A Study on Microbial Carbon Use Efficiency in Soil

YILU XU

B.Sc. in Environmental Science, Hangzhou Normal University M.Sc. in Ecology, East China Normal University

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD)



Global Center for Environmental Remediation (GCER)

Faculty of Science

University of Newcastle (UoN)

New South Wales, Australia

March 2018

TABLE OF CONTENTS

TABLE OF CONTENTS	I
LIST OF FIGURES	V
LIST OF TABLES	X
LIST OF PLATES	XII
LIST OF PUBLICATIONS	XIII
ABSTRACT	XV
DECLARATION	
ACKNOWLEDGEMENTS	
CHAPTER 1	
INTRODUCTION	1
1.1 Biogeochemical carbon dynamics and soil as carbon reservoir	
1.2 Soil carbon reservoir mediated by microorganisms	
1.3 Carbon sequestration in various land use systems	
1.4 Heavy metal toxicity and biochar remediation	
1.4.1 Heavy metal influence microorganisms	
1.4.2 Biochar remediation	
1.5 Thesis objectives	
1.6 Thesis structure	
CHAPTER 2	
LITERATURE REVIEW	11
2.1 Introduction	11
2.2 Soil microorganisms in relation to carbon cycling processes	
2.2.1 Soil carbon pools and microbial accessibility	
2.2.2 Soil properties in relation to microorganisms	
2.2.3 Microbial adaptation to elevated temperature	
2.2.4 Soil microbial activity in response to drought and rewetting cycle	
2.3 Microbial community composition and soil carbon dynamics	19
2.4 Priming effect	25
2.5 Incorporation of microbial parameters into soil carbon modelling	
2.6 Conclusions and future research directions	29
CHAPTER 3	

i

MICROBIAL MEDIATED SOIL CARBON DYNAMICS AND PRI EFFECT IN RELATION WITH ORGANIC CARBON AND NUTR	
AMENDMENT	31
3.1 Introduction	
3.2 Objectives	
3.3 Hypothesis	33
3.4 Experimental treatments and amendment characteristics	33
3.5 Materials and methods	
3.5.1 Soil collection and preparation	
3.5.2 Soil physicochemical characterization	
3.5.3 Isotopic glucose addition and microbial respiration	
3.5.4 Total and ¹³ C labelled microbial biomass carbon	
3.5.5 Microbial Phospholipid fatty acid analysis	
3.5.6 Calculations	
3.5.7 Statistical analysis	
3.6 Results and discussion	
3.6.1 Priming effect in relation to microbial growth strategies	40
3.6.2 Microbial respiration response to carbon and nitrogen amendments	
3.6.3 Microbial biomass and soil carbon storage	
3.6.4 Microbial community regulated soil carbon dynamics	
3.6.5 Microbial carbon use efficiency	53
3.7 Conclusions and recommendations	
CHAPTER 4	
LAND USE HISTORIES ALTER SOIL MICROBIAL COMMUNIT PREFERENCES FOR DEGRADATION OF DIFFERENT CARBO	NC
SOURCES	56
4.1 Introduction	
4.2 Objectives	57
4.3 Hypothesis	57
4.4 Materials and methods	
4.4.1 Soil sampling and site record	
4.4.2 Physicochemical characterization of soil and organic amendments	61
4.4.3 Microbial respiration monitoring during incubation	63
4.4.4 Microbial biomass and carbon use calculation	63
4.4.5 Microbial PLFA analysis	64
4.4.6 Statistic analysis	65
4.5 Results and discussion	

	4.5.1 Soil properties, total organic carbon and nitrogen in different land uses
	4.5.2 Microbial activity variation with organic amendments under different land uses 68
	4.5.3 Microbial biomass carbon and carbon use as affected by organic source and land use72
	4.5.4 Microbial community composition patterns in relation to organic amendments and land use systems
4	.6 Conclusions and recommendations

	5.1 Introduction	83
	5.2 Objectives	84
	5.3 Hypothesis	85
	5.4 Materials and methods	85
	5.4.1 Soil preparation and spiking	85
	5.4.2 Potential and bio-availability of heavy metals	86
	5.4.3 Microbial properties	87
	5.4.4 Statistic analysis	89
	5.5 Results and discussion	89
	5.5.1 Soil physicochemical characteristics	89
	5.5.2 Potential and bioavailable heavy metal concertation	91
	5.5.3 Effect of heavy metal pollution on microbial carbon use	94
	5.5.4 Microbial community composition features altered by heavy metal	99
	5.6 Conclusions and recomendations	109
(CHAPTER 6	
E	EFFECT OF BIOCHAR ON HEAVY METAL TOXICITY AND	

MICROBIAL CARBON USE EFFICIENCY IN SOIL	11
6.1 Introduction1	111
6.2 Objectives1	113
6.3 Hypothesis1	113
6.4 Materials and methods 1	113
6.4.1 Soil sampling and preparation1	113
6.4.2 Soil and biochar characterization1	114
6.4.3 Soil spiking, biochar amendment and incubation experiment 1	116
6.4.4 Bioavailability of heavy metals1	118
6.4.5 Microbial properties1	118

6.4.6 Statistical analysis121
6.5 Results and discussion 121
6.5.1 Influence of biochar on heavy metal availability121
6.5.2. Influence of biochar on soil microbiota under metal stress
6.5.3. Influence of biochar on soil and microbial carbon
6.6 Conclusions and recomendations 139
CHAPTER 7
SUMMARY AND CONCLUSIONS141
7.1 Research Concept141
7.2 Research components and processes involved142
7.2.1 Microbial mediation of soil carbon dynamics142
7.2.2 Microbial carbon use efficiency-Effect of organic amendments 143
7.2.3 Microbial carbon use efficiency-Effect of land use 144
7.2.4 Metal pollution and biochar remediation in relation to soil microorganisms 144
7.2.5 Microbial community composition and the interpretation on carbon use alteration
7.3 Application of this research146
7.4 Future research
REFERENCES150

LIST OF FIGURES

CHAPTER 1

Figure 1. 1	Graphical	representation of	of thesis outline and	l chapter l	ayout	3
-------------	-----------	-------------------	-----------------------	-------------	-------	---

CHAPTER 2

Figure 2. 2 Concept *r*- and *K*-strategy microorganisms mediating soil carbon dynamics. The thickness of and arrow of the arrow bars indicate the carbon flow volume and direction. The sum of extracellular enzyme and hyphae carbon loss and microbial biomass carbon are the carbon sequestrated by microorganisms...16

- **Figure 3. 2** Dynamics of total CO2 efflux rate in different treatments: (**a**) soil applied with low organic C (CL) and high organic C (CH), and (**b**) soil applied only with organic C (C) or mineral N (N), and soil with C:N ratio at 23 (CN23), 50 (CN50) and 10 (CN50), respectively, **inset** in (**a**) showed cumulative microbial respiration values by the end of incubation among the different treatments. Different letters in the same column indicate significant differences (p < 0.05). Bars show standard errors of the means (n = 3). Standard errors are not shown when less than the symbol size.
- **Figure 3. 4** Carbon and nitrogen contents in microbial biomass and soil among the different treatments. Treatments include control: soil applied only with water, CL and CH: soil applied with glucose at low and high quality, respectively; N: soil applied only with mineral nitrogen; CN23, CN50 and CN10: carbon:nitrogen ratio at 23, 50 and 10, respectively. Microbial biomass carbon as MBC, microbial biomass nitrogen as MBN, δ 13C in microbial biomass carbon as 13C-MBC, total carbon as TC, total organic carbon as TOC, total nitrogen as TN. Bars show standard errors of the means (n = 3).
- Figure 3. 5 Proportion of fatty acids represent four microbial species (%): Gram-positive bacteria, Gram-negative bacteria, fungi and actinomycetes. Treatments include

- Figure 3. 7 (a): Principal component analysis (PCA) score plot of the two first principal components of the microbial species data set, providing a map of how the C:N treatment sets related to each other, (b): shows loading values for the first two principal components of the individual microbial species. The specie located far from plot origin is less influential. Figure also shows all of the microorganisms are closely positively correlated.

- Figure 4. 3 Microbial biomass carbon in three land use systems during 31 days incubation. Data expressed as mean \pm SE (n = 4)......72

- Figure 4. 6 Soil Gram-positive: Gram-negative (G+:G-) bacteria ratio (a) and Fungi:Bacteria ratio (b) under different land use histories. Values are mean ± SE (n = 3). Different letters above bars indicate significant different among land uses.
 Figure 4. 7 Soil Gram-positive: Gram-negative (G+:G-) bacteria ratio (a) and Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Figure 4. 7 Soil Gram-positive: Gram-negative (G+:G-) bacteria ratio (a) and Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Figure 4. 7 Soil Gram-positive: Gram-negative (G+:G-) bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories. Values are mean + Fungi:Bacteria ratio (b) under different land use histories.

- Figure 5. 2 Bioavailable Cd (a) and Pb (b), potential Cd (c) and Pb (d) concentrations in different treatments. Data are displayed as means, bars indicate SE (n = 3). Control as uncontaminated soil; CL: soil applied with low Cd level; CH: soil applied with high Pb level; CPL: soil applied with low Cd + Pb level; CPH: soil applied with high Cd + Pb level.

- Figure 5. 6 Total microbial PLFA content with different heavy metal contamination. Data are displayed as means, bars indicate SE (n = 3). Control as uncontaminated soil; CL: soil applied with low Cd level; CH: soil applied with high Cd level; PL: soil applied with low Pb level; PH: soil applied with high Pb level; CPL: soil applied with low Cd + Pb level; CPH: soil applied with high Cd + Pb level. 103
- Figure 5. 7 The diversity index H rooted in microbial PLFA profile with different heavy metal contamination. Data are displayed as means, bars indicate SE (n = 3). Control as uncontaminated soil; CL: soil applied with low Cd level; CH: soil applied with high Cd level; PL: soil applied with low Pb level; PH: soil applied with high Pb level; CPL: soil applied with low Cd + Pb level; CPH: soil applied with high Cd + Pb level.

- Figure 6. 2 Bioavailable Cd (a) and Pb (b) concentrations in different treatments. Data are displayed as means, bars indicate SE (n = 3). CB: soil applied with Cd + biochar; CG: soil applied with Cd + glucose; PB: soil applied with Pb + biochar; PG: soil applied with Pb + glucose; CPB: soil applied with Cd + Pb + biochar; CPG: soil applied with Cd + Pb + glucose.

- Figure 6. 6 Microbial biomass carbon in different treatment soils. Data are displayed as means, bars indicate SE (n = 3). S: control soil without any amendment; B: soil applied with biochar; CB: soil applied with Cd + biochar; CG: soil applied with Cd + glucose; PB: soil applied with Pb + biochar; PG: soil applied with Pb + glucose; CPB: soil applied with Cd + Pb + biochar; CPG: soil applied with Cd + Pb + glucose.
- Figure 6. 7 Proportion of fatty acids representing five microbial species (%). G+: Grampositive bacteria; G-: Gram-negative bacteria; F: fungi; A: actinomycetes; S: control soil without any amendment; B: soil applied with biochar; CB: soil applied with Cd + biochar; CG: soil applied with Cd + glucose; PB: soil applied with Pb + biochar; PG: soil applied with Pb + glucose; CPB: soil applied with Cd + Pb + biochar; CPG: soil applied with Cd + Pb + glucose.
- Figure 6. 8 Score plot of principal component analysis (PCA) showing treatment variation based on phospholipid fatty acid (PLFA) patterns. S: control soil without any amendment; B: soil applied with biochar; CB: soil applied with Cd + biochar; CG: soil applied with Cd + glucose; PB: soil applied with Pb + biochar; PG: soil

applied with Pb + glucose; CPB: soil applied with Cd + Pb + biochar; CPG: so	oil
applied with Cd + Pb + glucose13	35

Figure 7. 1 Conceptual diagram containing the principal research components covered and processes addressed in various research chapters (Chapter 3-6) in this thesis.

LIST OF TABLES

CHAPTER Table 2. 1	Selected references on environmental factors and the influences on soil
	microorganisms
Table 2. 2	Techniques to identify microbial community structure
CHAPTER Table 3. 1	3 The major experiment treatments and selected characteristics of the amendments
Table 3. 2	Selected properties of sample soil
Table 3. 3	Biomarker phospholipid fatty acid (PLFA) used to characterize microbial communities in the experimental soils (Frostegård et al., 1993; Zelles, 1999). 38
Table 3. 4	Average mole percentages of individual PLFAs at the ending of incubation. Data displayed as mol% (mean \pm SE, n = 3) of PLFA
Table 3. 5	Principal component analysis (PCA) loading plot of the first two principal components of the individual PLFAs from the PLFA data set
Table 3. 6	Microbial carbon use efficiency based on various approaches. Data shows mean \pm SE (n = 4)
CHAPTER Table 4. 1	4 Land management records and soil information of sampling sites60
Table 4. 2	Selected soil properties
Table 4. 3	Soil element information
Table 4. 4	Selected biochar properties
Table 4. 5	Soil total organic carbon (TOC), total nitrogen (TN) content and C:N ratio in the three land use systems. Data shows mean \pm SE (n = 4)67
Table 4. 6	One factor ANOVA (land use) and significance of differences of soils chemical and biochemical properties (Tukey test, $p < 0.05$, $n = 4$)
Table 4. 7	Microbial carbon use efficiency (CUE) with different organic carbon addition in three land use systems. Data showed means \pm SE (n = 4). Different letters in one column indicate significant (p < 0.05) difference among treatment in one land use
CHAPTER	5
Table 5. 1	Soil spiking rate and final metal concentrations. Mean \pm SE, n = 3
Table 5 2	Soil organic carbon, nitrogen, C:N ratio and Cmin:Corg ratio in different metal

Table 5. 2Soil organic carbon, nitrogen, C:N ratio and Cmin:Corg ratio in different metal
contaminated soils by the end of incubation period. Means \pm SE (n = 3) 91

- **Table 5. 4** Comparison of Gram-positive bacteria (G+ bacteria), Gram negative bacteria (G- bacteria), fungi and actinomycetes as obtained through respective PLFA profile (nmol g⁻¹ dry soil). Means ± SE (n = 3) of total PLFA, PLFA diversity, ratio of Gram-positive and Gram-negative bacteria, ratio of bacteria and fungi 101

- Table 6. 2
 Soil spiking rate and final metal concentrations. Mean ± SE, n = 3

 117
- Table 6. 4Comparison of Gram-positive bacteria (G+ bacteria), Gram negative bacteria
(G- bacteria), fungi and actinomycetes as obtained through respective PLFA
profile (nmol g⁻¹ dry soil). Means \pm SE (n = 3) of total PLFA, PLFA diversity, ratio
of Gram-positive and Gram-negative bacteria, ratio of bacteria and fungi. Mean
values followed by the same letter are not significant according to ANOVA (p >
0.05)131
- Table 6.5
 Detected microbial PLFA data under different treatments after 49 days of incubation (nmol g⁻¹ soil)
 133
- Table 6. 6
 Comparison of total organic carbon (TOC), total nitrogen (TN), and ratio of C:N in soils. Means ± SE (n = 3)

 137

LIST OF PLATES

Plate 4.1	Soil sampling location landscape in the three land use systems of South
	Australia: (a) Cropping land, (b) Pasture land, (c) Natural forest
Plate 4. 2	Set up of solid phase extraction column for different PLFA fractions separation.

LIST OF PUBLICATIONS*

Journal papers and book chapters

- Xu, Y., Seshadri, B., Sarkar. B., Rumpel C., Sparks, D., Bolan, N.S. 2017. Microbial Control of Soil Carbon Turnover. *Advances in Agronomy*.
- Xu, Y., Seshadri, B., Sarkar, B., Wang, H., Rumpel, C., Sparks, D., Farrell, M., Hall, T., Yang, X., Bolan, N.S. 2017. *Science of the Total Environment* 621, 148-159.
- Xu, Y., Sarkar, B., Seshadri. B., Farrell, M., Rumpel, C., Sparks. D., Hall, T., Bolan, N.S. Microbial functional diversity and carbon use efficiency in soil as impacted by heavy metal contamination. *Journal of Environmental Management*. In preparation.
- Xu, Y., Sarkar, B., Seshadri. B., Farrell, M., Yang X., Zhang, W., Rumpel, C., Sparks. D., Shilpi, S., Bolan, N.S. Microbial community properties and carbon degradation patterns under different land use systems. In preparation.
- Xu, Y., Sarkar, B., Seshadri. B., Farrell, M., Yang X., Rumpel, C., Sparks. D., Shilpi, S., Bolan, N.S. The priming effect: Fresh carbon and nitrogen inputs determine soil microbial community and carbon use. *Soil Biology and Biochemistry*. In preparation.
- Yang, X., Xu Y., Wang, W., Duan, L., Lv, G., Bolan, N.S., Guo, Y, Zibibula. S., Yang, S. 2017. Exploration of plants diversity and distribution against flooding in diluvial fan in arid desert region, NW, China application of a low altitude UAV. In preparation.
- Yang, X., Xu, Y., Bolan, N.S., Ali, A., Lv, G., Yang, S., Guo, Y., Zibibula, S. 2017. Groundwater level forecasting in arid region using tree maximum height. In preparation.
- Cheng, Q., Li, H., Xu, Y., Chen, S., Liao, Y., Deng, F., Li J. 2017. Study on the absorption of nitrogen and phosphorus from biogas slurry by NaCI-modified zeolite. PLoS One e0176109.
- Yang, X., Duan, L., Xu, Y., Chen, Y., Bolan, N.S., Lv, G. 2017. Short-term response of degradation of polycyclic aromatic hydrocarbons to nitrogen fertilization in the temperate grassland of arid desert region. In preparation.
- Yang, X., Yan, K., **Xu, Y.,** Li, Y, Xu, Zhang, X., Liu, Y., Duan, L., He, X., Lv, G. 2017. Soil water potential caused shift in hydraulic lift of Populus euphratica across growth season in arid desert region. In preparation.
- Yan, Y., Qi, F., Seshadri, B., Xu, Y., Hou, J., Ok, YS., Dong, X., Li, Q., Sun, X., Wang, L., Bolan, N.S. 2016. Utilization of phosphorus loaded alkaline residue to immobilize lead in a shooting range soil. *Chemosphere* 162, 315-323.
- Kunhikrishnan, A., Thangarajan, R., Bolan, N.S., Xu, Y., Mandal, S., Gleeson, D.B., Seshadri, B., Zaman, M., Barton, L., Tang, C., Luo, J., Dala, R., Ding, W., Kirkham, M.B., Naidu, R. 2016. Functional Relationships of Soil Acidification, Liming, and Greenhouse Gas Flux. Advances in Agronomy 139, 1-71.
- Wijesekara, H., Bolan, N.S., Vithanage, M., Xu, Y., Mandal S., Brown, S.L., Hettiarachchi, G.M., Huang, L., Ok, Y.S., Kirkham, M.B., Saint, C.P., Surapaneni, A. 2016. Utilization of biowaste for mine spoil rehabilitation. *Advances in Agronomy* 138, 97-173.

^{*} All published content that been used in this thesis was permitted by the publishers.

Conference proceedings

- Xu, Y., Bolan, N.S., Khan, N., Farrell, M. 2015. Implication of microbial carbon use efficiency in contaminated soil. 6th International Contaminated Site Remediation Conference. (Oral presentation)
- Xu, Y., Bolan, N.S., Seshadri, B., Farrell, M. 2016. Microbial carbon use efficiency (CUE) in heavy metal contaminated soil. Best Practice Ecological Rehabilitation of Mined Lands 2016 Conference. (Best student poster winner)
- Xu, Y., Bolan, N.S., Farrell, M. 2015. Microbial carbon use efficiency in different land use systems and the implication on global carbon sequestration. Smart Future Cities 2015 Conference. (Poster presentation)
- Xu, Y., Bolan, N.S., Seshadri, B., Farrell, M., Anthony, H. 2016. Microbial carbon use efficiency in heavy metal contaminated soil remediated with biochar. 3rd Asia Pacific biochar conference 2016. (Oral presentation)
- Xu, Y., Bolan, N.S., Seshadri, B., Farrell, M. 2017. Microbial indicator of heavy metal toxicity. Best Practice Ecological Rehabilitation of Mined Lands 2017 Conference. (Poster awards)
- Xu, Y., Seshadri, B., Thangavel, R., Farrell, M., Bolan, N.S. (2017). Biochar reduces metal bioavailability using microbial CUE as indicator. Soil science Australia new branch hunter regional meeting. (Oral presentation)
- Xu, Y., Seshadri, B., Bolan, N.S., Farrell, M. 2017. Impact of land use on soil organic carbon fractions and CO₂ efflux. 7th International contaminated site remediation conference incorporating: The 1st International PFAS Conference. (Oral presentation)
- Xu, Y., Bolan, N.S., Seshadri, B., Farrell, M. 2017. Soil Chemical and Microbial Properties in Heavy Metal Contaminated soils as Remediated with Biochar. 2017 ASA & CSSA International Annual Meeting: Managing Global Resources for a Secure Future. (Oral presentation)
- Thangavel, R., Wijesekara, H., **Xu, Y.,** Seshadri, B., Bolan, N.S. 2017. Impact of land use on soil organic carbon fractions and CO₂ efflux. 7th International contaminated site remediation conference incorporating the 1st International PFAS Conference.
- Bolan, N.S., Mandal, S., Wijesekara, H., **Xu, Y.,** Karunanithi, R., Qi, F., Kunhikrishnan A., Seshadri, B. 2016. Biochar-nutrient interaction in soil. 3rd Asia Pacific biochar conference 2016.

ABSTRACT

Soil organic carbon (SOC) plays a critical role in soil health and also in maintaining its ecological service. The stabilization of SOC involves physical, chemical, and biological processes in soil. Soil microorganisms serve as a carbon (C) biological sink as well as biochemical agents in C transformation in soil. The plant litter inputs and root exudates provide microorganisms with both labile and recalcitrant C sources. The C availability and soil habitat environment alter microbiota, consequently impacting the organic C decomposition processes in soil. Anthropogenic disturbances such as organic amendments, contaminants, tillage and grazing practices impact soil 'biophysicochemical' properties. The addition of organic C sources such as manure composts and biochar can lead to processes such as priming effect and microbial population shifts. In metal contaminated soils, organic-metal bonding can be beneficial to the immobilization of heavy metals, thereby reducing their bioavailability and biotoxicity. Microorganisms also develop strategies for the purpose to adapt to soil environment stress conditions. These stress tolerance processes include alteration of microbial community composition, and the redistribution of energy between catabolism (respired CO_2) and anabolism (biomass C).

Although a number of studies have examined soil C biogeochemical dynamics, very few comprehensive studies have been reported on the role of soil microorganisms in relation to the mobilization and immobilization ((im)mobilization) processes of organic C dynamics. In this research, soil microbial function and community composition in relation to C dynamics as affected by environmental factors were investigated. The definition of 'microbial carbon use efficiency' (CUE) was introduced for the purpose of assessing the fraction of microbially decomposed organic C that is subsequently assimilated into microbial biomass. The specific objectives of this research include: (i) to determine microbial CUE involving different approaches in relation with various sources of C and nitrogen (N) inputs; (ii) to investigate the influence of land use practices on soil microbial functions in relation to CUE; (iii) to evaluate metal stress on microbial function in relation to CUE; and (iv) to examine the influence of biochar on metal toxicity in relation to microbial CUE.

The first experiment was aimed to compare four approaches to measure microbial CUE using isotopic labelled glucose as an organic C source. The first approach (C_s) for microbial CUE measurement was based on monitoring C depletion, while the second (C_m) and third (C_p) approaches were based on detecting of microbial biomass accumulation, the forth approach (C_r) was aimed at calculating the ratio of the increased microbial biomass to the decreased C content. The microbial CUE values varied amongst the four approaches, and the C_m values

ΧV

were generally higher than other measurements. Because the main aims of the subsequent experiments were to understand the microbial mediation of soil C and the accumulation of C in microbial community, the microbial CUE measurement based on the accumulation of microbial biomass C (C_m) was used in the remaining chapters. In the first experiment, the ¹³C labelled glucose was evenly applied to soils to trace the C flow as measured by the release of CO₂, C incorporation into microbial biomass, and C remaining as undecomposed C input. Microbial phospholipid fatty acids (PLFAs) were extracted and analysed as biomarkers in order to identify the microbial community composition. Results revealed that organic amendment coupled with mineral N [(NH₄)₂SO₄] stimulated both microbial activity and biomass, leading to a positive priming effect (PE). However, as different C:N ratios were introduced in this experiment, the PE intensity stimulated by different exogenous C and mineral N sources showed variation amongst C sources, similar to microbial CUE values as determined by above approaches. The labile C source (glucose) with low N contributed to relatively higher microbial PE. Microbial community varied with C input sources, the readily available C source (glucose) favoured bacteria community growth over fungi, while fungi population increased with mineral N application. In conclusion, microbial CUE measurements are related to the methods and parameters used, and the C use preference and community composition are highly dependent on the exogenous C and mineral sources.

Based on the microbial CUE measurement results of the first experiment, the second experiment used soils from three land use systems: cropping, pasture and natural forest soil. Three types of organic amendments were introduced: glucose as a labile C source, and wheat straw and macadamia nutshell biochar as a relatively recalcitrant C material. Microbial biomass C, and basal and substrate-induced respiration were measured to determine microbial CUE. Microbial community composition was determined based on the measurement of PLFAs. Land use history generally affects soil physiochemical and microbial properties. The natural forest soil had the highest organic C content while having relatively low soil nutrient contents. Because of constant disturbance and management, cropping soil had relatively lower values in microbial activity and biomass. Although there were no significant differences of microbial CUE values in soils from different land systems, the organic amendments lead to distinct microbial CUE values. Therefore, the exogenous C source applied to cropping land during cultivation played a more important role in terms of microbial C use preference. Glucose input significantly (p > 0.05) increased microbial respiration with less biomass formation, thereby resulting in a decrease in microbial CUE, while wheat straw and biochar inputs increased microbial CUE compared to glucose. However, microbial community composition differed among land use systems. Fungi was dominant in natural forest soil while bacteria population was larger in cropping and pasture soils. The type of organic amendment inputs

xvi

also altered microbial community composition. The addition of an easily degradable C source such as glucose stimulated a growth in Gram-positive bacteria, while biochar input favoured fungi population growth.

The biotoxicity of heavy metal(loid)s was evaluated by monitoring microbial CUE and community composition in soil samples spiked with Cd(II) and Pb(II), both individually and in combination. The bioavailable metal concentrations, soil properties, and microbial parameters including microbial respiration, biomass and microbial PLFAs were determined at two sampling periods during the 49 days incubation experiments. Microbial CUE was determined as the ratio of accumulated biomass to decomposed C amount. Metal contamination had no significant effect on (p > 0.05) on soil properties such as pH and EC, while significantly (p < 0.05) inhibiting microbial activity and biomass formation. Notably, the microbial CUE decreased due to metal contamination, and the higher heavy metal concentration lead to lower microbial CUE values. Both total PLFAs and PLFA diversity decreased under metal stress. The microbial community composition and PLFA patterns also differed among treatments. Heavy metal pollution had greater negative influences on fungi population compared to bacteria. This might result in a vulnerable soil ecosystem with less resilience ability.

Based on the third experiment, biochar was introduced as an effective method for the remediation of metal contaminated soils. In this fourth experiment, Cd and Pb spiked soils treated with macadamia nutshell biochar (5% *w/w*) were monitored during a 49 days incubation period. Soil properties, metal bioavailability, microbial respiration, and microbial biomass C were measured after the incubation period. Microbial CUE was calculated from the ratio of C incorporated into microbial biomass to the C mineralised. Microbial community composition was determined by measuring microbial PLFAs. Results showed that total PLFA concentration decreased to a greater extent in metal contaminated soils than uncontaminated soils. Microbial CUE also decreased due to metal toxicity. However, biochar addition alleviated the metal toxicity, and increased total PLFA concentration. Both microbial respiration and biomass C increased due to biochar application, and CUE was significantly (*p* < 0.01) higher in biochar treated soils than untreated soils. Heavy metals reduced the microbial CUE through biochar addition in the contaminated soils could be attributed to the decrease in metal bioavailability, thereby mitigating the biotoxicity to soil microorganisms.

In conclusion, microbial properties are essential indicators in the determination of soil health. The microbial CUE values vary depending on the measurement adopted. As such, there is a need for a comprehensive conceptual understanding and unified method of determination of microbial CUE. For the purpose of this research, the microbial CUE measured based on the accumulation of microbial biomass was more appropriate to examine microbial function in

xvii

terms of microbial C utilization. Land use histories, organic amendments and environmental factor all alter the direction and dimension of microbial CUE, as well altering the microbial community composition. Especially certain microbial species such as bacteria and fungi could reveal soil functional status because of the difference in C use and allocation preference among these communities. Biochar could be beneficial to microbiota under metal stress, not only because of its high C content, but also because of its remediation ability as metal sorbents.

DECLARATION

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains published scholarly work of which I am a co-author. As the author of this Elsevier article, I retain the right to include it in a thesis or dissertation, provided it is not published commercially. The article in Science of the Total Environment Journal is the original source.

The thesis contains no other material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Yilu Xu

Signed____

Date___03/03/2018_____

ACKNOWLEDGEMENTS

With all my immense gratefulness, I express first and foremost my gratitude and respect to my principal supervisor, Professor Nanthi Bolan, a great mentor and one of the world leading scientists in soil science research. All through my candidature, he has been available and promptly responding to my research queries, inspiring and encouraging me to achieve high quality research work. I cannot image my career life without his invaluable support and guidance. Any words would not be enough to express my thanks to him. Professor Nanthi Bolan has been and still is an exceptional role model to me, in scientific area and human virtue.

Great thanks to my co-supervisor Dr. Balaji Seshadri and Dr. Mark Farrell, both are experienced senior research scientists, for their excellent guidance and assistance. I always value the ideas and suggestions that were raised up during our meetings.

It is with great honour for me to study and complete my research in Global Center for Environmental Remediation (GCER) and University of Newcastle. The valuable support and outstanding facilities enabled me to have this opportunity to pursue this high degree research. And a special acknowledgement to University of South Australia, where I began my Ph.D journey, for the support of most parts of my experiments.

To Professor Ravi Naidu, a world leading scientist and a real gentleman in life. Thanks to Professor Petra Marschner. I am extremely thankful to her constructive suggestions during my experiments preparation and for her technical support.

To my dearest friends and staffs Dr. Morrow Dong, Dr. Jason Du, Dr. Fangjie Qi, Dr. Yanju Liu, Mr. Kenny Yan, Dr. Luchun Duan, who took me in as part of their families, who diverted my attention when I was frustrated with writing. It was them that have helped me and companied me through all the difficulties, I will never forget the times I spend in Australia because they have made the time here more precious and memorable. When I look back, I realize I have grown up so much and turned into a person that I have never thought I could become. And you are all part of it.

I wish I could have the opportunity to express my appreciation person by person for their priceless participation in my life. Thanks also to Mr. Stuart, who kindly provided his land for my soil sampling. To Dr. Qiaoqi Sun, Dr. Jack Zeng, Dr. Li Yu and Mr. Congling Cheng, who have helped me at the beginning of my Ph.D.

I would like to dedicate this Ph.D thesis to my dear parents, Mrs. Meifang Jin and Mr. Shiming Xu. They are my backbone and my home. Although I used to say I am 'homeless' in Australia, I never really felt afraid or regret because of their caring and supporting. 致我亲爱的父母, 谢

谢你们. A special 'Dankeschön' to Florian Faulenbach. There were many dark nights with tears and frustration, the faith and companionship that he gave me helped me to pick up the courage and stand up again from the hopelessness. When you close the cover of a book, you have to fade out of one story and drift away from the characters. That is why life is measured by nodes and time points. Nevertheless, the beloved ones will stay a lifetime long. And I am eternally grateful to those people in my life.

One of my favourite German philosophers Friedrich Wilhelm Nietzsche wrote "Was mich nicht umbringt, macht mich stärker". I believe a 'life' should be a constant flow contains both of happiness and sorrow. Those two parts complete me and make me the person who I am, and I would never want a life without hardness and sadness.