of HCl used successfully hydrolysed the $CaCO_3$ added, with a recovery rate of 84.8-117.0 % (means = 99.7 %). In addition, there was a positive linear correlation between the measured amounts of C and the amounts of IC added (R^2 = 0.997), without significant interference from the tailings matrix or the organic material (sucrose) spiked in the tailings.

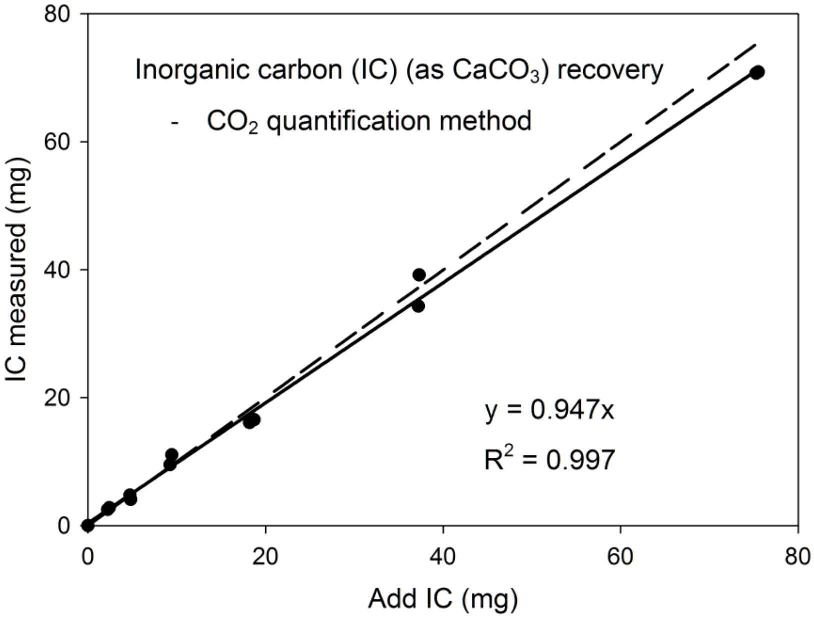

Fig. A-1 The linear relationship between inorganic carbon (IC) content added as $CaCO_3$ in the tailings samples and measured IC content in IC quantification method measured by titration. The dashed line is a 1:1 line, indicating a 100 % recovery of the added IC.

The mean recovery (%) of IC from calcium carbonate, magnesium carbonate, calcite, magnesite, and dolomite using the IC quantification method were 103.0, 91.9, 91.4, 87.5, and 90.6 % respectively (Table A-3). As a result, the IC in base metal mine tailings can be satisfactorily quantified as the amount of CO_2 released from the HCl induced hydrolysis of carbonate minerals by using the setup of acid reaction vessel and base trapping method.

Nevertheless, the recovery of the added IC may be underestimated when the IC content is > 60 mg g^{-1} (Fig. A-1), because of the lower efficiency of HCl to release CO_2 when there were high contents of carbonate. A quantitative recovery of IC could be obtained with less sample or increase the volume of HCl used, but this possibility was not tested.

Table A-3 Mean recovery (%) of inorganic carbon (IC) in carbonate materials in IC quantification (measured by titration) and organic carbon (OC) in typical organic materials in direct OC measurement based on dry combustion method.

IC quantification		TC measurement after HCl treatment	
Carbonate material	Recovery (%)	Organic material	Recovery (%)
Calcium carbonate	103.0	Sucrose	40.3
Magnesium carbonate basic	91.9	EDTA	76.2
Calcite	91.4	Cellulose	81.1
Magnesite	87.5	Litter	91.6
Dolomite	90.6	Charcoal	85.8

A.3.2 Organic carbon recovery and acid pretreatment for IC removal

The recovery of added OC in the tailings was satisfactory, except for the water-soluble OC, sucrose. The recovery rate of the added sucrose C varied with the amount of sucrose C spiked in the tailings samples.

The recovery rate of added OC in the form of sucrose in the tailings spiked with $CaCO_3$ declined from 76.4 to 28.3 %, when the amount of OC added increased from 2 to 70 mg C. However, in tailings free mixture of $CaCO_3$ and sucrose, the recovery of OC in the form of sucrose was as high as 93.9 %. This suggested that there may be impacts of the tailings matrix (rich in minerals) on the recovery of added soluble OC in the form of sucrose through the loss as CO_2 during the acid pretreatment phase. Other studies found that water-soluble OC (such as sucrose) would be quickly oxidised and lost as CO_2 by chloride and co-formed chlorine when concentrated HCl interacted with manganese dioxide (MnO_2) at elevated temperature on a hot plate (Bisutti et al., 2004; Furlani et al., 2006). However, possible reasons were not examined in this study. It is suggested the loss of organics during acid pretreatment could be minimised by keeping the temperature moderate (e.g., 40 °C) (Heron et al., 1997). As shown in Table A-4, except for sucrose, average recovery of C in most of organic compounds ranged from 76-91 % using the direct OC quantification by acid pretreatment method. Therefore, it could be a mean to quantifying OC in base metal mine tailings.

Table A-4 Mean recovery (%) of IC in carbonate materials in IC quantification (measured by titration) and OC in typical organic materials in direct OC measurement based on dry combustion method.

IC quantification		TC measurement after HCl treatment	
Carbonate material	Recovery (%)	Organic material	Recovery (%)
Calcium carbonate	103.0	Sucrose	40.3
Magnesium carbonate basic	91.9	EDTA	76.2
Calcite	91.4	Cellulose	81.1
Magnesite	87.5	Litter	91.6
Dolomite	90.6	Charcoal	85.8

The direct OC quantification method with acid pretreatment had a generally lower recovery rate compared to the added OC standard materials. Several critical factors should be closely controlled when using this method for OC quantification in base metal mine tailings, including (1) the possible loss of soluble C by mineral oxidization induced by the acid pretreatment; (2) the accuracy in weighing the samples before and after acid pretreatment; and (3) the representativeness of subsampling acid-pretreated samples. In general, direct OC quantification after the HCl pretreatment method in this study achieved better performance in the removal of carbonates, with superior time efficiency and greater OC recoveries for most of organic compounds compared to methods reported in literature, which involve acid pretreatment followed by washing. For example, hydrofluoric acid (2 and

10 %) or hydrochloric acid (0.5 M) wash resulted in about 20 % of OC loss as the acid dissolved OC in the discarded wash (Mathers et al., 2002; Schmidt et al., 2012). The loss of OC could be up to 80 % with H_2SO_4 and nitric acid (HNO_3). H_2SO_3 (6 %) wash and HCl vapour fumigation methods were unable to remove 100 % of carbonate present, particularly in highly calcareous soils with more than 50 % carbonate (Bisutti et al., 2004; Harris et al., 2001). Although the acid fumigation method could remove up to 80 % carbonate if the sample was wet, it took much longer time to achieve similar effects (32 mg IC required at least 6 h) compared to the liquid HCl treatment (Yamamuro and Kayanne, 1995).

A.3.3 Analytical Precision

The precision of different methods was characterized by RSD (%), which is calculated according to Table A-5. The upper limit for OC quantification was 7 %, which is generally greater than the values found for most base metal mine tailings. The lower limit was assessed by LOQ. The standard deviation of 10 blank analytical values in IC quantification by titration method was 0.22 g kg^{-1}, and total C analysis based on dry-combustion method was 0.03 g kg^{-1} (Table A-5).

RSD (%) was less than 10 % (Fig. A-2a) in the tailings samples with IC contents of 5 - 30 g kg^{-1}, whereas RSD (%) rose to 17.5 % when IC contents were less than 5 g kg^{-1}. Similarly, when using the dry combustion method to quantify C content in both acid-pretreated samples and untreated samples, RSD (%) values were less than 10 % when C contents in tested samples were larger than 5 g kg^{-1} (0.5 %), which were 3-10 folds of instrument detection and quantification limits (Fig. A-2b).

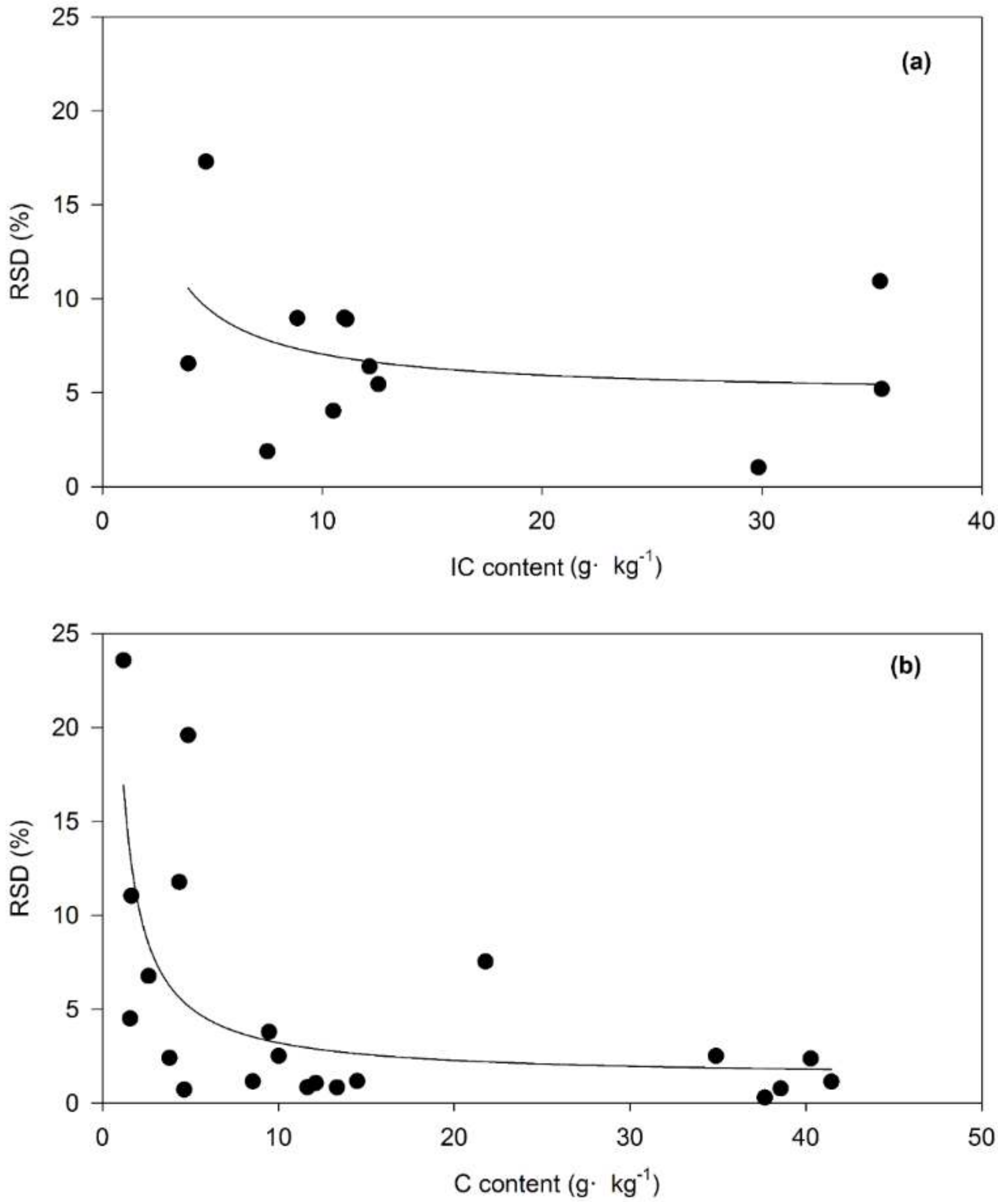

Fig. A-2 Analytical precision at different levels of C content in the mine waste samples: (a) the analysis of IC in carbonate materials as CO_2 quantified by titration and (b) direct OC quantification method after acid pretreatment to remove the IC. The data were pooled from all the tests conducted from the present study.

Table A-5 Limits of determination (LOD) and limits of quantification (LOQ) in IC quantification procedure in IC subtracting method and TC quantification based on dry combustion method

	IC quantification measured by titration ($g\ kg^{-1}$)	TC measured by dry combustion ($g\ kg^{-1}$)
S_{bl}[a]	0.22	0.03
LOD	0.66	0.09
LOQ	2.2	0.3

a: the number of blank sample analysis n = 10.

A.3.4 Method comparison in OC quantification of base metal mine tailings

TC contents in the 7 base metal mine tailings samples were determined by dry combustion and ranged from 3.91-38.57 $g\ kg^{-1}$ (Table A-6).

Table A-6 OC content obtained from IC subtraction method and direct OC method. TC and IC contents were also presented.

Tailings Sample No [a]	IC subtraction method			Direct OC method
	Total C ($g\ kg^{-1}$)	Inorganic C ($g\ kg^{-1}$)	OC estimated ($g\ kg^{-1}$)	OC($g\ kg^{-1}$)[b]
1	14.48 (0.12)	12.55 (0.49)	1.93(0.60)	1.20(0.20)
2	21.78(1.16)	8.86(0.56)	12.92(0.60)	9.47(0.25)*
3	37.67(0.08)	35.37(2.73)	2.30(2.30)	1.27(0.03)
4	38.57(0.21)	35.45(1.3)	3.12(1.51)	4.36 (0.36)
5	34.89(0.62)	29.84(0.22)	5.05(0.40)	4.64 (0.03)
6	3.82(0.06)	4.71(0.57)	-0.89(0.64)	1.65 (0.13)
7	4.88(0.67)	3.91(0.18)	0.97(0.49)	2.62 (0.13)*

a: details of the tailings samples have been described in Table A-2; b. OC values followed with asterisks (*) indicated there are significant differences between the two methods following the paired T- test ($P < 0.05$).

The majority of TC was present in the form of carbonate C in these samples. The IC subtraction method based on the difference between TC and IC measured separately was unsuccessful in tailings 3, which is pure tailings without neither plant biomass mulch nor vegetation cover. The problem with this method is that by subtracting IC from TC, when there is a relatively small difference (OC content less than 5 g kg^{-1}), there is a much larger error occuring in 2 separate measurements. By comparing the OC values, no significant differences between the two methods for 5 of the 7 samples. However, sample 2 with high OC content showed a significant differences between these two methods.

As shown in Fig. A-3, the regression relationship indicated both of the methods were comparable (R^2 = 0.818) with a general lower values in the direct OC quantification with acid pretreatment method. It may be the reason that part of readily soluble/oxidisable OC in tailings samples were lost during acid-pretreatment procedure.

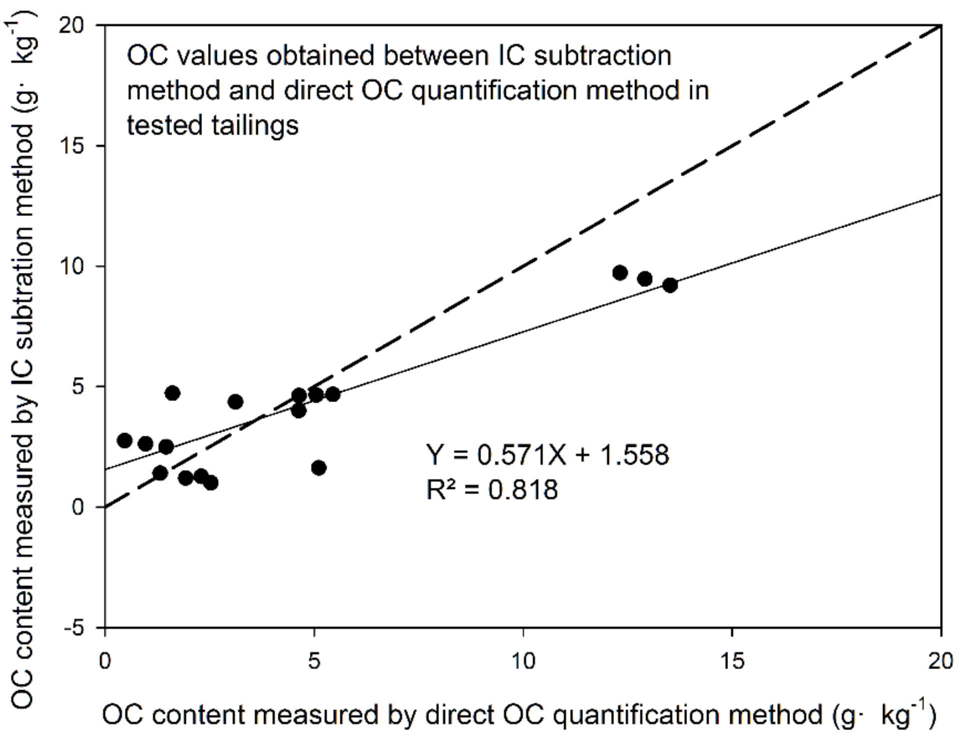

Fig. A-3 The linear relationship of OC values obtained by IC subtraction method and direct OC quantification method in tested tailings samples. The dashed line is a 1:1 line, indicating a same value obtained by both methods.

A.4 General discussion and conclusions

The acid treatment in the IC subtraction method effectively solubilized (quantified as CO_2 released) a range of carbonate compounds and minerals such as $CaCO_3$, $MgCO_3$, dolomite, magnesite, and calcite with recovery rates of 87-103 %. In the direct OC determination method, the pretreatment with 5 M HCl brought about a favorable recovery (76-92 %) of a range of added organic materials, such as EDTA, cellulose, plant litter, and charcoal. However, the water-soluble sucrose had only a 40 % recovery rate. The precision of both IC and OC quantification methods declined when the C contents were less than 5 g kg^{-1}, with RSD greater than 10 %. The OC values in 5 of the 7 tested samples of base metal mine tailings were comparable by these two methods. However, the IC subtraction method was unsuccessfully applied in samples with very low OC contents (less than 5 g kg^{-1}). Direct OC quantification with acid pretreatment method will result in a significantly lower OC values compared to the IC subtraction method if there is high OC content.

Based on results above, two suggestions are raised for OC quantification methods for base metal mine tailings. The IC subtraction method is applicable for tailings samples with relative high OC content, such as tailings amended with organic matter (e.g., litter or mulch) or under vegetation cover, with an expected OC content greater than 5 g kg^{-1}. The direct OC quantification method with acid pretreatment is preferred when the samples contained very low levels of C (e.g., IC < 5 g kg^{-1} and OC < 5 g kg^{-1}). This is because the IC subtraction method is not applicable and often results in negative values. However, because the recovery of OC varies greatly with the nature of organic materials spiked in the tailings samples, a significantly large portion of water-soluble OC may be lost during acid pretreatment for direct OC measurement. As such, this method is not recommended for OC quantification for mine wastes containing large amounts of soluble/small molecules of organic compounds (e.g., carbohydrates, proteins, low weight organic acids, etc.), such as tailings frequently amended with manure and biosolids. When there is less 5 g kg^{-1} OC in mine waste (such as pure tailings), both methods may need further refinement to increase the analytical precision, for example, by adjusting sample weight and increasing the number of replications.

A major uncertainty from IC subtraction method was the effect of removal of all types of carbonate minerals in tailings. Carbonate minerals such as siderite ($FeCO_3$), malachite ($Cu_2(CO_3)(OH)_2$), cerussite ($PbCO_3$), and smithsonite ($ZnCO_3$) are quite common in base metal mine tailings (Blowes et al., 1998). Reactions of acid and these carbonate minerals vary. For example, compared to malachite, which strongly reacts with hydrochloric acid, siderite and smithsonite only slowly react with it (Bisutti et al., 2004). Therefore, it is recommended to investigate the ore mineralogy of tailings and forms of major carbonate minerals. Although a stronger or more oxidisable acid or additional heating procedure will increase acid hydrolysation ability and result in the induced oxidisation of organic C as well. In addition to effectiveness of acid to remove all types of carbonate minerals, for direct OC quantification method, the various recoveries of different organic standard materials used in this study indicate the acid pretreatment procedure results in a difference of loss of OC with different forms. Further analysis is required to test the effects of acid pretreatment on OC recovery from different OC forms.

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Appendix B Biochemical properties of highly mineralised and infertile soil modified by Acacia and Spinifex plants in northwest Queensland, Australia

B.1 Introduction

In many remote regions of Australia (such as Mt Isa and Cloncurry, Northwest Queensland) where base metal mines (e.g., Cu, Pb-Zn mines) are located, the lack of adequate volumes of topsoil to cover large area of mined landscapes (e.g., 100s-1000s hectares of waste rock and tailings dams) has made necessary to engineer growth media and root zones for revegetation purposes (Huang et al. 2014). Wrong choices of engineering options such as the common practice of applying high rates of N and P inputs via (in)organic fertilizers in root zones would result in significant deviation of developmental trajectory of revegetated plant communities from the expected, in terms of species diversity and weed competitions (as we have observed in field trials at Mt Isa Mine) (Huang et al. 2011; 2012). This is because there is a close feedback between soil and plant systems in terrestrial ecosystems through linkages of functional microbial community and associated biochemical processes (Kardol et al. 2010; Wardle et al. 2004). High fertility in root zones favour forest and crop species of high productivity (Liu et al. 2012; Waldrop et al. 2000), while slow-growing native plant species with low productivity and low nutrient requirements dominate infertile and arid landscapes (Burns et al. 2013). Soil biochemical properties and key biological processes (e.g., organic matter decomposition, nutrient cycling) (Caravaca et al. 2005; Marschner et al. 2001) in the root zone are results from the long-term plant colonization through species-specific litter feedback and root zone modification (Quideau et al. 2001). Therefore, understanding physicochemcial and biochemical properties in soils colonized by the keystone native plant species in target plant communties will provide benchmarks for engineering growth media and root zones in mined land rehabilitation.

At Mt Isa, Northwest Queensland where many copper-gold (Cu-Au) and lead-zinc (Pb-Zn) mines are located, native plant communities distributed in the colluvial plains are dominated by slow-growing and water-nutrient efficient spinifex and acacia species (Specht and Specht 1999; Fox et al. 2001), due to their highly adaptive ecophysiological traits in infertile and arid landscapes (Diagne et al. 2013; Reid and Hill 2013). For example, Chisholm's Wattle (*Acacia chisolmii,* C_3 plant, N_2-fixing legume shrub) and Spinifex (*Triodia spp,* C_4 grass) are two keystone native plant species widely present on stony and lateritic soils in the colluvial and well drained land of Mt Isa region (Northwest, Queensland, Australia) (Specht and Specht 1999; Fox et al. 2001). Leguminous species (e.g., *Acacia* spp.) are critical N sources in infertile soil systems (Wardle et al. 2004). In addition, legume-microbes interactions have been observed for *Acacia* spp. with abundant propagules, AM hyphae and infectivity, which are not common for spinifex (Jasper et al. 1989a; b). Spinifex with unique drought adaptive leaf anatomy is tolerant of high temperature and radiation and extreme water deficit conditions (Winkworth 1967). The canopy characteristics and physiological traits of these species are ideal for phytostabilizing mined land under semi-arid climatic conditions (Murphy et al. 2010; Nicholas et al. 2009). However, the lack of information about biogeochemcial properties of natural soils colonized by target native plant communities hinders decision-making at local base metal mines (such as Mt Isa Mines), about growth media and root zone reconstruction options in rehabilitation programs.

The present study is aimed at characterizing physicochemical and biochemical properties in infertile soils colonized by the acacia (*A. chisholmii*) and spinifex (*T. pungens*) species in a colluvial plain at George Fisher, Mt Isa, Northwest Queensland, Australia. Differences of microbial biomass, structure (PLFAs profile) and functions (respiration, mineralization, enzymatic activities) in root zone soils were compared between the two species. Carbon (C) isotope signature of plant litters and soil organic carbon (TOC) were used to confirm species' contribution to TOC over long periods (Dalal et al. 2005). The analysis of community-level phospholipid-derived fatty acids (PLFAs) profiles (Zelles et al. 1992) and enzymatic activities (Badiane et al. 2001) was conducted to characterize biochemical properties of soils colonized by the two native species. Possible associations were explored between the physicochemical properties and microbial community structure and functions in root zone soils. The specific objective of this study is to provide fundamental information about the biochemical properties and processes in native soils and its interaction with native plant species, with the purpose to aid decision-making in engineering growth media and root zones, which are consistent with ecophysiological requirements of these native plant species for phytostabilizing mined land, such as fertility management and amendment options.

B.2 Materials and methods

B.2.1 Site description and sampling

Mt Isa (20.73 °S, 139.5 °E) is located in Northwest Queensland, Australia. Local climatic conditions can be found at Australian Metrological Bureau (http://www.bom.gov.au/qld/mt_isa/). In summary, its climate can be classified as subtropical and semi-arid, with annual pan evaporation of 2800 mm and average annual rainfall of 427 mm, and average yearly temperature of 25.5 °C (19 – 32 °C). Local rainfalls are highly variable between wet season (during November to February) and dry season and can vary significantly from year to year. For example, above average rainfalls were recorded for the years 1991 (618 mm), 1997 (799 mm) and 2011 (1113 mm), but as low as 201 mm in 2008. Plant and soil sampling was conducted in February, 2013 when there was monthly rainfall of 90.9 mm and average temperature of 23.7 °C (Bureau of Meteorology 2013).

The soil in the region is classified as Rudosols (Isbell 2002). Soils are shallow red duplexes, red-brown loams and red earths (Christian et al. 1954). The dominant vegetation in Mt Isa area is low open woodland (*Eucalyptus-Acacia*) in combination with open hummock grassland (*Triodia pungens*) (Perry et al. 1964).

Soil and plant samples were collected from a selected area of 50 × 70 m at George Fischer colluvium plain with similar topographic feature to Mt Isa Mine tailings landscapes, about 26 km north of the Mt Isa Mine tailings impoundments. Two transacts of 30 m length with dominant species stands were chosen for each species. The distance between the two transacts was maintained at least 20 m apart from each other. Three representative sites

(30 x 30 cm quadrat under dense species stand) along each transact were sampled for soil and litters beneath mono-dominance stands of acacia (*A. chisholmii*) and spinifex (*T. pungens*), respectively. Plant litters and the corresponding surface soil at the depth of 0-5 cm was sampled within each quadrat. At each site, 3 representative subsamples of soil were taken across each quadrate and pooled into a composite sample which was evenly divided into two sets of subsamples. One set of sub-sample was transferred in plastic bags in the field and stored at 4 °C before transportation back to the laboratory, and then, dried at 40 °C and sieved < 2 mm for physicochemical analyses. The other set was snap-frozen in liquid nitrogen in the field, transported back to the laboratory in a cryo-shipper and freeze-dried prior to PLFA analysis. Plant materials were rinsed in 3 changes of deionized water and dried at 65 °C until a constant weight and ball milled for further analysis.

B.2.2 Plant analysis

Total elements of plant litter were analysed as described in Chapter 7.2.3. The ratio of isotope ^{13}C and ^{12}C ($\delta^{13}C$) were analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS, Tracer Mass, Europa Scientific). Solid-state cross-polarisation magic angle spinning ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C-NMR) for plant litter was done using A Bruker Advance 300 high-resolution NMR spectrometer interfaced to a 7.05 Tesla ULTRASHIELD bore magnet system. Material was placed in the 4 mm zirconium rotor and rotated at 7 kHz. Usual parameters included 42 ms acquisition time with sweep width of 30 kHz; 2 K data points were collected. Cross-polarisation time was between 1 and 4 ms. High-power decoupling was applied using tppm15 scheme. Between 4 and 10 K scans were collected. The spectra were plotted between -15 and 265 ppm, and peaks in the spectrum were assigned to four main chemical shift regions: alkyl C (0-50 ppm), O-alkyl C (50-110 ppm), aromatic C (110-160 ppm) and carboxyl C (160-210 ppm) (Webster et al., 2000). In general, Alkyl C represents lipids and other aliphatics, whereas O-alkyl represents more labile carbohydrates such as cellulose (Mathers et al., 2007). Relative intensity for each region was determined by integration using the Varian NMR software package

(Version 6.1c, Varian Inc., Palo Alto, CA). Areas of each chemical shift regions were measured and calculated as percentage of the total area.

B.2.3 Soil physicochemical properties analysis

The ratio of isotope ^{13}C and ^{12}C ($\delta^{13}C$) were analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS, Tracer Mass, Europa Scientific). As no carbonate was detected in any of the soil samples, TOC and TN concentrations in soils were determined by dry combustion method with a LECO CNS-2000 analyser (LECO Corporation, MI, USA). Selective physicochemical properties were measured as described in Chapter 3.2.2, including pH, EC, WHC, CEC, WSOC and total and water-soluble elements in aqua-regia digest. Hot-water extractable organic carbon (HWOC) was determined according to the method from Sparling et al. (1998). In brief, 10 g air-dried soil was saturated in 20 ml cold water (20 °C) for 30 min. The supernatant solution was then discarded and the change of sample weight was recorded to correct the actual sample: water ratio applied for hot water extraction. The mixture of sample and deionised water with a ratio of 1: 2 was incubated in water bath at 80 °C for 16 h. After then, the mixture was centrifuged at 4000 rpm for 10 min and filtered through 0.45 µm glass-fibre filter. HWOC was determined by dichromate digestion method (Bremner and Jenkinson, 1960).

Bioavailable organic carbon (Bioavailable OC) was determined using the incubation method (Chen et al., 2004). In brief, 50 g soil was adjusted to 50 % WHC and incubated aerobically at 25 °C for 4 weeks. All containers were covered with plastic film perforated with several pinholes for gas exchange but avoiding rapid water loss. Deionized water was added to the mixture every 2-3 days during incubation to compensate for water loss via evaporation. Samples were placed in a closed chamber attached with infrared gas carbon dioxide (CO_2) analyser (Q-Box SR1LP soil respiration package, Oregon, Canada). The atmosphere accumulated in the chamber were collected twice per second for 10 minutes for the analysis of CO_2 concentrations. Tests were conducted at 1, 3, 7, 14, 21 and 28 days of incubation.

The bioavailable OC was estimated by calculating the cumulative production of CO_2 from soils during 28 days incubation.

B.2.4 Estimation of plant derived organic carbon in soil

The OC in soil derived from C_3 plant (acacia) and C_4 plant (spinifex) was estimated by a mixed model mass balance following the equations below (Balesdent et al., 1996).

$$\text{Soil } C_4 \text{ -derived C} = (\delta^{13}C_{soil} - \delta^{13}C_{C_3})/(\delta^{13}C_{C_4} - \delta^{13}C_{C_3}) \times TOC \quad \text{Eq. B-1}$$

$$\text{Soil } C_3 \text{-derived C} = TOC - \text{Soil } C_4 \text{ -derived C} \quad \text{Eq. B-2}$$

Where $\delta^{13}C_{soil}$ is the $\delta^{13}C$ value of TOC, and $\delta^{13}C_{C4}$ and $\delta^{13}C_{C3}$ are the $\delta^{13}C$ values of the pure C_4 and C_3 plant litter in the study site, respectively.

B.2.5 Microbial biomass, basal respiration, net mineralisation rate and enzyme assays

The microbial biomass, basal respiration, net mineralisation and enzymatic activities were measured as described in Chapter 4.2.4 and 5.2.4.

B.2.6 Microbial community using PLFA analysis

The soil samples were freeze dried prior to phospholipid fatty acids (PLFAs) extraction (Bossio and Scow, 1998). Briefly, soil was extracted in a single-phase mixture of chloroform, methanol and 0.05 M phosphate buffer (pH 7.4) at the ratio of (1: 2: 0.8 v/v/v) from 5 g soils. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction column (SPE-SI; Bond Elute, Varian, Palo Alto, USA). Neutral lipids and glycolipids were eluted with 5 ml chloroform, followed by 10 ml acetone. Polar lipids were eluted with 5 ml methanol, and dried under nitrogen gas at 32 °C. Afterwards, samples were subjected to mild alkaline methanolysis by methanol: toluene mixture and potassium hydroxide (KOH). Resulting phospholipid fatty acid methyl esters (PLFA-ME) were extracted with hexane and acetic acid. Prior to analysis with gas chromatography-flame ionization detector (GC-FID) (Agilent Technologies, Santa Clare, USA), HP Ultra 2 column, hexane containing methyl nonadecanoate fatty acid (19:0) were added as the internal standard. To identify the PLFA-

ME, the gas chromatograph was coupled to an ion trap mass spectrometer (GCQ, Thermoquest, Germany). After measurement all values were corrected for the methyl carbon. Standard fatty acid nomenclature was applied (Frostegård et al., 1993). Individual PLFA biomarkers were used to quantify relative abundance of specific microbial groups. The abundance of individual PLFA was determined as nmol per g soil. Concentrations of each PLFA were calculated based on the 19:0 internal standard concentration. In this study, representative fatty acids for typical microbial community were summarised as follows (Bååth and Anderson, 2003; Frostegård and Bååth, 1996): A set of fatty acids represented bacterial PLFAs, including 14.0, 15:0, i15:0, a15:0, 16:0, i16:0, 16:1ω7c, a17:0, i17:0, br 17:0, cy17:0, 18:1ω7c and cy19:0. Sum of i15:0, a15:0, i16:0, a17:0, i17:0 and br17:0 was used an indicator of gram-positive (G+) bacteria. Gram-negative (G-) bacteria were identified by the PLFAs: 16:1ω7c, cy17:0, 18:1ω7 and cy19:0. The fungi was identified using the PLFAs 18:2ω6,9c, 18:1ω9c and 18:1ω9t. PLFAs 16:1ω5c were used as a biomarker for arbuscular mycorrhizal fungi (AMF). The actinomycetes were identified by the PLFAs 10Me 18:0. Other PLFAs such as 11:0, 18:0, and 10Me 19:0 were also used to analyse the composition of microbial community. Taken together, all of the PLFAs indicated above were considered to be representative of the total PLFAs of soil microbial community (Zelles et al., 1992) (Table B-1).

Table B-1 Summary of representative fatty acids for typical microbial community

Microbial community		Representative PLFAs
Actinomycetes		10Me 18:0
AMF		16:1ω5c
Fungi		18:2ω6,9c, 18:1ω9c and 18:1ω9t.
Bacteria	G+ bacteria	i15:0, a15:0, i16:0, a17:0, i17:0 and br17:0
	G- bacteria	16:1ω7c, cy17:0, 18:1ω7 and cy19:0.
	Other bacteria	14.0, 15:0, 16:0,
Others		11:0, 18:0, and 10Me 19:0

B.2.8 Statistical methods

Primary data processing was performed using Microsoft® Excel. One-way ANOVA was carried out after normality check to test differences between acacia and spinifex on general plant chemistry, soil physicochemical properties, OC and N fractions, microbial properties, PLFAs biomarkers and enzymatic activities in the soils. Means were compared using the Tukey honest significant difference (HSD) test at P = 0.05. All statistical analyses were conducted using the SPSS software package (SPSS Statistics 20.0, Chicago, IL, USA). Redundancy analysis (RDA) were made using CANOCO software for Windows 4.5 (Biometris-Plant Research international, Wageningen, The Netherlands). RDA-environment analysis was performed for microbial community composition (relative abundance of individual PLFAs, expressed as % mol of the total) and environmental variables (including soil and litter parameters). RDA-function analysis was for linkage between microbial structure (relative abundance of individual PLFAs) and functions (basal respiration, net mineralisation, enzyme assays) in the examined soils.

B.3 Results

B.3.1 Litter chemistry

As shown in Table B-2, TOC and TP were similar in the acacia and spinifex litter, which were 40.2-41.8 % and 0.44 % respectively. TN in the acacia litter was 8-fold greater than that in the spinifex litter, resulting in a lower C: N ratio in the acacia litter (38) than the spinifex (268). $\delta^{13}C$ values of the acacia and spinifex litters were -26.36 ‰ and -14.12 ‰ respectively, within the range of typical C_3 and C_4 plants.

Chemical composition of acacia and spinifex litter based on characteristic peaks on the CP/MAS ^{13}C-NMR spectra was showed in Fig. B-1. Overall, in both acacia and spinifex litter, O-alkyl C (the sum of methoxyl, carbohydrate and di-O-alkyl C, 63.3-77.5 %) was the highest among the C functional groups, followed by alkyl C (12.3-22.6 %), aromatic C (the sum of aryl C and phenolic C, 7.7-8.0 %) and carboxyl C (the sum of carboxylic, amide and ester C, 2.5-6.1 %). No difference of the relative intensity of aromatic C was found between the acacia litter and spinifex litter, yet the former was characterised with higher intensities of alkyl C and carboxyl C, and lower intensity of O-alkyl C compared to the latter.

Table B-2 Chemical properties of the acacia and spinifex litter

Parameters	Acacia	Spinifex	F	Sig.
TOC (%)	40.2 (1.0)	41.8 (0.2)	7.09	0.056
TN (%)	1.05 (0.03)	0.16 (0.01)	2527.00	0.000
TP (%)	0.44 (0.05)	0.44 (0.01)	0.059	0.820
C:N ratio	38 (2)	268 (27)	210.91	0.000
$\delta^{13}C$ (‰)	-26.36 (0.58)	-14.14 (0.02)	1317.604	0.000

Values are means (n = 3) with standard errors in brackets.

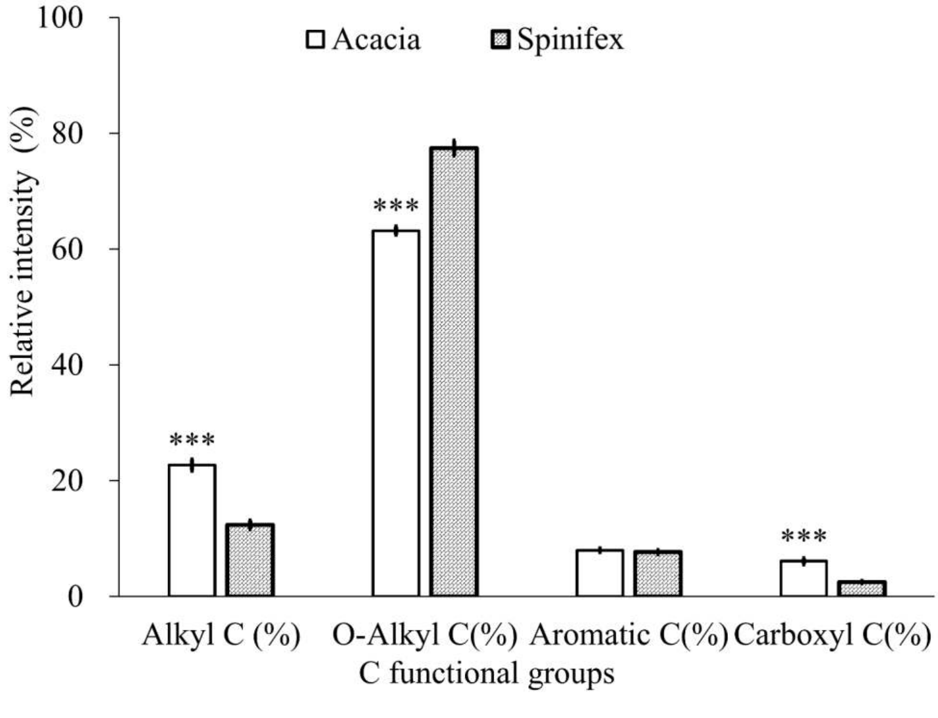

Fig. B-1 CP/MAS ^{13}C-NMR intensities for alkyl C, O-alkyl C, aromatic C and carboxyl C determined by spectra integration for the acacia and spinifex litter. Values are means (n = 3); error bars indicate standard deviations; bars above labelled with '***' suggests significant differences between the soils beneath acacia and spinifex at the level of $P < 0.001$.

B.3.2 Soil physicochemical properties

The soils beneath acacia and spinifex plants shared some similar basic properties (Table B-3), such as WHC and C: N ratio. Other physicochemical properties appeared to be different in the soils beneath the two species, such as pH, TOC, TN and CEC. Specifically, pH in the acacia soil was lower than the spinifex. The levels of TOC, TN and CEC in the soils beneath acacia were 2-3 folds compared to those in the soils beneath spinifex. Labile fractions of TOC, WSOC, HWOC and Bioavailable OC were considerably higher in the acacia soil than the spinifex soil.

Table B-3 Selective physicochemical properties in the soils beneath acacia and spinifex

Parameters	Acacia	Spinifex	F	Sig.
WHC (%)	32.2 (1.8)	27.9 (2.2)	7.277	0.054
pH	7.2 (0.0)	7.5 (0.0)	60.500	0.001
EC ($mS \cdot cm^{-1}$)	0.052 (0.001)	0.071 (0.001)	406.125	0.000
CEC ($cmol_c \cdot kg^{-1}$)	15.0 (3.6)	6.5 (0.8)	15.814	0.016
TOC ($g \cdot kg^{-1}$)	14.17 (1.37)	6.03 (0.39)	97.331	0.001
TN ($g \cdot kg^{-1}$)	1.11 (0.10)	0.47 (0.02)	118.361	0.000
$\delta^{13}C$(‰)	-21.29 (0.36)	-19.31 (0.45)	34.423	0.004
C: N ratio	12.8 (1.3)	12.7 (0.3)	0.043	0.846
TP ($g \cdot kg^{-1}$)	0.45 (0.08)	0.43 (0.09)	0.111	0.756
Cu ($mg \cdot kg^{-1}$)	94.3 (14.9)	93.1 (7.8)	0.016	0.907
Pb ($mg \cdot kg^{-1}$)	18.0 (8.3)	22.1 (2.5)	0.686	0.454
Zn ($mg \cdot kg^{-1}$)	371.4 (24.4)	295.5 (75.1)	2.776	0.171
WSOC ($mg \cdot kg^{-1}$)	17.8 (1.6)	8.6 (2.2)	33.488	0.004
HWOC ($mg \cdot kg^{-1}$)	205.1 (30.4)	114.2 (19.3)	19.144	0.012
Bioavailable OC ($mg \cdot kg^{-1}$)	768.0 (36.8)	436.0 (35.8)	125.333	0.000

Values are means (n = 3) with standard errors in brackets.

B.3.3 Microbial biomass, basal respiration, net mineralisation rate and enzymatic activities

Microbial biomass and activities in the surface soils were contrastingly different between acacia and spinifex (Table B-4). MBC in the soils beneath acacia and spinifex were 219.2 and 104.3 mg·kg^{-1}, respectively. Similar patterns were found for MBN, basal respiration rate and net mineralization rate, which were 2-fold in the former compared to the latter.

Specific enzymatic activities related to litter decomposition and nutrient cycling in the soil were compared between the acacia and spinifex. Except for the neutral phosphatase, activities of dehydrogenase, invertase and urease activities were higher in the acacia soil than the spinifex soil. Specifically, the activities of dehydrogenase and invertase in the acacia soil were about twice as much as that in the spinifex and urease activity about 3-fold higher in the acacia soil than that in the spinifex soil.

Table B-4 Microbial biomass, basal respiration, and net mineralisation rate, and enzymes including dehydrogenase, invertase, urease and neutral phosphatase activities in the soils beneath acacia and spinifex

Microbial properties	Acacia	Spinifex	F	Sig.
MBC (mg·kg^{-1})	219.2 (25.9)	104.3 (9.3)	52.439	0.002
MBN (mg·kg^{-1})	36.4 (10.8)	17.2 (1.5)	9.446	0.037
Basal respiration rate (mg CO_2-C·kg^{-1} d^{-1})	27.4 (1.3)	16.8 (2.9)	33.617	0.004
Net mineralisation rate (mg Mineral N kg^{-1} d^{-1})	2.5 (0.2)	1.1 (0.0)	177.281	0.000
Enzymatic activities				
Dehydrogenase (μg TPF g^{-1} h^{-1})	37.2 (8.7)	15.1 (4.9)	14.582	0.019
Invertase (μg glucose g^{-1} h^{-1})	1746.0 (216.4)	656.8 (180.3)	44.846	0.003
Urease (μg NH_4-N g^{-1} h^{-1})	51.2 (7.4)	14.2 (4.1)	57.970	0.002
Neutral phosphatase (μg phenol g^{-1} h^{-1})	93.7 (4.4)	82.9 (7.3)	4.850	0.092

Values are means (n = 3) with standard errors in brackets.

B.3.4 Microbial profiles with PLFA biomarker

A general structure of soil microbial communities was reflected by PLFA biomarkers. In total, 27 PLFAs were identified in the analysis. The numbers of PLFAs (24-27) were within similar range in soil underneath acacia and spinifex. Total PLFAs were higher in the acacia soil than the spinifex soil, which were 64.0 and 27.2 nmol g^{-1}, respectively (Table B-5). In general, abundance of all the microbial groups of PLFAs was greater in the acacia soil than the spinifex soil.

The relative abundance of the individual PLFAs (mol %), as ratios of specific PLFAs to total PLFAs, suggested that bacteria is the most abundant (76.2-79.6 %), followed by fungi (13.6-18.5 %), AMF (3.5-4.4 %) and actinomycetes (1.9-2.4 %) in the soils colonized by the acacia and spinifex plants (Fig. B-2). Although no differences were observed in the distribution of bacteria and actinomycetes (mol %) and G+: G- bacteria ratio in the soil between the two species, the relative abundance of AMF and fungi and the fungal: bacterial ratio were greater in the acacia soil than the spinifex soil (Fig. B-2 and Table B-5).

Table B-5 PLFA profiles of microbial community in the soils beneath acacia and spinifex

PLFAs profile	Acacia	Spinifex	F	Sig.
Numbers of PLFAs	27 (1)	24 (2)	6.050	0.070
Total PLFAs ($nmol \cdot g^{-1}$)	64.0(9.7)	27.2(6.1)	30.759	0.005
Actinomycete PLFA ($nmol \cdot g^{-1}$)	1.2 (0.2)	0.7 (0.2)	8.548	0.043
AMF PLFA ($nmol \cdot g^{-1}$)	2.2 (0.3)	1.2 (0.4)	11.685	0.027
Bacteria PLFA ($nmol \cdot g^{-1}$)	48.8 (7.7)	21.4 (4.2)	28.943	0.006
Fungi PLFA ($nmol \cdot g^{-1}$)	11.8 (1.6)	3.8(1.3)	44.213	0.003
Bacteria G + PLFA ($nmol \cdot g^{-1}$)	24.8 (3.0)	11.6 (3.3)	25.955	0.007
Bacteria G – PLFA ($nmol \cdot g^{-1}$)	6.0 (0.8)	3.0 (0.6)	27.026	0.007
Bacteria G +/G- ratio	4.2 (1.0)	3.8 (0.4)	0.395	0.564
Fungal: Bacterial ratio	0.24 (0.01)	0.17 (0.03)	12.358	0.025

Values are means (n = 3) with standard deviation in brackets.

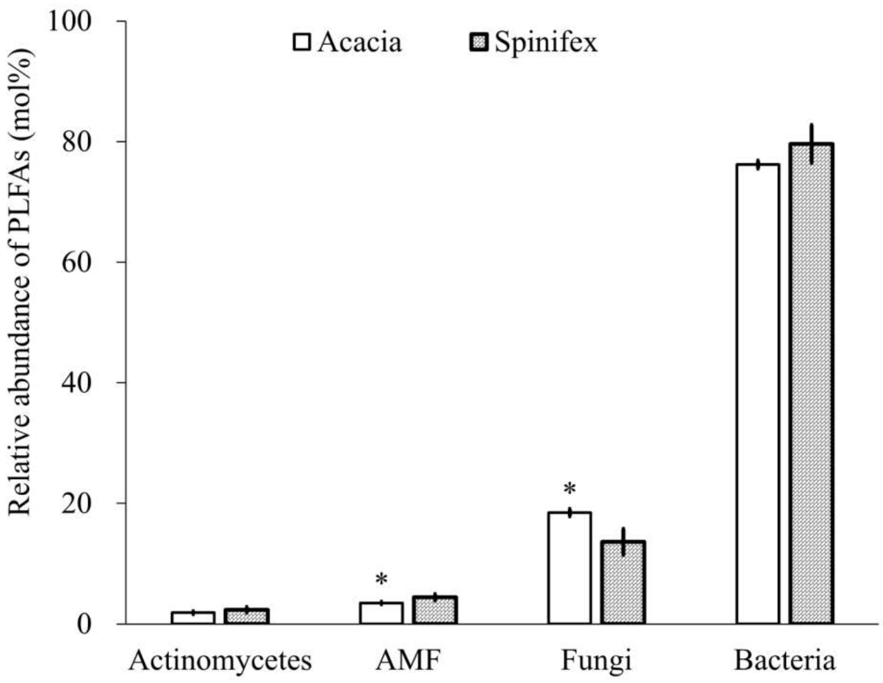

Fig. B-2 The relative abundances of the individual phospholipid fatty acids (PLFAs) (mol %), including Arbuscular mycorrhizal fungi (AMF), actinomycetes, fungi and bacteria in the soils beneath acacia and spinifex. Values are means (n = 3); error bars indicate standard deviations; bars above labelled with '*' suggests significant differences between the soils beneath acacia and spinifex at the level of $P < 0.05$.

B.3.5 Interactions among plants, soil and microorganisms

The levels of TOC in the acacia soil were higher than the spinifex soil (Fig. B-3). About half (58.5 %) of TOC in the acacia soil was derived from C_3 plant, while 57.7 % of TOC in the spinifex soil was derived from C_4 plant, indicating the dominant influence of *in situ* litters from each species on the soil TOC composition.

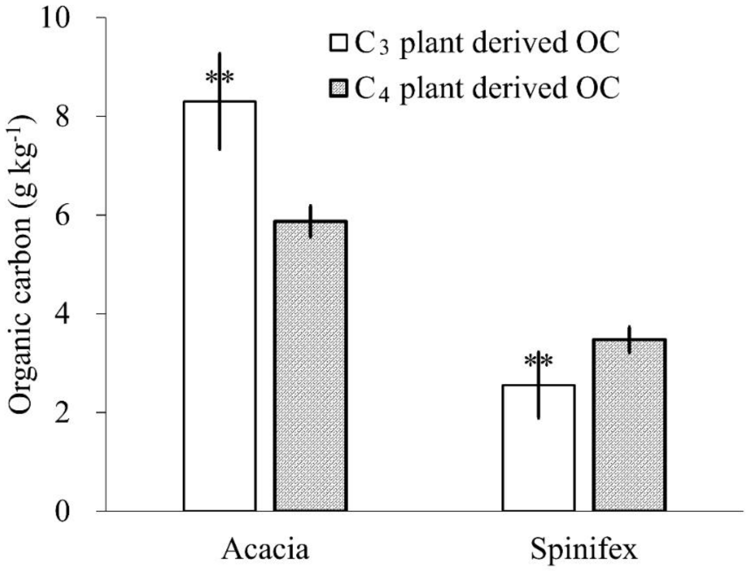

Fig. B-3 C_3 and C_4 plant derived organic carbon in the soils beneath acacia and spinifex. Values are means (n = 3); error bars indicate standard deviations; bars above labelled with '**' suggest significant differences between the soils beneath acacia and spinifex at the level of $P < 0.01$.

The RDA-environment ordination biplot showed specific associations between dominant site factors and individual PLFAs (Fig. B-4). Several biotic and abiotic factors, including litter C: N ratio, pH, EC, CEC, WHC, TOC and its labile fractions were identified to be closely related to the soil microbial community composition.

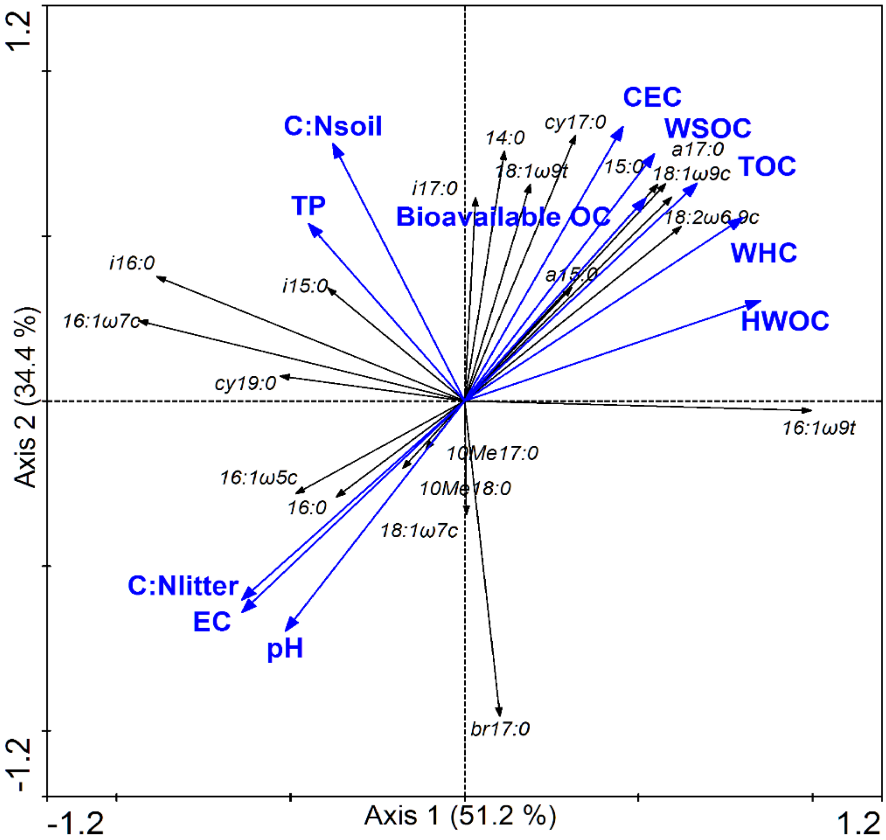

Fig. B-4 Redundancy analysis (RDA)-environment ordination biplot of relative abundance of individual phospholipid fatty acids (PLFAs) and dominant site factors. $C:N_{litter}$, litter carbon to nitrogen ratio; $C:N_{soil}$: soil carbon to nitrogen ratio; Abbreviations are indicated in Table B-3 and Fig. B-2.

In particular, TOC and its labile fractions were positively related to the relative abundance of fungi and majority of G+ bacteria groups. The $C: N_{litter}$ ratio, EC and pH were positively associated with the relative abundance of AMF (16:1ω5c) and actinomycetes (10Me 18:0), but negatively associated with the abundance of fungi (18:2ω6,9c, 18:1ω9c, 18:1ω9t) and bacteria. The microbial groups were also closely related to the biochemical processes in the soils examined (Fig. B-5). Despite the relatively low abundance, AMF (16:1ω5c) and fungi (18:2ω6,9c, 18:1ω9c, 18:1ω9t) were positively correlated with the N cycle processes (net mineralization and urease activities).

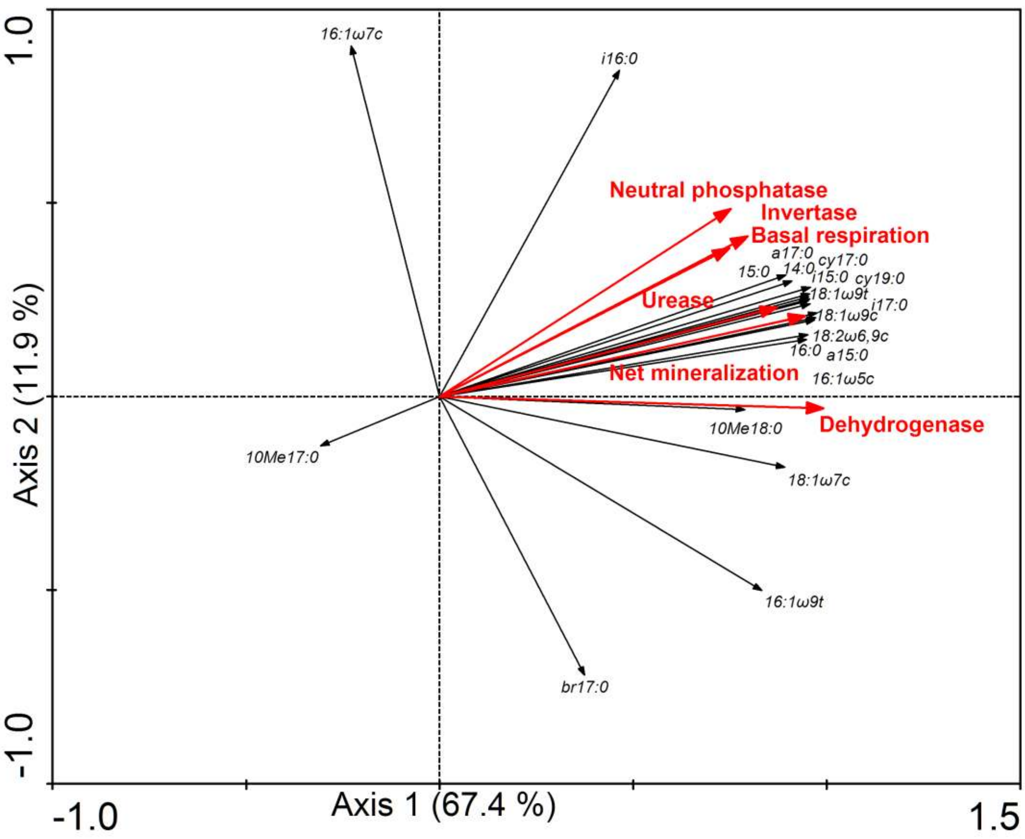

Fig. B-5 RDA-function ordination biplot of individual phospholipid fatty acids (PLFAs) and biochemical processes.

B.4 Discussion

Native plant communities in arid landscapes are of low productivity and overall patchy distribution pattern of examined native plant species colonizing infertile landscapes under semi-arid climatic conditions (e.g., Mt Isa area) (Reid and Hill 2013). From the present findings, nutrient (mainly N and P) status in the soils colonized by the native *A. chisholmii* and *T. pungens* plants was poor compared to that in productive crop/plantation soils (refer to Peverill et al. 1999). The nutrient supply, especially N supply, in the root zones seemed to rely on N_2-fixing native acacia (*A. chisholmii*) and microbe-driven litter decomposition. Within the native plant community, surface soils underneath acacia and spinifex had been modified by *in situ* litter return, based on the $\delta^{13}C$ values, in terms of TOC, structure and functions of microbial community, though it was unclear about the history of the colonization of the two species at the sites sampled. The levels of MBC, MBN, basal respiration rate and net mineralization rate were more than 2 times in the acacia soil than the spinifex soil. Microbial communities in the acacia soil had a greater fungal: bacterial ratio than the spinifex soil. On this basis, the strategy for engineering growth media and root zones for revegetating native acacia-spinifex communities to cover mined lands at local mines may be based on remediation with plant organic matter to supply available nutrients and native acacia as host plants to rehabilitate native microbial communities for *in situ* litter decomposition and nutrient cycling, rather than nutrient-rich organic/inorganic fertilizer inputs. Biogeochemical properties in soil systems are closely coupled with the long-term development of the above ground plant communities. Modification of soil system properties induces changes of species diversity and abundance in the plant community through feedback mechanisms of *in situ* quantity and quality of plant litter and the abundance of functions of decomposer microbes in the rhizosphere (Wardle et al. 2004).

B.4.1 Importance of organic matter in infertile soil colonized by native plant species

The present soil (regardless of colonizing plant species) contained much lower TOC compared to those reported in forest soils in semi-arid area or those forest/pasture soils located in eastern Queensland with higher rainfalls than Mt Isa (Richards et al. 2007; Spain and Feuvre 1987; Xu et al. 2008). In general, TOC concentrations in the soil in this study were within similar ranges (2.7-16.6 g kg^{-1}) of those found in the soils beneath natural shrub-grass plant community located in other semi-arid regions (Bastida et al. 2006; Shang and Tiessen, 1998; Zhao et al. 2010; Brid et al. 2002; Emmerich, 2003). The annual input of plant litter was assumed to be low for both acacia and spinifex, due to water limitation for plant biomass production in semi-arid regions (Facelli and Brock 2000). Even though detailed information about annual/seasonal patterns of plant litter inputs for both species was yet to be determined, it is assumed that higher litter inputs from the acacia stands might have occurred, compared to spinifex, based on the levels of TOC and TN. Soil organic matter is essential to the long-term soil fertility for sustainable plant biomass production

(Tiessen et al. 1994), but its mineralization rate and associated nutrient release in rate and composition should be in line with the ecophysiological requirements of target plant communities, due to the closely feedback between soil and plant systems (Wardle et al., 2004).

Nitrogen enrichment was also observed in the soil beneath acacia and spinifex with similar C: N ratio (13), much lower than those in the acacia litter (38) and spinifex litter (268). The majority of TOC in the examined soil was composed of microbial biomass or microbial by-products rather than the initial state of plant litter (Plaza et al. 2013). This might be the reason that the soil underneath both species have a similar C: N ratio, regardless of N concentrations of corresponding plants litter.

In natural ecosystems, plant litter and roots (not reported here) are the main sources of TOC, as shown by the isotope evidence of relative contribution of *in situ* plant litter and root inputs to TOC in the soil underneath the two plant species in the present study (Fig. B-3). However, acacia species are generally short-lived (10-20 years) (Fox et al., 2001; also see http://www.herbiguide.com.au/Descriptions/hg_Cootamundra_Wattle.htm) and the presence of acacia stands at specific sites may also be impacted by bush fire (unfortunately without specific fire and ecology records available for the location). In our own field observations, we have also noticed the fact that new emergence of spinifex plants tended to concentrate around sites where old acacia plants have died off. This may be one of the reasons for the present patterns of $\delta^{13}C$ values ($^{13}C/^{12}C$ isotope ratios) in the surface soils which were influenced by the standing acacia and spinifex plants, rather than exclusively dominated by individual species at the sites sampled. Given the fact that litter decomposition rate in terrestrial ecosystems generally increases with initial litter N concentrations (Aerts 1997), higher N concentration in acacia litter than the spinifex may have enhanced the decomposition rate of acacia litter, contributing to enhanced nutrient cycling processes in the surface soils. This implies the importance of acacia species in local soil remediation either in the form of pioneer plants and/or plant mulch (organic matter) to engineer growth media with nutrient supply potentials in line with growth rates of native plant species to be revegetated across mined landscapes (including the >1000 ha tailings) at Mt Isa Mines.

B.4.2 Structure and functions of microbial community with acacia and spinifex species

Both soil and plant affect microbial community and associated biochemical processes (Buyer et al. 2002). Our assessment of structure and functions of microbial community was based on coarse PLFAs biomarkers in combination with enzyme assay evaluation. Although not allowing a very detailed characterization of microbial community, it permits to understand the relationship between structure and functions of soil microbial community and *in situ* plant species. The number of PLFAs and relative distribution of major groups in microbial community in the root zone soil associated with both plant species were similar (Table B-5), regardless of the different levels of microbial biomass and activities (Table B-3). Overall, microbial community associated with both plant species were found to be highly bacteria dominant (> 75 %), especially G+ bacteria, which might be attributed to a high input of C from rhizodeposition of easily decomposable litter (e.g., sugar, carboxylic acid, amino acids) (Kuzyakov et al. 2007), promoting bacterial proliferation rather than fungal microorganisms (Buyer et al. 2002). A broad range of bacteria would be required to mediate soil biochemical processes such as C acquisition (DeAngelis et al. 2008), N_2 fixation (Evans and Ehleringer 1993), organic phosphorous mineralization and dissolution (Rodríguez and Fraga 1999), contributing to overall organic matter decomposition and nutrient cycling processes (Fig. B-5).

The fungal: bacterial ratio is a widely used index to indicate the relative contribution of fungi and bacteria in soil microbial community. The values (0.14-0.25) in the present study, were within the similar range of grassland (0.19-0.22) (Breulmann et al. 2011), but lower than those reported in coniferous forest ecosystem (0.26-0.80) with relatively greater productivity under favorable fertility conditions (Frostegård and Bååth 1996; Pennanen et al. 1999). Although AMF and total fungi biomass comprised only a minor proportion (< 20 %)

of microbial communities in the examined soil, they are not only important to decomposition of more lignified organic compounds (e.g., lignin, phenol) (Araujo et al. 2012), but also play a vital role in improving plant growth through nutrient acquisition and tolerance resistance to drought (Michelsen and Rosendahl 1990), salts and metals (Ortega-Larrocea et al. 2010).

As a result, rehabilitation of microbial communities in engineered growth media would be critical to initiate and maintain litter decomposition and nutrient cycling for supporting the establishment and development of native acacia-spinifex communities revegetated across local mined land in the long term. We have demonstrated the use of root zone soils from natural plant communities as carriers of native microbial communities in a parallel study to investigate factors and processes in biogeochemical rehabilitation of engineered technosols from weathered Cu-Pb-Zn tailings (Li et al., 2014; 2015).

B.5 Conclusions

In summary, the soils from native plant communities consisting of leguminous *Acacia chisolmii* and Spinifex grasses (*Triodia* spp.) were characterized by relatively low levels of soil organic carbon (6.03-14.17 g kg^{-1}), microbial biomass (104.3-219.2 mg kg^{-1}) and enzymatic activities, which have sustained the growth and recruitment of these native plant species in the infertile and dry landscapes of northwest Queensland. Surface soils underneath acacia and spinifex were also modified by *in situ* litter return, in terms of TOC, structure and functions of microbial community. Overall, the soil underneath *Acacia chisholmii* contained greater levels of TOC and N, microbial biomass and enzymatic activities with greater fungal: bacterial ratio than under spinifex. This initial investigation has highlighted the greater contribution of native acacia species than spinifex in terms of organic matter, nutrient supply and fungi development. This has provided a general guidance for our further studies on remediation options for mine tailings rehabilitation.

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Appendix C Mount Isa tailings history, properties and phytostabilisation

C.1 Tailings history, properties and climatic conditions in Mount Isa

Tailings for phytostabilisation in this study are from Mount Isa Mine (MIM) and Ernest Henry Mine (EHM) located at Mount Isa, northwest Queensland, Australia (20.73 °S, 139.5 °E) (Fig. C-1). MIM has been operating since 1923, and operating 2 separate mining and processing streams, copper (Cu) and zinc-lead-silver (Zn-Pb-Ag). The ore body is originated on sedimentary Cu-Pb-Zn-Au deposit containing 3.3 % Cu, 5.4 % Pb, 6.5 % Zn and 154 ppm Ag (Guilbert and Park, 1986). In EHM, commercial production started in March 1998. Current core contains 1 % Cu, 0.5 ppm Au and 23 % Fe_3O_4. Intensive mining and processing activities occurring in MIM and EHM has brought about huge volumes of tailings to be rehabilitated from both history and current expansion of underground operation.

The mineralogy of MIM tailings are comprised of 20-60 % quartz, 18-25 % carbonate and ankerite, 6-35 % pyrite, 10-15 % chlorite and feldspar, and less than 5 % minor phases (i.e., chalcopyrite, sphalerite, galena, cobaltite, pyrrhotite, muscovite, talc/serpentine and biotite) (Forsyth, 2014). Current tailings impoundments include a decommissioned section that is excluded from the operation and a much larger on-going discharge section, which is predicted to be expanded to 1500 ha (Longbin Huang, personal communication).

Tailings from MIM Tailings dam 5 (TD5) were mixed Cu-Ag and Pb-Zn tailings, which were deposited more than 40-year ago with obvious oxidised zone formed. The tailings from MIM tailings dam 7 (TD7) have been recently (less than 2-year) deposited from mixed stream of Cu-Ag and Pb-Zn tailings, which is still active. EHM is located approximately 160 km northeast from MIM. In addition to quartz, EHM tailings contain abundant magnetite, pyrite and calcite and minor phase (e.g., microcline, orthoclase and kaolinite). EHM tailings dam continuously receive on-going discharge streams from Cu-ore processing.

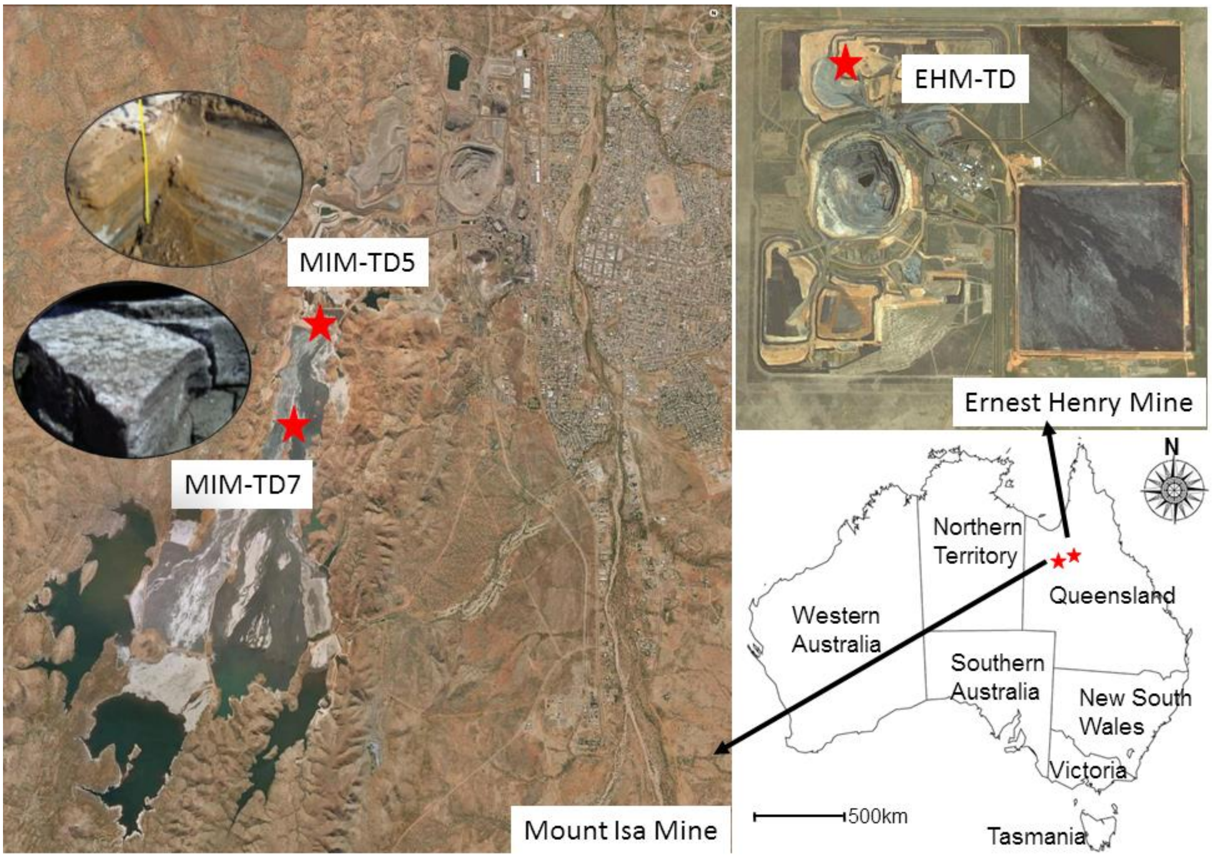

Fig. C-1 Location map of Mount Isa Mine (MIM) and Ernest Henry Mine (EHM) and tailings impoundments located in Mount Isa, Northwest, Queensland, Australia. Photos showed an excavated pit on MIM-TD5 (smooth surface, salt efflorescence) and MIM-TD7 (moist surface with salt efflorescence when surface water became evaporated).

The temperature at Mount Isa is generally warm to hot (17-32 °C). The climate condition is described as subtropical semi-arid with an annual pan evaporation of 2800 mm, and an average rainfall of 467 mm (Bureau of Meteorology Australian Government, 2015). Rainfall is highly variable between wet season and dry season, of which 80 % occurs during the period between December and February. Native soils in the region are shallow red duplexes, red-brown loams and red earths, formed from the geology background ranging from sandy to clay, with pH ranging from 4 to 9. The natural soil adjacent to MIM and EHM are classified as Rudosols (Isbell, 2002). Surface water surrounding (Leichhardt River) is slight-moderate alkaline with slight salinity (probably in the dry season). The dominant vegetation is low open woodland (*Eucalyptus-Acacia*) in combination with open hummock grassland (*Troidia pungens*) (Perry et al., 1964). These keystone native plant species are characterised by large root systems and slow growth rates but are highly competitive and capable of self-sustaining in landscapes deficient in nutrients and water as well as drought and salinity (Diagne et al., 2013).

C.2 Phytostabilisation practices and unsolved problems

Investigation of MIM tailings phytostabilisation could be traced back to late 1960s. Various amendments, ranging from simple ripping, organic amendment, and fertilizer application to limited soil or coarse sand cover together with total deep capping, were evaluated individually or combined as a whole. In total, there are 20 research greenhouse/field trials conducted, with most extensive investigations on vegetation establishment in tailings from tailings dam 3 (TD3) and 5 (TD5) (Table C-1).

Table C-1 Amendment strategies and tests of native and naturalised plant species in the past phytostabilisation trials in MIM tailings under field or greenhouse conditions, reproduced from Dorjsuren (2014)

NO	TD	Amendments	Tested plant species	Outcomes	Reference
1	3	Mulch and quarried rock low in N and P.	200 native and naturalised species	Pea bush (*Sesbania benthamania*), Tumble weed (*Salsola kali*), Polycarpaea glabra, Native amaranth (*Amaranthus interruptus*), Gomphrena brownie, Golden beardgrass (*Chrysopogon fallax*), Blue pea (*Clitoria ternatea*), Pink Mulla or Lamb tails (*Ptilotus exaltatus*) survived.	Ruschena et al., 1974
2	3	7 cm fly ash incorporated into 15 cm surface layer; Basal fertiliser: 600 kg ha^{-1} super-P; 400 kg ha^{-1} blood and bone and 90 kg ha^{-1} urea; Maintain weekly with 22.5 kg ha^{-1} urea.	Natural Colonisation	No detailed report available.	Hunter, 1974
3	3	Basal fertiliser: 1270 kg ha^{-1} super-P; 1270 kg ha^{-1} blood and bone and 90 kg ha^{-1} urea;	*Sesbania* spp., *Vicia* spp., Cereal rye (*Secale cereale*), Buffel grass (*Cenchrus ciliaris*), Wimmera rye (*Lolium rigidum*),	Dominant growth: *Cenchrus ciliaris, Cynodon dactylon* and *Chloris gayana*.	

			Rhodes grass (*Chloris gayana*), Couch grass (*Cynodon dactylon*), Sudan grass (*Sorghum x drummondii*), Siratro (*Macroptilium atropurpureus*)		
4	3	7 cm fly ash incorporated into 15 cm surface layer of tailings; Basal fertiliser: 1270 kg ha^{-1} super-P; 90 kg ha^{-1} blood and bone and 90 kg ha^{-1} urea; Maintain weekly with 25 kg ha^{-1} urea	Rhodes grass (*Chloris gayana*)	No detailed report available.	
5	3	7 cm fly ash incorporated into 15 cm surface layer of tailings; Basal fertiliser: 90 kg ha^{-1} blood and bone and 90 kg ha^{-1} urea; Super-P 250-400 kg ha^{-1}	Rhodes grass (*Chloris gayana*)	No detailed report available.	
6	3	15 cm ripping with 7 cm siltstone fines spread on surface or incorporated; Basal fertiliser: 115 kg ha^{-1} P and 57 kg ha^{-1} N;	Rhodes grass (*Chloris gayana*)	Surface siltstone fines more effective in biomass production.	

		Maintain bimonthly with 44 kg ha^{-1} urea			
7	3	Super-P supply ranging from 432-4320 kg ha^{-1}	Phasey bean (*Macroptilium lathyroides*)	Highest dry biomass at 1728 kg ha^{-1} super-P but 1728-4320 kg ha^{-1} was not significantly different; Optimum super P: 1296-1728 kg ha^{-1}; 0-400 kg ha^{-1} caused extreme P deficiency; Cu, Zn uptake reduced with increasing P addition.	
8	3	Cu-furnace slag mixing with tailings	Phasey bean *(Macroptilium lathyroides*)	Poor growth.	
9	3	Straw or bagasse furnace or mixed in 15 cm layer at the rate of 5 t ha^{-1}; Irrigation and fertiliser	Winter crops: Vetch and Oats; Summer crop –*Sorghum* spp.	Organic amendments increased plant growth; Incorporation of straw is the best treatment.	
10		1: 3 fly ash vs tailings incorporation; Basal fertiliser: Blood and Bone, NH_4NO_3 and Super-P	Phasey bean (*Macroptilium lathyroides*)	Good growth response if fertilisers were added	
11	3	15 cm ripping followed by rotary hoeing;	Couch grass (*Cynodon dactylon*), Rhodes grass (*Chloris gayana*), salt bush (*Atriplex*	Amendments improved infiltration rate, decreased EC, and increased P	

		Fly ash (121-162 t ha^{-1}) in the 8-10 cm surface layer; Basal fertiliser: blood and bone 27 kg ha^{-1}; NH_4NO_3 27 kg ha^{-1} and super-P 2160 kg ha^{-1};	spp.), Stylo (*Stylosanthes guyanensis* cv Schofield), Pea bush (*Sesbania benthamania*), Amaranthus (*Amaranthus interruptus*), Townsville stylo (*Stylosanthes humilis*), Buffel grass (*Cenchrus ciliaris*), Siratro (*Macroptilium atropurpureus*), Ptilotus (*Ptilotus* spp.)	supply; In pure tailings, only *Cynodon dactylon* survived with very low biomass and high levels of Cu (> 220 ppm), Zn (153 ppm), Pb (205 ppm) in plant tissues; In fly ash-amended plots, only *Cenchrus ciliaris Chloris gayana* and native *Sesbania benthamania* grew well.	
12	3	Mulching or fly ash amendments; Basal fertiliser: blood and bone 27 kg ha^{-1}; NH_4NO_3 27 kg ha^{-1} and super-P 2160 kg ha^{-1}; Maintain with 11 kg ha^{-1} urea per 4 weeks	Couch grass (*Cynodon dactylon*), Rhodes grass (*Chloris gayana*), salt bush (*Atriplex* spp.), Stylo (*Stylosanthes guyanensis* cv Schofield), Pea bush (*Sesbania benthamania*), Amaranthus (*Amaranthus interruptus*), Townsville stylo (*Stylosanthes humilis*), Buffel grass (*Cenchrus ciliaris*), Siratro (*Macroptilium atropurpureus*), Ptilotus (*Ptilotus* spp.) cereal rye (*Secale cereale*), wimmera rye (*Lolium rigidum*), vetch (*Vicia sativa*) and Sudan grass (*Sorghum sudanense*).	Outstanding growth performance: *Cenchrus ciliaris*, *Chloris gayana*, *Sorghum sudanense*, and *Lolium rigidum*. Fly ash far better than mulching, which was better than the control.	

13	3	15 cm ripped in tailings; 7 cm fly ash blade-mixed into the 15 cm surface tailings; Basal fertilizer: 55 kg ha^{-1} N in form of urea; 30-250 kg ha^{-1} super-P; Maintain with 27.5 kg ha^{-1} urea bimonthly	Rhodes grass (*Chloris gayana*) and *Sorghum* spp.	Best growth at super P 250 kg ha^{-1}; In fly ash-amended tailings, main limiting factor is salinity rather than P deficiency.	Ison, 1976
14	3	Urea: 150-600 kg ha^{-1} y^{-1} Basal fertilizer: 55 kg ha^{-1} urea and 1270 kg ha^{-1} super-P; Monthly application of urea	Rhodes grass (*Chloris gayana*)	150 kg ha^{-1} urea was still better than the control; Best growth at 300 and 600 kg ha^{-1} urea application; Monthly application of 300 kg ha^{-1} y^{-1} urea is far better than 600 kg ha^{-1} y^{-1} as high rates of urea application resulted in significant N loss.	
15	3	7 cm layer of siltstone fines (3.24 t ha^{-1}) surface/blade-mixed into 15 cm tailings; Irrigation sewerage.	Rhodes grass (*Chloris gayana*)	Surface application is better than incorporation as it provided protection for seedlings and help to reduce surface evaporation thus conserving water	

16	5	1 m ripping; Surface application of hay and cattle grazing.	Biloela buffel (*Cenchrus ciliaris* cv. Biloela), Bambatsi panic (*Panicum coloratum* cv. Bambatsi), Silk sorghum (*Sorghum hybrid* cv. Silk), Red Flinders grass (*Iseilema vaginiflorum*), Bundle (*Dichanthium fecundum*), *Acacia* spp. and *Senna* spp.,*Eucalyptus* spp., *Triodia* spp., *Ptilotus exaltatus, Atalaya hemiglauca*; Sudan grass (*Sorghum sudanense*).	Ripping is necessary to minimise water stress and most suitable treatment for native species with high plant survival rates during water logging and summer drought; Good germination and diversity of seeded species, with the dominance of *seilema vaginiflorum*, *Dichanthium fecundum*, *Sorghum hybrid Sorghum sudanense* and significant recruitment of *Acacia* spp.	Hodge, 1997
17	5	1 m deep ripping; 4 rates of sewage sludge treatments; Organic amendments (legume hay, mitchell and red flinders grass; garden refuse, compost green wastes).	*Eucalyptus* spp., *Acacia* spp, *Triodia spp*, *Ptilotus exaltatus*, Whitewood (*Atalaya hemiglauca*)		
18	5	Ripping to 1 m deep and 2 m wide with rock island constructed; Sewage sludge injection	*Eucalyptus* spp., *Acacia* spp, *Triodia spp*, *Ptilotus exaltatus*, Whitewood (*Atalaya hemiglauca*), *Stylosanthes guyanensis* cv. Schofield, Legume tree, *Cenchrus*	Produced the best and most consistent vegetative growth of native seed mix and *Leucaena Cunningham*; Good establishment of *Acacia*	

			ciliaris, *Atriplex* spp., White paperbark (*Melaleuca leucodendra*)	*salicina*and *Cenchrus ciliaris*; Natural colonisation was observed with Kapok (*Ceiba pentandra*)*,* *Ptilotus* spp. and Tomato; Less of *Eucalyptus* spp. in fine hay and sewage treatment.	
19	5	Deep ripping; 0.5-1 m quarried rock capping	*Atalaya hemiglauca*, *Eucalyptus* spp., *Acacia* spp., *Triodia* spp.*,* and *Ptilotus. exaltatus*	In 2004, *Acacia* spp. dominant, with grass (*Aristida pruinosa, Cenchrus ciliaris, Triodia pungens)*; In August 2008*, Acacia* spp., *Cenchrus ciliaris* and *Triodia* spp. showed good growth and species diversity with low ground cover.	
20	7		Kapok bush (*Averva javanica*), *A. hemiglauca*, Turpentine wattle (*Acacia chisholmii*), *A. cunninghamii*, *Solanum ellipticum*, *Sida cunninghamii*	No detailed report available	Duff, 2001

Past phytostabilisation trials in MIM tailings employed agronomic techniques to establish plants with high fertiliser input and irrigation in addition to various amendment options (e.g., topsoil, siltstone fines, sewage sludge, straw, fly ash etc.). Among more than the 200 native species tested in the trials, native grass, *Triodia* spp., Rhodes grass, buffel grass and woody shrub and tree species (e.g., *Acacia* spp. and *Atriplex* spp. whitewood) showed overall high survival rates and longevity in various trials with minimum amendment input (Hodge, 1997; Hunter, 1974; Ruschena et al., 1974). Past trials also showed coarse capping material significantly improve plant growth conditions with alleviated compaction, capillary rise of salts and improved water storage (Hodge 1997). However, application of capping materials onto large scale revegetation trials on existing tailings impoundments at MIM with approaching 1500 ha is cost prohibitive. Moreover, the established plants might not be sustainable without regular input of nutrients and water (Hodge, 1997). Revegetation practice conducted in TD3 showed no recruitment of second generation seedlings emerged among the 6500 trees planted in 30 ha area of tailings. Ridge trials (0.5 m topsoil capping and sewage sludge irrigation) and Silica capping trial (1 m thick cover of coarse stones) conducted in TD5 showed satisfying plant recruitment, but with patchy, inadequate and poor growth due to visible stresses from drought, salinity and poor fertility conditions (Hodge, 1997). Although no conclusive amendment strategies were derived from these trials, these past trials provide useful information about predominant constraints in tailings and possible amendment treatments for initial germination and establishment of various native plant species.

C.3 Reference

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