

Phosphorus Dynamics in Soils Amended with Recycled Organics

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**Phosphorus Dynamics in Soils Amended with Recycled
Organics**

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Abstract

Soils organic amendments are increasingly being used as an alternative to conventional inorganic fertilizer to reduce deterioration of soil quality brought about by high inputs of chemicals and to counteract the depletion of mineral phosphorus (P) sources. The organic wastes, now referred to as recycled organics (RO), have been used in recent years as soil amendments to improve soil quality and crop growth. However, continuous application of RO amendments may result in the accumulation of P in soil and can eventually lead to eutrophication in surface water through excessive P runoff.

Bioavailability of P from applied RO is not only related to the P content of the RO and the soil total P content but also to the particular P forms present in soil. Phosphorus in soil occurs in multiple forms and fractions which differ in their availability for plant uptake. The distribution, dynamics and availability of P are controlled by a combination of chemical and physical processes as well as by microbial activity. To date, there is little information available on the dynamics of P in soil amended with RO and the associated microbial processes. This study thereby focuses on RO as a source of P in soil using two application rates.

The aims of this study were to: (i) examine the effect of RO application on soil P fractions and availability as well as investigating its interaction on soil microbial properties in agricultural soil, (ii) understand biochemical and microbial mineralization processes in soils amended with RO as well as identifying its effects on the availability of P in incubated soils, (iii) investigate the effect of RO on soil P fractions and associated soil microbial properties in rhizosphere soil as well as on the P uptake and plant biomass (roots and shoots).

A series of three experiments including field experiment, incubation, and pot trials were undertaken to examine the P dynamics and microbial properties in soil following

the application of different types of RO collected throughout Australia. The P sequential fractionation method was used to investigate P availability and sizes of different soil inorganic and organic P fractions. Soil microbial biomass carbon (MBC) and phosphorus (MBP) were determined using the fumigation-extraction method, while microbial activity was measured by determining cumulative CO₂-C evolution.

A screening test was initially conducted on 22 RO from varied sources across Australia to characterise their basic properties. From these, five RO with different characteristics and carbon to phosphorus (C: P) ratios were selected for the incubation and pot experiments, namely: biosolids (BS), broiler litter (BL), green waste biochar (BC), green waste compost (CG) and mixed compost (CM).

(1) Soil samples were collected from a large field study site at the Centre for Recycled Organics (CROA) in New South Wales to investigate the effects of repeated organic green waste compost amendments on soil properties and P fractions and availability to vegetable crops. Results showed that compost treatments significantly increased labile Pi indicating that the continuous application of the compost treatment accumulated labile P in soil. The concentrations of hot water extractable organic C (HWEOC), MBC, soil respiration and metabolic quotient were significantly higher in all compost treatments ($P < 0.05$), while concentrations of microbial biomass P (MBP) were also higher in all compost treatments. In conclusion, this study revealed that the current practice of periodically applying large amounts of compost based on plant N requirements has led to high levels of available P similar to the conventional practice.

(2) The aim of the second study, an incubation experiment, was to understand biochemical and microbial mineralization processes in soils amended with RO and its effects on the availability of P following the amendment of two different soils (sandy loam and loam) with the five selected RO types using two different P-based application rates. The CM, BL and CG treatments significantly increased ($P < 0.05$) the soil Pi and Po

fractions as well as microbial biomass compared to the control. The BS treatment led to the greatest increase in the labile Pi fraction, in both soils, but had minimal effect on the microbial biomass compared to the other RO treatments in both soils due to its high Cu content. The BC treatments amended in sandy loam soil resulted in small increases in labile Pi extracts, and unexpectedly increased the microbial biomass (MBC and MBP) and respiration ($P < 0.05$) showing the potential of BC in increasing soil P cycling as well as increasing soil C sequestration.

(3) In the third study, ryegrass and wheat grass were grown in a greenhouse pot experiment over 12 weeks. The results indicated that the BL applications (HP and LP) is the most effective treatment in increasing P availability, plant P uptake, plant yield and microbial biomass, followed by CM and CG treatments (HP and LP). The BC treatment increased all parameters tested in HP rate under wheat, but did not showed significant impact on MBP, plant P uptake and biomass yield under ryegrass. It was attributed to the release of root exudates, larger root systems and biochemical properties of wheat compared to the ryegrass. The minimal but significant effect of BS treatments on the increase of microbial properties was attributed to the root exudation although this treatment was high in heavy metal content too. This study indicates that root exudations play an important role in P transformation in rhizosphere soil.

Overall, these studies have highlighted the potential role of RO in increasing P availability, particularly in soils with low available P and in acidic soils. The results also provide important information on the response of microbial biomass and activity and P pools following RO amendment. This information enable us to accurately predict the required P inputs to soils to achieve optimum growth of crops. The results achieved in this project will help land users and managers to increase P efficiency in soil without further increase in P inputs to soil.

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university.

To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

NOR ASHIKIN AHMAD

Table of Contents

Abstract	i
Statement of Originality.....	iv
Table of Contents.....	v
List of Figures	x
List of Tables.....	xiii
Acknowledgement.....	xvii
Chapter 1 Introduction	1
1.1 Background	1
1.1.1 Research Questions, Hypotheses and Objectives.....	4
1.1.2 Research framework.....	4
1.2 Phosphorus in soil	7
1.2.1 Soil organic phosphorus	7
1.2.2 Soil inorganic phosphorus (Pi).....	8
1.2.3 Soil P availability and cycling.....	9
1.2.4 Soil P characterisation	11
1.3 Application of Recycled Organics (RO).....	13
1.3.1 Recycled organics production	14
1.3.2 Recycled Organics and Soil Phosphorus.....	17
1.4 Environmental Concerns.....	22
1.5 References	23
Chapter 2 Materials and Methods	36
2.1 Soils used in the study	36
2.2 Recycled organics (RO) used in the study	36
2.2.1 Chemical Analyses used for RO and soils	37

2.2.2	Characterisation of the selected RO	39
2.2.3	Selection of Recycled Organics for Experiments	42
2.3	Plants used in the study	42
2.4	P fractionation for soil samples	43
2.5	Hot water extractable organic C (HWEOC) and inorganic P (HWP)....	45
2.6	Microbial Analysis	45
2.6.1	Microbial Biomass Carbon (MBC)	45
2.6.2	Microbial Biomass Phosphorus (MBP).....	46
2.6.3	Soil respiration (CO ₂ -C).....	46
2.7	References	47
Chapter 3 Soil phosphorus fractionation and associated microbial processes		
under contrasting treatments of green waste compost.....		
3.1	Abstract.....	49
3.2	Introduction.....	51
3.3	Materials and methods	54
3.3.1	Experimental site description	54
3.3.2	Treatments and experimental design.....	55
3.3.3	Soil sampling.....	59
3.3.4	Soil analyses	59
3.3.5	Statistical Analysis	60
3.4	Results	61
3.4.1	Soil pH and EC.....	61
3.4.2	Soil P fractionation.....	62
3.4.3	Soil microbial biomass C and P	68
3.4.4	Soil respiration and metabolic quotient.....	70

3.4.5	Hot water extractable organic carbon and hot water extractable phosphorus	71
3.4.6	Relationships between soil biochemical properties	73
3.5	Discussion.....	75
3.5.1	Effects of organic amendments on soil P availability	75
3.5.2	Effects of organic amendments on soil chemical and microbial properties.....	78
3.6	Conclusion	81
3.7	References.....	81
Chapter 4	Soil phosphorus availability and microbial dynamics under amendments of recycled organics- an incubation study	91
4.1	Abstract.....	91
4.2	Introduction.....	93
4.3	Materials and methods	95
4.3.1	Soils and Recycled Organics (RO).....	95
4.3.2	Experimental treatments and design	96
4.3.3	Analytical methods.....	97
4.3.4	Statistical Analysis	98
4.4	Results	99
4.4.1	Chemical and physical properties of soils and RO	99
4.4.2	Soil P fractionation and availability	100
4.4.3	Microbial biomass carbon and phosphorus	107
4.4.4	Microbial respiration and metabolic quotient	111
4.4.5	Relationship between soil P availability and microbial properties	115
4.5	Discussions	119
4.5.1	Effects of recycled organics on soil P fractions and availability	119

4.5.2	Effects of recycled organics amendments on soil microbial properties.....	121
4.5.3	Interaction between soil type and recycled organic amendments	125
4.6	Conclusions.....	127
4.7	References	128
Chapter 5 Effects of amendment of recycled organics on soil P availability and microbial biomass in the rhizosphere		
5.1	Abstract.....	135
5.2	Introduction.....	137
5.3	Materials and Methods.....	139
5.3.1	Soils and Recycled Organics.....	139
5.3.2	Pot trial preparations	140
5.3.3	Experimental design.....	141
5.3.4	Plant harvest and measurements	141
5.3.5	Soil Analyses.....	145
5.3.6	Statistical Analysis	145
5.4	Results	146
5.4.1	Plant biomass and phosphorus uptake in roots and shoots.....	146
5.4.2	Soil P fractionation and availability in bulk and rhizosphere soils....	150
5.4.3	Soil microbial biomass C and P in bulk and in rhizosphere soils	159
5.5	Discussions	162
5.5.1	Effects of the RO on soil P fractions and availability in the rhizosphere.....	162
5.5.2	Effects of RO on microbial biomass C and P in the rhizosphere under ryegrass and wheat.....	164
5.5.3	Effects of RO on plant P uptake and plant biomass yield.....	167

5.6	Conclusions	169
5.7	References	170
Chapter 6	General Discussion, Conclusion and Future Research	176
6.1	Summary	176
6.2	General Discussion and Conclusion	179
6.3	Future research	180

List of Figures

Figure 1.1 General framework of the research which covers the research questions, objectives, hypotheses and chapters of the research.....	6
Figure 1.2 The general fate of P on agroecosystems.....	8
Figure 1.3 The P cycle in soil with different organic and inorganic forms of P in different pools based on their availability to plants. The soil solution pools and microbial biomass pool containing both Pi and Po can fluctuate in size as indicated by dashed borders. The physicochemical sorption/desorption processes including precipitation/dissolution processes are indicated by thin arrow. Immobilization/remineralization processes are indicated by thick arrow.	10
Figure 3.1 Field site at the Centre for Recycled Organics in Agriculture (CROA), New South Wales (NSW).	54
Figure 3.2 Response of P fractions (% of total P) of different lability in soil under different treatments. Pi= inorganic P; Po= organic P. APi= NH ₄ Cl-Pi; BPi= NaHCO ₃ -Pi; BPo= NaHCO ₃ -Po; N ₁ Pi= NaOH-Pi (first extraction); N ₁ Po= NaOH= Po (first extraction); HPi= HCl-Pi; N ₂ Pi= NaOH-Pi (second extraction); N ₂ Po= NaOH-Po (second extraction); Res-P= residual (non-extracted) P. Treatments, T1= HP-Conventional; T2= HP-Compost; T3= HP-Mixed; T4= LP-Conventional; T5= LP-Compost; T6= LP-Mixed; T7= Control.....	67
Figure 3.3 Means values for MBC, MBP and microbial C:P ratio in the soil under different treatments. Mean values followed by different letters for each treatment are significantly different from each other. Barred lines indicate the standard error of the means.	69
Figure 4.1 Percentage of P fractions in the sandy loam and loam soils under different organic amendments. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG= compost green waste, CM= compost mixed and BC= green waste biochar.	105

Figure 4.2 Microbial biomass carbon (MBC), microbial biomass phosphorus (MBP) and microbial C:P ratio under different treatments for two soil types. Different letters above each column for each treatment mean indicate significant differences ($P < 0.05$) from each other. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG= compost green waste, CM= compost mixed and BC= green waste biochar..... 109

Figure 4.3 Respiration rate in $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ and cumulative respiration in $\mu\text{g CO}_2\text{-C g}^{-1}$ (b) as affected by amendments of different RO for the sandy loam soil. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG=compost green waste, CM= compost mixed, BC= green waste biochar, HP= high phosphorus treatment, LP= low phosphorus treatment. 113

Figure 4.4 Respiration rate in $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ and cumulative respiration in $\mu\text{g CO}_2\text{-C g}^{-1}$ as affected by the amendments of different RO in the loam soil. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG=compost green waste, CM=compost mixed, BC= green waste biochar, HP= high phosphorus, LP= low phosphorus. 114

Figure 5.1 Ryegrass plants in the soil amended with RO at week 12. A) high P rate (HP) and B) low P rate (LP). CK= control; CG= green waste compost; BL= broiler litter; BC= biochar; BS= biosolids; CM= Mix compost..... 143

Figure 5.2 Wheat plants in the soil amended with RO at week 12. A) high P rate (HP) and B) low P rate (LP). CK= control; CG= green waste compost; BL= broiler litter; BC= biochar; BS= biosolids; CM= Mix compost..... 144

Figure 5.3 Percentage of P fractions in rhizosphere and bulk soil following RO amendments under ryegrass and wheat determined after 12-weeks of growth. Treatments are CK= control, BS= biosolids, BL= broiler litter, BC= green waste biochar, CM= compost mixed and CG= compost green waste; Treatments marked with LP are low P (50 kg P ha^{-1}) and marked with HP are high P (100 kg P ha^{-1}). 154

Figure 5.4 MBC, MBP and MBC:P Ratio for all treatments in rhizosphere and bulk soils following RO and P amendments under ryegrass and wheat after 12-weeks of growth. Treatments are CK= control, BS= biosolids, BL= broiler litter, BC= green waste biochar, CM= compost mixed and CG= compost green waste. Treatments marked with LP are low P (50 kg P ha^{-1}) and marked with HP are high P (100 kg P ha^{-1}). 161

Figure 6.1 Conceptual diagram on the general conclusion of the research. 183

List of Tables

Table 1.1 Selected wastes handled by organic processors by region during 2014-2015 (tonnes).	15
Table 2.1 Basic characteristics of the twenty-two recycled organics used for screening purposes.	41
Table 2.2 Selected properties of recycled organics.	42
Table 3.1 Properties of the surface soil (0-10 cm) at the CROA site prior to the field experiment.	55
Table 3.2 Properties of poultry manure and composts used in the CROA study.	57
Table 3.3 Mean soil pH and EC with different organic treatments. Values followed by different letters for each treatment indicate significant differences ($P < 0.05$). Standard errors are shown in parentheses.	61
Table 3.4 Mean values for P fractions (mg P kg^{-1}) in soil under different treatments. Mean values followed by different letters for each treatment are significantly different from each other. Standard errors are shown in parentheses.	65
Table 3.5 Mean P values (mg P kg^{-1}) for total extractable inorganic P (TPi), total extractable organic P (TPo) and total P (TP) fractions in soil under different P treatments. Standard errors are shown in parentheses.	66
Table 3.6 Mean values for microbial respiration and metabolic quotient in soil under different P treatments. Mean values followed by different letters for each treatment are significantly different from each other. Standard errors are shown in parentheses.	71
Table 3.7 Soil hot water extractable organic C (HWEOC) and inorganic P (HWP) in different treatments. Mean values followed by different letters for each treatment means are significantly different from each other. Standard errors are shown in parentheses. ..	72
Table 3.8 Correlation coefficient (r) between soil chemical and biological properties determined in different amendments.	74

Table 4.1 Mean values for basic properties of soils used in the incubation experiment.	95
Table 4.2 Mean chemical properties of recycled organics used in the incubation experiment.	96
Table 4.3 Application of RO (in g pot ⁻¹) based on 50 kg P ha ⁻¹ (LP) and 100 kg P ha ⁻¹ (HP).	97
Table 4.4 Soil phosphorus fractions (mean, mg P kg ⁻¹) as affected by amendments of different recycled organics (RO) in sandy loam (SL) and loam (L) soils and P values from analysis of variance (ANOVA) on the effects of soil types, RO treatments and their interactions. The mean values followed by different letters indicate significant differences from each other within a column. Treatments are CK = control, BS = biosolids, BL = broiler litter, CG =compost green waste, CM =compost mixed, BC = green waste biochar.	102
Table 4.5 Soil total inorganic and organic P fractions (mean, mg P kg ⁻¹) as affected by amendments of different recycled organics (RO) in sandy loam (SL) and loam (L) soils and P values from analysis of variance (ANOVA) on effects of soil types, RO treatments and their interactions. The mean values followed by different letters indicate significant differences from each other within a column. Treatments are CK = control, BS = biosolids, BL = broiler litter, CG =compost green waste, CM =compost mixed, BC = green waste biochar.	103
Table 4.6 P values from analysis of variance (ANOVA) on the effects of soil types, RO types and P rates and their interactions on various soil microbial properties determined over 140 days of incubation (MBC = microbial biomass C; MBP = microbial biomass P).	110
Table 4.7 Soil microbial metabolic quotients (<i>q</i> CO ₂) as affected by the amendements of different recycled organics (RO). The mean values followed by different letters indicate significant differences from each other with a column. Treatments are CK = control, BS=	

biosolids, BL= broiler litter, CG= compost green waste, CM= compost mixed, BC= green waste biochar, HP= high phosphorus, LP= low phosphorus.....	115
Table 4.8 Correlation coefficient (r) among key variables in the sandy loam soil.....	117
Table 4.9 Correlation coefficient (r) among key variables in the loam soil.....	118
Table 5.1 Basic properties for the sandy loam soil used in the pot trial. Results are a mean of 3 replicates (n=3).....	139
Table 5.2 Mean chemical properties of recycled organics used in the pot trials experiment.	140
Table 5.3 Actual amount of recycled organics applied (in g) based on low and high P rates (w/w).....	141
Table 5.4 Root, shoot and total biomass (root + shoot) (dry weight, g pot ⁻¹) and shoot: root ratios (S: R) of ryegrass and wheat determined after a 12 week period of growth. The mean values in the column followed by different letters indicate significant differences from each other at <i>P</i> <0.05 level.	148
Table 5.5 Phosphorus concentration and P uptake by ryegrass and wheat plants determined after a 12-week period of growth. The mean values in the column followed by different letters indicate significant differences from each other at <i>P</i> <0.05 level..	149
Table 5.6 Mean values of labile Pi fractions (mg kg ⁻¹) in bulk and rhizosphere soils under ryegrass (R) and wheat (W) following RO amendments. Data in brackets are the standard deviation of the mean.	155
Table 5.7 Mean values of moderately labile inorganic phosphorus (Pi) (NPi) and stable inorganic Pi fractions (HPi) (mg P kg ⁻¹) in bulk and rhizosphere soils under ryegrass (R) and wheat (W) following RO amendments. Data in brackets are the standard deviation of the mean.....	156

Table 5.8 Mean values of organic phosphorus (Po) (mg P kg^{-1}) and residual P (Res-P) (mg P kg^{-1}) fractions in rhizosphere and bulk soils under ryegrass (R) and wheat (W) following RO amendments. Data in brackets are the standard deviation of the mean. 157

Table 5.9 Mean values of soil total inorganic and organic P fractions (mean, mg P kg^{-1}) as affected by amendments of different recycled organics (RO) in sandy loam (SL) and loam (L) soils and P values from analysis of variance (ANOVA) on effects of plant types, RO treatments and their interactions. The mean values followed by different letters indicate significant differences from each other. Treatments are CK = control, BS = biosolids, BL = broiler litter, CG = compost green waste, CM = compost mixed, BC = green waste biochar. Data in brackets are the standard deviation of the mean. 158

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Chapter 1

Introduction

1.1 Background

Phosphorus (P) is an essential ingredient of the energy metabolism of all living things and is an essential plant macronutrient that is limiting for plant growth in most soils (Mackey and Paytan 2009). The P biogeochemical cycle includes important links between the living and non-living components of the environment and between inorganic and organic forms of P. Continuous human activities such as cultivation and land use changes have disrupted the global biogeochemical cycles of P which may contribute to causing environmental problems (Ceulemans et al. 2011). These anthropogenic activities have resulted in soil degradation leading to soil organic matter losses and alteration of soil microbial properties which in turn lead to P deficiency.

For decades, soil application of organic and inorganic amendments such as manures, plant residues, mineral rock phosphates, composts and other fertilisers had been a common practice in agriculture in order to counteract the low plant available P in soils (Ghosh et al. 2011; Damon et al. 2014). Consequently, the use of inorganic fertilisers in agriculture has become common practice to counteract the P depletion in soils and to increase crop production. One of the drawbacks of using inorganic fertilisers is the depletion of finite and non-renewable sources of mineral phosphate. Liu et al. (2009) reported that global P reserves are depleting, with a global consumption of all mineral P fertilisers of ~13.8 million metric tonnes of P in the year 2003-2004, and 78% of this was from mining of phosphate rock. Based on the estimation of the annual mining rate of the world's identified phosphate reserves, mineral P mined from the phosphate rock has been estimated to be exhausted in about 100 years (Cordell et al. 2009, Liu et al. 2009, Childers et al. 2011). In recent years, organic wastes have been used as an alternative to conventional inorganic fertiliser and one of the reasons for this, apart from the increasing

volume of organic wastes produced, is the depleting source of mineral phosphates (Cordell et al. 2009; Liu et al. 2009; Childers et al. 2011). It has been reported that only less than 20% of the applied P from inorganic fertilisers are taken up by plants, while the rest become unavailable to plants through adsorption via precipitation with soil iron (Fe), aluminium (Al) and calcium (Ca), leaving a large unavailable P pool in the soil (Holford 1997; Kashem et al. 2004). Studies on the inorganic fertiliser application have found that such fertiliser with high inputs of chemicals can deteriorate soil quality (Ylivainio et al. 2008). Also, inorganic fertiliser is said to have no ability to improve soil physical condition (Ferrerias et al. 2006), while the accumulation of hazardous trace elements such as cadmium (Cd), lead (Pb) and arsenic (As) in croplands due to long-term mineral P fertiliser application have been observed (Jiao et al. 2012). Because of the above mentioned problems with the excessive use of inorganic P fertilisers in agriculture the use of inorganic fertilisers has gained popularity in recent years as an alternative to conventional inorganic fertilisers. However organic fertiliser, such as RO need to be further studied in order to make a significant contribution to available plant P in soils and soil fertility, which is the objective of this research work. Therefore, the use of organic wastes is considered as a good alternative to inorganic fertiliser where organic P resources are available and economic. This needs to be widely explored and developed in restoring soil nutrients as well as reducing the amount of solid wastes generated.

The use of organic wastes for p supply is a good sustainability practice, provided it is safe to do so in terms of environmental health and safety associated with the wastes. There are numerous types of organic amendment mainly from organic wastes that have been recycled. These are known as recycled organics (RO) and they are being used as soil amendments in recent agricultural practices. These RO have the potential to improve soil quality and structure, increase organic carbon (C) storage in soil as well as increasing soil nutrients such as nitrogen (N) and P. A large number of studies have focused on the

benefit of RO-amended in soils as a nutrient source for plants. However, most of the studies only focused on the benefit and fate of N in soil whereas the impacts of RO on soil P availability and its cycling dynamics have been much less understood.(O'Connor et al. 2004; Wu et al. 2007; Vadas et al. 2007). Despite organic amendments being reported to have more benefits than inorganic fertiliser applications, continuous application of organic materials can result in the increase of soluble P concentration thus increasing the potential of P mobility in the form of organic P, thus negatively affecting water quality of receiving environments. Runoff transport of P from agriculture lands contribute to the eutrophication in surface water bodies (Anderson et al. 2002). It is therefore important to conduct research on the P fractions to understand its dynamics in soils amended with RO, to optimise the use of RO, and minimise the loss of P from soils to water bodies.

The P in soil occurs in multiple pools or forms and fractions which differ in their availability for plant uptake. Sequential P fractionation (Hedley et al. 1982; Chen et al. 2003) have been used to separate different P pools. Fractionation method, based on chemical extraction of increasing power of extractants, is an important method to characterise the P pools, to understand the transformation of P forms in soils affected by the amendment with RO. The transformation of P pools in soils usually occurs from a combination of physicochemical and biochemical processes which can also be mediated by microorganisms, and organic material (Marschner et al. 2011). Therefore, the microbial biomass content and microbial activity are believed to be sensitive and reliable indicators that can be used to monitor the effects of the RO amendment on soil health. To date, little work has been done on P fractions and availability as affected by the RO and associated soil microbial properties.

Although various studies suggest potential benefits to plant and soil from the utilisation of RO, but their application rates in agriculture are commonly based on

required nitrogen (N) rates and not (P). This often results in concomitantly and unnecessarily high application rates of P which eventually leads to P runoff into water bodies (Eghball and Gilley 1999; Sharpley and Moyer 2000). Therefore, studies on P-based application rates of RO in agriculture are very much needed. Also, information is scarce on the simultaneous influences of type and application rate of RO applied on the soil P fractions and microbial properties. Additionally, optimal use of RO in agroecosystem for both improved P sustainability and environmental benefits will require the understanding of the chemical and biological mechanisms responsible for changes in soil P dynamics, associated microbial processes and plant P uptake following RO application particularly in the rhizosphere soil.

This research focuses on the utilisation of RO as soil amendments and sources of P nutrients. The aim of this study is to examine the effect of various RO on soil P availability and dynamics as well as associated soil microbial properties in soils with initially low pH and low available P.

1.1.1 Research Questions, Hypotheses and Objectives

The general framework of this research is depicted in Figure 1.1, which covers the research questions, objectives, hypotheses and chapters of this research.

1.1.2 Research framework

The response of RO as soil amendment in field and in laboratory have to be determined as a key to understanding the dynamics of P and microbial properties in RO-amended soil, thus we hypothesize that:

- a) The current method of using compost as amendment are more suitable compared to the conventional treatment.

- b) The RO amendment can change and improve P fractions and microbial properties.
- c) The rhizosphere might have affect the P dynamics and microbial biomass response differently.

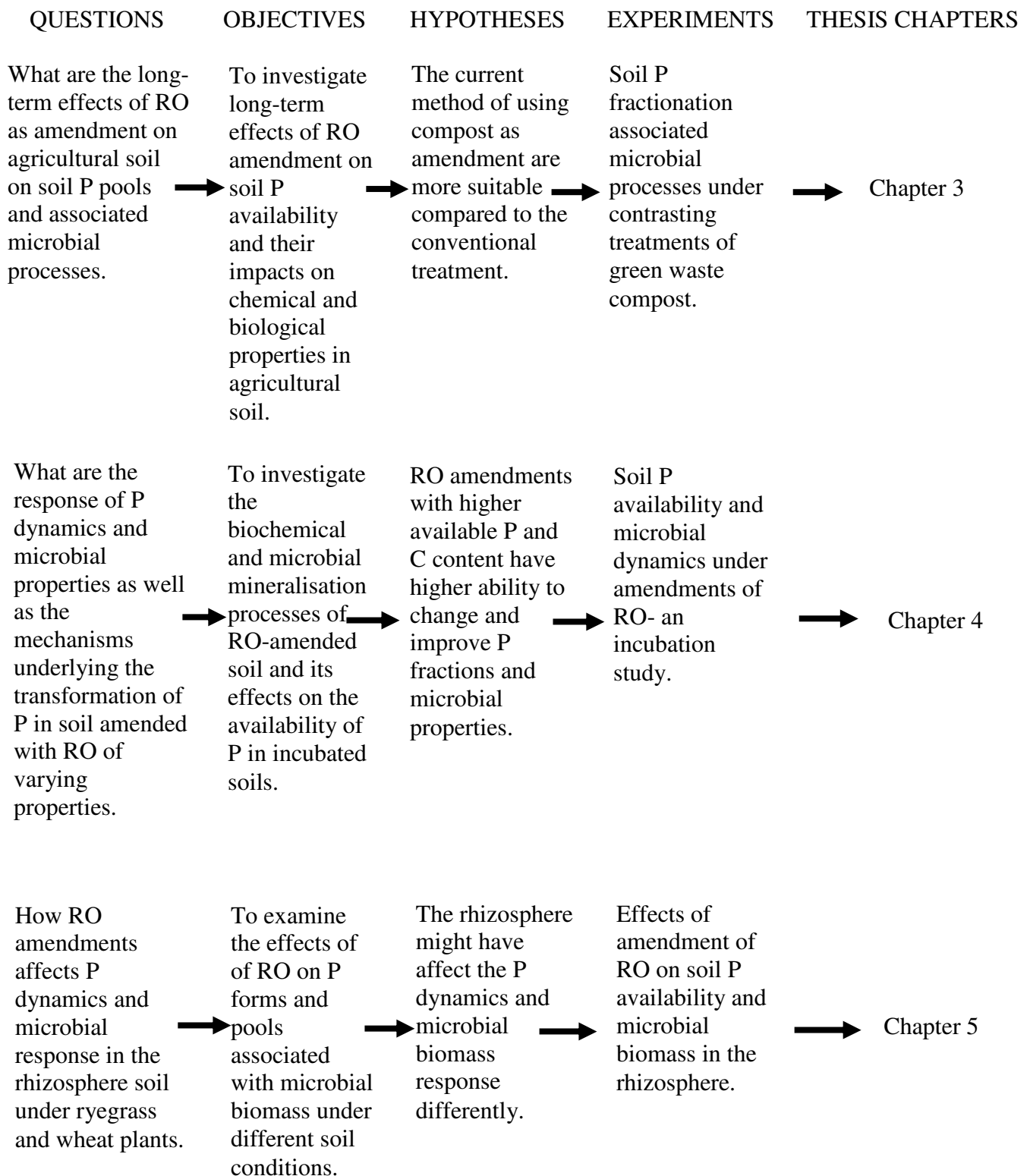


Figure 1.1 General framework of the research which covers the research questions, objectives, hypotheses and chapters of the research.

1.2 Phosphorus in soil

Soil P can be divided into two forms, organic and inorganic (Bünemann 2015). Inorganic P (Pi) is usually associated with iron (Fe) and aluminium (Al) and calcium (Ca) compounds, while organic P (Po) includes readily available undecomposed organic residues including microbes (Frossard et al. 1995; Sharpley 1995). The P in soil also can be further classified into three pools that vary in their availability to plants. They include labile P (P as phosphate in the soil solution and available to plants), moderately labile P (moderately available to plants, P that moves in and out of solution depending on soil pH, temperature, moisture and removal by plant roots), and stable (recalcitrant) P (highly insoluble minerals and organic P that are not available to plants) (Damon et al. 2014; Bünemann 2015). Despite the fact that vast quantities of total P can be present in soils, only a small portion of it is immediately available to plants (Richardson 1994) and this is in the orthophosphate forms of H_2PO_4^- or HPO_4^{2-} (hydrogen phosphate).

1.2.1 Soil organic phosphorus

Organic P (Po) is broadly defined as the pool of P associated with organic compounds or materials either structurally or nonstructurally. Pools of Po consist of crop residues, organic matter and microbial biomass. The content of Po varies among soils, but it can represent up to 80% of total soil P (Dalal 1977). The general fate of P on agroecosystems were illustrated in Figure 1.2. In Australian soils, the amount of Po ranges from 40 to 900 mg kg^{-1} (Stevenson and Cole 1999). Soil Po may include phosphate esters, phosphonates and phosphoric acid anhydrides, based on the nature of the phosphorus bond (Turner et al. 2005). Most of the soil Po is stabilised by association with mineral components, because negatively charged Po compounds attach to minerals such as aluminosilicates (clay) and aluminium oxides or hydrous iron (Doolette et al. 2011). The Po can also be transferred from soil to water bodies and become a source of nutrition for aquatic

organisms (Whitton et al, 1991). The Po derived from soil organic matter, plant residues and soil microorganisms becomes available to plants after Po is converted to available Pi (inorganic P) through hydrolysis and mineralisation processes (Frossard et al. 1995). Phosphatase excreted by plants and microorganisms is also found to mediate the mineralisation of Po (Tian et al. 2016). The rate of P release from the Po forms depends partly on the specific Po compounds present where different pools of Po such as crop residues, native organic matter, and microbial biomass have different mineralisation rates and therefore supply Pi at different rates (McLaughlin et al. 1988).

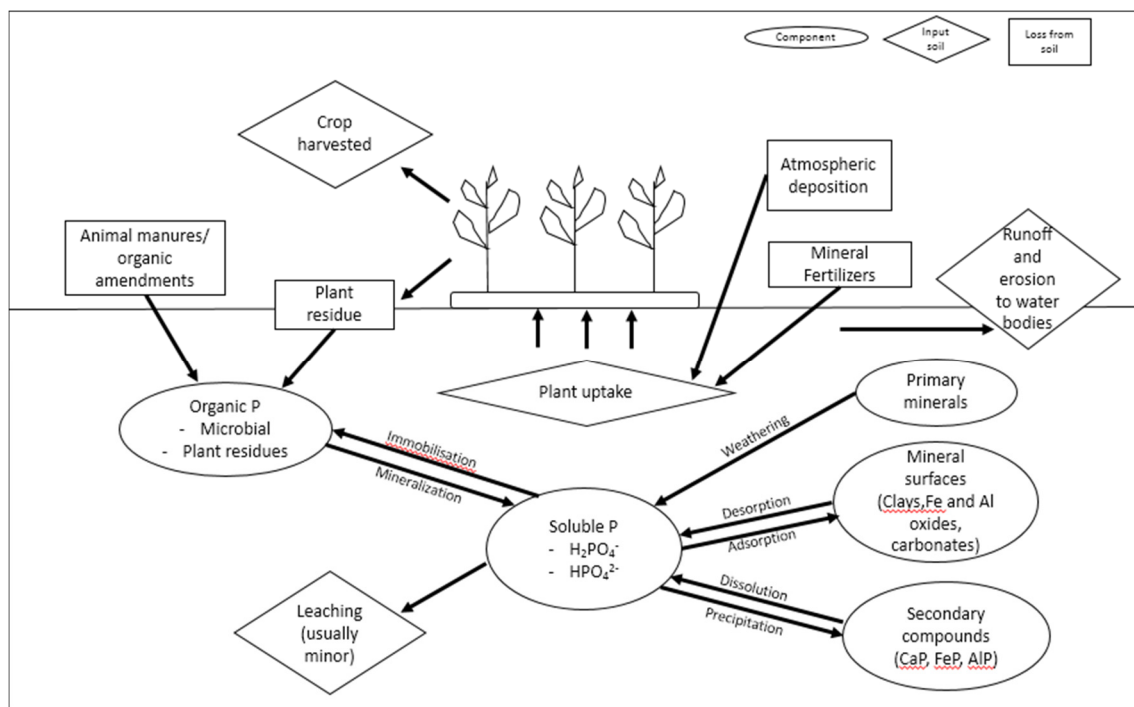


Figure 1.2 The general fate of P on agroecosystems.

1.2.2 Soil inorganic phosphorus (Pi)

Inorganic P (Pi) occurs in soils in variable forms such as mineral, adsorbed and precipitated. The Pi is also known as a pool of chemical compounds which cannot be derived immediately from living organisms which can occur in both soluble and insoluble forms (Damon et al. 2014). The main source of Pi in agricultural ecosystems is mineral P fertiliser, usually from rock-derived P-fertilisers. The solubility of the most abundant

form of Pi, orthophosphate, is determined by the pH and the cation (e.g. Ca^{2+} , Mg^{2+} , Fe^{2+} , Fe^{3+} , and Al^{3+}) in soil (Damon et al. 2014). The solubility of Fe and Al phosphates increases, while the solubility of Mg and Ca phosphate decreases, with increasing soil pH except for pH values more than 8 (Hinsinger, 2001). The variation in the quantities of Pi found in the various pools in different soils can be attributed to a combination of factors including parent material, original vegetation cover and fertiliser history (Chen et al. 2003).

1.2.3 Soil P availability and cycling

Only a small proportion in total soil P is immediately available to plant roots despite the large amounts of total P present in soils (Richardson, 1994) due to the P reaction with the soil matrix (Chen et al. 2003). Phosphorus can be released into soil solution and become available to plants by desorption or dissolution of Pi associated with the solid phase of the soil or by the mineralisation of Po (Tiessen and Moir 1993). Sharpley (1995) found that microbial P plays an important role in short-term dynamics of the mineralisation of Po and also the management of soil P availability. The Po is mineralised to Pi by different groups of microorganisms in the environment (Chen et al. 2000), immobilisation is the reverse reaction of mineralisation where it forms more stable organic P from Pi for example during the immobilisation process, microorganisms convert Pi to Po which will be incorporated into their living cells (Mackey and Paytan 2009). However, there are many factors affecting the mineralisation and immobilisation processes of Po. Soil pH is one of the main properties controlling the mineralisation and immobilisation process as well as the availability of Al, Fe, and Ca in soil solution for P fixation (Liu and Chen 2008). In acid soils, Al and Fe dominate P fixation process, while Ca fix P in alkaline soils. As a result, the P availability is greatest at soil pH values of between 6 and 7. However, for the mineralisation process, the rate depends partly on the specific existing

Po compounds, as different pools of Po such as crop residues, native organic matter, and microbial biomass have different mineralisation rates and therefore supply Pi at different rates (McLaughlin et al. 1988).

The removed P from solution by root absorption needs to be replenished in order for plants to continue receiving the P supply. In response to P removal by plant roots, the concentration of orthophosphate ions is maintained by the replenishment from the soil solid phase. The supply of P to the plants will depend on the ability of the soil to replenish the P concentration in solution, and the replenishment ability depends on the quantity of P in the source (the labile pool) and the P absorption or buffering capacity of the soil.

The major processes of the P cycle are illustrated in Figure 1.3 which is adapted from Stevenson and Cole (1999). The immobilisation, mineralisation and redistribution of P in soil depends on soil physical and chemical properties, such as P sorption by colloidal surfaces, as well as microbial, mycorrhizal or plant uptake of P. A portion of soluble Pi will be precipitated as secondary P minerals and eventually will be converted to stable (unavailable) forms in more weathered soils (Mackey and Paytan 2009).

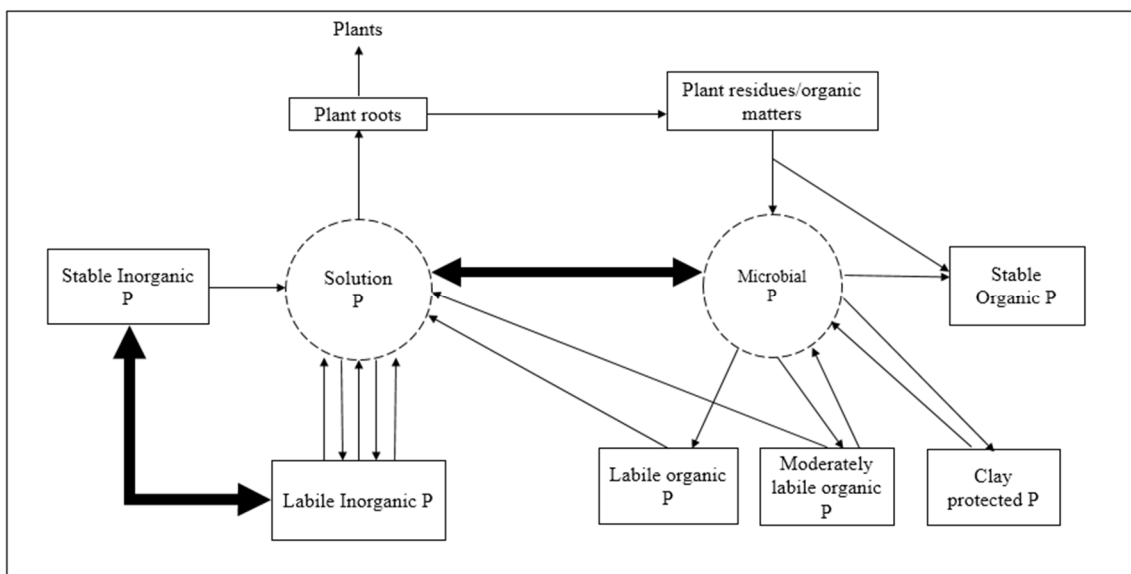


Figure 1.3 The P cycle in soil with different organic and inorganic forms of P in different pools based on their availability to plants. The soil solution pools and microbial biomass pool containing both Pi and Po can fluctuate in size as indicated by dashed borders. The physicochemical sorption/desorption processes including precipitation/dissolution

processes are indicated by thin arrow. Immobilization/remineralization processes are indicated by thick arrow.

1.2.4 Soil P characterisation

There are numerous extraction methods to characterise various forms of P in soil. Extraction methods aim to characterise soil P into different pools (Pi and Po) that respond similarly to the biochemical change of P pools in the soil environment. Single extractions are used to give an estimate of a certain pool of P while sequential extractions aim to characterise P in more detail and separate it into pools with different chemical properties.

The characterisation of soil Po can be estimated by ignition of organic matter or by using extractants that are known to efficiently extract soil organic matter. The Po is calculated as a difference between total P (TP) and Pi in the extract. The ignition method developed by Saunders and Williams (1955) and later modified by Walker and Adams (1958) are found to overestimate the concentration of total Po (TPo) in soil due to the increased extractability of Pi at temperatures above 160°C (Williams et al. 1970). In recent years, the Nuclear Magnetic Resonance (NMR) spectroscopy or ³¹P-NMR analysis provides more detailed descriptions of Po forms in soil after decades of Po values being calculated using the difference between total P and Pi in soil. However, the NMR spectroscopy often used in soil analysis still requires extraction of soil P into solution. The advantage of NMR spectroscopy is its ability to measure Po directly from an extract. Also, NMR spectroscopy allowed screening of Po forms in different types of soil and ecosystem which is useful for monitoring transformations due to land use changes in different environment (Cardoso et al. 2003) as well as studying effect of temperature and precipitation gradients on soil Po (Turrión et al. 2008). However the NMR method has some disadvantages as well, one being the high pH condition required for this method can result in hydrolysis of some labile Po species (Lienweber et al. 1997). Also, the NMR method is expensive to perform on a large number of samples.

In order to characterise the soil Pi in detail, Chang and Jackson (1957) developed a sequential fractionation procedure based on the ability of selected extractants to separate P in different pools. The method distinguishes different Pi pools according to the surface the P is bound to. The chemical extraction is used to divide soil Pi into labile P using ammonium chloride extract (NH_4Cl), aluminium-bound P using ammonium fluoride (NH_4F), iron-bound P using sodium hydroxide extract (NaOH), calcium-bound P using hydrochloric acid (HCl), reductant-soluble P using sodium dithionite citrate extract ($\text{Na}_2\text{S}_2\text{O}_4$ -citrate) and refractory P using NaOH extract. However, Williams et al. (1967) discovered that the Chang and Jackson fractionation procedure was not suitable to be applied on calcareous soils. Because of this problem, the Chang and Jackson fractionation procedure was further modified on calcareous soils (William et al. (1967). Another widely used fractionation procedure was developed by Hedley et al. (1982). Their method aims to categorise P into pools based on biological availability. This extraction procedure uses a series of chemical extractants to sequentially remove P in various operationally defined pools. Mild extractants such as deionised water and sodium bicarbonate (NaHCO_3) are used to remove the more labile P fractions, followed by stronger extractants such as sodium hydroxide (NaOH) and HCl to remove less labile fractions. Water extractable P in manure has been found to be a good indicator of P loss by runoff from manure applied soils (Sharpley and Moyer 2000). The NaOH also is one of the common chemicals used for extracting soil organic matter. Phosphorus in the extracts is then analysed for inorganic and organic P forms. In recent years, the sequential fractionation scheme developed by Hedley et al. (1982) has been modified and adapted for characterising organic amendments (Sharpley and Moyer 2000; He and Honeycutt et al. 2001) as well as soil treated with organic and inorganic amendments (Ippolito et al. 2007; Waldrip et al. 2009). The modified P fractionation scheme has been classified as H_2O - or resin-, NaHCO_3 -, NaOH - and HCl - or H_2SO_4 - extracted P as well as residual-P (Chen et al. 2000;

Ajiboye et al 2004). The P_o in each extractant is estimated by the difference between TP and P_i . Although the P fraction method has its own limitations such as it is very time consuming and high complexity, the P fractionation procedure is a cheap method and could provide rapid estimation of P solubilities and labilities. Also, the fractionation method extracts P forms from labile until non-labile P forms and the information obtained from P fractionation is useful for managing P in agricultural lands, maximizing soil productivity, and minimizing the environmental impacts of this chemical (Condrón and Newman, 2011; Dodd et al. 2013). The P fractionation method also helps in interpreting the direction of soil P distribution. For instance, the method provides the information on P status such as whether P_i has been immobilized into organic forms or P_o has been mineralized into plant-available forms. Such information is useful in evaluating the effects of RO addition such as P dynamics in soil and thus answering the research questions outlined in Figure 1.2.

1.3 Application of Recycled Organics (RO)

Due to the depletion of mineral phosphate reserves and the sustainable utilisation of this non-renewable materials, the use of RO as a source of fertiliser has markedly increased in many parts of the world in recent years (Grigatti et al. 2004). The RO products comprise of a broad range of composted and non-composted organic wastes from agricultural, industrial, and municipal operations (Queensland EPA 2002; Quilty and Cattle 2011).

The application of the RO in soil could: (i) improve crop growth and yield by supplying nutrients; (ii) control agricultural pests and diseases; (iii) improve soil structure via enhancement of the C sink in soil, and; (iv) reduce the amount of organic waste ending up in landfills or get incinerated thus reducing the emission of greenhouse gasses such as methane (CH_4), carbon dioxide (CO_2), and nitrogen oxides (NO_x) into the atmosphere

(Rondon et al. 2005; Zhang et al. 2010). The incorporation of RO products into agricultural soils has also increased rapidly as there is increasing consumer demand for organic produced agricultural products in recent years. The use of RO has resulted in economic and environmental benefits even though these organic amendments need to be certified prior to its application in organic farming. This is because the amendments may not meet the criteria of the certifying bodies, such as the National Association of Sustainable Agriculture Australia (NASAA 2008). However, the sewage sludge or biosolids cannot be used in organic farming because they do not meet the criteria of the certifying bodies such as National Association of Sustainable Agriculture Australia (NASAA 2008).

The RO containing essential macro and micronutrients have also been used in forest soils in many countries including United States and New Zealand (Henry et al. 1993; USEPA, 1997; Magesan and Wang, 2003). According to USEPA (1997), the sewage sludges or biosolids contain organic matter that can improve the condition of forest soils by increasing the aggregation and macropores in fine-textured clayey soil and the water-holding capacity Sewage sludge can be applied in all stages of forest plantation, from recently cleared land prior to planting, to newly established plantations (about 3 to 10 years old) and fully established forests (USEPA 1997). In addition, research by Magesan and Wang (2003) on the application of municipal and industrial residuals in New Zealand radiate pine forest found that biosolid treatments significantly enhanced tree growth and resulted in increased economic return (Kimberley et al. 2002).

1.3.1 Recycled organics production

Organic materials such as food waste, paper and cardboard, and yard trimmings are the largest component of municipal solid wastes (MSW) (USEPA 2012). In Asian countries, around 70% of the total wastes generated is organic while it is around 65% in European

countries (IPCC 2006). In the United States, around 87 million tonnes of total wastes were generated and only 75% of MSW were recovered, recycled and composted (USEPA 2012). In Australia, Recycled Organic Unit (2010) reported that in the year 2010 to 2011, around 14 million tonnes (Mt) of organic waste (excluding paper and cardboard and primary production waste) was generated and it was estimated that around 12 Mt of organic waste was recovered nationally. The sources of RO in Australia mainly consist of green waste from parks, gardens and orchards, residues from food production and consumables, residual wood and timber, sewage sludge from wastewater treatment plants or biosolids, manures, plant residues and other wastes from organic materials (Quilty and Cattle 2011).

In Queensland, the reported total quantity of compostable organic elements that have been processed into profitable RO products has increased by around 17% from 2010 to 2011, with a total of 1,997,339 tonnes of raw materials processed in 2011 compared to 1,702,335 tonnes in 2010 (Recycled Organics Unit 2012). Selected wastes handled by organic processors by region in 2014-2015 in Queensland is given in Table 1.1 (Department of Environment and Heritage Protection (Queensland), 2016). All types of organic wastes, such as grease trap, sewage sludges, manures and other agricultural residuals are processed using the aerobic windrow composting (hot composting) method. Also in Queensland, the RO products such as biosolids are treated by chemical treatments or digestion in wastewater treatment plants and can be applied directly to land (Recycled Organics Unit 2012). Guidelines for land application of biosolid products in Queensland (Recycled Organics Unit 2012) is the same as those in New South Wales guidelines (Queensland EPA 2000).

Table 1.1 Selected wastes handled by organic processors by region during 2014-2015 (tonnes).

	Green waste	Forestry residuals	Agricultural residuals	Food waste	Biosolids	Grease trap and other organic sludges	Ash	Drilling mud
South East Queensland	247345	128636	100913	56480	111	92712	39776	1251
Darling Downs-Maranoa	4587	-	106577	40	65542	5374	324	35920
Wide Bay	68184	34927	24127	100	450	-	250	-
Fitzroy	50152	-	17108	782	930	7192	2706	8834
Mackay	7591	-	-	-	-	-	-	-
Townsville	10060	-	9655	-	-	2400	250	1500
Cairns	19000	-	1	-	1700	902	-	-
Remote Queensland	1510	-	13426	1981	-	-	-	-

According to LAWMAC (1995), typical domestic wastes in Queensland are usually recovered at source and does not enter the waste stream. The chemical composition of the organic materials varies with the origin and source of the waste materials and also depending on location and climate (Queensland EPA 2002). The contamination factor is of concern in recycling the green and organic wastes. Green wastes may be contaminated with glass, plastic and steel which should be processed (shredded, screened), prior to further use. Usually, green wastes will be composted before they can be used as a soil amendment to destroy weed seeds and pathogens (Standards Australia, 2003). Organic wastes such as biosolids, which contain pathogenic organisms, pesticides and heavy metals, are also not suitable as stockfeed. Such material can only be used in land amendments provided that the pathogen and contaminant concentrations are within regulatory requirements.

Organic waste materials are required to be pasteurised and converted microbially under thermophilic and aerobic conditions in the composting process for a period not less than six weeks (Standards Australia, 2003). The final products are stabilised materials with high carbon content and are free from pathogens and weed seeds (Department of Environmental Conservation (NSW) 2005). In Australia, the quality of compost is defined by an Australian Standard 4454-2012 (Standards Australia, 2012) for compost, soil conditioners and mulches, which is based on specified properties such as contaminant limits, maturity and stability criteria and physical properties for instance, moisture content, bulk density and particle size distribution.

1.3.2 Recycled Organics and Soil Phosphorus

1.3.2.1 Effects of recycled organics on soil P dynamics

Various studies have shown that the RO application increases concentrations of soil total P as well as specific P forms including soluble P and stable P in soil (Erich et al. 2002, Waldrip et al. 2009). Also, the application of RO may decrease P fixation, and improve P availability (Ayaga et al. 2006). Studies of the effects on soil P following RO applications have been reported mostly in comparison with inorganic fertilisers (Sugito et al. 2010; Wickramatilake et al. 2010) or a combination of RO and inorganic fertiliser (Bulluck III et al. 2002). An incubation study by Malik et al. (2012), found that all treatments (plant residue, composts and inorganic fertiliser) increased total P but microbial activity only increased in soil amended with RO leading to greater formation of Po pool in the soil. Studies by Osborne (1995) and He et al. (2010) found that the biosolids they used contained more inorganic P forms than organic P forms. This finding is supported by Pritchard (2005) who found that biosolids from Beenyup wastewater treatment plant (WWTP), contained 92% of inorganic forms of P and only 8% of P in organic forms.

Effects of RO on soil P availability depend on the type, composition and source of RO. For instance, the contents of P in manures are reported as depending on differences in animal diets (Dou et al. 2000; Ajiboye et al. 2004). The contents of Pi and Po pools in biosolid-amended soils were dependent on the biosolid treatment processes (Barnett 1994; Sharpley and Moyer 2000; and Ebeling et al. 2002). The responses of soils/plants to different types, rates of application, composition and sources of RO as well the interactions of these factors on soil P availability and the role of RO in soil P transformation needs to be further studied due to the limited knowledge on such interactions.

Numerous studies examining soil amendment by RO (compost and manures) have been based on plant-required N applications while at the same time such amendment also increases plant P availability in soils (Eghball and Gilley 1999; Sharpley and Moyer 2000). However, the RO applications based on high N requirements may contribute to soil P accumulation above the levels needed for optimum crop growth, which may increase the risk of P export to water body and environmental contamination (Erich et al. 2002; Maguire et al. 2000). Therefore, it is important to study the effects of different RO using P-based application rates to understand the potential of different types and rates of RO in supplying nutrient P, while at the same time, minimizing the risk of P runoff or leaching into surrounding environments such as rivers and lakes. Although there have been several studies on P availability in RO-amended soil using P based application rates, most of such studies have used manure-based RO and there are very limited studies on the effect of P forms and availability associated with microbial biomass and its activity (Pagliari et al. 2010; Tlustoš et al. 2016).

1.3.2.2 Effects of recycled organics on soil microbial biomass

Soil microbial biomass, as a source and sink of available P, plays a critical role in the transformation of soil Po (Dalal 1977, Damon et al. 2014). Unlike inorganic fertilisers, most RO contain nutrients in organic molecular structures which means they are not readily available for plants uptake (Jeng et al. 2006). Thus, the organic molecules need to be mineralised by soil microorganisms before they become available to plants (Frossard et al. 1995, Oehl et al. 2004). Organic amendments improve soil available P and biological fertility via microbial growth and increase the process of the mineralisation of Po in soil. The microbial biomass C (MBC) content can be used more effectively than the total organic C (TOC) content as an indicator of variations in soil fertility since it responds more rapidly and with a greater degree of sensitivity to changes of carbon in soil (Garcia

2000). Therefore, short-term measurements of microbial biomass can reflect the long-term effects of the organic matter on soil mineralisation (Plaza et al. 2004). The growth of microbial biomass is proportional to the amount of C in organic amendments since C-substrate availability is the primary factor limiting microbial activity in soil (Bünemann et al. 2004).

The increase in soil biological abundance and activity, due to the presence of higher levels of organic matter in the soil, positively influences soil structure and nutrient cycling. In most agricultural soils, the amount of microbial biomass P (MBP) varies between 1 and 10% of total soil P (Richardson 1994). The Po in microbial biomass consists of nucleic acids, inositol phosphates, and polyphosphates (Condrón et al. 2005). The MBP is potentially available for plants via microbial turnover to supply inorganic P to plant roots (Chen et al. 2008). Soil microorganisms are directly involved in mineralisation of Po due to their demand for growth and for C to provide energy (Bünemann 2015). The high soil microbial C:P ratio could indicate the P immobilisation in soil. Microorganisms and plants excrete phosphatase enzymes to mediate the mineralisation process and this phosphatase excretion causes hydrolysis of Po. The mineralisation process is intensified under low Pi availability (Bünemann et al. 2004, Chen et al. 2008). On the other hand, it is known that immobilisation of P is influenced by the growth-death cycle of microorganisms. The immobilisation occurs when the microbial C:P is high (Oehl et al. 2004; Damon et al. 2014).

There are number of studies on the effects of RO on microbial properties. A study by Khan and Joergensen (2009) on the effects of inorganic fertiliser combined with biosolids found that biosolids increased the MBP, stimulated the synthesis of soil microbial biomass and released labile P during microbial turnover. Similarly studies by Reddy et al. (2005) and Ayaga et al. (2006) compared this to fertiliser application without the combination of RO and found that the RO were responsible for stimulating microbial

biomass growth and activities thus influencing P mineralisation and immobilisation processes. However, most of the studies did not look at the P forms and availability and its interaction with the soil microbial properties. Understanding the microbial processes and mechanisms associated with P availability following the addition of different types of RO can provide insight into the soil P cycle and the potential to manipulate the utilisation of RO for better organic P fertiliser management.

1.3.2.3 Effects of recycled organics on plant P uptake and the rhizosphere

An important aspect of the RO addition to soil is that it can provide nutrients such as available P for plant uptake and maintain crop yields at the same level as inorganic fertilisers in addition to other soil physical, chemical and biological benefits. A study carried out by Waldrip et al. (2011) on ryegrass P uptake following poultry manure amendment found increased total P uptake via the stimulation of root phosphatase enzyme and microbial production, while a study by Ghosh et al. (2008) found that organic amendments including cattle manure, composted gin trash and liquefied vermicompost increased nutrient uptake by mature cotton plants. Study done by Brechin and McDonald (1994) study on barley yield found that the nutrient uptake increased following the amendment of swine manure in slurry form compared to the solid form. All these studies showed that the effect of RO on plant P uptake differs depending on the RO type and composition as well as plant types.

According to Richardson (2001), in order to increase P uptake from the soil solution, plants have developed mechanisms which include root morphological adaptations and varying kinetics of P uptake by the roots. The rhizosphere, according to Bais et al (2006) and Kamaludeen and Ramasamy (2008), is the soil area that is surrounded by plant roots, contains abundance of microbial communities and activity due to the presence of plant exudates and rhizodeposits. Plant roots can influence rhizosphere

nutrient cycling by nutrient uptake, rhizodeposition and interactions with microorganism (Marschner et al. 2006). Chen et al. (2002) also noted that the P availability for plant uptake is influenced by root-soil interactions in the rhizosphere that include the rate of diffusion of phosphate anions and the rate of its replenishment. Also, organic acid exudations by plant roots and microorganism in the rhizosphere may solubilise nutrient ions which have been immobilised in soil particles (Landeweert et al. 2001). Organic acid exudation from roots varies both quantitatively and qualitatively depending on genotype, distance from the root tip and environmental factors. Also, organic acids may be derived from organic sources such as microbial and plant sources (Richardson 2001). Therefore, the study of P availability in the rhizosphere in soils amended with RO is important as this causes various interactions beneficial to plant health and growth such as nutrient uptake in general and P uptake in particular.

Microorganisms in the rhizosphere and bulk soil play a critical role in mediating the availability of soil P to plants and maintaining plant growth especially in soil with low available P (Phillips and Fahey 2007). Microorganisms may directly affect P solubilisation and mineralisation and have effects on root structure and function or immobilise readily available sources of P making them unavailable to plants (Oehl et al. 2001; Richardson et al. 2011). The microbial P is a dynamic component of the soil P cycle and can be influenced by soil fertility status, seasonal factors and management practices (Stewart and Tiessen, 1987; Mackey and Paytan, 2009). Most studies have found significant changes in soil microbial communities following the addition of organic or inorganic amendments (Marschner 2003). Study done by Nziguheba et al. (1998) and Malik et al. (2012) found that soil microorganisms are capable of rapidly immobilising significant amounts of P following the application of organic or inorganic amendments.

Studies have been done on processes affecting nutrients in the rhizosphere under controlled or natural conditions by Chen et al. (2002), Zhao et al. (2010), Waldrip et al.

(2011) and Marschner et al. (2011). However, the relationship between soil microorganisms such as microbial P and C with plants and its role on soil P availability is still poorly understood, particularly for rhizosphere organisms that are in close association with plant roots. However, there are very limited studies on soil P availability associated with microbial biomass in rhizosphere soils as affected by RO applications (Waldrip et al. 2011; Alvarez et al. 2012). As most investigations have been conducted at a bulk soil scale (Nziguheba et al. 1998; Malik et al. 2012), little information is available on smaller scale rhizosphere effects on P pools transformation and microbial organisms.

1.4 Environmental Concerns

To achieve beneficial effects from the RO applications, it is important to have stringent quality control on the RO materials to be applied, so that these amendments do not deliver potentially harmful materials to soil. According to the EU Commission, the main limiting factors of amendment applications to soils are related to the eventual addition of organic and/or inorganic pollutants and microbial contaminants (Pedra et al. 2007).

Soil application of RO or organic wastes and effluents of any type requires these materials to be treated appropriately prior to application. Most common treatments of organic wastes include aerobic and anaerobic digestions, various fermentation processes and composting (Quilty and Cattle, 2011). These processes can destroy pathogens and phytotoxic organic substances originally present in the organic wastes, as well as reducing or, possibly, eliminating polluting and/or phytotoxic trace metals and organic contaminants (Senesi et al. 2007). The use of RO has raised some concern with respect to eutrophication problems, as high RO application rates and multiple applications could lead to increases in organic forms of P and Po mobility and loss in soil runoff and seepages. The use of municipal waste such as biosolids in agriculture may also cause an increase in the concentration of heavy metals such as Cd and Cu in treated soils which

could present risks to human and animal health if they enter the food chain (Misra et al. 2009).

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Chapter 2

Materials and Methods

Every “result” chapter of this thesis is prepared in a journal paper format with its own specific materials and methods section. This chapter presents general methods and materials applicable to the entire thesis and not specific to any particular chapter.

2.1 Soils used in the study

Soils for the experiments were collected from two different locations in Australia. The two soils comprised a sandy loam and a loam with low pH values of 4.5 and 5.2 respectively. The soils were selected due to their low available phosphorus (P) contents, as plants growth on these soils varies and likely to be limited by soil P availability. The low P availability is due to the high P fixation in soil (Shen et al. 2011). The Colwell P value for sandy loam soil was $4 \mu\text{g g}^{-1}$ and for loam $29 \mu\text{g g}^{-1}$. The sandy loam soil was collected from Griffith University, Nathan Campus, Queensland. The soil is classified as a Podsol (Isbell 1996). The loam soil was obtained from the Centre for Recycled Organics in Agriculture (CROA) in New South Wales (NSW) and classified as Chromosol/Dermosol inter-grade (Isbell 1996). Further properties of the two soils are listed in Table 4.1 (in Chapter 4) and the methods used to determine these properties are shown in section 2.2.1.

2.2 Recycled organics (RO) used in the study

Recycled organics (RO) comprise a broad range of composted and non-composted organic wastes from agricultural, industrial, and municipal operations (EPA 2002, Quilty and Cattle 2011). The wastes are usually pasteurised and composted, using various techniques such as thermophilic and mesophylic aerobic treatments, prior to being used as RO.

An initial screening was carried out to select a suitable range of RO for the experiments. Twenty-two different RO were therefore collected from NSW and Queensland. Some of the organic amendments were supplied by Centre for Recycled Organics in Agriculture (CROA) in New South Wales (NSW), while others were collected around the state of Queensland. The 22 collected RO were then characterised as explained in the following section 2.1. The selected RO for this study were the common organic wastes that can be easily obtained by farmers throughout Australia, particularly in Queensland. For the incubation and pot experiments (Chapter 4 and 5), five RO were subsequently selected from the list of 22 RO, based on their wide range of the C: P ratio.

All 22 RO were air dried at room temperature, ground with soil grinder and passed through a 2 mm sieve before being analysed for their basic properties such as pH, EC, total carbon (C), total nitrogen (N), and Colwell P as follows:

2.2.1 Chemical Analyses used for RO and soils

2.2.1.1 Electrical conductivity EC and pH (1:5 water)

A 5 g sample of air-dried RO or soil was dispersed in 25 ml of deionised water, shaken for 1 hour, and its pH and EC measured using a pH meter and EC probe (Chen et al. 2003).

2.2.1.2 Organic P- Ignition method

The organic P content was measured by extraction before and after ignition as proposed by Saunders and Williams (1955). Soil samples (0.5 g) were ignited in a furnace at 550°C for 1 hour or left at air temperature (un-ignited). The ignited and un-ignited samples were then extracted with 50 mL of 0.5 M H₂SO₄ for 16 hours. The samples were subsequently centrifuged for 15 minutes before being filtered through Whatman 42 filter paper. An aliquot of samples were finally analysed colorimetrically.

2.2.1.3 Total P- Nitric-perchloric acid digestion method

Finely ground samples were weighed onto weighing paper to 4 or 5 decimal places (1.0 g for soil samples and 0.5 g for roots, shoots and RO samples) and transferred to labeled digestion tubes. Then 8 mL of concentrated nitric acid was added to each tube and the tubes were left overnight in the fume cupboard to allow for partial breakdown of organic materials in the samples. Concentrated perchloric acid (2 mL) was then added to each tube and the samples were digested for 5.5 hours. The remaining acid in the digestion tubes was diluted with distilled water and the sample mixed well by vortex. The diluted acid solution was then transferred into a 50 mL volumetric flask and made to volume with distilled water. The solution was filtered through a Whatman 42 paper and extracts were collected in a clean 70 mL plastic container for colorimetric analysis.

2.2.1.4 Total C and N and stable isotope composition

Total C and N were analysed by Isotope Ratio Mass Spectrometer (GV Isoprime, Manchester UK) with a Eurovector Elemental Analyser inlet (EA 3000, Milan, Italy). Between 1 to 10 mg of RO were packed into a tin capsule. The capsules were then loaded into the auto-sampler and the total C and N, $^{13}\text{C}/^{12}\text{C}$ ratio, and $^{15}\text{N}/^{14}\text{N}$ ratio determined which were then used to calculate the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) as:

$$\delta (\text{‰}) = (R_{\text{sample}}/ R_{\text{standard}} - 1) * 1000$$

Where R_{sample} and R_{standard} are the isotope ratios of the sample and standard respectively.

2.2.1.5 Elemental analysis using Inductively Coupled Plasma (ICP-OES)

Metal elemental analysis was used to determine magnesium (Mg), iron (Fe), aluminium (Al), calcium (Ca) and Copper (Cu) using Inductively Coupled Plasma (ICP). Finely ground (<2mm) RO samples (0.5 g) and soil (1.0 g) samples were treated with 8 mL of concentrated nitric acid, followed by 2 mL of concentrated perchloric acid. The samples

were then digested in a digestion block for 5.5 hours. The remaining acid in the digestion tubes was diluted with distilled water and the sample mixed well by vortex. The diluted acid solution was transferred into a 50 mL volumetric flask and made to volume with distilled water. The solution was filtered through a Whatman 42 paper and extracts were collected in a clean 70 mL Sarstedt plastic container for ICP analysis.

2.2.1.6 Colwell P (Bicarbonate extractable-P)

The method, as proposed by Colwell (1963), was followed for this measurement. Briefly soil and RO samples (<2 mm) were weighed into extracting bottle with the ratio of 1:25 samples to extractant. Extracting solution (0.5 M NaHCO₃) was then added and the bottle was shaken for 16 h at 25°C before being centrifuged and filtered with Whatman No 42-tested P free. Aliquots of supernatant were added into 100 mL volumetric flasks. Then, 2 mL of 1 M H₂SO₄ was added and mixed and after effervescence has ceased, a further 5 mL 1 M H₂SO₄ was added. The samples were left to stand overnight, to complete the removal of CO₂. Samples were then analysed colorimetrically using the molybdate blue method.

The Colwell P analysis for 22 RO samples were found to be challenging as some of the RO were high in P. Therefore samples need to be diluted and analysis needs to be repeated for several times.

2.2.2 Characterisation of the selected RO

The pH of the selected RO ranged from 4.74 to 9.84 (Table 2.1). The poultry manure biochar was the most alkaline and green waste biochar the most acidic RO used for screening purpose. The electrical conductivity (EC) of the RO ranged from 2.14 to 80.30 dS m⁻¹ with the highest EC in green waste biochar and lowest in blood and bone pellet. The percentage of total C ranged from 17 to 65 % with highest in green waste biochar

and lowest in compost consisted of green waste (80%) and chicken manure (20%) (Table 2.1). The percentage of total N ranged from 0.1 to 8.2 %. The bicarbonate extractable P (Colwell P) was highest in aerobically digested biosolids with 18,200 mg P kg⁻¹ whereas the green waste biochar had only 43 mg Colwell P kg⁻¹.

Table 2.1 Basic characteristics of the twenty-two recycled organics used for screening purposes.

No.	Type of recycled organic material	pH	EC (dS m ⁻¹)	Total C (%)	Total N (%)	Colwell P (mg P kg ⁻¹)
1)	Biosolids- Granulated	5.65	4.76	32	4.9	1900
2)	Biosolids- Thermal pyrolysis	7.40	7.85	29	6.5	8600
3)	Biosolids - Aerobically digested	7.11	9.79	30	6.0	18200
4)	Biosolids- Anaerobically and aerobically digested	7.09	5.52	35	6.4	9800
5)	Manure- Chicken	6.32	13.01	31	3.4	2640
6)	Manure- Dairy (cow)	8.27	6.38	36	2.9	6600
7)	Manure- Dairy (cow)	7.15	3.29	39	0.5	1000
8)	Manure- Swine	7.10	7.79	39	3.0	11000
9)	Manure- Broiler litter	7.12	11.91	39	3.9	4200
10)	Compost- Broiler litter	7.16	10.12	36	3.9	4000
11)	Compost- Cow manure	7.86	10.24	29	2.3	5100
12)	Green waste- Compost	6.94	2.93	26	1.3	840
13)	Compost- Nitro-humus	6.43	3.01	24	1.3	1400
14)	Compost- Mix organic wastes	7.04	2.89	20	1.5	3600
15)	Compost- Green waste (80%), Chicken manure (20%)	7.73	3.62	17	1.0	920
16)	Compost- mixed with biosolids	6.64	7.91	31	2.0	640
17)	Blood and bone (Meal)- Pellet	6.90	2.14	36	8.2	1800
18)	Poultry manure- Pellet	6.52	14.77	39	3.9	3200
19)	Chicken manure- Pellet	6.34	15.36	33	3.6	3100
20)	Biochar- Poultry manure	9.84	4.29	37	2.2	6000
21)	Biochar- Green waste biochar	4.74	80.30	65	0.1	43
22)	Biochar- Chicken litter	9.56	12.51	41	4.6	8000

2.2.3 Selection of Recycled Organics for Experiments

From the list of 22 characterised RO presented in Table 2.1, five were selected to be used as amendments in incubation and pot trials as shown in Table 2.2. This selection was based on the diversity of their C to P ratios.

Table 2.2 Selected properties of recycled organics.

Property	Biosolids (BS)	Broiler litter (BL)	Compost (green waste)(CG)	Compost mix (CM)	Biochar (green waste) (BC)
pH	7.40	7.12	6.94	7.04	4.74
EC (dS m ⁻¹)	7.85	11.91	2.93	2.89	80.30
Total C (%)	28.9	39.0	26.0	19.7	65.0
Total N (%)	4.9	3.0	1.3	1.5	0.1
Total P (%)	4.52	1.36	0.22	0.28	0.06
C: N ratio	6	13	20	13	650
C: P ratio	8	29	118	70	1083
Colwell P (mg P kg ⁻¹)	8600	4000	840	3600	43
Total Mg (%)	0.5	0.5	0.3	0.2	0.09
Total Ca (%)	1.2	2.2	1.5	3.9	0.22
Total Fe (mg kg ⁻¹)	8000	1300	11000	189	6500
Total Al (mg kg ⁻¹)	12000	1100	11000	148	1800
Total Cu (mg kg ⁻¹)	830	150	66	180	5.3

2.3 Plants used in the study

Two different types of plant were used in the pot trial whose results are given in Chapter 5. The plants used were Wimmera ryegrass (*Lolium rigidum*) and wheat (*Triticum aestivum*). The seeds were obtained from a seed supply shop in Queensland, Australia. The plants were chosen because they are commonly grown in Australia, easily grown from seed, fast to germinate and fast growing. The ryegrass were chosen because it is

found to be P-efficient based on the high root to shoot ratio and has an external P requirement of only 50 mg P kg⁻¹ (Fohse et al. 1998). Ryegrass also have a potential to remediate high P soils and the yield of ryegrass and the ryegrass P uptake increase with increasing P application rate (Szogi et al. 2010).

2.4 P fractionation for soil samples

For P fractionation, the soil samples (1 g) were extracted by sequential using of different types of extracting solutions according to soil P fractionation schemes described by Hedley et al. (1982) and Condron et al. (1996). The method was, however, modified as described by Chen et al. (2000) by including an analysis of the residual P using the nitric acid (HNO₃)- perchloric acid (HClO₄) digestion (Olsen and Sommers, 1982). The concentration of inorganic P in the extracts was determined after the precipitation of organic matter by acidification, and the concentration of total P was determined after persulphate oxidation, while the concentration of organic P in the extracts was calculated as the difference between total P and inorganic P (Tiessen and Moir 1993).

The modified soil P fractionation method involved the determination of various forms of inorganic (Pi) and organic (Po) soil P by sequential extraction of finely ground (<150 µm) soil with 1 M ammonium chloride (NH₄Cl) for APi, 0.5 M sodium bicarbonate (NaHCO₃) at pH = 8.5 for BPi, BPo, 0.1 M sodium hydroxide (NaOH) for N(I)Pi and N(I)Po, 1 M hydrochloric acid (HCl) for HPi and finally 0.1 M NaOH for N(II)Pi and N(II)Po. The fractions separated by the above procedure generally correspond to the following soil P pools:

- NH₄Cl extractable Pi (APi) – solution Pi;
- NaHCO₃ extractable Pi (BPi) – labile Pi, adsorbed on the surface of more crystalline P compounds, sesquioxides or carbonate;

- First NaOH extractable Pi ((N(I)Pi) – moderately labile Pi, associated with amorphous and some crystalline Al, Fe hydrous oxides;
- HCl extractable Pi (HPi) – less labile Pi, associated with primary calcium minerals (e.g. apatite).
- Second NaOH extractable Pi (N(II)Pi) – moderately stable Pi, which is adsorbed into the mineral structure of soil components, or occluded by Fe and Al coatings.
- NaHCO₃ extractable Po (BPO) – labile Po, easily mineralisable.
- First NaOH extractable Po ((N(I)Po) – moderately labile Po, associated with Fe and Al hydrous oxides;
- Second NaOH extractable Po ((N(II)Po) – stable Po, adsorbed into the mineral structure of soil components, or occluded by Fe and Al coatings.
- Residual P – non-extracted/ recalcitrant Pi and Po.

To obtain the different P fractions, 1 g of soil was sequentially extracted using different types of extractants according to the P fractionation schemes described by Hedley et al. (1982) and Condron et al. (1996). The method was modified by including an analysis of the residual P using the nitric and perchloric acid digestion method of Olsen and Sommers (1982). The total P was measured as the sum of all extractable fractions and residual P. Based on this sequential procedure, soil P fractions were extracted by 1 M ammonium chloride (NH₄Cl) [APi]; 0.5 M sodium bicarbonate (NaHCO₃, pH 8.5) [BPI, BPO]; 0.1 M sodium hydroxide (NaOH) [N₁Pi, N₁Po]; 1 M hydrochloric acid (HCl) [HPi]; and 0.1 M NaOH [N₂Pi, N₂Po]. (Zhang et al. 2002; Sharpley et al. 2004). The APi is considered to be soluble Pi; BPI and BPO, readily mineralisable P; N₁Pi and N₁Po, associated with amorphous and some crystalline Al, Fe hydrous oxides; HPi, relatively stable Ca-bound P; N₂Pi and N₂Po, adsorbed into the mineral structure of soil components or occluded by

Fe and Al coatings; and residual P, non-extractable P. The concentration of inorganic P in the extracts was determined after the precipitation of organic matter by acidification, and the concentration of total P in the extracts was determined after persulfate oxidation, while the concentration of organic P in the extracts was calculated as the difference between total P and inorganic P (Tiessen and Moir, 1993).

2.5 Hot water extractable organic C (HWEOC) and inorganic P (HWP)

Hot water extractable organic C (HWEOC) and inorganic P (HWP) were determined by extracting 8g of oven-dry equivalent soil sample with 40 mL of hot distilled water by incubating the samples in the oven at 70°C for 16 h. Following incubation, the suspension was shaken in an end-over-end shaker for 5 min followed by centrifuging at 10 000 rpm for 10 min. The suspension was then filtered through a Whatman 42 filter paper followed by 0.45µm filter membrane through a 33 mm Millex syringe-driven. The HWEOC concentrations of filtered solutions were measured using a SHIMADZU TOC-VCPH/CPN Analyser and the concentration of HWP was measured using a UV Spectrophotometer according to the method of Chen and Xu (2005).

2.6 Microbial Analysis

2.6.1 Microbial Biomass Carbon (MBC)

Microbial biomass carbon (MBC) was estimated based on the difference between organic C extracted with 0.5 M K₂SO₄ from chloroform-fumigated and unfumigated soil samples using a K_c factor of 0.45 (Vance et al. 1987; Brookes et al. 1982). Fumigation was carried out in a dessicator using alcohol-free chloroform for 16 h at 25 °C under a fume hood, while non-fumigated soils were incubated in the dark for the same period of time and temperature at the same time. Fumigated and non-fumigated soils (4 g dry weight equivalent) were then extracted with 20 mL of 0.5 M K₂SO₄ (soil/extractant ratio 1:5).

Samples were then shaken for 1 h using an end-to-end shaker and filtered through a Whatman 42 filter paper. The C in the extractant was determined using a Shimadzu TOC-5000 analyser.

2.6.2 Microbial Biomass Phosphorus (MBP)

Soil microbial phosphorus (MBP) was also measured using the fumigation-extraction method (Vance et al. 1987; Brookes et al. 1982), but the soil samples were first divided into 3 sets of soils for fumigated, non-fumigated and spiked samples. All samples were extracted with 0.5 M NaHCO₃ for 16 hrs before they were centrifuged using a high-speed centrifuge at 10000 rpm for 10 minutes. The P concentration was determined colorimetrically. The P flux upon the fumigation was calculated from the difference between the quantity of inorganic P in 0.5 M NaHCO₃ extracts of fumigated and unfumigated soils. Fumigation was carried out using alcohol-free chloroform for 24 h at 25 °C. The MBP was calculated by using a conversion factor, K_P value of 0.40 because only about 40% of the P in the original biomass is release to NaHCO₃ reagent following extraction (Brookes et al. 1982). The recovery efficiency of MBP was determined by spiking soil with 250 µg P ml⁻¹ and the recovery rate at 60%.

2.6.3 Soil respiration (CO₂-C)

Soil respiration was measured using the NaOH trap method as described by Chen et al. (2000). Briefly the moist soil was aerobically incubated in a 1 L sealed glass jar at 25°C for 7 days before being weighed into a 70 mL plastic container for each sample for a total of 88 samples. The sample was then placed in the glass jar together with 10 mL of 0.1 M NaOH. The samples were aerated every 2 days. The carbon dioxide (CO₂) evolved from soil was trapped in 0.1 N NaOH and measured by back titration. At the end of the incubation period, the container was opened and the amounts of CO₂ produced were

determined by titration with 0.1 M HCl in the presence of 2 M BaCl₂ together with phenolphthalein as an indicator. The metabolic quotient ($q\text{CO}_2$) was calculated as the ratio of microbial respired C to microbial biomass C and this was used as an indicator of microbial metabolism in the soil (Iglesias-Jiménez and Alvarez 1993).

2.7 References

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Chapter 3 Soil phosphorus fractionation and associated microbial processes under contrasting treatments of green waste compost

3.1 Abstract

Addition of organic materials, such as compost, can improve soil chemical, biological and physical properties as well as providing nutrients such as phosphorus (P) to plants. As a part of a project led by the Centre for Recycled Organics in Agriculture (CROA) in New South Wales (NSW), soil samples were collected to investigate the effects of recycled organics (RO) on P availability and associated microbial properties in a loamy Chromosol/Dermosol from a vegetable cropping field. Seven treatments were applied in a fully randomised block design in the sample field trial. Treatments included full compost applied with high and low P rates; half-compost and half- chemical fertiliser; conventional practice and a control. The treatments were: HP-Conventional = high P, conventional practice (½ poultry manure and ½ chemical fertiliser); HP-Compost = high P, full compost; HP-Mixed = high P, compost and chemical fertiliser (½: ½); LP-Conventional = low P, conventional (½ poultry manure and ½ chemical fertiliser); LP-Compost = low P, full compost; LP-Mixed = low P, compost and chemical fertiliser (½: ½); Control = Nil inputs. The samples were taken from the CROA plots after the third application of compost and prior to the planting of the twelfth crop of cabbage. The soils samples were tested for hot water extractable carbon (C) (HWEOC), hot water extractable P (HWP), microbial biomass P (MBP), microbial biomass C (MBC) and soil respiration under laboratory conditions. The P fractions were also analysed using a modified fractionation method with an analysis of the residual P fraction using nitric-perchloric acid digestion. Results indicated that compost treatments increased the soil P availability

under both high and low P rates of P application. Concentrations of HWEOC, MBC, MBP and soil respiration were higher in all compost treatments ($P < 0.05$), in the order of compost > mixed > conventional > control. The HP-Compost treatment had the highest labile inorganic P (Pi) (184 mg P kg^{-1}) but this was not significantly different from the HP-conventional treatment (143 mg P kg^{-1}) compared to the control (35 mg P kg^{-1}). The highest labile Pi in the HP-Compost treatment was mediated by microorganisms through the mineralisation process. This was indicated by the low MBC:P ratio and labile organic P (Po) data measured in the HP-Compost treatment. The HP-Compost application increased moderately labile and stable Pi compared to the control but these fractions can readily be changed into labile P and might not accumulate as recalcitrant P in soil due to the high microbial activity in this treatment. However, the high labile Pi in the HP-Compost indicated that high P input of compost treatment could have the same environmental risk as the conventional treatments after multiple applications. In conclusion, this study revealed that in this field trial, the current practice of periodically applying large amounts of compost based on required N supply, has led to high levels of available P similar to conventional practice. Therefore, it is crucial to determine both the amount of available P in the compost amendment prior to its incorporation into soil and also the P loading rate equally as much as the N loading rate.

3.2 Introduction

The increased demand for food production due to population growth has led to increased use of mineral P fertilisers in agriculture. However, increasingly large applications of mineral P fertilisers to farming lands is of worldwide concern, due to economic and environmental implications (Jiao et al. 2012; Walan et al. 2014). High applications of inorganic (mineral) P fertilisers are depleting finite and non-renewable sources of mineral phosphate, leading to worldwide concerns of P resource sustainability. It has been reported that global P reserves are depleting and are expected to be exhausted in the next 100 years due to accelerated extraction and the non-replenishment of mineral phosphate sources (Cordell et al. 2009; Liu et al. 2009; Childers et al. 2011). When the maximum global P production rate is reached and there is a peak in use of P fertilisers, the availability and affordability of P fertiliser may begin to limit food and commodity production (Childers et al. 2011). To meet the high demand for food production and to avoid the forthcoming P crisis, more sustainable agriculture practices therefore need to be adopted by integrating organic P in RO wastes into the P-sustainability strategy.

Another concern regarding large applications of inorganic fertiliser is resultant runoff transport of P and other nutrients from agricultural land to surface water bodies, contributing to their accelerated eutrophication (Bechmann et al. 2005; Grizetti et al. 2012). For example, a study reported by Hollinger et al. (2001), found approximately 11kg/ha P losses in typical vegetable farm in western Sydney, per year. Due to these concerns, an alternative and potentially environmentally friendly approach is the use of RO to provide P (and other nutrients) for agriculture and to help restore soil quality. Additionally, the use of RO may be beneficial, as utilising a waste material reduces the cost of landfill and recycles otherwise lost P, thus 'closing the loop'. Utilisation of RO also plays an important role in combating global warming as incorporation of organic matter can increase the levels of organic carbon (C) stored in soils (Pedra et al. 2007).

Phosphorus is an essential plant macronutrient however many soils are P-deficient which puts constraints on agriculture production. Many studies have focused on the benefits of organic products as a nutrient source for plants (Entry et al. 1997; Ghosh et al. 2008; Singh et al. 2009; Reziq et al. 2014). The application of organic wastes to the soil therefore has the potential to provide a source of P for plant growth. However, little research has been done to understand the effect of RO on P availability and dynamics in soils. Further study on the fate of P in RO applied to the soil is essential to refine the use of such organic fertilisers and achieve optimum outcomes for agricultural production and environmental protection.

Many types of organic materials have been recycled worldwide and used as soil amendments in agriculture (Quilty and Cattle 2011). Composts produced from plant and animal residues have been widely used by farmers to improve soil quality and fertility, thereby increasing crop production. In New South Wales (NSW) Australia alone, over 600,000 tonnes of organic green wastes were collected annually from domestic and municipal areas and used to produce composted organic green wastes (DEC 2004). However, only a small proportion of this compost is used in Australian agricultural production systems (Chan et al. 2007) because it is very expensive to transport compost from urban peripheral areas to cropping fields.

Source-separated green waste compost, which is mostly produced from grass clippings and green garden trimmings, is high in organic matter and often rich in plant nutrients (Albiach et al. 2000). As such, this type of amendment has high potential for improving soil fertility as it is nutrient-rich and low in contaminants (plastics and heavy metals) unlike low quality composted municipal solid wastes. Source-separated green waste compost is thus more suitable as a fertiliser for growing vegetables. Green waste compost also plays an important role as source of C in the soil, thus improving soil quality

in the long-term by increasing water holding capacity, microbial activity and aggregate stability (Dorahy et al. 2005).

There has been considerable interest in the nutrient aspect of composting, in particular nitrogen (N) dynamics in compost-amended soils (Gil et al. 2011; Vaughan et al. 2011; Weber et al. 2014). There are several studies on the effects of composts on soil P, but the studies have mostly focussed on composted manures (Sharpley and Moyer, 2000; Erich et al. 2002; Zvomuya et al. 2006). There are therefore few studies on the use of other composted organic wastes such as green waste, despite the high amount of green wastes collected throughout Australia each year. Also, compared to other composts such as composted manures, there are few studies on the P dynamics of green waste compost. In particular, there is little information on the forms of P in vegetable-cropped soils amended with green waste compost and the effect of this compost on soil biochemical properties. In addition, a survey of the soils of vegetable farms in the Sydney basin area by Chan et al. (2007b) found that current conventional farming practices have resulted in elevated levels of available P and depleted levels of C in the soils. Therefore, research is needed to provide more information on the effects of applying composted green waste and to investigate its use as a potentially more sustainable P nutrient source for vegetable growers than inorganic P fertiliser.

A vegetable field experiment was established near Camden in Sydney, Australia, in 2005, to evaluate the effectiveness of green waste compost as an alternative to mineral fertiliser for vegetable production (Chan et al. 2008). This field experiment examines the agronomic and environmental effects of using green waste compost compared to conventional P fertilisation practices under low and high P application rates. High and low initial levels of soil extractable P were used because it was considered important to assess ways of reducing the P runoff risk, as vegetable farm in the Sydney basin typically have high P levels in their soils (Chan et al. 2007b and Chan et al. 2008).

This experiment was carried out on soils collected from the CROA site at the planting of the twelfth crop (cabbage), following the third application of compost. The objectives of this study were to further investigate the long-term effects of green waste compost as a soil amendment on soil P pools and associated microbial processes (e.g. microbial biomass C and P, respiration and metabolic quotient).

3.3 Materials and methods

3.3.1 Experimental site description

The experimental field site was located at the NSW Department of Primary Industries Centre for Recycled Organics in Agriculture (CROA), near Camden, (latitude $-34^{\circ} 05' 46.0313''$ S and longitude $150^{\circ} 42' 32.0861''$ E). The site has a long history of cropping and intensive forage production and it was chosen due to its soil type which is a typical one used for vegetable production in the Sydney region. The soil is classified as Chromosol/Dermosol (Isbell 1996).



Figure 3.1 Field site at the Centre for Recycled Organics in Agriculture (CROA), New South Wales (NSW).

Table 3.1 presents the basic properties of surface soil at the site, before the commencement of the field experiment (Chan et al. 2010). The initial Colwell extractable P was low (29 mg kg⁻¹) as the field site had not been used for vegetable production before.

Table 3.1 Properties of the surface soil (0-10 cm) at the CROA site prior to the field experiment.

	Soil
pH	5.2 ^A
EC (dS m ⁻¹)	0.13 ^B
TOC (g 100 g ⁻¹)	1.1
Total N (g 100 g ⁻¹)	0.11
Colwell P (mg kg ⁻¹)	29
Exchangeable cations (cmol _c kg ⁻¹):	
Na	0.12
K	0.29
Ca	5.35
Mg	1.25

^A 1:5 soil: 0.01 M CaCl₂.

^B 1:5 soil: water extract.

3.3.2 Treatments and experimental design

The field trial consisted of seven treatments in a randomised complete block design with 4 replicates of each treatment. The treatments were:

HP-Conventional = high P, conventional practice (½ poultry manure and ½ chemical fertiliser);

HP-Compost = high P, full compost;

HP-Mixed = high P, compost and chemical fertiliser (½: ½);

LP-Conventional = low P, conventional practice (½ poultry manure and ½ chemical fertiliser);

LP-Compost = low P, full compost;

LP-Mixed = low P, compost and chemical fertiliser (½: ½);

Control = Nil inputs.

The individual plots were 5 by 6 m with a 1 m buffer between plots, with three beds for each plot, each 1.2 by 6.0 by 0.15 m. All plots were rotary hoed to a depth of

0.10 m to incorporate added amendments prior to forming the plots areas. For the high P treatments (HP-Conventional, HP-Compost and HP-Mixed), triple superphosphate was applied to each plot at the start of the experiment (2005) at a rate equivalent to 680 kg P ha⁻¹ and incorporated to 0.10 m, to raise the soil extractable P concentrations to levels similar to those observed in vegetable farm soils (~250 mg kg⁻¹ in 0.10 m) (Chan et al. 2007b). The soil at this site had a low initial concentration of bicarbonate extractable P (29 mg P kg⁻¹, Table 3.1) and as such ensured the other treatments (LP-Conventional, LP-Compost, LP-Mixed and control) were representative of new vegetable farms with no prior history of high fertiliser inputs.

The compost used in this field experiment was obtained from source-separated garden organics blended with 10% poultry manure that was composted in compliance with the Australian Standard AS 4454-2003 (Standard Australia, 2003) from a commercial supplier in the Sydney basin (Table 3.2). The compost was applied as a single application at the beginning of the trial in 2005 at a rate of 125 dry t ha⁻¹ (250 mg P/kg) for the full compost treatments (HP- and LP-Compost) and 62.5 dry t ha⁻¹ in the mixed compost treatments (HP- and LP-Mixed) and incorporated into the soil to a depth of 10 cm. A repeat of this compost application to these treatment plots was applied in 2008 prior to crop 6, and then again in 2011 prior to crop 11. Thus, each large application of compost was followed by 5 vegetable crops over 3 years, in a repeating pattern.

Before the planting of each crop, poultry manure was applied to the conventional practice treatment plots (HP- and LP-Conventional) and triple superphosphate was applied to the conventional practice treatment and was incorporated to a soil depth of 10 cm. Potassium as muriate of potash and N as urea were applied by hand to four treatments (HP-Conventional, LP-Conventional, HP-Mixed and LP-Mixed) for each crop. These were applied as a split side-dressing to the surface (without Incorporation).

Table 3.2 Properties of poultry manure and composts used in the CROA study.

	pHw	EC dS m ⁻¹	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C: N ratio	TP (g kg ⁻¹)	Colwell P (mg kg ⁻¹)
Compost 1 (Crop 1)	5.6	3.14	210	11	19.1	3.8	1200
Poultry manure 1 (Crops1-10)	8.1	9.20	320	31	10.3	26	7500
Compost 2 (Crop 6)	6.9	5.30	300	13	18.8	7.2	2200
Compost 3 (Crop 11)	6.9	3.50	170	10	17.0	3.6	720
Poultry manure 2 (Crop 11-12)	6.3	13.00	390	39	10.0	13	3400

pHw and electrical conductivity EC in 1:5 soil: water extract; TOC= total organic carbon; TN = total nitrogen; TP= total phosphorus.

The nutrient requirements of each crop were based on industry-expert recommendations for agronomic rates of NPK fertiliser from the NSW Agriculture (1997) and district horticulturalist advice. For the conventional practice treatments, half of the required N was applied as the inorganic fertiliser urea (split surface applications over the cropping season) and the other half as poultry manure (incorporated into the soil prior to planting). The amount of required poultry manure was calculated from its total N content assuming an availability index of 0.60 (i.e. 60% of poultry manure total N is available to the crop) as per Evanylo and Sherony (2002). For P and K fertiliser rates in the conventional treatment, it was assumed that half of the P and K was also supplied by the poultry manure rate (determined by total N calculation). So on this basis, only half of the recommended agronomic rates of P and K were applied as inorganic fertiliser for this treatment.

The full rate for compost treatments was determined to be 125 dry t ha⁻¹. This was based on the recommended agronomic rate for N for the first crop (broccoli) and the total N content of the compost, assuming an availability index of 0.10 (Evanylo and Sherony

2002). The half compost rate for HP-Mixed and LP-Mixed treatments was thus 62.5 dry t ha⁻¹. The compost was applied as one single application at the beginning of the experiment for all compost treatments (i.e. HP- and LP-Compost and HP- and LP-Mixed). The half compost treatments (HP- and LP-Mixed) received half their NPK for each crop as inorganic fertilisers, identical to that for the conventional treatments (HP- and LP-Conventional).

For the full compost treatments, plant sap tests for N were carried out on the sap from the petioles of each crop to monitor N nutrition in comparison to conventional practice treatments. Urea was only applied to the compost treatments when sap test results confirmed crop observation of low nitrate levels. Applications of urea were not required for the compost treatments for the first two crops but were necessary for crops 3 to 5. No K or P fertiliser applications were required for the full compost treatment plots for the 5 vegetable crops.

Crops were managed following recommendations from the NSW Agriculture (1997) and industry handbook (Salvestrin 1998). The crops were drip irrigated with irrigation scheduling based on soil moisture monitoring using gypsum blocks (Gbug) in the plots. Irrigation was applied when soil water potential at 20 cm depth was < -30 kPa.

The first five crops following the initial application of compost were: 1) broccoli, 2) eggplant, 3) cabbage, 4) capsicum, and 5) leek. The five vegetable crops in the second phase of the experiment (i.e. crops 6 to 10) following the second application of compost were: 6) capsicum, 7) broccoli, 8) lettuce, 9) cabbage and 10) sweet corn. The third phase (following the third application of compost in 2011 included crops 11) capsicum and 12) cabbage. The soil samples studied in this chapter were sampled at the planting of crop 12, which was cabbage.

After the harvesting of each crop, all of the non-harvestable crop residues on each plot were incorporated into the soil by rotary hoeing. Further details on the field experiment are provided in Chan et al. (2008; 2010).

3.3.3 Soil sampling

Fresh soil samples were collected from the treatments at the field site on 8th June 2012, prior to the transplanting of the cabbage crop (crop 12). The samples were composite samples of 7 cores with 5 cm diameter, all from 0-10 cm depth in each plot. The samples were well-packed in a cool box before they were sent through overnight shipment to be analysed in the biogeochemical laboratory at Griffith University, Queensland. Upon arrival, the samples were then immediately sieved through a 2 mm screen sieve at room temperature and stored in a refrigerator at 4°C prior to analyses.

3.3.4 Soil analyses

Four replicates for each treatment were analysed. Soil pH determined in 1:5 (w/w) soil/water extracts using combination glass electrodes. Soil gravimetric moisture was determined by drying in the oven for 24 h at 105°C (Chen et al. 2003).

Hot water extractable organic C (HWEOC) and hot water extractable inorganic P (HWP) were determined from extracts as follows: 8 g (oven dry equivalent) soil samples were mixed with 40 mL of distilled water and the samples were incubated in the oven at 70°C for 16 h. Following incubation, the suspension was shaken by an end-over-end shaker for 5 min followed by centrifuging at 10 000 rpm for 10 min. The suspension was then filtered through a Whatman 42 filter paper followed by a 0.45µm filter membrane using a 33 mm Millex syringe. The HWEOC concentrations of filtered solutions were measured using a SHIMADZU TOC-VCPH/CPN Analyser and the concentration of

HWP was measured using a UV Spectrophotometer according to the method of Chen and Xu (2005).

Soil P fractionation and availability were measured using the modified fractionation method developed by Hedley et al. (1998) and Condon et al. (1996) including the residual P analysis using the nitric perchloric acid digestion method as described by Olsen and Sommers (1982).

Soil microbial biomass C (MBC) was measured using the fumigation-extraction method as described by Vance et al. (1987) and Brookes et al. (1982). Soil microbial biomass P (MBP) was measured using the fumigation-extraction method as described by Brookes et al. (1982) and modified by Wu et al. (2000). Soil samples were divided into 3 sets of soils for extraction: (1) fumigated, (2) non-fumigated and (3) spiked samples. The recovery efficiency of MBP was determined by spiking soil with $250 \mu\text{g P mL}^{-1}$ with the recovery rates ranging from 50 to 70%.

Soil respiration was measured by the NaOH trap method (Chen et al. 2000). In brief, CO_2 respiration was analysed using an incubation technique in a 1 L sealable jar at 25°C for 7 days. Metabolic quotient ($q\text{CO}_2$) was calculated from the ratio of the rate of microbially respired C (mg kg^{-1} over the 7 days) to microbial biomass C and used as an indicator of microbial metabolism in the soil (Iglesias-Jiménez and Alvarez 1993).

3.3.5 Statistical Analysis

Statistical significance of the effects of different treatments on soil properties, P fractions and soil microbial processes were determined separately by analysis of variance (ANOVA) and randomized complete block design was used using Statistix for Windows version 8.0 (Analytical Software, Tallahassee, FL). Least significance difference (LSD) was used to test whether these differences were significant ($P < 0.05$). Pearson correlation

analysis between soil chemical and biological properties was performed using Statistix for Windows version 8.0.

3.4 Results

3.4.1 Soil pH and EC

Soil pH and EC after amendment with the different compost treatments were showed in Table 3.3. All organic treatments had significant effects on these soil properties. Soil pH increased in all amendment treatments compared to the control, but remained in the acidic range of pH 5.9 - 6.5. Soil EC also increased in all treatments compared to the control, ranging from 0.26 to 0.37 dS m⁻¹. Compost treated soils (HP- and LP-Compost) had the highest pH and EC values amongst amended treatments, followed by the half-compost treatments (HP- and LP-Mixed), and then conventional practice (HP- and LP-Conventional). The control soil (without amendment) had the lowest value for both EC and pH compared to soil with amendments.

Table 3.3 Mean soil pH and EC with different organic treatments. Values followed by different letters for each treatment indicate significant differences (P < 0.05). Standard errors are shown in parentheses.

Treatment	pH ^A	EC ^A (dS m ⁻¹)
HP-Conventional	5.96 (0.20) d	0.31 (0.03) d
HP-Compost	6.35 (0.02) b	0.33 (0.03) ab
HP-Mixed	5.97 (0.12) d	0.27 (0.01) cd
LP-Conventional	5.82 (0.12) c	0.26 (0.07) e
LP-Compost	6.50 (0.05) a	0.37 (0.02) a
LP-Mixed	6.11 (0.04) bc	0.29 (0.01) bc
Control	5.77 (0.06) e	0.24 (0.04) e
<i>Significance</i>	***	***

^A 1:5 soil:water extract, ***P<0.05

3.4.2 Soil P fractionation

3.4.2.1 Soil inorganic P (Pi) fractions

Details on soil P fractions are presented in Table 3.4. The less labile Pi (HPi) fraction is predominant in the inorganic P pools, ranging from 59 to 200 mg P kg⁻¹, accounting for 33 - 40% of total Pi. The HPi in all amended soils were significantly higher than the unamended soils ($P < 0.001$). The concentrations of HPi fraction was greater in the higher P rate (HP) treatments compared with the lower P rate (LP) treatments (Table 3.4). The effects of amendments on the HPi fraction generally follow the order: Conventional > Compost > Mixed > control (Table 3.4). However, the mixed and compost treatments were not significantly different in the HP rate.

The NaOH-extractable Pi [N_1 Pi] ranged from 59 to 150 mg P kg⁻¹, accounting for 22 - 23% of total Pi. The N_1 Pi in soil with amendments increased significantly compared to the control ($P < 0.001$) (Table 3.4). The P concentration in this fraction also followed the same trend as the HPi fraction where the conventional treatments had higher values than the compost or mixed treatments. The second NaOH-extractable Pi [N_2 Pi] was highest in the HP conventional treatment with 43 mg P kg⁻¹ followed by the compost treatment, with the lowest value in the mixed treatments. In the LP treatments, N_2 Pi values for compost and conventional were similar and slightly higher than the mixed treatment. The labile Pi fraction, NaHCO₃-extractable Pi [BPi], ranged from 28 to 144 mg P kg⁻¹, which is 16 - 29% of total Pi. The P concentration in this fraction was highest in the HP-Compost and HP-Conventional treatments followed by the HP-Mixed and all LP treatments. Finally, the least dominant Pi fraction was the soluble Pi pool –the NH₄Cl-extractable Pi [APi]. The APi in all treatments increased significantly ($P < 0.001$) compared to the control. The APi results for all amended soils did not show significant difference to one another (Table 3.4).

3.4.2.2 Soil organic P (Po) fractions

The N₁Po was the most dominant organic P fraction, ranging from 150 to 206 mg P kg⁻¹ and accounting for 46 – 66% of total Po, followed by the BPo fraction (55 – 173 mg P kg⁻¹, 18 – 42% of total Po). The BPo fractions were highest in conventional treatments in both high and low P inputs with 173 and 140 mg P kg⁻¹ respectively. The N₂Po fractions were the smallest Po ranging from 23 – 57 mg P kg⁻¹ and accounting for less than 18% of total Po (Table 3.4). The compost treatments were found to be highest in this stable Po fraction [N₂Po] with 18% and 19% of total Po in high and low input P compost treatments respectively.

3.4.2.3 Total soil Pi and Po fractions

The conventional treatments had the highest soil total Pi, total Po and total P contents, followed generally by compost treatments and the mixed treatments (Table 3.5). Total soil Pi and Po were in this order: conventional > compost > mixed > control for both high and low P input treatments. The highest amount of total P was found in the HP-conventional treatment at 974 mg P kg⁻¹ which was approximately twice the amount of total P in the control, while the lowest compared to the control was from the LP-mixed treatment with 591 mg P kg⁻¹ of total P.

3.4.2.4 Relative proportions of the soil P fractions

The fractions (% of total P) of different lability found in the different P amendment treatments are shown in Figure 3.2 compared to the control. The Pi fractions in total, were slightly more dominant than the organic P fractions in treated samples, with the most stable Pi fraction [HPi + N₂Pi] comprising the largest proportion of total Pi (25 - 27% of total soil P). The next largest Pi fraction was the most labile one [APi + BPi] with 11 - 23% of total soil P, and the least dominant Pi fraction was the moderately labile Pi [N₁Pi] with 14 - 18% of total soil P. On the other hand, the organic P was less dominant in the

labile Po fractions [BPo + N₁Po] comprising of 28 - 40% of total soil P and recalcitrant Po fraction N₂Po was 3 - 7% of total soil P. The residual fraction ranged was the same in all treatments with 2% except for LP-Mix with 3% compared to 4% in the control.

It was observed that the HP-Compost had the highest labile Pi [APi + BPi] proportion with 23% of total P compared to only 11% of total P in the control. The proportion of moderately labile Pi [N₁Pi] was the highest in the LP-Compost and LP-Mix with 16% of total P in both treatments. The recalcitrant Pi [HPi + N₂Pi] was highest in the LP-Compost and LP-Mixed the 27% of total P in both treatments, and lowest in the HP-Conventional with 24% of total P. In the Po fractions of treated soils, the labile Po [BPo + N₁Po] was highest in the LP-Conventional treatment with 40% of total P and lowest was in the HP-Compost with 28% of total P. However overall, the largest labile Po fraction was in the control. The recalcitrant Po [N₂Po] was highest in the HP-Compost and LP-Compost with 7% of total P in both treatments while the lowest was in the LP-Conventional with 3% of total P.

Table 3.4 Mean values for P fractions (mg P kg⁻¹) in soil under different treatments. Mean values followed by different letters for each treatment are significantly different from each other. Standard errors are shown in parentheses.

Treatments	APi	BPi	BPo	N ₁ Pi	N ₁ Po	HPi	N ₂ Pi	N ₂ Po	Res-P
HP- Conventional	39 (2) a	144 (10) a	173 (29) a	150 (12) a	193 (22) a	200 (4) a	43(11) a	50 (8) ab	23 (3) a
HP-Compost	40 (3) a	144 (14) a	57 (13) b	108 (11) bc	201 (23) a	165 (5) b	33 (4) abc	57 (21) a	18 (1) b
HP-Mixed	34 (4) a	94 (6) b	130 (25) a	112 (9) b	184 (6) ab	172 (3) b	31 (3) bc	32 (4) bc	16 (2) b
LP-Conventional	33 (2) a	65 (10) d	140 (24) a	106 (10) bc	206 (6) a	156 (5) bc	34 (5) ab	24 (6) c	18 (3) ab
LP-Compost	31 (2) a	63 (9) d	78 (14) b	105 (5) bc	171 (15) ab	138 (12) cd	35 (5) ab	47 (6) ab	16 (1) b
LP-Mixed	27 (7) a	65 (15) d	55 (23) b	88 (10) c	174 (19) ab	120 (15) d	28 (3) bc	34 (13) bc	15 (1) b
Control	7 (1) b	28 (1) e	60 (9) b	59 (3) d	150 (9) b	59 (2) e	24 (3) c	23 (8) c	14 (1)b
<i>Significance</i>	***	***	***	***	*	***	*	**	*

* Indicates significant differences at $P<0.05$, ** $P<0.01$, *** $P<0.001$

Pi = inorganic P; Po = organic P. APi = NH₄Cl-Pi; BPi = NaHCO₃-Pi; BPo = NaHCO₃-Po; N₁Pi = NaOH-Pi (first extraction); N₁Po = NaOH=Po (first extraction); HPi = HCl-Pi; N₂Pi = NaOH-Pi (second extraction); N₂Po = NaOH-Po (second extraction); Res-P = residual (non-extracted) P.

Table 3.5 Mean P values (mg P kg⁻¹) for total extractable inorganic P (TPi), total extractable organic P (TPo) and total P (TP) fractions in soil under different P treatments. Standard errors are shown in parentheses.

Treatments	TPi	TPo	TP
HP-Conventional	576 (31) a	416 (43) a	992 (35) a
HP-Compost	490 (21) ab	315 (37) c	805 (49) b
HP-Mixed	443 (6) bc	346 (15) bc	789 (10) b
LP-Conventional	394 (21) cd	370 (19) ab	764 (23) b
LP-Compost	372 (2) cd	296 (18) cd	668 (17) c
LP-Mixed	328 (7) d	263 (35) de	591 (28) c
Control	177 (7) e	234 (24) e	410 (23) d
<i>Significance</i>	***	***	***

*** Indicates significant difference at $P < 0.001$

TPi (total extractable Pi) = APi + BPi + N₁Pi + HPi + N₂Pi;

TPo (total extractable Po) = BPo + N₁Po + N₂Po; TP (total soil P) = TPi + TPo + Res-P.

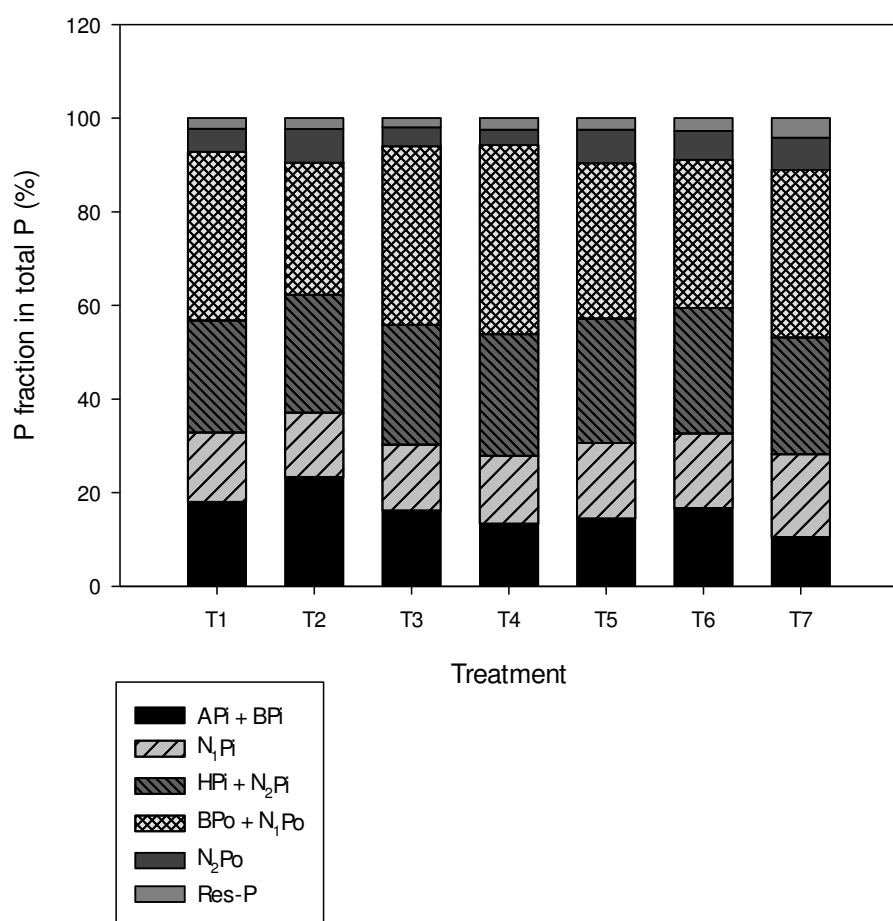


Figure 3.2 Response of P fractions (% of total P) of different lability in soil under different treatments. Pi= inorganic P; Po= organic P. APi= NH₄Cl-Pi; BPi= NaHCO₃-Pi; BPo= NaHCO₃-Po; N₁Pi= NaOH-Pi (first extraction); N₁Po= NaOH= Po (first extraction); HPi= HCl-Pi; N₂Pi= NaOH-Pi (second extraction); N₂Po= NaOH-Po (second extraction); Res-P= residual (non-extracted) P. Treatments, T1= HP-Conventional; T2= HP-Compost; T3= HP-Mixed; T4= LP-Conventional; T5= LP-Compost; T6= LP-Mixed; T7= Control.

3.4.3 Soil microbial biomass C and P

The effects of amendments on MBC and MBP (Figure 3.3) varied with soil P status. There was a significant difference in both MBC and MBP between high P and low P rate treatments ($P < 0.001$). The conventional, compost and mixed treatments all significantly increased MBC for both high and low P status compared to the control ($P < 0.001$). The highest MBC concentration was observed in the HP-Compost treatment (310 mg kg^{-1}), while the lowest concentration in the treatments was observed in LP-Conventional (209 mg kg^{-1}) compared to the control value of 143 mg kg^{-1} .

It was observed that the concentration of MBP increased significantly in all treatments compared to the control (Figure 3.3). The highest soil MBP was found in the HP-Compost treatment soil (13 mg kg^{-1}) and the lowest was in the HP-Conventional (6 mg kg^{-1}), the order of: HP-Compost > LP-Compost > HP-Mixed > LP-Conventional > LP-Mixed > HP-Conventional > LP-Mixed > HP-Conventional > control.

Microbial biomass C to P ratio (MBC:P) was significantly lower in all seven treatments compared to the control ($P < 0.001$). The MBC:P ranged from 26 to 57 in all treatments in comparison to 91 for the control. The highest MBC:P values were in HP-Conventional, HP-Mixed and LP-Mixed treatments as they were not significantly different from each other. The lowest MBC:P were in HP-Compost, LP-Conventional and LP-Compost treatments compared to the control (Figure 3.3).

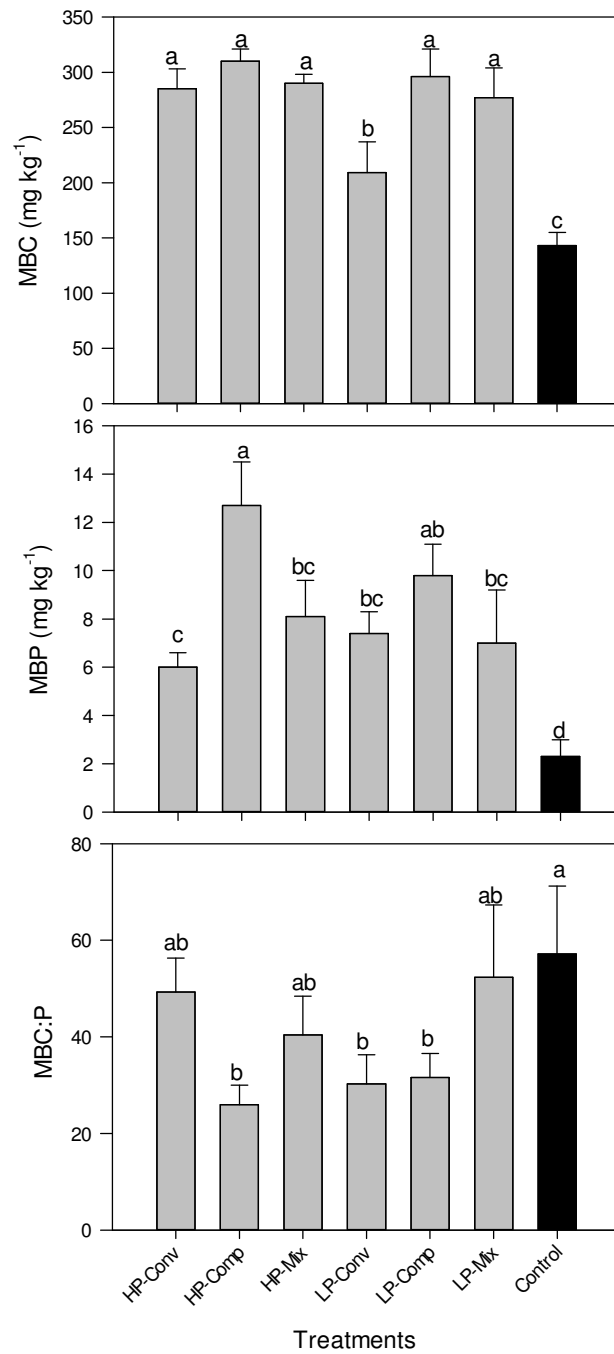


Figure 3.3 Means values for MBC, MBP and microbial C:P ratio in the soil under different treatments. Mean values followed by different letters for each treatment are significantly different from each other. Barred lines indicate the standard error of the means.

3.4.4 Soil respiration and metabolic quotient

Soil respiration over a 7 day period (Table 3.6) significantly increased in all treatments relative to the control ($P<0.001$). The cumulative respiration ranged between 153 to 184 $\text{CO}_2\text{-C g}^{-1}$ in all treatments compared to 117 $\text{CO}_2\text{-C g}^{-1}$ in the control. The highest cumulative respiration ($\text{CO}_2\text{-C}$) was observed in the compost-treated soils (HP and LP) with 184 $\mu\text{g CO}_2\text{-C g}^{-1}$ and 178 $\mu\text{g CO}_2\text{-C g}^{-1}$ values respectively.

Likewise, the respiration rates were also highest in HP-Compost and LP-Compost, with values of 1.10 and 1.06 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$, respectively. The other treatments showed lower respiration rate and showed no significant difference between each other. Rates in all of the treatments increased significantly compared to the control ($P<0.001$). The metabolic quotient was the highest in soil without any amendment (control) with 4.93 $\mu\text{g CO}_2\text{-C mg microbial C}^{-1} \text{ h}^{-1}$. It was also observed that metabolic quotient ($q\text{CO}_2$) in all treatments were significantly lower than the control except for LP-Conventional ($P<0.001$) (Table 3.6).

Table 3.6 Mean values for microbial respiration and metabolic quotient in soil under different P treatments. Mean values followed by different letters for each treatment are significantly different from each other. Standard errors are shown in parentheses.

Treatments	Cumulative respiration ($\mu\text{g CO}_2\text{-C g}^{-1}\text{in 7 days}^{-1}$)	Respiration rate ($\mu\text{g CO}_2\text{-C g}^{-1}\text{ h}^{-1}$)	Metabolic quotient ($q\text{CO}_2$) ($\mu\text{g CO}_2\text{-C mg microbial C}^{-1}\text{ h}^{-1}$)
HP-Conventional	154 (10) b	0.95 (0.07) b	3.23 (0.07) b
HP-Compost	184 (11) a	1.10 (0.06) a	3.54 (0.19) b
HP-Mixed	160 (12) b	0.95 (0.02) b	3.28 (0.17) b
LP-Conventional	159 (4) b	0.92 (0.06) b	4.40 (0.69) a
LP-Compost	178 (6) a	1.06 (0.03) a	3.73 (0.46) b
LP-Mixed	153 (5) b	0.91 (0.03) b	3.29 (0.11) b
Control	117 (5) c	0.69 (0.03) c	4.93 (0.40) a
<i>Significance</i>	***	***	***

*** indicates significant differences at $P < 0.001$

3.4.5 Hot water extractable organic carbon and hot water extractable phosphorus

Significant differences were observed between the treatments for HWEOC and HWP ($P < 0.001$) (Table 3.7). The HWEOC is an indicator of labile organic C and the highest HWEOC was observed in the HP-compost treated soil (488 mg kg^{-1}), followed by LP compost-treated soil (486 mg kg^{-1}), both being about twice that of the control. The HWEOC concentrations were found to have the same trend as MBC, in the order of compost > mixed > conventional > control within each of the respective high and low P treatments.

In this study, it was observed that all of the treatments significantly increased HWP compared to the control ($P < 0.001$). The HP-Conventional treatment greatly increased labile inorganic P measured by (HWP) with 6.63 mg P kg^{-1} compared to the

control with 3.42 mg P kg⁻¹. The smallest increase in HWP relative to the control was in the LP-Mixed treatment with 5.28 mg P kg⁻¹. The HWP was in the order of conventional > compost > mixed > control were found to have the same trend as MBC, in the order of compost > mixed > conventional > control within each of the respective high and low P treatments.

Table 3.7 Soil hot water extractable organic C (HWEOC) and inorganic P (HWP) in different treatments. Mean values followed by different letters for each treatment means are significantly different from each other. Standard errors are shown in parentheses.

Treatments	HWEOC (mg kg ⁻¹)	HWP (mg kg ⁻¹)
HP-Conventional	345 (19) bc	6.63 (0.29) a
HP-Compost	488 (23) a	5.96 (0.48) ab
HP-Mixed	385 (12) b	5.86 (0.58) ab
LP-Conventional	330 (24) c	6.23 (0.34) ab
LP-Compost	486 (29) a	5.37 (0.14) b
LP-Mixed	381 (7) b	5.28 (0.56) b
Control	217 (4) d	3.42 (0.09) c
<i>Significance</i>	***	***

*** indicates the significant differences at $P < 0.001$

3.4.6 Relationships between soil biochemical properties

Correlation coefficients between the various soil parameters discussed earlier in this chapter are presented in Table 3.8. As expected, CO₂ respiration was significantly related ($P<0.001$) (Table 3.8) to MBC and MBP with r values of 0.79 ($P<0.001$) and 0.62 ($P<0.001$) respectively. The MBC was significantly and positively related to soil properties such as HWEOC, MBP and HWP, with a significant negative correlation for $q\text{CO}_2$. It was observed that MBP was also significantly related ($P<0.001$) to soil HWEOC ($r= 0.69$) and HWP ($r= 0.44$). The CO₂ respiration was found to be positively correlated with HWEOC and HWP across all treatments r values of 0.89 ($P<0.001$) and 0.76 ($P<0.001$) respectively.

Table 3.8 Correlation coefficient (r) between soil chemical and biological properties determined in different amendments.

r	MBC	MBP	MBC:P	CO ₂ respiration	qCO ₂	HWEOC	HWP
MBC	1						
MBP	0.5374***	1					
MBC:P	0.2911	-0.5991***	1				
CO ₂ respiration	0.7893***	0.6162 ***	0.0340	1			
qCO ₂	-0.8438***	-0.3262	-0.3975*	-0.3861*	1		
HWEOC	0.8634***	0.6881***	0.0052	0.8929***	-0.5548***	1	
HWP	0.6888***	0.4393**	0.1820	0.7630***	-0.4360**	0.6625***	1

* indicates the significant differences at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

3.5 Discussion

3.5.1 Effects of organic amendments on soil P availability

Soil P availability and dynamics are controlled by a combination of physico-chemical and biological processes including dissolution-precipitation, adsorption-desorption, and mineralisation-immobilisation processes (Frossard et al. 2000). Sequential-fractionation procedures have been adopted to separate P in organic and inorganic amended soils into various functional inorganic and organic fractions (Dou et al. 2000; Sharpley and Moyer 2000). In this research, organic and inorganic amendments increased concentrations of most Pi and Po fractions in these soils relative to the control. This is consistent with previous studies by Nziguheba et al. (1998), Motavalli and Miles (2002) and Zhang et al. (2009). In this study, the application of inorganic and organic amendments generally increased the proportion of total P present in the Pi fractions extracted by NH_4Cl , NaHCO_3 , NaOH and HCl (Table 3.4). The content of these Pi fractions in the control was significantly lower ($P < 0.001$) than those in the amended soils.

Among all treatments, the most labile Pi forms (APi and BPi) were highest in the HP-Compost treated soils but the Pi concentrations were not significantly different from the HP-Conventional in comparison to the control. Three applications of the HP-Compost were made in the CROA experiment: the first addition was for crops 1 to 5, the second application for crops 5 to 10, and the final phase was added for the crop 11 until 12. These APi and BPi results were obtained after the third compost application, at the planting of crop 12 (cabbage). Previous studies on the same field by Chan et al. (2008) and Chan et al. (2010) found that the conventional practice had the most available P (Colwell P), while compost treatments were significantly lower than the other treatments. The recommended P inputs used for the conventional practice treatment are continually exceeding crop requirements as demonstrated in Chan et al. 2010 for the first 5 crops where <10% of the P applied was removed in all treatments. They also found that for the first five crops,

compost and half-compost treatments produced similar yields to those achieved by conventional practice regardless of their P input rates even though the lowest Colwell P recorded in low compost treatment was less than 100 mg P kg⁻¹. It was apparent that the compost treatment had lower levels of water soluble and available P in the soil and posed less risk to water quality (Chan et al. 2010) after the first phase of compost application. As for the second phase of compost application, it was found that the crop yields for compost treatments exceeded or matched the yields for the conventional treatment consistently for the second set of 5 crops as well (Eldridge et al. 2014). However by crop 8, following a repeated application of compost (second phase of the compost application), it was apparent that the difference of available P between treatments was less pronounced (Dougherty and Chan 2014). Following the third application of compost (including crop 12), it was apparent that the current compost system, based on compost N loading rates, is not sustainable either given the levels of available P obtained in this study. Therefore, it could be concluded that at high P rate, compost additions are no different to conventional amendments in terms of supplying available P to crops in the long-term. It was interesting to see that the high labile P_i was found in the HP-Compost. This was unexpected because the compost treatment was lower in available P compared to the conventional and mixed treatments. The result therefore suggests an important cumulative effect of compost on these labile forms of P and it may be attributed to: (i) multiple application of compost leads to high decomposition of compost which increased reduction of soil P adsorption thus releasing labile P in soil, over time; (ii) rapid biological mineralisation of labile P_o and the conversion of P_o into P_i in soil. The apparent increase in labile P_o mineralisation as indicated by lower soil BPO (Table 3.4), is consistent with the enhanced microbial activities in the same treatment (Table 3.6). The enhanced microbial activities could partly be due to improved soil quality in the long-term through increasing aggregate stability and water holding capacity from the compost amendment

(Wells and Chan, 2000). Also, it could be due to the high C content in compost treatments. The labile Po concentration was high in the HP-Conventional treatment due to the long-term application of poultry manure and mineral fertiliser in the treatment as well as low soil P removal by crops (Dougherty and Chan, 2014). The high labile Po in HP-Conventional corroborates with the findings of Reddy et al. (2000). They found that addition of poultry manure together with fertiliser P promoted the accretion of soil labile Po via the inhibition of phosphorylase activity, leading to the suppressed mineralisation process in soil. However, this fraction could be a source of available P as it could be easily transformed into labile Pi.

The high moderately labile Pi fractions (N_1Pi and N_2Pi) and high recalcitrant Pi (HPi) in the HP-Conventional treatment suggested that these fractions have a potential to provide available P for a longer time compared to other treatments. In the moderately labile Po fractions, the N_1Po and N_2Po were highest in the HP-Compost compared to the conventional and mixed treatments (Table 3.4). The direct input of Po through the compost application, in part, explain the increase in the NPo fractions. Also, the ability of compost to stimulate microbial population might have partly increased the Po in the soil. The high N_1Po and N_2Po in the HP-Compost indicate that this fractions have the potential to be mineralised into labile Pi and can continuously supply available P in the future. The low recalcitrant P concentration in HPi and Res-P fractions in the HP-Compost suggest that stable P in this fractions have transformed into less stable P fractions such as the concentrations of the moderately labile Po fractions (N_1Po and N_2Po). Previous studies also have reported that Res-P fraction may be depleted by plant uptake and transformed biologically by soil microorganisms (Oberson et al. 1996; Chen et al. 2000).

The LP-Compost showed a similar trend with the HP-Compost on the P fractions, but with lower magnitude. This indicates that lower P rate might have the potential to be

used in the agriculture field. However, the results obtained showed that some consideration to the P based loading rate in the compost material should be taken into account. Therefore, compost P application rates need to be determined by performing soil tests for available P prior to soil application. This is because the soil P levels are one of the first parameters that come into effect in defining the environmental limit for large compost applications in such a system. This is due to the ability of excessive P to be transported from land into waterways through the surface runoff.

3.5.2 Effects of organic amendments on soil chemical and microbial properties

In this experiment, all treatments with both high and low P status have significantly increased pH and EC (Table 3.3) as well as increasing soil microbial biomass and CO₂ respiration (Figure 3.3 and Table 3.6) compared with the non-treated (control) soil. Erich et al. (2002) also reported similar increases in pH, EC and total P for their soil amendment experiment which also included compost, manure and inorganic fertiliser. However, the highest pH and EC values in this study were observed in the compost treated soils, in both the HP and LP treatments compared to conventional and mixed treatments. The highest pHs were found in both compost treatments (high and low P status) (Table 3.2). Other researchers, Iyamuremye et al. (1996), Erich et al. (2002) and Narambuye and Haynes (2006) also found that soil pH was significantly elevated by the addition of organic amendments such as compost, as they contain many carboxylic, phenolic, and enolic groups associated with humic material. These groups have the ability to consume protons and are thus able to control the buffering characteristics and neutralise acidity when added to soils (Wong et al. 1998, Tang et al. 1999).

Hot water extractable organic carbon (HWEOC), is a soil organic C pool which correlates strongly with microbial biomass and therefore is known to be labile (Sparling et al. 1998). Loss of HWEOC in any given soil system indicates a decrease of the

microbial biomass pool and organic labile nutrients (Ghani et al. 2003). The highest concentrations of HWEOC were measured in the compost-amended soil for both high and low P status compared to other amendments. It is well understood that the application of organic inputs such as compost enhances organic C storage in soil (Singh et al. 2009). In this study, HWEOC was found to have a positive correlation with MBC (Table 3.7), in agreement with research on added organic amendments by Ghani et al. (2003). The hot water P (HWP) was found to be the highest in the HP-Conventional and lowest in LP-Mixed treatments compared to the control. The HWP results showed similar trend with the APi in Table 3.4. However, the HP-Compost is significantly lower in HWP than the HP-Conventional indicating that the HP-Compost was less water soluble than the HP-Conventional.

Microbial biomass, being a dynamic component of organic matter, is largely responsible for the assimilation and transformation of organic matter in soil as well as affecting the labile pool of nutrients such as P (Chen et al. 2000). The utilisation of organic matter such as compost supplies substrate for microorganisms, and therefore increases the activity of the indigenous soil microbial biomass (Wardle 1992; Goyal et al. 1999; Dinesh et al. 2010; Malik et al. 2013). The MBC for both high and low P status compost treatments were the highest compared to the conventional practices. Respiration rate also exhibited a similar trend to MBC in the compost treated soil. A study done by Chan et al. (2008) on the first three crops at the field site also found that the compost treatments had the highest MBC and respiration rates compared to the other treatments. This is supported by Fauci and Dick (1994) and Goyal et al. (1999) as they found that soil microbial biomass is generally correlated with the amounts of compost added to soil. This is because the added compost supplies readily decomposable organic matter which contains high labile C for the growth of the microbial community. This also accords with the findings observed in this study, which showed that both of compost treatments (LP

and HP) increased labile C found in HWEOC extractions (Table 3.7). However, in a study done by Ghosh et al. (2010) on soil amended with green waste compost, they found that there was little effect on microbial activity. However, this could be due to their use of low compost application rates (13.9 t ha^{-1}) in comparison to this study (125 and 62.5 t ha^{-1}). This finding indicates that microbial populations are generally influenced only by higher doses of compost as it contains higher labile C needed for microbial growth. Perucci (1992) also concluded that MBC contents in compost-amended soil, increased with the increasing rate of application. In this study, it was observed that the compost treated soils, both HP-Compost and LP-Compost, greatly increased MBP compared to other treatments. The high MBP in these treatments was due to their high organic C component whereby they could supply sufficient C for microbial growth. The high MBP in the compost treatments indicate that they could promote higher level of P cycling in soil.

The MBC:P ratio is an important factor that controls the availability of P in soil. As the MBC:P ratio decreases, microbes have a higher potential to release P into the soil solution during mineralisation and microbial turnover (He et al. 1997). In this study, the LP-Mixed treatment was found to have the highest MBC: P compared to other treatments. However, the HP-Conventional was not significantly different with both of HP and LP-Mixed compost treatments. The wide ratio of MBC: P indicates that if an amendment is high in C, it therefore has high potential to immobilise more P (Chen et al. 2003, Kwabiah et al. 2003). This could further explain the presence of low labile P_i in our mixed treatments as shown in Table 3.4.

Metabolic quotient, $q\text{CO}_2$, was significantly higher in the control and the LP-Conventional treated soil compared to the other treatments. This indicates that more of the C consumed in these soils was evolved as CO_2 rather than being incorporated to microbial biomass. In the present study, the use of compost has increased soil microbial C and P, soil respiration and decreased metabolic quotient compared to the other

treatments (Table 3.6). These parameters are important biological indicators of soil fertility and these measured improvements in them indicate an overall increase in soil quality (Elliot et al. 1996) for the compost treatment.

3.6 Conclusion

This study has revealed significant and positive effects on the soil microbial properties and soil P availability following long-term addition of green waste compost in a vegetable production soil. Higher contents of labile organic C in compost treatments with both high and low P status was found to increase microbial populations and microbial activities, thus increasing soil fertility. However, the results obtained in this study also revealed that repeated compost applications could increase available P similar to the conventional treatment particularly at high P rate. The findings indicated that in the long-term, the compost treatment is not different from the conventional treatments (at high P rate) as it could enhance the possibility of P being transported to nearby surface waters from runoff and leaching. Therefore, it could be concluded that it is crucial to determine available P in compost amendment prior to incorporation into soil and to consider the use of a P-based loading rate in addition to the N-based loading rate. This leads to the use of P rate in the next investigations of RO effects in soils. More studies on effects of RO, including compost, were conducted on the chemical and biological properties in laboratory using incubation method and looking at the rhizosphere soil to further understand the effects of RO, in the following chapters.

3.7 References

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Chapter 4 Soil phosphorus availability and microbial dynamics under amendments of recycled organics- an incubation study

4.1 Abstract

Application of recycled organic (RO) materials as amendments has been proposed as an option to improve soil quality and increase soil carbon (C) stock and phosphorus (P) availability in soil, as an attractive alternative to depleting phosphate rock. However, there is little understanding of the simultaneous impacts of RO application on soil P dynamics and the associated microbial processes especially with RO of varying properties. This study therefore aims to determine the effect of varying types of RO under controlled condition on soil P fractions, P availability and the associated microbial properties to further understand the effect of RO from what have been observed in the previous chapter. An incubation experiment was conducted for 20 weeks under controlled temperature (22°C) using two different acidic soils (sandy loam and loam) amended with five different RO and compared to control soils with no RO amendments. The RO comprised: biosolids (BS), green waste biochar (BC), broiler litter (BL), green waste compost (CG) and mixed compost (CM). The RO addition was based on two commonly used field P application rates, low P rate (LP) at 50 kg P ha⁻¹, and high P rate (HP) at 100 kg P ha⁻¹. The P fractions in the soils were subsequently analysed using a modified Hedley et al. (1982) and Condon et al. (1996) fractionation method by including an analysis of the residual P using nitric-perchloric acid digestion (Olsen & Sommers 1982). Results indicated that the various RO amendments affected soil P pools and soil microbial properties differently and these effects varied with soil types and P rates. The CM, BL and CG treatments significantly increased ($P<0.05$) the soil Pi and Po fractions as well as microbial

properties compared to the control where no amendment was applied. The BS treatment led to the greatest increase in the labile Pi fraction, with 23.5 mg P kg⁻¹ and 47.1 mg P kg⁻¹ in the sandy loam and loamy soil respectively, but had minimal effect on the microbial properties compared to the other RO treatments in both soils. Although BC is known as an inert substance, its application in sandy loam soil unexpectedly increased the microbial biomass (MBC and MBP) and respiration ($P < 0.05$), indicating that it could enhance P cycling in soil with lower clay contents. This study has shown the potential of green waste-based amendments (CG and BC) for the enhancement of soil nutrient cycling. Importantly, this study has highlighted that an understanding of the mechanisms involved in P dynamics and microbial properties following RO addition may provide important information to maximise the potential benefits of RO as effective fertilising practices, thus improving agro-ecosystems. The nature of RO amendments therefore appears to have profound effects on P availability and soil microbial dynamics, hence careful consideration is needed when selecting the most appropriate RO for a specific application objective and soil type.

4.2 Introduction

The RO amendments provide microbes with P and C and thereby are likely to induce different changes in P pools compared to conventional fertilisers (Malik et al. 2012). These RO have been used as an alternative to inorganic fertiliser in restoring nutrients, including P and C. They have the added benefit of being more environmentally-friendly and reducing the production of greenhouse gases by increasing C storage in soil as well as reducing the generation of solid wastes (Marinari et al. 2000; Pedra et al. 2007; Quilty and Cattle 2011). There are numerous types of organic wastes that have been recycled and used as soil organic amendments in recent agricultural practice. However, in previous study (Chapter 3), it was found that the P availability of organic amendment to crops and their impact on available P pools are no difference compared to the inorganic fertilisers. Therefore, the effects of RO on soil P dynamics is further investigated in this study.

Soil P transformation occurs through a combination of processes including physicochemical (adsorption/desorption and precipitation/dissolution) and biochemical processes (mineralisation/immobilisation). The physicochemical process can also be mediated by microorganisms (Bünemann 2015). Soil microbial biomass is among the most labile pools of organic matter and it serves as an important reservoir of plant nutrients in the soil P cycle (Chen et al. 2003; Molero et al. 2006). Phosphorus is also released and made available for plant uptake from microbial biomass following microbial cell death, in response to physical and environmental changes (Bünemann et al. 2015).

The addition of organic amendments can increase the availability of soil nutrients such as P and N (Iyamuremye et al. 1996; Hosseinpur and Pashamokhtari 2013). It also affect micronutrient availability (Richards et al. 2011); improve soil pH (Marschner and Noble 2000; Zhao et al 2015); increase carbon storage (Pedra et al. 2007; Sánchez-Monedero et al. 2008; Jones et al. 2010). The RO addition also enhance microbial

population and activities (Ferrerias et al. 2006; Teklay et al. 2006; Criquet et al. 2007; Bünemann et al. 2008; Liu et al. 2009) as well as improve soil structure (Ghosh et al. 2011). However, most of these studies on organic amended soils were based on addition of manures and there is very little information on the effects of RO such as green waste-based amendments. However, information is still scarce on the chemical and biological mechanisms responsible for changes in soil P dynamics and associated microbial processes following applications of different types and rates of RO.

It is important to understand the relationship between the P in different RO amendments, microbial activity and changes in soil P pools, as well as the mechanisms underlying the transformation of P in soil for the sustainable management of soil P. Therefore, this study leads to further investigation of P dynamics in soil amended with RO of varying properties. The influence of the RO on microbial properties as well as the P fractions were examined. The aims of the study were therefore to investigate the effects of RO type and application rates on: 1) soil P fractions and availability; 2) biochemical and microbial mineralisation processes of RO-amended soil such as microbial biomass carbon (MBC) and microbial biomass phosphorus (MBP), microbial activity (CO_2 respiration) and metabolic quotient ($q\text{CO}_2$). The metabolic quotient represents the quantity of substrate that is mineralised per unit of microbial biomass C per unit of time (Anderson and Domsch, 1985). The $q\text{CO}_2$ was used as an indicator of microbial metabolism in the soil (Iglesias-Jiménez and Alvarez, 1993).

4.3 Materials and methods

4.3.1 Soils and Recycled Organics (RO)

The two different soils used for this study were a sandy loam and a loam. The loam soil was obtained from the Centre for Recycled Organics in Agriculture (CROA), in New South Wales, (NSW) which is classified as a Chromosol/Dermosol (Isbell 1996). The sandy loam soil was collected from Griffith University, Nathan Campus, Queensland and is classified as a Podosol (Isbell 1996). The basic properties of the two soils are given in Table 4.1. Soils and the RO were air-dried at room temperature and passed through a 4 mm sieve to remove any coarse material, prior to the incubation trial.

Table 4.1 Mean values for basic properties of soils used in the incubation experiment.

	Sandy loam	Loam soil
pH	4.5	5.2
EC(dS m ⁻¹)	0.29	0.37
Total C (%)	1.89	2.04
Total N (%)	0.09	0.19
C: N ratio	21	11
Colwell P (mg kg ⁻¹)	4	29
Clay (%)	14	20
Sand (%)	75	41
Silt (%)	11	39
Total Ca (mg kg ⁻¹)	18	25
Total Mg (mg kg ⁻¹)	8	19
Total Na (mg kg ⁻¹)	2	17
Total K (mg kg ⁻¹)	21	40
Total Fe (mg kg ⁻¹)	263	336
Total Al (mg kg ⁻¹)	281	330

Five types of RO were used for the incubation study as outlined in Table 4.2 including biosolids (BS), broiler litter (BL), compost green waste (CG) compost mix (CM) and biochar from green waste (BC). The compost mix amendment was a mixture of composted household solid wastes, farmyard wastes and garden wastes.

Table 4.2 Mean chemical properties of recycled organics used in the incubation experiment.

Recycled organics	Biosolids (BS)	Broiler litter (BL)	Compost green waste (CG)	Compost mix (CM)	Biochar (green waste) (BC)
pH ^A	7.40	7.12	6.94	7.04	4.74
EC ^A (dSm ⁻¹)	7.85	11.91	2.93	2.89	80.30
Total C (%)	28.9	39.0	26.0	19.7	65.0
Total N (%)	4.9	3.0	1.3	1.5	0.1
Total P (%)	4.52	1.36	0.22	0.28	0.06
C: N ratio	6	13	20	13	650
C: P ratio	8	29	118	70	1083
Colwell P (mg P kg ⁻¹)	8600	4000	840	3600	43
Total Mg (%)	0.5	0.5	0.3	0.2	0.09
Total Ca (%)	1.2	2.2	1.5	3.9	0.22
Total Fe (mg kg ⁻¹)	8000	1300	11000	189	6500
Total Al (mg kg ⁻¹)	12000	1100	11000	148	1800
Total Cu (mg kg ⁻¹)	830	150	66	180	5.3

^A 1:5 soil: water extract

4.3.2 Experimental treatments and design

The experimental treatments included the five selected RO (biosolids, broiler litter, green waste biochar, green waste compost and mixed compost), the two soils described above, and three application rates (Control with no amendment, low P (LP) at 50 kg P ha⁻¹ and high P (HP) at 100 kg P ha⁻¹). The experiments took place under laboratory conditions, which made it possible to assess the effects of the RO on P availability and microbial properties without environmental interference. Each soil was mixed with the selected RO based on total P content at different application rates by weight (w/w) which were based on the agronomic P rates commonly used for manure (broiler litter) in agricultural practices. Air-dried soil (50 g) was placed in a pot (100 mL plastic container from Sarstedt

Australia) and then the required amount of RO was mixed thoroughly with the soil (Table 4.3). The treatments comprised: 5 RO treatments, 2 soils and 2 P levels with 4 replicates per treatment. They were incubated in 1L glass containers for 20 weeks at 22°C in the incubator. The samples were aerated by opening the container lid every 2 days. Water was added once a week to maintain the moisture level at 60% of water holding capacity. After 20 weeks of incubation, the soil samples were removed from the pots and the samples were stored at 4°C prior to analysis of selected microbiological and chemical properties.

Table 4.3 Application of RO (in g pot⁻¹) based on 50 kg P ha⁻¹ (LP) and 100 kg P ha⁻¹ (HP).

Treatments	Sandy loam		Loam	
	LP	HP	LP	HP
BS	0.079	0.158	0.092	0.184
BL	0.263	0.525	0.306	0.613
BC	5.952	11.905	6.944	13.889
CG	1.623	3.247	1.894	3.788
CM	1.276	2.551	1.488	2.976

4.3.3 Analytical methods

All the methods for characterisation of soil chemical and RO amendments (Table 4.1 and 4.2) were explained in detail in Chapter 2. Microbial biomass C (MBC) was measured by a fumigation-extraction [soil: extractant (0.5 M K₂SO₄) ratio 1:4] method using a conversion *K_c* factor of 0.45 (Wu et al. 1990). Microbial biomass P (MBP) was also measured by fumigation-extraction [soil extractant (0.5 M NaHCO₃) with the ratio of 1:20] method using a *K_p* factor of 0.4 (Brookes et al. 1982). Soil respiration was measured using the method described by Chen et al. (2000). Metabolic quotient (*q*CO₂) was calculated as the ratio of microbial respired C (µg g⁻¹ 140 days⁻¹) to MBC. Sequential P fractionation was performed only on soil samples treated with the HP application rate (100 kg ha⁻¹) due to limited laboratory capacity and time. All soil analysis results are

reported on an oven-dry basis (24 h at 105° C). Soil P fractions and availability were measured on high P treatments using the modified fractionation method developed by Hedley et al. (1982) and Condon et al. (1996) by including an analysis of the residual P using the nitric perchloric acid digestion by Olsen and Sommers (1982).

4.3.4 Statistical Analysis

All results of the measured variables are expressed on the basis of the oven-dry soil as means (n=4), and their standard error (s.e). Statistical significance of the effects of different treatments on soil chemical and microbial properties and P fractions were determined separately by analysis of variance (ANOVA) using Statistix for Windows version 8.0 (Analytical Software, Tallahassee, FL). The least significant difference (LSD) test was used to separate the means when differences were significant at $P < 0.05$.

4.4 Results

4.4.1 Chemical and physical properties of soils and RO

Some of the main chemical and physical characteristics of the two soils before adding amendments and those of the RO are shown in Table 4.1 and Table 4.2, respectively. The pH was acidic for both soils but the pH of the sandy loam was lower than that of the loam soil. The electrical conductivity (EC) for both soils was low with values less than 0.4 dS m⁻¹. Soil total carbon (TC) was 1.9% and 2.0% in the sandy loam and loam soil respectively (Table 4.1). The soil total nitrogen (TN) content was very low for both sandy loam (0.09%) and loam (0.19%) soil (Table 4.1). Soil available P (Colwell P) was very low in the sandy loam soil with 4 mg P kg⁻¹ compared to 29 mg P kg⁻¹ in the loam soil. The total Ca, Mg, Na and K concentrations in the sandy loam soil were lower than the loam soil, while total Fe and Al were higher in the loam soil compared to the sandy loam soil.

For the amendments, the pH of the compost amendments (CG and CM) were close to neutral, while BS and BL amendments were slightly alkaline and the BC amendment was the most acidic among all RO. The highest EC was in the BC which was 28-times higher than the lowest EC found in the CM. The EC of both compost amendments (CM and CG) were similar. Total C in RO ranged from 19.7% to 65.0%, while total P in RO ranged from 0.1 to 4.5% (Table 4.2). Total N varied between 0.1 to 4.9% across all 5 RO (Table 4.2). Colwell P was highest in the BS amendment followed by BL, CM, CG and BC. The C: N ratio was higher in the sandy loam compared to the loam soil with values of 21 and 11 respectively. In all 5 RO, the C: N and C: P ratios ranged from 6 to 650 and 8 to 1083 respectively. Total magnesium (Mg) ranged from 0.09 to 0.50%, while total calcium (Ca) ranged from 0.22 to 3.90% in RO (Table 4.2). The total iron (Fe) ranged from 189 to 11,000 mg kg⁻¹ and total aluminium (Al) ranged from 148 to 12,000 mg P kg⁻¹

¹ in RO. Total copper (Cu) was the highest in BS with 830mg Cu kg⁻¹ and lowest in BC amendment with 5.3mg Cu kg⁻¹.

4.4.2 Soil P fractionation and availability

4.4.2.1 Soil inorganic P (Pi)

The soil inorganic P (Pi) fractions are shown in Table 4.4. Overall, the NaOH-extractable Pi (NPi) was the dominant Pi fraction in both un-amended and amended soils with RO ranging from 58 - 100 mg P kg⁻¹ in the loamy soil (16 - 22% of total P) and 9- 38 mg P kg⁻¹ in the sandy loam soil (16 - 30% of total P), with the highest concentration of NPi fraction found in the BS treatment in both soils. All organic treatments significantly increased NPi fractions in both soils, except for the BC treatment in the loam soil with 58 mg P kg⁻¹ compared to 71 mg P kg⁻¹ in the un-amended control. However, the NPi increased in the BC treatment in the sandy loam but was not significantly different from the control.

The next most dominant fraction was the NaHCO₃-extractable Pi (BPi) ranging from 17 to 43 mg P kg⁻¹ (accounting for 5 to 11 % of total P) and 3 to 22 mg P kg⁻¹ (accounting for 5 to 18% of total P) in the loam and sandy loam soil, respectively. All treatments significantly increased ($P<0.001$) BPi compared to the control in the sandy loam soil except for the BC treatment in the loam soil. The BS treatment strongly increased BPi in both soils with 22 and 43 mg P kg⁻¹ in the sandy loam and loam soils respectively. Concentrations of the labile Pi fractions (APi + BPi) were in the order of BS>BL>CM>CG>BC>CK in the sandy loam soil, and BS>CM>BL>CG>CK>BC in the loam soil.

The stable Pi fraction, HCl-extractable Pi (HPi), was the third most dominant Pi fraction with 17 to 30 mg P kg⁻¹ in the loam soil (accounting for 5 - 10% of total P) and 2 to 9mg P kg⁻¹ (accounting for 3 - 6% of total P) in the sandy loam soil. The HPi fraction

increased in all treatments compared to the control in both soils. The highest increase of H_Pi fraction in the sandy loam soil was in CM treatment with 8.5 mg P kg⁻¹ and the highest H_Pi fraction in the loam soil was in CG treatment with 30.2 mg P kg⁻¹. The H_Pi fraction was marginally increased with the BS amendment in both soils.

The NH₄Cl-extractable P_i (A_Pi) was the smallest P pool in the soil with ~1 to 5 mg P kg⁻¹ and ~1 to 2 mg P kg⁻¹ in the loam and sandy loam soils, respectively. The effects of RO on the soil A_Pi fraction varied with soil type. In the sandy loam soil, only the applications of the BS and CM treatments have significantly increased the concentrations of A_Pi fraction (1.54 mg P kg⁻¹ and 1.43 mg P kg⁻¹) in comparison with the control. For the loam soil, the concentrations of soil A_Pi in all organic amendments treatments were 2-3 times higher than in the control except for the BC treatment (Table 4.4). The BC treatments did not have significant effects on A_Pi in both soils. In general, the sizes of the soil P_i fractions in the loamy soil were larger than the sandy loam soil. Among all treatments, the concentrations of all soil P_i fractions were highest in the CM treatments, and the P_i fractions were lowest in the BC treatments for both soils among the RO except for the H_Pi fraction.

Table 4.4 Soil phosphorus fractions (mean, mg P kg⁻¹) as affected by amendments of different recycled organics (RO) in sandy loam (SL) and loam (L) soils and P values from analysis of variance (ANOVA) on the effects of soil types, RO treatments and their interactions. The mean values followed by different letters indicate significant differences from each other within a column. Treatments are CK = control, BS = biosolids, BL = broiler litter, CG =compost green waste, CM =compost mixed, BC = green waste biochar.

RO Treatments	APi		BPi		BPo		NPi		NPo		HPi		Res-P	
	SL	L	SL	L	SL	L	SL	L	SL	L	SL	L	SL	L
CK	0.95 b	1.4 c	3 e	19 c	8.1 a	46.6 b	9 c	71 c	15.6 d	145 bc	1.8 d	17.3 c	20 d	81
BS	1.54 a	4.1 b	22 a	43 a	7.9 a	44.6 b	38 a	100 a	22.1 c	154 bc	1.9 cd	20.2 bc	27 cd	86
BL	1.11 b	5.4 a	20 ab	39 b	6.9 a	56.3 a	27 b	92 a	44.0 a	182 a	3.2 b	20.4 bc	35 b	85
BC	1.08 b	2.4 c	8 d	17 c	5.7 a	42.1 b	10 c	58 d	16.5 d	136 c	3.1 bc	25.8 ab	34 bc	82
CM	1.43 a	4.6 ab	19 b	41 ab	9.6 a	55.7 a	34 a	91 a	30.5 b	163 ab	8.5 a	29.5 a	41 a	88
CG	1.06 b	3.7 b	14 c	38 b	9.7 a	48.6 ab	29 b	79 b	29.3 b	183 a	4.0 b	30.2 a	53 b	90
Mean	1.2	3.6	14.2	32.7	8.0	49.0	24.7	84.9	26.3	160.3	3.6	25.6	34.7	85.5
S.E	0.1	0.9	3.7	5.7	1.5	3.7	5.9	8.3	5.4	12.4	1.1	6.0	7.1	3.6
Two-way ANOVA (<i>P</i> value):														
RO Treatment	<0.001		<0.001		<0.001		0.030		0.073		0.015		0.002	
Soil type	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
RO Treatment x Soil	0.003		0.065		0.637		0.623		0.075		0.193		0.115	

Pi = inorganic P; Po = organic P. APi =NH₄Cl-Pi; BPi = NaHCO₃-Pi; BPo = NaHCO₃-Po; NPi = NaOH-Pi; NPo = NaOH-Po; HPi = HCl-Pi; Res-P = residual (non-extracted) P.

Table 4.5 Soil total inorganic and organic P fractions (mean, mg P kg⁻¹) as affected by amendments of different recycled organics (RO) in sandy loam (SL) and loam (L) soils and P values from analysis of variance (ANOVA) on effects of soil types, RO treatments and their interactions. The mean values followed by different letters indicate significant differences from each other within a column. Treatments are CK = control, BS = biosolids, BL = broiler litter, CG =compost green waste, CM =compost mixed, BC = green waste biochar.

RO Treatments	TPi		TPo		TP	
	SL	L	SL	L	SL	L
CK	15 d	108 c	24 c	192 bc	58 d	381 b
BS	64 a	168 a	30 b	199 bc	120 ab	452 a
BL	50 b	156 ab	51 a	243 a	138 b	480 a
BC	22 c	130 bc	22 c	178 c	78 c	363 b
CM	63 a	167 a	40 b	217 ab	143 a	473 a
CG	48 b	152 a	39 bc	231 a	140 ab	473 a
Mean	46.6	151.7	35.0	209.9	112.2	441.4
S.E	11.9	16.0	5.9	15.3	18.9	27.4
Two-way ANOVA						
(P value):						
RO Treatment	<0.001		0.003		<0.001	
Soil type	<0.001		<0.001		<0.001	
RO x Soil type	0.786		0.074		0.284	

TPi (total extractable Pi) = APi + BPi + N₁Pi + HPi + N₂Pi; TPo (total extractable Po) = BPo + N₁Po + N₂Po; TP (total soil P) = TPi + TPo + Res-P.

4.4.2.2 Soil organic P (Po)

Soil organic P (Po) accounted for 23 - 41% and 41 - 50% of soil total P in the sandy loam and loam soils respectively (Table 4.4). Among the extractable Po fractions, the NPo was the dominant fraction in both soils, accounting for 17 - 32% of soil total P in the sandy loam and 30 - 38% of soil total P in the loam. The highest NPo fraction in the sandy loam soil was in the BL treatment with 44 mg P kg⁻¹ and the CG treatment was the highest in the loam soil with 183 mg P kg⁻¹. All amendments increased the NPo in the sandy loam and in the loam soil except for the BC treatment. In the loam soil, the BC value was lower than that in the control.

The BPo fraction was the least dominant Po fraction accounting for only 5 - 18% and 10 - 18% of total P in the sandy loam and loam respectively. The BPo fraction in the sandy loam soil ranged from 5.7 to 9.7 mg P kg⁻¹ while in the loam soil it ranged from 42.1 to 56.3 mg P kg⁻¹. In the loam soil, the BL and CM treatments significantly increased the concentrations of the BPo fraction compared to the control, while other treatments did not significantly change the BPo fraction. It was observed that the BPo fraction in the BC treatment was the lowest in both soils and in fact, the BC treatment was lowest in all Po fractions in both soils. In relation to the control, the concentrations of all soil Po fractions (NPo and BPo) were highest in the BL treatment and lowest in the BC treatment in both soils.

4.4.2.3 Total soil P

Analysis of variance (ANOVA) results showed that the different RO treatments and soil type affected the total P fractions significantly ($P < 0.01$) in both soils (Table 4.5). However, there were no significant interactions between the RO treatments and soil type on total inorganic P (TPi), total organic P (TPo), and total P (TP). Generally, it was observed that the BS treatment had the highest TPi while TPo was highest in the BL

treatment. Among the treated soils, the BC treatments had the lowest TPi and TPo values in both soils. For the TP, where all fractions were combined, it was observed that the BL treatment had the highest value and BC the lowest among all RO in both soils. Overall, the sandy loam was more affected by the RO amendments compared to the loam soil as the increments of P from the RO addition were more profound.

4.4.2.4 Proportion of soil P fraction over total P

The percentage of soil P fractions (% of total P) of different lability under different RO treatments is shown in Figure 4.1. The labile Pi fraction (APi and BPi) consisted of 7 - 20% and 5 - 10% of the total soil P in the sandy loam and loam soils respectively.

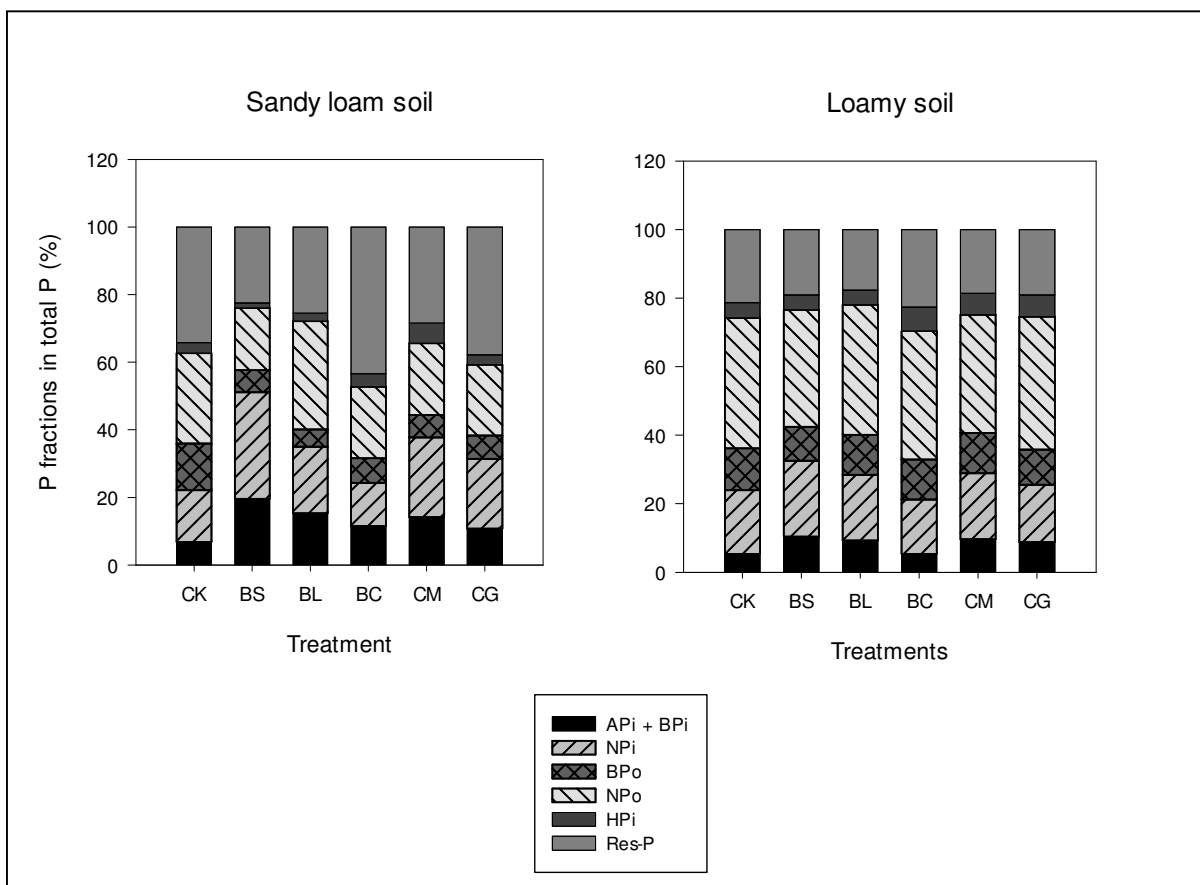


Figure 4.1 Percentage of P fractions in the sandy loam and loam soils under different organic amendments. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG= compost green waste, CM= compost mixed and BC= green waste biochar.

The moderately labile Pi (NPi) accounted for 8 - 32% (sandy loam soil) and 16 - 22% (loam soil) of total soil P and the recalcitrant Pi fraction (HPi) accounted for 2 - 6% (in the sandy loam) and 4 - 7% (in the loam soil) of total soil P. The most labile organic P fraction (BPo) consisted of 5 - 7% (sandy loam soil) and 10 - 12% (loam soil) of total P and moderately labile Po (NPo) accounted for 18 - 32% (sandy loam soil) and 34 - 39% (loam soil) of total soil P. The residual P accounted for 22 - 43% and 18 - 23% of total P in the sandy loam and loam soil, respectively.

As indicated by the darkest column in Figure 4.1, the BS treatment had the highest proportion of labile Pi (APi + BPi) in the sandy loam soil which accounted for 20% of total P compared to only 11% in the BC treatment. While in the loam soil, the highest percentage of labile Pi was in the BS and BL with 10% for both treatments. The moderately labile Pi (NPi) was the highest in the BS treatment in both soils with 32% and 22% of total P in the sandy loam soil and loam soil respectively. The lowest NPi in the sandy loam and loamy soil was in the BC treatment with 13% and 16% of total P, respectively. The recalcitrant Pi (HPi) was highest in the CM treatment in the sandy loam soil and the BC treatment was highest in the loam soil. The BS treatment was the lowest in HPi for both soils with 2% and 4% of total P in the sandy loam and loam soil, respectively.

As for the labile Po fraction (BPo) all treatments showed a lower proportion of BPo percentage compared to the control (accounted for 14% of total P) with less than 10% of total P in the sandy loam soil. For the loamy soil, all RO had lower BPo than the control except for the BL, BC and CM treatments with 12% of total P. The moderately labile Po (NPo) fraction was more than twice the labile Po with the highest percentage was in the BL and CG treatments with 32% and 39% of total P respectively.

4.4.3 Microbial biomass carbon and phosphorus

Microbial biomass results are presented in Figure 4.2. The MBC in the loam soil was higher than in the sandy loam soil in all treatments. There were statistically significant differences ($P<0.01$) in the MBC concentration among different treatments in both soils. In the sandy loam soil, with the exception of the BS treatments, all treatments with RO increased MBC compared to the control with the highest respective values of 114 (for LP) and 151 mg kg⁻¹ (for HP) in the BL treatments. In the loam soil, the CM treatments increased the MBC by the highest amount with 337 (LP) and 466 mg kg⁻¹ (HP). The lowest MBC was found in the BS treatments, with 56 (LP) and 57 mg kg⁻¹ (HP) in the sandy loam, and with 173 (LP) and 174 mg kg⁻¹ (HP) in the loam soil respectively. The BC treatments significantly increased MBC in the sandy loam soil but did not significantly increase MBC in the loam soil compared to the control ($P<0.01$).

All treatments with RO also showed a significant increase ($P<0.01$) in MBP with respect to the control soils except for the BS treatments in both soils and for the BC treatments in the loam soil. It was observed that the MBP in the sandy loam soil was lower than that in the loam soil for all treatments. As expected, the MBP was higher in the treatments with a high P rate compared to a low P rate in both soils. There were statistically significant differences ($P<0.01$) between MBP concentrations for different treatments in both soils. The MBP in both soils showed the same trend whereby the MBP in BS treatments (LP-BS and HP-BS) had the lowest values (1.3 – 1.6 mg P kg⁻¹ in the sandy loam and 3.1 – 3.6 mg P kg⁻¹ in the loam soil). In contrast, the the HP-CM had the highest MBP value in both soils with 7.6 mg P kg⁻¹ and 10.8 mg P kg⁻¹ in the sandy loam and loam soil, respectively. The values of MBP for other treatments, BL, BC, CG for both high and low P rates, were intermediate between the HP-CM and BS treatments (Figure 4.2).

In general, the microbial C:P ratio was lower in all treatments in the sandy loam (16 – 53 mg P kg⁻¹) compared to the loam soil (32 – 60 mg P kg⁻¹). Microbial C:P ratio was significantly lower in all treatments in relation to the control ($P<0.01$), except for the HP-CM treatment under loam soil. The microbial C:P ratio values were lowest in green waste compost, CG treatments (LP-CG and HP-CG) in both soils, ranging from 16:1 to 12:1 in the sandy loam and 32:1 to 20:1 in the loamy soil. The highest microbial C:P ratio reached 53:1 and 60:1 in the control treatment in the sandy loam and loam soil, respectively.

The analysis of variance (ANOVA) (Table 4.6) performed on the three varied factors (soils types, RO types, P rates) on the microbial biomass data showed that different soil types had a significant effect on microbial biomass (MBC and MBP) (Figure 4.2) and microbial activities (cumulative respiration and $q\text{CO}_2$). The individual microbial activities are discussed in more detail in the following section (4.3.4). Changes in microbial biomass and cumulative respiration were significantly ($P<0.001$) influenced by different types of RO apart from the metabolic quotient ($P>0.05$). The P application rates were only significant ($P<0.05$) for MBC, MBP and cumulative respiration. There were significant interactions between soils and treatments in all microbial properties ($P<0.05$). Interactions between soils and P rates were only significant in MBC ($P<0.05$). However, there were no significant interactions between RO types and P rates in all microbial properties.

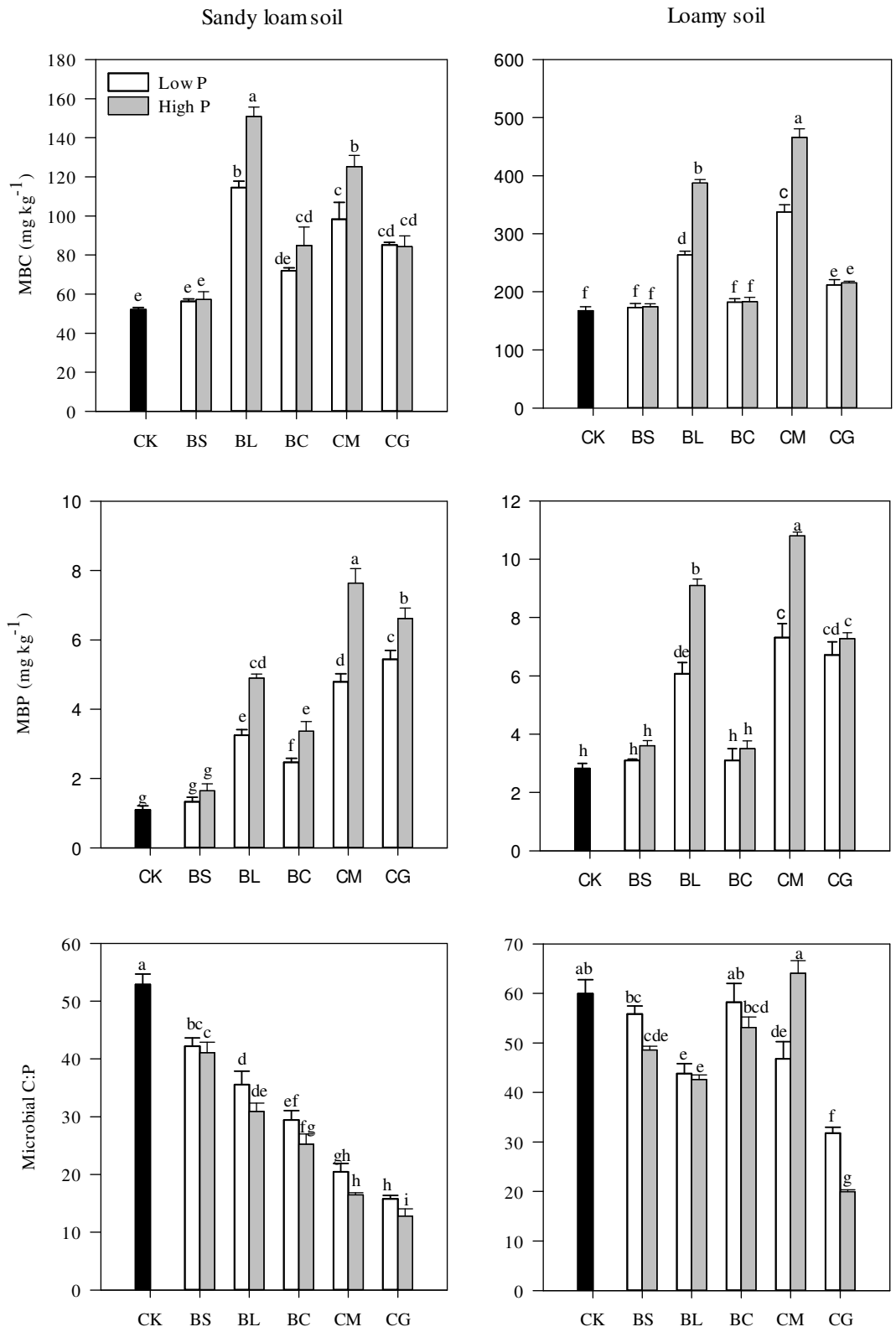


Figure 4.2 Microbial biomass carbon (MBC), microbial biomass phosphorus (MBP) and microbial C:P ratio under different treatments for two soil types. Different letters above each column for each treatment mean indicate significant differences ($P < 0.05$) from each other. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG= compost green waste, CM= compost mixed and BC= green waste biochar.

Table 4.6 *P* values from analysis of variance (ANOVA) on the effects of soil types, RO types and P rates and their interactions on various soil microbial properties determined over 140 days of incubation (MBC = microbial biomass C; MBP = microbial biomass P).

Factors	MBC (mg kg ⁻¹)	MBP (mg kg ⁻¹)	Microbial C:P ratio (mg kg ⁻¹)	Cumulative respiration (μg CO ₂ -C g ⁻¹)	Metabolic quotient (μg CO ₂ -C mg ⁻¹ microbial C h ⁻¹)
A (Soils)	<0.001	<0.001	<0.001	<0.001	<0.001
B (RO treatments)	<0.001	<0.001	<0.001	<0.001	0.078
C (P rates)	0.002	<0.001	0.849	0.026	0.350
A x B (Soils x RO treatments)	<0.001	0.009	<0.001	0.002	<0.001
A x C (Soils x P rates)	0.027	0.847	0.204	0.531	0.075
B x C (RO treatments x P rates)	0.777	0.059	0.559	0.241	0.621
A x B x C (Soils x RO treatments x P rates)	<0.001	<0.001	<0.001	<0.001	<0.001

4.4.4 Microbial respiration and metabolic quotient

The production of CO₂ by respiration was measured as an indicator for microbial activity. The organic amendments increased soil microbial respirations compared to the control (Figure 4.3 for the sandy loam and Figure 4.4 for the loam). Significant differences were observed among all RO in the CO₂-C evolution ($P < 0.001$) after 20 weeks (140 days) of incubation (Figures 4.3 and 4.4). Compared to the un-amended soil, cumulative respiration on day 140 was significantly higher in all RO-amended soils. It was observed that the application of all RO amendments in both soils induced a significant effect on the cumulative respiration of CO₂-C production ($P < 0.05$). Also, the total amount of CO₂-C released was higher in the loamy soil compared to the sandy loam soil for all treatments. In this study, HP treatments increased cumulative respiration more than the LP treatments. In the sandy loam soil, CO₂ respired from HP-BL treated soils was the highest with the cumulative respiration being 1109 $\mu\text{g CO}_2\text{-C g}^{-1}$ and the LP-BS treated soil being the lowest with 374 $\mu\text{g CO}_2\text{-C g}^{-1}$ compared to the control. However, cumulative CO₂ respired from both BS-amended treatments (HP and LP-BS) were found to be the lowest compared to other treatments and they were not significantly different from the control. In the loam soil, CO₂ respired from HP-CM was the highest with the cumulative respiration being 1630 $\mu\text{g CO}_2\text{-C g}^{-1}$ while LP-CG treatments were the lowest with 895 $\mu\text{g CO}_2\text{-C g}^{-1}$.

It was observed that the maximum respiration rate occurred in the first day of incubation for both soils. At this time, the highest CO₂ respiration rate in the sandy loam soil (Figure 4.3) was observed in the HP-BL-amended soil (3.9 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) and the lowest in the LP-BS-amended soil (0.7 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) in relation to the control (0.6 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$). Among the treatments in the loam soil, the CO₂ released on the first day was highest in the HP-CM-amended soil (4.0 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) and lowest in the LP-BL-amended soil (1.9 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) (Figure 4.4), compared to the control at 2.8 $\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$.

$\text{C g}^{-1} \text{ h}^{-1}$, The higher P input induced larger respiration rates throughout the incubation in both soils ($P < 0.05$). The $\text{CO}_2\text{-C}$ respiration rate in soil with different types of RO was greater during the first week of incubation compared to the control. A gradual decrease in rate was observed in all treatments before becoming constant after 80 days and the respiration rate become constant earlier in the sandy loam soil (~60 days) compared to the loam soil (~80 days) (Figures 4.3 and 4.4). In the sandy loam, the respiration rate at the end of the incubation period was highest in HP-CG-amended soil and lowest in the soil amended with HP-BS, whereas in the loam soil, the rate was highest in HP-CM and lowest in LP-BS.

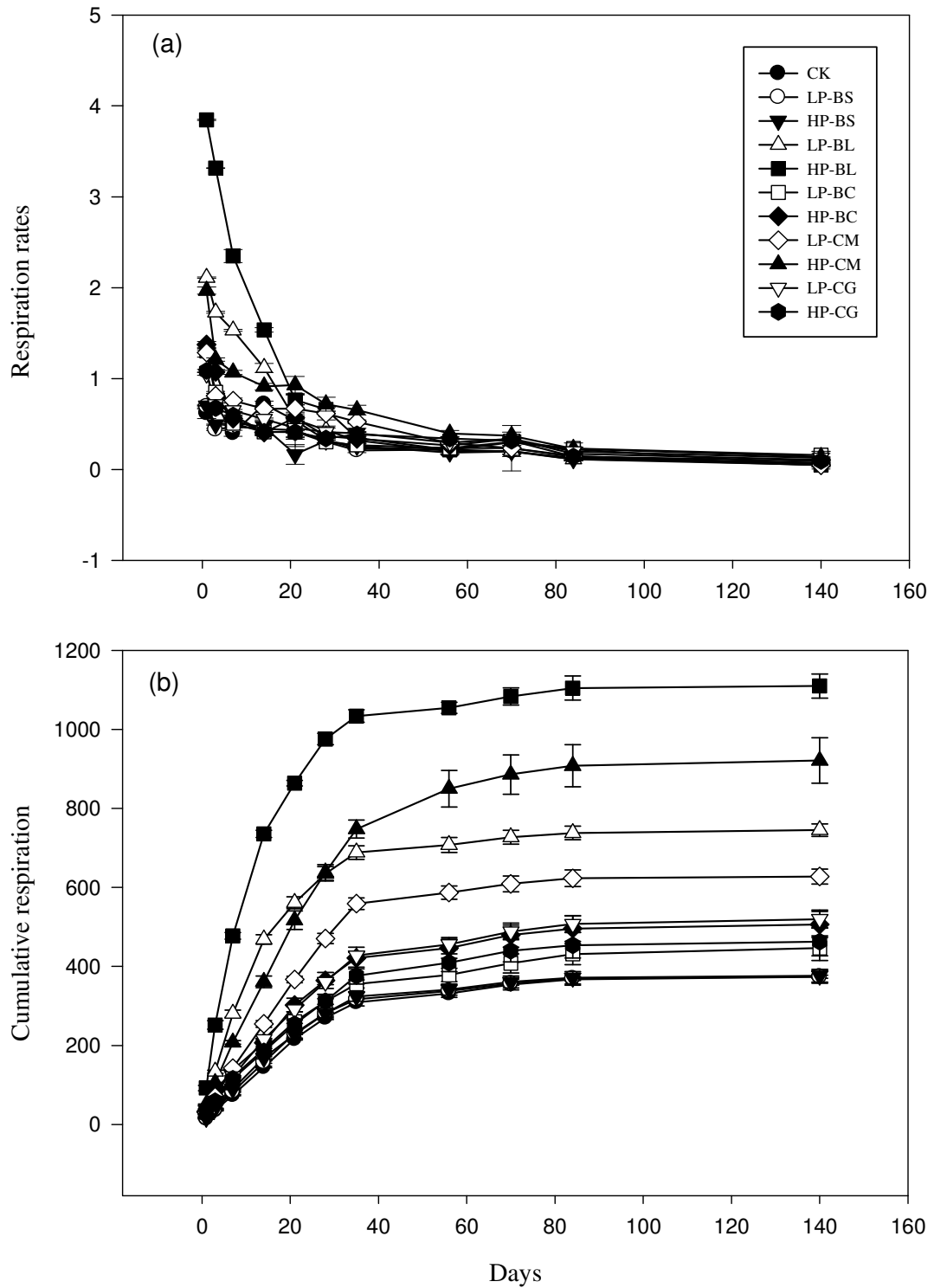


Figure 4.3 Respiration rate in $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$ and cumulative respiration in $\mu\text{g CO}_2\text{-C g}^{-1}$ (b) as affected by amendments of different RO for the sandy loam soil. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG=compost green waste, CM= compost mixed, BC= green waste biochar, HP= high phosphorus treatment, LP= low phosphorus treatment.

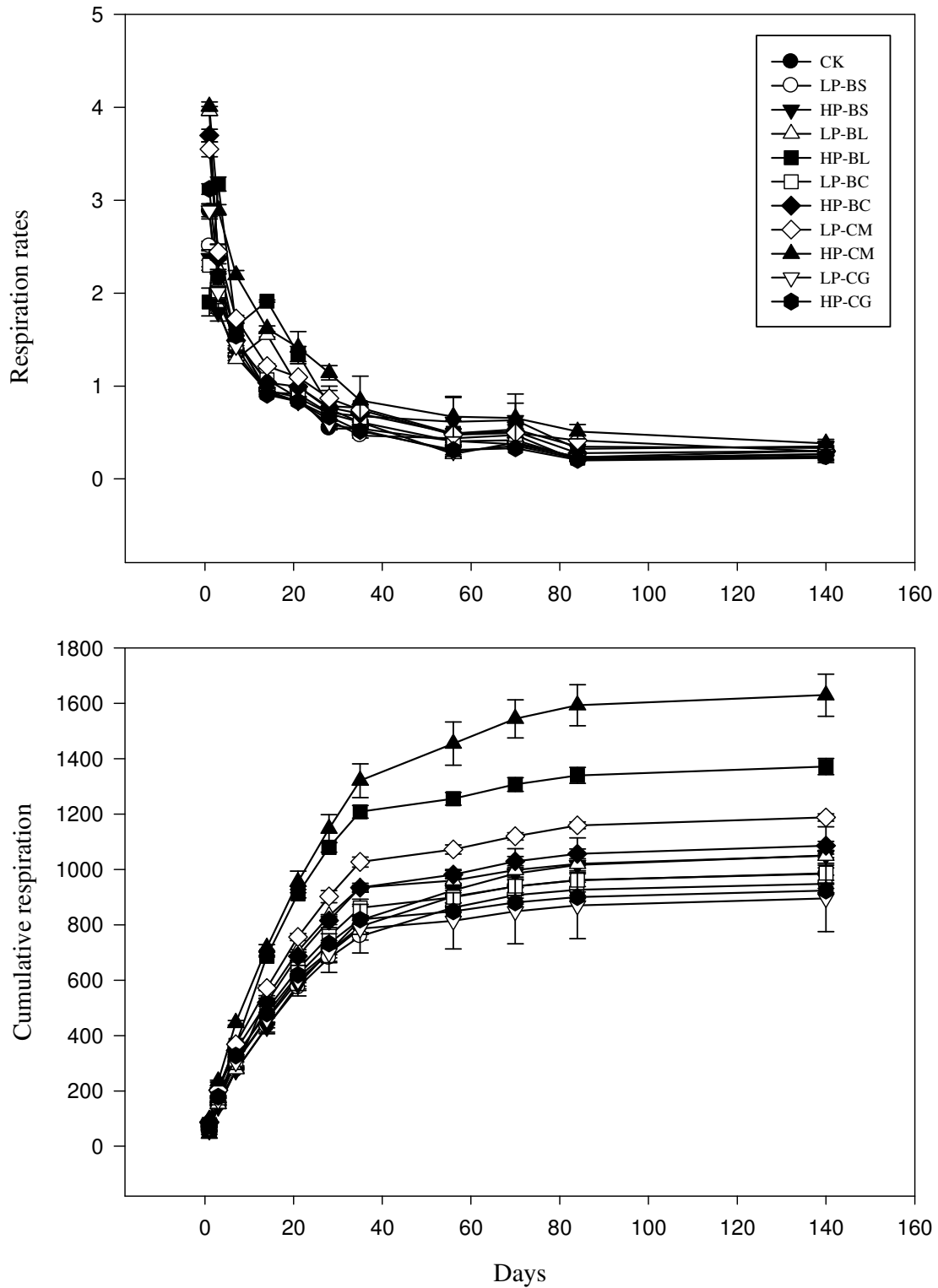


Figure 4.4 Respiration rate in $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$ and cumulative respiration in $\mu\text{g CO}_2\text{-C g}^{-1}$ as affected by the amendments of different RO in the loam soil. Treatments are CK= control, BS= biosolids, BL= broiler litter, CG=compost green waste, CM=compost mixed, BC= green waste biochar, HP= high phosphorus, LP= low phosphorus.

The $q\text{CO}_2$ in this study was calculated from the ratio of the rate of microbially respired C ($\mu\text{g g}^{-1}$ over the 140 days) to MBC. Results in Table 4.7 shows that the metabolic quotient ($q\text{CO}_2$, $\mu\text{g CO}_2\text{-C mg}^{-1}$ microbial C h^{-1}) was higher under the sandy loam soil compared with the loam soil. The HP-CM and HP-BL treatments had higher $q\text{CO}_2$ than the control in sandy loam soil, while in loam soil the control had highest $q\text{CO}_2$ compared to other treatments. However, the $q\text{CO}_2$ in both CM treatments in the loam soil were not significantly different from each other.

Table 4.7 Soil microbial metabolic quotients ($q\text{CO}_2$) as affected by the amendements of different recycled organics (RO). The mean values followed by different letters indicate significant differences from each other with a column. Treatments are CK = control, BS= biosolids, BL= broiler litter, CG= compost green waste, CM= compost mixed, BC= green waste biochar, HP= high phosphorus, LP= low phosphorus.

Properties	Treatment	Soil types	
		Sandy loam soil	Loam soil
Metabolic quotient ($q\text{CO}_2$) ($\mu\text{g CO}_2\text{-C mg}^{-1}$ microbial C h^{-1})	CK	2.13 (0.11) abc	1.74 (0.05) a
	LP-BS	1.97 (0.07) abc	1.62 (0.05) a
	HP-BS	1.86 (0.08) abcd	1.15 (0.14) cd
	LP-BL	1.93 (0.04) abcd	1.18 (0.03) cd
	HP-BL	2.18 (0.06) ab	1.05 (0.04) d
	LP-BC	1.84 (0.14) bcd	1.62 (0.04) a
	HP-BC	1.81 (0.20) cd	1.21 (0.08) cd
	LP-CM	1.94 (0.19) abcd	1.05 (0.03) d
	HP-CM	2.19 (0.17) a	1.04 (0.06) d
	LP-CG	1.83 (0.13) bcd	1.41 (0.17) b
	HP-CG	1.62 (0.09) d	1.27 (0.02) bc
		<i>Significance</i>	*

* indicates the significance at $P < 0.05$ level; while *** at $P < 0.001$ level

4.4.5 Relationship between soil P availability and microbial properties

The labile Pi fractions (APi and BPi) were positively correlated with each other In the sandy loam treatments (Table 4.8) and these fractions were also positively correlated with moderately labile Pi fraction (NPi) ($P < 0.001$). The BPi fraction was also observed to be positively correlated with both the inorganic and organic moderately labile P

fractions (NPi and NPo) ($P < 0.01$). The HPi fraction was positively correlated with residual P. All labile Pi fractions (APi + BPi) and NPi were found to have significant and positive correlation with MBC and MBP and negative correlation with microbial C: P ratio. There were no correlations observed between any P fractions and $q\text{CO}_2$ (Table 4.8). On the other hand, MBC and MBP were significantly correlated with CO_2 respiration ($P < 0.05$) as expected.

In the loam soil (Table 4.9), all labile P fractions (APi, BPi and BPo) were positively correlated with each other ($r = 0.41 - 0.63$, $P < 0.05$). The APi and BPi were also correlated positively with moderately labile P (NPi and NPo), while BPo only correlated with NPo. The stable P fraction (HPi), however, was not correlated with any variables measured. Residual P was positively correlated with BPi, BPo and NPo. The MBC and MBP were correlated well with CO_2 , $q\text{CO}_2$, all labile P and NPi (Table 4.9). However, all microbial properties did not correlate with any stable P and residual P fractions.

Table 4.8 Correlation coefficient (r) among key variables in the sandy loam soil.

r	MBC	MBP	MBC:P	CO ₂	qCO ₂	APi	BPi	BPo	NPi	NPo	HPi	Res-P
MBC	1											
MBP	0.6162*	1										
MBC:P	-0.2097	-0.7779***	1									
CO ₂	0.9429***	0.5024*	-0.0937	1								
qCO ₂	0.1937	-0.1506	0.3373	0.5009*	1							
APi	0.0318	0.3087	-0.4998*	0.0675	0.0595	1						
BPi	0.4721*	0.5775**	-0.6526***	0.4614	0.0453	0.6905***	1					
BPo	0.0698	0.3244	-0.1593	0.0082	-0.1068	0.2518	0.1718	1				
NPi	0.3100	0.6368***	-0.6778***	0.2784	-0.0779	0.6278***	0.8702***	0.3233	1			
NPo	0.7461*	0.5485**	-0.2921	0.7695***	0.2651	0.0710	0.6098**	-0.0199	0.4335*	1		
HPi	0.5384**	0.7515***	-0.4156*	0.5249**	0.1706	0.1794	0.2292	0.2002	0.3657	0.2506	1	
Res-P	0.3288	0.6301**	-0.5240**	0.2606	-0.1277	0.0572	0.2576	0.1217	0.2807	0.3881	0.4186*	1

*, ** and *** indicate the significance at P < 0.05, P < 0.01 and P < 0.001 levels, respectively.

MBC = microbial biomass carbon; MBP = microbial biomass phosphorus; MBC:P = MBC to MBP ratio; CO₂ = soil respiration; qCO₂ = metabolic quotient; Pi = inorganic P; Po = organic P. APi = NH₄Cl-Pi; BPi = NaHCO₃-Pi; BPo = NaHCO₃-Po; NPi = NaOH-Pi; NPo = NaOH-Po; HPi = HCl-Pi; Res-P = residual (non-extracted) P.

Table 4.9 Correlation coefficient (r) among key variables in the loam soil.

r	MBC	MBP	MBC:P	CO ₂	qCO ₂	APi	BPi	BPo	NPi	NPo	HPi	Res-P
MBC	1											
MBP	0.8960***	1										
MBC:P	0.3563	-0.0405	1									
CO ₂	0.9049***	0.7621***	0.5244**	1								
qCO ₂	-0.7445***	-0.8016***	0.1425	-0.4221*	1							
APi	0.6005**	0.6702***	-0.1648	0.3914	-0.6733***	1						
BPi	0.4895*	0.7320***	-0.3453	0.3468	-0.5776**	0.6306**	1					
BPo	0.5142*	0.5505**	0.0615	0.5660**	-0.2832	0.4699*	0.4128*	1				
NPi	0.4799*	0.5890**	-0.1073	0.3685	-0.4560*	0.6106**	0.8408***	0.3951	1			
NPo	0.2180	0.4503*	-0.4205*	0.0554	-0.3671	0.4509*	0.5016*	0.4428*	0.3937	1		
HPi	0.2244	0.3680	-0.1971	0.1949	-0.3169	0.2047	0.1970	0.2419	0.0293	0.2342	1	
Res-P	0.1371	0.3417	-0.3569	0.0740	-0.2682	0.2819	0.4859*	0.4217*	0.3284	0.5668**	0.3568	1

*, ** and *** indicates the significance at P < 0.05, level; while ** at P < 0.01 and *** at P < 0.001 levels, respectively .

MBC = microbial biomass carbon; MBP = microbial biomass phosphorus; MBC:P = MBC to MBP ratio; CO₂ = soil respiration; qCO₂ = metabolic quotient; Pi = inorganic P; Po = organic P. APi =NH₄Cl-Pi; BPi = NaHCO₃-Pi; BPo = NaHCO₃-Po; NPi = NaOH-Pi; NPo = NaOH-Po; HPi = HCl-Pi; Res-P = residual (non-extracted) P.

4.5 Discussions

4.5.1 Effects of recycled organics on soil P fractions and availability

The impact of RO on P fractions is much more pronounced at high P rates, therefore only the effect of HP application rate on P fractions is discussed. In this study, it was confirmed that the different RO types affected the P dynamics in both soils at high P rate. Generally, it was observed that all RO increased P availability and increased the size of APi and BPi (defined as labile Pi) fractions compared to the control after 20 weeks of incubation, except for the BC treatment in both soils. This implied that all RO except for BC could supply P even after 20 weeks. The labile Pi maintained in the low P content of RO such as the CG was not expected. It might have been due to the net P immobilisation at the initial stage of incubation and then went through the remineralisation process at the later stage of incubation. This is supported by the C to P ratio of the CG amendment which is >300 indicating that net immobilisation has occurred at the initial stage of incubation (Reddy et al. 2005). The low labile Pi in the BC treatments in both soils is consistent with the low level of available P from this source (Table 4.2). The highest increase of labile Pi in the BS treatment compared to other RO is due to this amendment's high available P (Colwell extractable P) content (Table 4.2). This suggests that the BS treatment could act as a source of plant available P in short-term.

The labile Po (BPo) concentrations in all RO treatments showed no significant differences in the sandy loam soil and the variation of BPo fractions among RO were small in the loam soil (Table 4.4). The possible explanation for this result is that this fraction has gone through rapid mineralisation throughout the incubation process as the diester PO₄, which is a major component of labile Po (Tiessen et al. 1984) and prevents it from binding strongly to soil minerals. This is supported by the study of Chauhan et al. (1979) who found that labile Po is very sensitive to microbial activity and has a fast

turnover rate hence the small variation of P_o among RO (Trasar-Capeda and Carballas 1991; Chander et al. 1997; Reddy et al. 2005).

The NaOH pool which represents moderately labile organic and inorganic P pool are associated with Al and Fe on soil surfaces (Hedley et al. 1982; Tiessen and Moir, 1993). Increases in the organic pool in the moderately labile (NPo) fraction following RO amendments have been reported after the addition of cattle manure (Kashem et al. 2004), wheat residue (Reddy et al. 2005) and compost and residue (Malik et al. 2012). The build up of NPo pools in the previous studies were reported due to the increasing activity of the soil microorganisms following addition of organic amendments. The decomposition process of the organic amendments used in these studies may have caused desorption of sorbed P and the decomposition process may have solubilised some recalcitrant P in the soil and favoured the formation of P_o . However, in this study, all RO were found to increase the NPo pool except for the BS and the CM treatments in the sandy loam soil (Table 4.4). This was due to: (i) the high concentration of NP_i in the BS treatment originating from the amendment itself due to its high Fe and Al content (Table 4.2). The Fe and Al oxides have a high P sorption potential, fixing P in the highly acidic soil in the sandy loam soil. The findings are corroborated by the findings by Iyamuremye et al. (1996) who also reported an increased NP_i fraction in manure and alfalfa amendments with high Fe and Al contents; (ii) NPo increased in CM under the loam soil was due to high microbial activity and this is supported by respiration data. The low respiration data in the CM treatment in the sandy loam soil is due to its low pH as biological activity becomes inhibited, thus lowering the formation of P_o ; (iii) the microorganisms in the loamy soil are more efficient in the formation of P_o than those in the sandy loam soil. This might be due to the difference in the soil texture where the finer textured loam soil has a higher organic carbon content. The high organic carbon content provides more substrate for soil microorganism growth, thus higher microbial activity and consequently

higher Po. The high NP_i fraction in the BS and CM treatments suggest that these treatments could supply immediate P_i to plants in the sandy loam soil where other treatments could act as a P_i supply in the long-term due to the mineralisation of Po over time.

From the comparison among the P_i fractions, it was observed that NP_i fractions were higher when compared to the BP_i fractions in both soils (Table 4.4). The trend where NP_i increases is consistent with numerous short- and long-term incubation studies suggesting that NaOH-extractable P_i acts as sink for BP_i in soil (Buehler et al. 2002, He et al. 2004a). As for the recalcitrant P_i fraction (HP_i), the CM treatment increased this fraction the most compared to other treatments in both soils. This is indicative of the high Ca levels of this amendment (Table 4.2) which enhanced precipitation and adsorption of P, resulting in higher P in this Ca-bound fraction. Also, the increased Po fractions and recalcitrant P pools in this treatment indicates that these treatments are suitable for slow release of P, thus promoting P maintenance in soil.

4.5.2 Effects of recycled organics amendments on soil microbial properties

It has been well established that the addition of organic amendments improves various soil properties including microbial biomass and microbial activity. In this study, the mean values for MBC in all sandy loam treatments were lower compared to the values obtained by other authors (Dick 1992; Plaza et al. 2004). The low MBC values indicate that sandy loam soil had a very low available C content compared to the loamy soil (Table 4.1) and this limits the increase of MBC as available C provides substrate for microbial growth in the sandy loam soil (Bünemann et al. 2008). The high P application rate (HP) treatments showed higher MBC compared to the low P application rate (LP) in both soils, indicating that soil microbes may be P-limiting in these soils. A number of incubation studies have found that soil amended with BC did not affect soil biological properties and soil

microbial respiration (Kuzyakov et al. 2009; Jones et al. 2010; Dempster et al. 2012). There was a significant increase in MBC and MBP in the BC treatments in the sandy loam soil. This was due to the acidic nature of the soil and the well known fact that BC is a biologically inert substance with low N and P values. One possible explanation to the increase of MBC and MBP in the BC treatments is that the BC improved the structure of the sandy loam soil in relation to microbial activities. BC is a form of charcoal which has a high surface area and high numbers of micropores (Peitikäinen et al. 2000; Keiluweit et al. 2010). This improves the water holding properties and retains moisture of sandy loam soil and thus provides additional habitat for microbial communities and increases their activities. However this is not the case for loam soil, as the moisture in the soil decreases with the addition of BC due to hydrophobicity of the BC as suggested by Glaser et al. (2002), making it unsuitable for microbial habitat thus explaining the low and insignificant increase of MBC and MBP in the loam soil (Figure 4.2). This increase in microbial properties following BC amendment implies that there is the possibility of maximising the potential benefits of biochar in enhancing nutrient cycling in soil.

On the other hand, the concentration of MBC in CM and BL treatments (both LP and HP) were highest in the loam and sandy loam soil respectively (Figure 4.2). The highest MBC in the CM treatment in the loam soil can be explained by the high supply of readily metabolisable C in the treatment supported by the high amount of total C added in the CM treatment (Alamgir et al. 2012). In the sandy loam soil, the highest amount of MBC in the BL treatment (Figure 4.2) is indicative of the stimulated microbial activities and growth due to the organic substrates added with the amendment in the soil (Sakamoto and Oba 1994; Marinari et al. 2000; Ghosh et al. 2011).

Soil microbial respiration, measured through CO₂ production, is considered a direct indicator of microbial activity and could be an indicator of organic C availability (Gomez et al. 2001). In this study, it was observed that microbial respiration had a strong

relationship with MBC (Tables 4.8 and 4.9) thus explaining the same trend in the MBC and microbial respiration between the BL and CM treatments in both soils. The strong relationship between the soil microbial respirations and MBC suggest that the large amounts of readily available C (or C substrates) in these treatments are mineralised more rapidly and are more susceptible to mineralisation, acting as an immediate energy source for soil microbial growth (Tejada et al. 2006). This result was also supported by the low $q\text{CO}_2$ concentrations in these treatments (Table 4.7) which indicates that the decomposition of readily available C in these treatments has stimulated the microbial growth. The high $q\text{CO}_2$ in HP-CM and HP-BL in sandy loam soil indicated microbial stress and it was due to high acidity of the soil. Although the BS amendment significantly increased the labile Pi fraction as mentioned previously, it was however found to have low MBC and low CO_2 respiration in both soils (Figure 4.2 and 4.3). One possible explanation is that it can be attributed to the high Cu content in the BS (Table 4.2) and its associated microbial toxicity compared to the other treatments. This is in agreement with the results of previous works by Filip (2002) and Gil-Sotres et al. (2005). They observed that the presence of high concentrations of heavy metals such as Cu and Cd are toxic to soil microorganisms and can be responsible for the inhibition of microbial population growth in soils. Therefore, it is vital to limit the possible hazards associated with large-scale use of this treatment. The low MBC concentration and CO_2 respiration in BS has also been attributed to lower concentration of labile organic C in the BS treatments, since organic C content measured in the unfumigated K_2SO_4 extract was low in the BS treatment (data not shown) (Tejada et al. 2006). Thus, it can be confirmed that the increase in labile P following BS amendment is not mediated by microbial biomass and is solely from the amendment itself.

The MBP was found to have a positive relationship with the labile Pi (APi and BPi) in the loam soil and in the sandy loam soil (BPi) (Table 4.9). Literature often

indicates that an increase of microbial P usually decreases the labile P in soil as the soil microorganisms use up the available P for their cell growth (Reddy et al. 2005). However, the increased of MBP when P availability is high, has been reported in other studies (Iyamuremye et al. 1996; Malik et al. 2012). The increased MBP could be explained by the increased microbial activity and the mineralisation of recalcitrant fractions of P or from the replenishment by the other P pools (Agbenin and Goladi, 1998).

Among the organic treatments, the CM treatments strongly increased MBP suggesting that the increase in the MBP was from the available P uptake by microbes in both soils. The increase in MBP content in RO amended soils demonstrates the potential of these treatments to enhance P supply in soil in the longer term as P may be released slowly from MBP during the biomass turnover process.

Literature suggests that low microbial C:P ratio is an indication of a higher level of labile P_i as microorganisms in soil with high P contents usually assimilate more P than in soils with low P content (Hedley et al. 1982). The C: P ratio of the soil microbial biomass is therefore an important factor that controls the availability of P in soil. As the microbial C: P ratio decreases, microbes have a higher potential to release P into the soil solution during mineralisation and microbial turnover, whereas with increased microbial C: P ratios, microbes immobilise more P (He et al. 1997; Chen et al. 2003). The CG treatments (LP-CG and HP-CG) had the lowest microbial C: P ratio in both soils (Figure 4.2) suggesting that this treatment could increase the P mineralisation process. This is supported by the significant correlation between the cumulative respiration and the microbial C: P ratio in the loam soil (Table 4.9) which indicated that P availability was affected by microbial activity. However, the HP-CM treatment in the loam soil had the highest microbial C: P ratio (65:1). With such a wide C:P ratio, the soil microbial biomass in this treatment would have had a stronger potential to immobilise P compared to other treatments which explained the high P_o in this treatment.

4.5.3 Interaction between soil type and recycled organic amendments

The different soil textures and chemical properties of the two soils (Table 4.1) might be associated with the changes in P forms and concentrations (Table 4.4 and 4.5) in various treatments. In terms of interaction between RO and soil types, it was consistently observed that the increase in soil P concentration in the NH_4Cl , NaHCO_3 , NaOH and HCl fractions were greater in loam soil than in sandy loam soil. In fact, the P concentrations in NH_4Cl , NaHCO_3 , NaOH , HCl and residual fractions were two to four times higher in the loam soil than in the sandy loam soil (Table 4.4). This may be explained by the differences in the initial P status between the two soils where the loam soil had a higher available P contents (Colwell extractable P) than the sandy loam soil (Table 4.1). However, despite the higher concentrations of most P fractions observed in the loam soil, the sandy loam had a greater increase in P fractions (relative to the control) with the RO amendment compared to the loam soil. This could be explained by the properties of sandy loam soil which had a lower concentration of clay, which caused the soil to have less capacity to retain added P and making the soil more susceptible to loss of P (Penn and Sims 2002). The difference in pH between the soils also affects the relative reactivity of Fe and Al with regard to P, as Fe and Al become activated in very acidic soil. The high solubility of Fe and Al therefore increase their possibility to be fixed with phosphates, thus explaining the greater increase of P (relative to control) in the sandy loam compared to the loam soil. A study done by He et al. (2004b) found that the changes in H_2O -, NaHCO_3 -, and NaOH -extractable P were similar in amended and un-amended soils showing that soil properties played a major role in controlling P dynamics. However, the interactions between soil types and RO treatments as shown by the two way analysis of variance (ANOVA) (Table 4.4) were significant only in the most labile P fractions (APi).

This study showed that amending soils with BS not only affected the amount and solubility of soil P, but also the potential for soils to adsorb P. This is because BS are

known to be important in soil P retention due to their high Fe and Al contents. Previous researchers have shown that the Fe and Al content of BS can be important to P mobility and availability in BS-amended soils (Maguire et al. 2001). This could explain the high concentration of P in the APi fraction and NPi fraction under BS treatments (Table 4.4). The incubation of a BS amendment with high available P in alkaline soil by Hosseinpur and Pashamokhtari (2013) did not significantly increase available P content in the treated soil. This was due to the precipitation of available P as calcium phosphate during the incubation period. This proved that available P depends on the soil characteristics such as soil pH as well as the RO characteristics.

The low concentrations of BPi, BPo, NPi and NPo fractions in soil under BC treatments might be due to the transformation of P in these fractions to other forms by microbial activities. This was confirmed by the regression analysis (Table 4.8 and 4.9) where BPi and BPo fractions have a significantly positive relationship with MBC and MBP as well as with the microbial respiration.

In the present study, soil type appears to have a significant effect on soil microbial properties, following RO amendments. Even though the BC treatments did not increase the MBC concentrations in the loam soil as mentioned before, it was observed that there were higher concentrations of the MBC, MBP and cumulative respiration in the loam soil (Figure 4.2, 4.3 and 4.4) in other treatments. This may be explained by the properties of the soils where the higher organic C and available nutrients in the loam compared to the sandy loam soil, might increase the abundance and activity of microorganisms through the availability of more labile organic matter. The higher concentration of microbial properties observed in loam soil could also be due to the higher percentage of clay in loam soil (Table 4.1). This agrees with previous findings that showed strong correlation between soil microbial biomass C and the percentage of clay in soils (Saggar et al. 1994). Also, Baldock (2007) suggested that finer textured soils such as loamy soils could provide

more habitat for soil microorganisms and stabilise organic matter to a greater extent than coarse-textured soils.

4.6 Conclusions

In conclusion, the effects of RO application on soil P dynamics and P availability varied with RO as well as soil types. The extent of the effects of RO application on soil microbial properties also varied with the RO types, soil types and P application rates. The different effects of the RO observed between the two soils may be related to their different physical and chemical characteristics such as the Fe and Al contents, pH, readily available organic C and P contents as well as their clay contents. The BS treatment increased labile Pi fraction the most in both soils, but had minimal effect on the microbial properties due to the high proportion of heavy metals. The BC unexpectedly increased labile Pi and microbial properties in the sandy loam soil indicating the potential of this treatment in enhancing the P and C cycling in such soil. Overall, the CM treatment showed the greatest influence on P fractions and availability as well as microbial properties, followed by BL and CG treatment. However, the amount of P used in this treatment should be determined beforehand and should be monitored as there is possibility of excess P to be transported into water ways.

Importantly, this study has highlighted that it is vital to understand the mechanisms involved in P dynamics associated with microbial properties following RO addition. This understanding provides important information in maximising the potential benefits of RO. This includes the use of green waste amendments (such as biochar) as effective fertiliser practices, thus improving agricultural management particularly in acidic soils. The observations from this study suggest that there are possibilities of combining different types of RO to manipulate the different benefits and improve the utilisation of the organic wastes. In this particular case, the BC and CM treatment

increased labile P, recalcitrant P and C content as well as improving soil microbial properties. This potentially means that they could reduce negative effects such as excessive accumulation of P and heavy metals, increase the C sequestration in soil and be more beneficial for plant growth. In order to further understand the response of RO on plant growth, a rhizosphere pot experiment is warranted as the P cycling is different with the existence of plant roots.

4.7 References

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Chapter 5 Effects of amendment of recycled organics on soil P availability and microbial biomass in the rhizosphere

5.1 Abstract

Increases in plant growth and phosphorus (P) uptake have been reported following the amendment of soil with organic matter. However, there is very limited information on P pools and soil microbial biomass as affected by the addition of different types of recycled organics (RO), especially in the rhizosphere. Therefore, this study aimed at determining the effects of different types of RO on rhizosphere soil P availability and microbial biomass as well as on plant yield and P uptake. A 12-week greenhouse pot experiment was conducted with a sandy loam soil to examine the effects of five different types of RO (biosolids, BS; broiler litter, BL; green waste biochar, BC; green waste compost, CG; mixed compost, CM) on plant growth, soil P availability and microbial properties. The application rate was P-based and the RO were added at rates of 50 kg P ha⁻¹ (low P, LP) and 100 kg P ha⁻¹ (high P, HP) into the soil, respectively. Two plant species (Ryegrass and wheat) were grown for this study. RO amendments affected P pools, soil MBC and MBP, plant yield and plant P uptake differently and these effects varied with plant species ($P < 0.05$) and P rates ($P < 0.05$). All P fractions measured were observed to be higher in the bulk soil compared to the rhizosphere soil due to the higher plant absorption in the rhizosphere. The effect of RO on P fractions were in a similar pattern in rhizosphere and bulk soil at both P rate but the concentrations were more pronounced with the HP rate compared to the LP rate. In summary, the results indicated that the BL applications (HP and LP) are the most effective treatment in increasing P availability, plant P uptake, biomass yield and microbial biomass, followed by CM and CG treatments (HP and LP).

The BC treatments unexpectedly increased all parameters tested in HP rate under wheat, but did not show significant impact on MBP, plant P uptake and biomass yield under ryegrass. The BS treatment showed positive effect on plant growth (root and shoot), plant P uptake (root and shoot) as well as most of Pi fractions with a highest increase in the stable Pi fraction, indicating that the BS treatment could act as a source for soil Pi in the future selection of RO. The minimal effect of BS treatments on the increase of microbial properties was possibly attributed to the root exudation although this treatment was high in heavy metal content. This study indicates that root exudation plays an important role in P transformation in the rhizosphere soil. The findings of this study provide important information of biogeochemistry of P in RO-amended soils, particularly in the rhizosphere. The study also highlights the need to examine the rhizosphere dynamics of the P in soil amended with RO.

5.2 Introduction

Although the amount of total phosphorus (P) (TP) in the soil may not be limited, much of phosphorus (P) is present in the forms that are unavailable to plants and microorganisms, with only a small proportion being immediately available. The amounts of P present in different soil P pools and the fluxes of available P are influenced by physicochemical and biochemical factors such as sorption-desorption, dissolution-precipitation and immobilisation and mineralisation (Buehler et al. 2002). These reactions occur rapidly in soil, in contemplation of supplying available P for plants. Recently, the amendment of recycled organics (RO) has been used in agroecosystems as an alternative to conventional chemical P fertilisers. There are several factors influencing plant available P in soil by the application of the RO, such as the chemical composition of the RO (Erich et al. 2002), the application rate of RO, microbial and chemical properties of the soil, plant uptake, as well as rhizosphere processes involved (Waldrip et al. 2011; Marschner 2011). According to Richardson et al. (2001), in order to increase the P uptake from the soil solution, plants have developed mechanisms which include root morphological adaptations and varying kinetics of P uptake by the roots. Due to the close proximity of the plant roots, many studies have shown that the plant roots highly affects the chemical changes in the rhizosphere soil (Ai et al. 2012; Mat Hassan et al. 2012; McGahan et al. 2014) and plays an important role in plant growth and soil fertility (Marschner et al. 2011). Plant roots can also influence rhizosphere nutrient cycling by nutrient uptake (Chen et al. 2002), rhizodeposition and interactions with microorganism (Hinsinger 2001)). Most studies currently conducted on P amendment effects on plant uptake and growth have been focused on the bulk soil without looking into the dynamics of nutrients in the rhizosphere (Breachin and McDonald 1994; Ghosh et al 2008). As a consequence, little information is available on the effects of RO amendments on the P availability of rhizosphere soils. Therefore, in-depth knowledge on how the RO amendment affects the P dynamics and

microbial response in the rhizosphere is important for the sound soil nutrient management.

Microorganisms in soil play an important role in contributing to the availability of soil P to plants and thus maintaining plant growth, particularly in soil with low P lability (Phillips and Fahey 2007; Marschner et al. 2011). Soil microbial biomass acts as both a source and a sink for nutrients such as P, while microbial biomass P becomes available through the turnover of soil microorganisms (Gichangi et al. 2009). As organic P (Po) needs to be mineralised by microorganisms into inorganic P (Pi) prior to plant uptake, the soil microbial activity play a vital role in this process. The amounts of microbial biomass are commonly used to characterise the size of microbial community in soil and vary with the composition of the microbial community, growth stage of microorganisms, and environmental conditions. Therefore, measurements of changes in MBP can be used to understand nutrient P dynamics in response to RO amendments as well as the relationship between P uptake by plants and the microbial processes. The RO application has been reported to increase the concentrations of both soil total and soluble P (Erich et al. 2002). Various sequential soil P fractionation schemes have been proven to be useful to determine the shifts in soil P pools that occur during short-term experiments (Hedley et al. 1994; Chen et al. 2003). Therefore, in this study, the modified Hedley P fractionation scheme (Condon et al. 1996) was used for investigating the short-term effects of applications of different RO on soil P fraction and availability and associated microbial properties on plant growth (shoots and roots). The two types of plants used were Wimmera ryegrass (*Lolium rigidum*) and wheat (*Triticum aestivum*) under glasshouse conditions. The specific objectives were to examine: 1) the effects of application of RO on soil P fractions and availability in the rhizosphere under ryegrass and wheat growth; 2) the effects of RO amendment on plant biomass (shoot and roots)

and plant P uptake; and 3) the effects of RO amendment on microbial biomass C (MBC) and P (MBP) in the rhizosphere under ryegrass and wheat growth.

5.3 Materials and Methods

5.3.1 Soils and Recycled Organics

The sandy loam soil used for this study was collected from Griffith University, Nathan Campus, Queensland. The soil is classified as a Podosol (Isbell, 1996) and the basic soil properties are outlined in Table 5.1. The soil was chosen due to its low available P content. It was air-dried and passed through a 2-mm sieve prior to being used in the pot experiment.

Table 5.1 Basic properties for the sandy loam soil used in the pot trial. Results are a mean of 3 replicates (n=3).

Soil property	Value
pH	4.45 ^A
EC (dS m ⁻¹)	0.29 ^A
Total N (%)	0.09
Total C (%)	1.89
C: N ratio	21
Colwell P (mg kg ⁻¹)	4
Clay (%)	14
Sand (%)	75
Silt (%)	11

^A1:5 soil: water extract.

From the initial stage of the research (see Chapter 2) where twenty-two different types of ROs were characterised, five RO were selected to be used as amendments in this pot study, based on their P contents and diverse C to P ratios (Table 5.2).

Table 5.2 Mean chemical properties of recycled organics used in the pot trials experiment.

Recycled organics	Biosolids (BS)	Broiler litter (BL)	Compost green waste (CG)	Compost mix (CM)	Biochar (green waste) (BC)
pH	7.40	7.12	6.94	7.04	4.74
EC	7.85	11.91	2.93	2.89	80.30
Total C (%)	28.9	39.0	26.0	19.7	65.0
Total N (%)	4.9	3.0	1.3	1.5	0.1
Total P (%)	4.52	1.36	0.22	0.28	0.06
C: N ratio	6	13	20	13	650
C: P ratio	8	29	118	70	1083
Colwell P (mg kg ⁻¹)	8600	4000	840	3600	43
Total Mg (%)	0.5	0.5	0.3	0.2	0.09
Total Ca (%)	1.2	2.2	1.5	3.9	0.22
Total Fe (mg kg ⁻¹)	8000	1300	11000	189	6500
Total Al (mg kg ⁻¹)	12000	1100	11000	148	1800
Total Cu (mg kg ⁻¹)	830	150	66	180	5.3

5.3.2 Pot trial preparations

Prior to the pot experiment, soil and RO were passed through a 2-mm sieve to remove any coarse particles and foreign materials. Soil samples (total weight of 220 g) were then amended with the five different types of RO as listed in Table 5.2. Two different P rates were applied for each RO, namely low P (LP) at 50 kg P ha⁻¹ and high P (HP) at 100 kg P ha⁻¹ by weight (w/w). Amounts of each RO used in each pot are shown in Table 5.3. The control treatments were not amended with RO. Amended and un-amended soils were then placed in small plastic pots (200 mL, with dimensions 65 x 65 x 75 mm) (Sarstedt Australia). The pots were then covered using aluminum foil to make sure that the roots were not exposed to sunlight throughout the growing period. Soils were watered to maintain 30% of water holding capacity (WHC) using deionised water and then allowed to incubate at room temperature for three days prior to sowing.

Two plant species, Wimmera ryegrass (*Lolium rigidum*) and wheat (*Triticum aestivum*), were used in this pot experiment. Uniform seeds were selected and washed with distilled water for 15-20 minutes before being used for germination. The seeds were then germinated on moist filter papers in plastic trays over 2 days in the dark at room temperature, prior to sowing. For each plant species, approximately 20 germinated seeds were sown in each pot to a depth of 1.5 cm. No additional fertilisers were added to the pots at the beginning of the experiments.

Table 5.3 Actual amount of recycled organics applied (in g) based on low and high P rates (w/w).

Recycled organic amendment	LP (50 kg P ha ⁻¹)	HP (100 kg P ha ⁻¹)
BS	0.35	0.70
BL	1.16	2.31
BC	26.19	52.38
CG	7.14	14.29
CM	5.61	11.22

Treatments are BS = biosolids, BL = broiler litter, BC = green waste biochar, CG = compost green waste and CM = compost mixed.

5.3.3 Experimental design

All pots (as per 5.3.2) were placed in the glass house and soil moisture contents were maintained at 70% of WHC by weighing each pot at least every second day. The positions of the pots were re-randomised every two days throughout the experiment. No nutrients were subsequently added to the pots throughout the experiments. The average daily temperature throughout the experiment ranged from 25 to 35°C.

5.3.4 Plant harvest and measurements

The pot experiment was conducted in the glasshouse facility, Nathan campus, Griffith University, Queensland. Plants were harvested at the end of 12 week growth (Figure 5.1 and 5.2) by cutting the shoots at 1 cm above the soil surface in the pots and fresh shoot

weight was measured immediately. Roots were removed from the soil and bulk soil and rhizosphere soil were collected separately for each pot. The rhizosphere soil samples were collected by gentle hand-shaking of the soil attached to roots (Mat Hassan et al. 2012) and they were stored at 4°C until chemical analysis. All soil analyses were carried out using 4 replicates and the results were expressed on an oven-dry basis.

Roots were cleaned by washing them with deionised water and manual removal of adhering soil particles. Collected shoots and root samples were then dried at 60°C for 72 h in the oven. Whole plant shoots and roots were weighed and finely ground using a mill before they were analysed. Total P uptake was determined by multiplying dry plant weight by P concentration of shoot and root tissues. Phosphorus concentrations in plant shoots and roots were determined by using nitric-perchloric acid digestion as described in Chapter 2. Due to the low amount of plant shoot and root dry matter, approximately 0.15 g of ground shoot dry matter and 0.08 g of ground root dry matter were used for measuring total P concentration in shoot and root respectively.

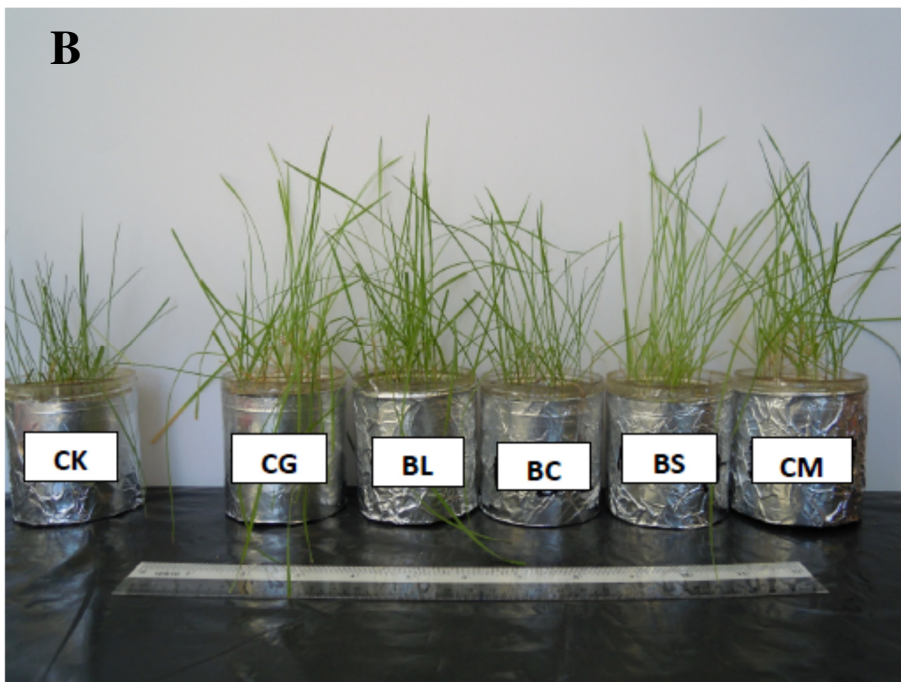
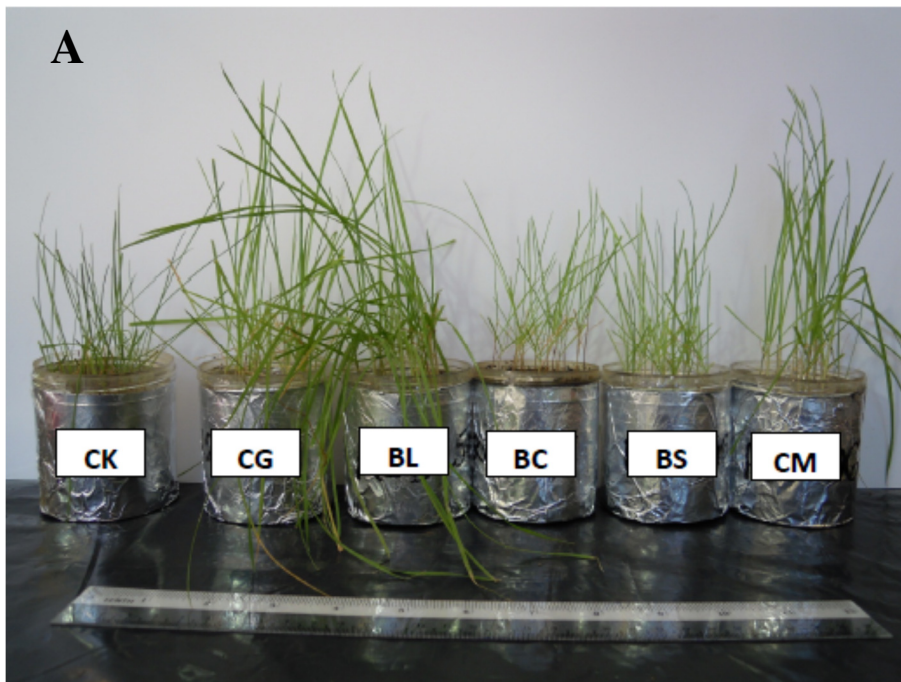


Figure 5.1 Ryegrass plants in the soil amended with RO at week 12. A) high P rate (HP) and B) low P rate (LP). CK= control; CG= green waste compost; BL= broiler litter; BC= biochar; BS= biosolids; CM= Mix compost.

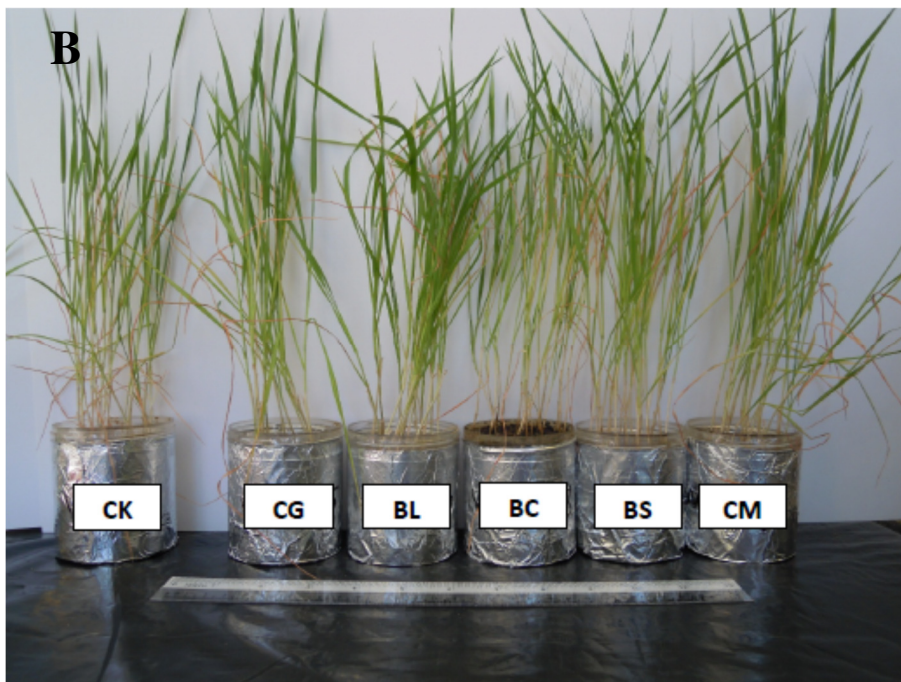
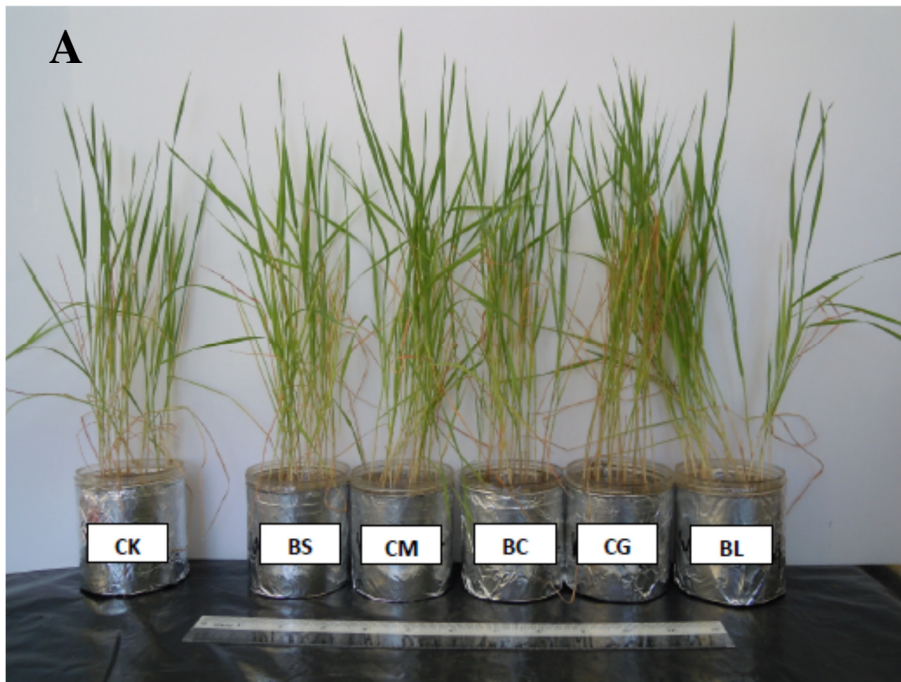


Figure 5.2 Wheat plants in the soil amended with RO at week 12. A) high P rate (HP) and B) low P rate (LP). CK= control; CG= green waste compost; BL= broiler litter; BC= biochar; BS= biosolids; CM= Mix compost.

5.3.5 Soil Analyses

The soil P fractionation scheme described by Hedley et al. (1982) and Condrón et al. (1996) was modified by analysing the residual P using HNO₃-HClO₄ digestion and carrying out sequential extraction of soil with 1 M NH₄Cl, 0.5 M NaHCO₃ (pH 8.5), 0.1 M NaOH, 1 M HCl and 0.1 M NaOH. The concentration of Pi in the extracts was determined after the precipitation of organic matter by acidification, and the concentration of total P (TP) in the extracts was determined after persulphate oxidation. The concentration of Po in the extracts was calculated as the difference between TP and Pi (Tiessen and Moir 1993). Total soil P was determined as the sum of extractable P fractions and residual P.

Microbial biomass C was measured by a fumigation-extraction method using a *K_c* factor of 0.45 (Vance et al. 1987; Wu et al. 1990). Soluble organic C in fumigated and non-fumigated soil samples was determined using a Shimadzu TOC-5000 analyser. Microbial biomass P (MBP) was also measured by a fumigation-extraction method using a *K_p* factor of 0.4 (Brookes et al. 1982). The details on the methods were presented in Chapter 2. .

5.3.6 Statistical Analysis

A two-way ANOVA was carried out using Statistix for Windows version 8.0 (Analytical Software, Tallahassee, FL) on the data (root and shoot biomass and P uptake, and soil chemical variables) to test significant effects of plant species and soil RO treatments. The least significant difference (LSD) was used to separate the means when the differences were significant. All results of the measured variables are expressed on the oven-dry soil basis.

5.4 Results

5.4.1 Plant biomass and phosphorus uptake in roots and shoots

There was a significant difference ($P < 0.001$) in total plant biomass between the control and amended soils for both plant species (Table 5.4). The total plant biomass (roots and shoots) increased in all treatments, except for ryegrass in the BC treatments where the plant biomass in the HP treatment and the LP treatment were not significantly different from the control (Table 5.4). The root biomass of ryegrass in all treatments ranged from 0.03 to 0.16 g pot⁻¹ compared to 0.04 g pot⁻¹ in the control. The HP-BL treatment significantly increased ($P < 0.001$) the root biomass of ryegrass with a mean value of 0.16 g pot⁻¹ compared to 0.03 g pot⁻¹ in the control. The effect of the CM, CG and BS treatments (LP and HP) were intermediate with root biomass of ryegrass ranging from 0.11 to 0.14, 0.09 to 0.14 and 0.06 to 0.08 g pot⁻¹, respectively. The LP-BC treatment had the lowest root biomass of ryegrass among the treatments and it was significantly lower than the control ($P < 0.001$). As for the wheat, the root biomass under different treatments ranged from 0.16 to 0.45 g pot⁻¹ compared to the control (0.13 g pot⁻¹). Similar to the root biomass of ryegrass, root biomass of wheat in the HP-BL treatment had the highest biomass with a three-fold increase compared to the lowest root biomass among the treatments and this was significantly higher than the control. Root biomass of wheat decreased in the order of: BL > CM > CG > BS > BC treatment. This trend for the root biomass was the same for the ryegrass.

The shoot biomass of wheat was higher compared to ryegrass in all treatments. The shoot biomass of wheat in different treatments ranged from 0.30 to 0.68 g pot⁻¹, while the shoot biomass of ryegrass ranged from 0.09 to 0.34 g pot⁻¹ in different treatments (Table 5.4). The shoot biomass under wheat was highest in the HP-BL treatment with 0.68 g pot⁻¹ in comparison with the control with 0.30 g pot⁻¹. The HP-BL treatment had the highest shoot biomass of ryegrass, being 3.8-fold greater compared to the control. The

BC treatments (LP and HP) did not have any significant effect on the ryegrass shoot growth as shoot biomass was not significantly different to the control. However, the BC treatments (LP and HP) significantly increased wheat shoot biomass. Among all treatments, the LP-BS treatment significantly increased the shoot biomass, but the least in comparison to the control for both plant species. The effects of all five RO treatments on shoot biomass of both plant species were similar to those on root biomass but with considerably different magnitude.

The P concentrations in shoots and roots ranged from 67 to 208 mg P kg⁻¹ for ryegrass, and from 116 to 220 mg P kg⁻¹ for wheat (Table 5.5). Generally, both root and shoot P concentrations increased significantly ($P < 0.001$) in all RO treatment compared to the control except for ryegrass in the LP-BC treatment (Table 5.5). It was observed that the P concentration was the highest in the HP-BL treatment and lowest in the LP-BC treatment for both plant species compared to the un-amended control.

The RO impact on P uptake was more profound in wheat where it generally took up more P than ryegrass in comparison with the control (Table 5.5). Results from this study showed that the total P uptake by the ryegrass ranged from 0.019 to 0.185 mg P pot⁻¹ and ryegrass P uptake was highest in the HP-BL treatment. Plant P uptake in the ryegrass did not increase in the BC treatments (HP and LP) compared with the control. The P uptake by wheat plants in different treatments ranged from 0.073 to 0.302 mg P pot⁻¹. The effect of RO on plant P uptake was highest for wheat in the HP-BL treatment with a 4 fold increase in P uptake compared to the control. Unlike the ryegrass where P uptake in the BC treatments (HP and LP) were not significantly different from the control, the LP-BC and HP-BC treatments significantly increased the P uptake by wheat plants with 0.096 and 0.107 mg P pot⁻¹, respectively.

Table 5.4 Root, shoot and total biomass (root + shoot) (dry weight, g pot⁻¹) and shoot: root ratios (S: R) of ryegrass and wheat determined after a 12 week period of growth. The mean values in the column followed by different letters indicate significant differences from each other at $P < 0.05$ level.

RO Treatment	Root		Shoot		Total biomass		S:R ratio	
	Ryegrass	Wheat	Ryegrass	Wheat	Ryegrass	Wheat	Ryegrass	Wheat
CK	0.03 cde	0.13 f	0.09 e	0.30 f	0.12 e	0.43 f	2.25	2.31 ab
LP-BS	0.06 f	0.19 de	0.15 d	0.35 ef	0.21 d	0.54 cde	2.50	1.84 abc
HP-BS	0.08 cde	0.22 d	0.21 bc	0.43 cde	0.29 c	0.65 de	2.63	1.82 abc
LP-BL	0.12 bc	0.30 c	0.21 bc	0.52bc	0.33 bc	0.82 bc	1.75	1.73 abc
HP-BL	0.16 ab	0.45 a	0.34 a	0.68 a	0.50 a	1.13 a	2.13	1.51 abc
LP-BC	0.03 cde	0.16 e	0.09 e	0.38 ef	0.12 e	0.56 ef	2.00	2.03 abc
HP-BC	0.04 def	0.18 de	0.09 e	0.42 cde	0.13 e	0.58 ef	2.25	2.38 a
LP-CM	0.11 ef	0.30 c	0.22 c	0.48 cd	0.33 bc	0.78 cd	2.00	1.60 abc
HP-CM	0.14 bc	0.41 b	0.24 b	0.61 ab	0.38 b	1.02 a	1.71	1.49 abc
LP-CG	0.09 cd	0.22 d	0.19 c	0.38 def	0.28 cd	0.68 cde	2.11	1.73 abc
HP-CG	0.14 bc	0.27 c	0.24 b	0.51 bc	0.38 b	0.96 ab	1.71	1.89 abc
Mean	0.09	0.26	0.18	0.46	0.29	0.68	2.11	1.73
S.E	0.02	0.05	0.04	0.06	0.06	0.11	0.15	0.22
Two way ANOVA (Significance)								
Plant	***		***		***		n.s	
Treatment	***		***		***		***	
Plant x Treatment	***		***		***		***	

*** $P < 0.001$, ** $P < 0.01$, $P^* < 0.05$

Treatments are: CK = control, BS = biosolids, BL = broiler litter, BC = green waste biochar, CM = compost mixed and CG = compost green waste; Treatments marked with LP are low P (50 kg P ha⁻¹) and marked with HP are high P (100 kg P ha⁻¹).

Table 5.5 Phosphorus concentration and P uptake by ryegrass and wheat plants determined after a 12-week period of growth. The mean values in the column followed by different letters indicate significant differences from each other at $P < 0.05$ level.

RO Treatment	P concentration (mg kg ⁻¹)				P uptake (mg P pot ⁻¹)				Total P uptake (mg P pot ⁻¹)	
	Root		Shoot		Root		Shoot		Ryegrass	Wheat
	Ryegrass	Wheat	Ryegrass	Wheat	Ryegrass	Wheat	Ryegrass	Wheat		
CK	67 f	116 e	176 h	192 g	0.002 g	0.015 g	0.016 e	0.058 f	0.019 fg	0.073 g
LP-BS	135 d	165 bc	256 f	210 e	0.008 f	0.031 ef	0.038 de	0.074 ef	0.047 ef	0.105 ef
HP-BS	143 cd	166 bc	295 e	231 cd	0.011 ef	0.037 de	0.062 cd	0.099 de	0.073 de	0.136 de
LP-BL	148 cd	176 b	364 bc	267 b	0.018 cd	0.053 c	0.076 bc	0.139 bc	0.094 bcd	0.192 c
HP-BL	208 a	220 a	446 a	298 a	0.033 a	0.099 a	0.152 a	0.203 a	0.185 a	0.302 a
LP-BC	79 f	126 de	185 h	194 fg	0.002 g	0.020 fg	0.017 e	0.074 ef	0.019 fg	0.094 fg
HP-BC	103 e	134 de	216 g	203 ef	0.004 g	0.024 fg	0.019 e	0.085 def	0.023 fg	0.109 ef
LP-CM	136 d	147 cd	328 d	240 c	0.015 de	0.044 d	0.072 bc	0.115 cd	0.087 cd	0.159 d
HP-CM	191 b	181 b	382 b	276 b	0.027 b	0.074 b	0.092 b	0.168 b	0.118 b	0.243 b
LP-CG	142 cd	137 de	319 de	206e	0.013 ef	0.041 d	0.061 cd	0.078 ef	0.073 de	0.119 ef
HP-CG	157 c	177 b	336 cd	226 d	0.022 bc	0.080 b	0.081 bc	0.115 cd	0.103 bc	0.195 c
Mean	137	159	300	231	0.013	0.041	0.062	0.099	0.073	0.136
S.E	20	14	41	17	0.01	0.01	0.02	0.02	0.03	0.03
Two way ANOVA (Significance)										
Plant	***		***		***		***		***	
Treatment	***		***		***		***		***	
Plant x Treatment	***		***		***		***		***	

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Treatments are CK = control, BS = biosolids, BL = broiler litter, BC = green waste biochar, CM = compost mixed and CG = compost green waste; Treatments marked with LP are low P (50 kg P ha⁻¹) and marked with HP are high P (100 kg P ha⁻¹).

5.4.2 Soil P fractionation and availability in bulk and rhizosphere soils

Generally, the soil under wheat had lower P concentrations in all fractions compared to that under ryegrass across all treatments (Tables 5.6 and 5.7). Generally, there were more profound changes in soil P concentrations under ryegrass compared with those under wheat, in relation to the control. The ANOVA analysis showed significant differences ($P < 0.01$) for plant species and RO treatment types and plant species x treatments interactions were apparent for all the extractable P fractions determined (Tables 5.6 and 5.7).

5.4.2.1 Soil inorganic P (Pi)

The labile inorganic P fractions (labile Pi) (APi and BPi) were shown in Table 5.6. The RO amendments significantly increased APi under both ryegrass and wheat in both bulk and rhizosphere soils ($P < 0.001$). It was observed that the labile Pi concentrations were higher in the bulk compared to the rhizosphere soil under both plant species in all treatments (Table 5.6). In soils under wheat, the most labile Pi as measured by NH_4Cl -extractable Pi (APi) was the smallest fraction among all Pi fractions, ranging from 0.71 to 2.41 mg P kg^{-1} and 0.22 to 1.48 mg P kg^{-1} in bulk and rhizosphere soil respectively. The BL treatments produced the highest increase in APi with 2.41 mg P kg^{-1} and 1.48 mg P kg^{-1} in the bulk and in rhizosphere soil respectively (Table 5.6). The similar impact of the HP-BL treatment on soil APi fraction under ryegrass was observed, with 3.1 mg P kg^{-1} and 1.8 mg P kg^{-1} in the bulk and rhizosphere soils, respectively. Other treatments such as CM, CG and BS influenced soil APi as well but to a lesser extent than the BL treatments under both ryegrass and wheat. The BC treatments, however, had the lowest impact on the APi fraction compared to the control in both rhizosphere and bulk soils, with the APi concentrations being less than 1.4 mg P kg^{-1} (Table 5.6).

The NaHCO_3 -extractable Pi (BPi) fractions ranged from 1.4 to 13.3 mg P kg^{-1} and 0.6 to 11.2 mg P kg^{-1} , in the bulk and rhizosphere soils under wheat, respectively (Table

5.6). While in soil under ryegrass, the BPi fractions ranged from 2.8 to 16.5 mg P kg⁻¹ and 1.4 to 14.0 mg P kg⁻¹ in bulk and rhizosphere soils, respectively. As with the APi fraction, the BL treatments (HP and LP) also had the greatest impact on BPi in soils under both plants in relation to the control. In the HP-BL treatment under wheat, BPi concentrations increased significantly in the rhizosphere ($P<0.001$) by eighteen-fold compared to the control, while LP-BL increased the BPi concentration by six-fold compared to the control. Generally, the increase of BPi in the BL treatment on the rhizosphere under ryegrass however, was lower than under wheat in relation to the control. The BC treatments increased BPi the least among other RO amendments.

The moderately labile Pi fraction from NaOH-extractable Pi (NPi) was the most dominant among Pi fractions in both soils under both plants across all RO treatments. Generally, among other Pi fractions, the BS treatments (LP and HP) increased P concentration of NPi fraction the most in both bulk soil and rhizosphere under both plants followed by the CM and CG treatments. On the other hand, the BC treatments did not impact the NPi fraction significantly compared with the control.

The less labile Pi fraction from the HCl-extractable Pi (HPi) increased significantly ($P<0.01$) with RO amendments in soils under both plants. Contrary to the other Pi fractions, the BS treatments caused the largest increase in HPi fractions compared to other RO treatments in rhizosphere under both ryegrass and wheat. In contrast, the LP-BC treatment did not affect the NPi compared to the control under both plants.

5.4.2.2 Soil organic P (Po)

The organic P (Po) fractions (BPo and NPo), were shown in Table 5.8. Overall, among the extractable Po fractions, the NaHCO₃-extractable Po (BPo) was the smallest fraction, ranging from 2.1 to 10.2 mg P kg⁻¹ and 0.9 to 8.5 mg P kg⁻¹ in the bulk under ryegrass and wheat respectively. In the soil under wheat, the highest concentration of BPo fraction in the bulk was observed in the HP-BC treatment with a mean value of 8.48 mg P kg⁻¹. The

trend was the same in the rhizosphere soil under the same plant. In the soil under ryegrass, the BPo fraction was the lowest in the HP-BL treatment (1.53 and 2.13 mg P kg⁻¹) and LP-BC (1.24 and 5.40 mg P kg⁻¹) in the rhizosphere and bulk soil respectively. In comparison with the control, the LP-BS treatment was higher in the bulk and rhizosphere soil with 10.23 and 8.53 mg P kg⁻¹ respectively.

The NaOH-extractable Po fraction (NPo) is the dominant Po fraction ranging from 7.1 to 21.4 mg P kg⁻¹ in rhizosphere soil and 10.2 to 29.0 mg P kg⁻¹ in the bulk under ryegrass and 8.0 to 30.2 mg P kg⁻¹ in rhizosphere and 9.5 to 41.2 mg P kg⁻¹ in the bulk soil under wheat (Table 5.8). The NPo under ryegrass was highest in the HP-BC treatment for both rhizosphere and bulk soils with mean values of 21.4 mg P kg⁻¹ and 29.0 mg P kg⁻¹, respectively. In the bulk soil under wheat, all RO treatments increased concentrations of the NPo fraction except for the LP-BS treatment in the relation to the control. Overall, the NPo fraction in the bulk soils was higher than in the rhizosphere soils under both plants.

5.4.2.3 Total soil P

Analysis of variance (ANOVA) results showed that the different RO treatments and plant types significantly affected the TP ($P < 0.001$) (Table 5.9). It was observed that the total Pi and Po in the bulk and rhizosphere soils increased significantly in all RO treatments ($P < 0.001$) in comparison with the control except for the LP-BC treatment under ryegrass (Table 5.9). It was found that the HP-BS treatment had the highest TPi in rhizosphere soil under both plants while the lowest TPi was found in the BC-amended soils. However, the HP-BC treatment had the highest TPo fraction in the rhizosphere soil under both plants. All RO amendments significantly increased ($P < 0.001$) the soil TP compared except for the LP-BC treatment under ryegrass. The TP fractions in bulk soils were highest in the HP-BS treatment with a mean value of 97 mg P kg⁻¹ under wheat, while in the bulk under ryegrass, the highest was observed in the HP-CM treatments (89 mg P kg⁻¹).

5.4.3.4 Proportion of soil P fraction over total P

The percentage of soil P fractions (% of total P) in different RO treatments was shown in Figure 5.3. The most labile Pi fractions (APi and BPi) comprised 3 to 10 % and 1 to 12 % of the total P in bulk soil and rhizosphere under ryegrass respectively, while the most labile Pi fractions under wheat from 2 to 14 % and 3 to 15 % and of total soil P in bulk soil and rhizosphere, respectively. As illustrated in Figure 5.3, the BL treatments had the greatest proportion of labile Pi, while the BC treatments had the lowest labile Pi. This trend was the same for both soils under both plants. It was observed that in relation to the control, the LP-BL treatment influenced labile Pi similarly in the bulk soil under ryegrass and under wheat. The moderately labile Pi (NPi) fraction accounted for and 7 to 22 % and 7 to 30 % of total soil P in bulk soil and rhizosphere under ryegrass and 7 to 27 % and 6 to 32 % of total soil P in bulk soil and rhizosphere under wheat, respectively. The most recalcitrant Pi was highest in the BC treatments in both bulk and rhizosphere under both plants.

The most labile Po fraction (BPo) accounted for 1 to 9 % and 2 to 9% in bulk and rhizosphere soil respectively under ryegrass, while the BPo fraction under wheat accounted for 1 to 10 % and 1 to 9 % of total soil P in bulk and rhizosphere soil respectively. The moderately labile Po (NPo) accounted for 9 to 27% and 7 to 28 % of total soil P in rhizosphere soil under ryegrass and under wheat, respectively. The percentage of residual P fraction was the most dominant in all treatments compared to other P fractions, both in rhizosphere and bulk soil under both plants.

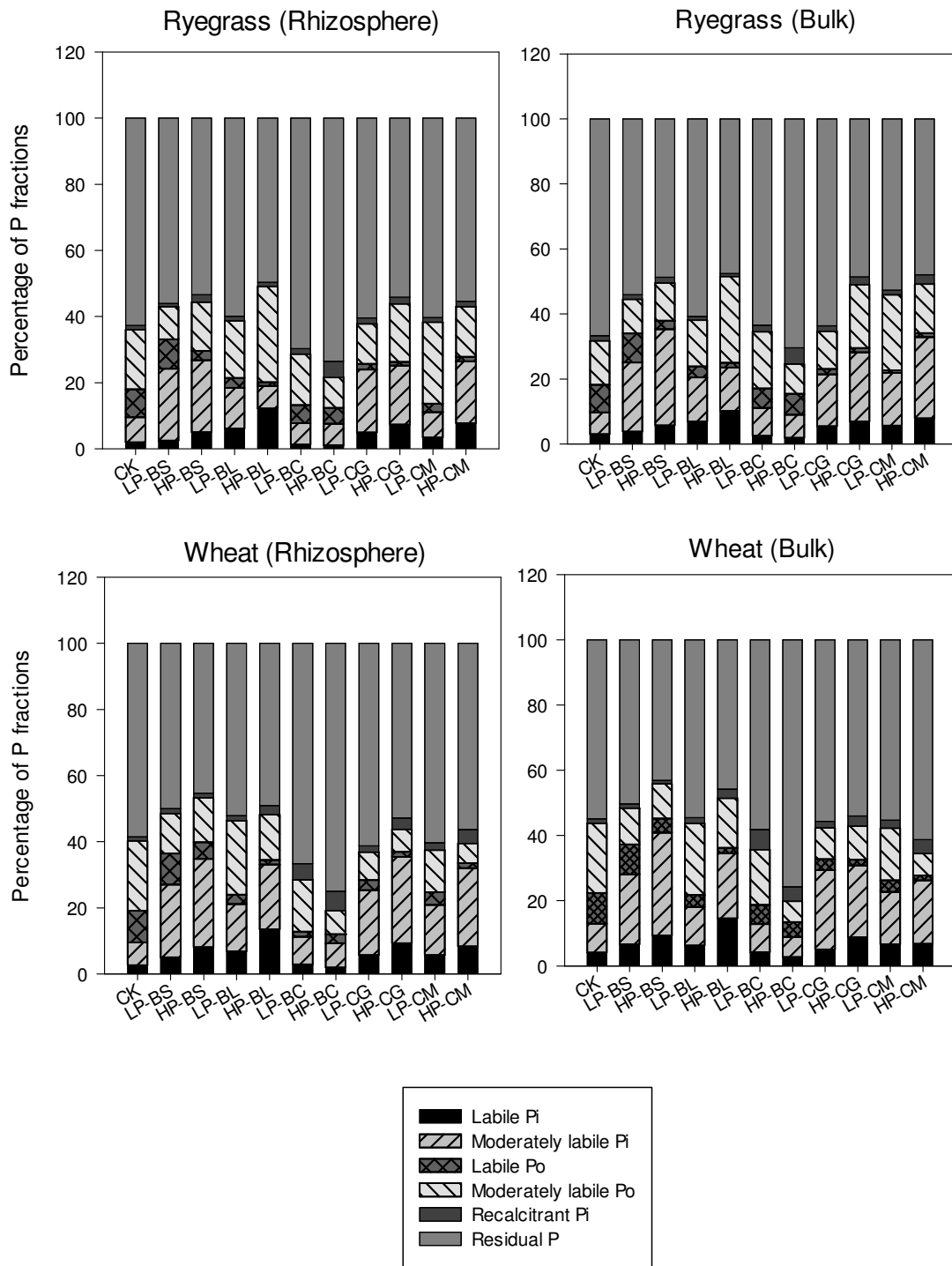


Figure 5.3 Percentage of P fractions in rhizosphere and bulk soil following RO amendments under ryegrass and wheat determined after 12-weeks of growth. Treatments are CK= control, BS= biosolids, BL= broiler litter, BC= green waste biochar, CM= compost mixed and CG= compost green waste; Treatments marked with LP are low P (50 kg P ha^{-1}) and marked with HP are high P (100 kg P ha^{-1}).

Table 5.6 Mean values of labile Pi fractions (mg kg⁻¹) in bulk and rhizosphere soils under ryegrass (R) and wheat (W) following RO amendments. Data in brackets are the standard deviation of the mean.

RO Treatment	APi				BPi				Labile Pi (APi + BPi)			
	Bulk		Rhizosphere		Bulk		Rhizosphere		Bulk		Rhizosphere	
	R	W	R	W	R	W	R	W	R	W	R	W
CK	0.78 (0.3) f	0.71 (0.2) g	0.61 (0.1) g	0.22 (0.03) g	2.81 (0.1) e	1.42 (0.2) f	1.35 (0.2) f	0.64 (0.1) f	3.59 (0.1) h	2.13 (0.2) e	1.96 (0.2) f	0.86 (0.1) e
LP-BS	1.21 (0.1) de	0.98 (0.1) ef	1.09 (0.6) cd	0.69 (0.03) d	6.22 (0.9) d	2.49 (0.4) ef	3.51 (0.3) e	1.42 (0.2) f	7.43 (0.9) efg	3.47 (0.4) de	4.60 (0.3) def	2.11 (0.2) de
HP-BS	1.41 (0.1) cd	1.22 (0.1) cd	1.46 (0.5) b	1.14 (0.05) b	12.70 (0.9) b	6.35 (0.3) bc	7.08 (0.8) c	3.54 (0.5) cd	14.11 (0.9) b	7.57 (0.3) bc	8.54 (0.8) bc	4.68 (0.5) c
LP-BL	1.34 (0.2) cd	1.13 (0.1) cde	1.08 (0.4) cd	0.91 (0.05) c	6.90 (0.5) d	6.30 (0.3) bc	5.49 (0.7) d	4.11 (0.4) c	8.24 (0.5) de	7.43 (0.3) bc	6.57 (0.7) cd	5.02 (0.4) c
HP-BL	3.10 (0.1) a	2.41 (0.1) a	1.79 (0.5) a	1.48 (0.01) a	16.49 (1.1) a	13.31 (0.9) a	14.02 (0.7) a	11.20 (0.5) a	19.59 (0.9) a	15.72 (0.9) a	15.81 (0.7) a	12.68 (0.5) a
LP-BC	1.19 (0.2) de	0.97 (0.02) ef	0.78 (0.1) fg	0.27 (0.01) fg	3.01 (0.2) e	2.30 (0.1) ef	1.56 (0.1) f	1.04 (0.1) f	4.20 (0.2) gh	3.27 (0.1) de	2.34 (0.1) ef	1.31 (0.1) e
HP-BC	1.30 (0.1) cd	1.03 (0.03) de	0.84 (0.6) ef	0.39 (0.12) ef	3.63 (0.2) e	2.48 (0.1) ef	1.46 (0.1) f	2.50 (0.6) de	4.93 (0.3) gh	3.51 (0.1) de	2.30 (0.1) ef	2.89 (0.2) cd
LP-CM	1.22 (0.1) d	1.07 (0.02) de	1.09 (0.4) cd	0.73 (0.10) d	4.13 (0.4) e	3.88 (0.6) de	3.84 (0.3) e	2.81 (0.4) de	5.35 (0.4) ef	4.95 (0.6) bcd	4.93 (0.3) d	3.54 (0.6) d
HP-CM	1.75 (0.1) b	1.31 (0.1) c	1.54 (0.1) b	1.18 (0.02) b	9.94 (0.8) c	7.62 (1.5) b	8.54 (0.4) bc	6.48 (0.1) b	11.69 (0.8) cd	8.93 (2.2) b	10.08 (0.3) b	7.66 (0.4) b
LP-CG	1.36 (0.1) cd	1.05 (0.04) de	0.99 (0.1) de	0.52 (0.04) e	6.10 (0.4) d	4.95 (0.5) cd	4.34 (0.2) de	2.39 (0.1) e	7.46 (0.3) fgh	6.00 (0.4) cde	5.33 (0.2) de	2.91 (0.1) cd
HP-CG	1.49 (0.2) c	1.55 (0.2) b	1.20 (0.5) c	0.82 (0.06) cd	9.01 (0.7) c	7.77 (0.6) b	8.83 (1.5) b	6.58 (0.5) b	10.50 (0.8) bc	9.32 (0.5) b	10.03 (1.5) b	7.40 (0.5) b
Two-way ANOVA (Significance)												
Plant	***		***		***		***		***		***	
Treatment	***		***		***		***		***		***	
Plant x Treatment	**		*		**		*		*		***	

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Treatments are CK = control, BS = biosolids, BL = broiler litter, BC = green waste biochar, CM = compost mixed and CG = compost green waste; treatments marked with LP are low P (50 kg P ha⁻¹) and marked with HP are high P (100 kg P ha⁻¹).

P fractions are: APi = NH₄Cl-Pi; BPi = NaHCO₃-Pi; Labile Pi = APi + BPi.

Table 5.7 Mean values of moderately labile inorganic phosphorus (Pi) (NPi) and stable inorganic Pi fractions (HPi) (mg P kg⁻¹) in bulk and rhizosphere soils under ryegrass (R) and wheat (W) following RO amendments. Data in brackets are the standard deviation of the mean.

RO Treatment	NPi				HPi			
	Bulk		Rhizosphere		Bulk		Rhizosphere	
	R	W	R	W	R	W	R	W
CK	7.7 (1.3)e	4.6 (0.7)f	5.1(0.4) e	3.4 (0.4)e	1.21 (0.03)g	1.08 (0.1)e	0.86 (0.1)e	0.57 (0.1)e
LP-BS	24.3 (2.5)c	19.6 (1.3)c	19.8 (0.6)bc	18.6 (0.5)a	5.58 (0.3)c	1.80 (0.1)de	3.82 (0.1)c	1.26 (0.1)cd
HP-BS	47.8 (3.7) a	39.2 (2.3)a	28.2 (1.9)a	20.0 (1.8)a	7.33 (0.4)a	6.68 (0.7) a	6.61(1.1) a	5.10 (0.3)a
LP-BL	15.8 (1.2) d	14.6 (1.4)d	13.8 (1.2)d	9.9 (0.8)c	2.42 (0.2)ef	1.23 (0.1)cd	1.52 (0.2) de	1.13 (0.1)cd
HP-BL	26.8 (1.1)bc	20.6 (1.9)c	23.0 (2.9)b	7.1 (0.9)cd	3.69 (0.2)d	1.45 (0.1)cd	3.18 (0.1)c	1.29 (0.1)cd
LP-BC	8.0 (0.4)e	7.8 (0.6)ef	6.6 (1.1)e	5.0 (1.0)de	1.44 (0.04)g	1.21 (0.1)e	1.26 (0.04)de	0.77 (0.1)de
HP-BC	10.2 (0.8)e	9.5 (0.9)e	8.4 (0.6)e	6.7 (1.1)d	1.50 (0.1) g	2.34 (0.1)cd	1.36 (0.1)de	2.03 (0.3)b
LP-CM	26.0 (2.1) bc	14.5 (1.7)d	17.0 (1.9)cd	13.8 (0.7)b	2.72 (0.2)e	1.48 (0.1)de	2.11 (0.2)d	1.18 (0.1)cd
HP-CM	29.1 (1.6)b	27.4 (2.1)b	28.4 (1.9)a	18.5 (1.7)a	6.57 (0.3)b	3.47 (0.1) b	4.99 (0.5)b	1.55 (0.4)bc
LP-CG	18.2 (0.9)d	17.0 (1.1)cd	14.1 (1.1)d	6.5 (0.8)d	2.11 (0.2)f	1.45 (0.1) de	1.58 (0.3)de	1.30 (0.1)cd
HP-CG	30.1 (1.3)b	29.3 (1.3) b	28.1 (0.7)a	17.7 (0.8)a	4.20 (0.4)d	3.00 (0.2) bc	3.69 (0.2)c	2.03 (0.3)b
Two-way ANOVA (Significance)								
Plant	***		***		***		***	
Treatment	***		***		***		***	
Plant x Treatment	*		**		***		*	

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Treatments are CK = control, BS = biosolids, BL = broiler litter, BC = green waste biochar, CM = compost mixed and CG = compost green waste; treatments marked with LP are low P (50 kg P ha⁻¹) and marked with HP are high P (100 kg P ha⁻¹).

P fractions are: NPi = NaOH-Pi; HPi = HCl-Pi.

Table 5.8 Mean values of organic phosphorus (Po) (mg P kg⁻¹) and residual P (Res-P) (mg P kg⁻¹) fractions in rhizosphere and bulk soils under ryegrass (R) and wheat (W) following RO amendments. Data in brackets are the standard deviation of the mean.

RO Treatment	BPo				NPo				ResP			
	Bulk		Rhizosphere		Bulk		Rhizosphere		Bulk		Rhizosphere	
	R	W	R	W	R	W	R	W	R	W	R	W
CK	8.20 (0.2) b	5.94 (0.03) b	7.07 (0.2) b	3.75 (0.1) c	18.7 (0.2) c	9.5 (0.2) f	15.6 (0.3) abc	8.0 (0.3) f	13.9 (1.3) h	12.9 (1.3) g	5.5 (1.0) f	4.1 (1.2) f
LP-BS	10.23 (0.2) a	8.18 (0.1) a	8.53 (0.2) a	7.60 (0.2) a	15.7 (0.7) d	15.6 (0.7) d	12.4 (0.8) bc	11.7 (0.8) def	28.7 (2.4) b	27.4 (1.9) ab	19.6 (1.5) a	17.3 (2.2) ab
HP-BS	6.53 (0.2) c	3.46 (0.1) d	5.37 (0.3) c	2.63 (0.1) d	16.2 (0.2) d	15.4 (0.2) d	14.2 (1.5) abc	13.5 (1.5) cde	32.5 (3.1) a	29.2 (1.9) a	20.8 (2.1) a	18.5 (1.1) a
LP-BL	4.72 (0.1) e	3.66 (0.2) d	2.67 (0.3) def	2.44 (0.2) de	10.2 (0.2) f	10.6 (0.2) ef	7.3 (1.5) c	8.8 (1.5) ef	16.4 (1.6) fg	14.5 (1.6) fg	6.7 (0.8) e	5.7 (1.4) f
HP-BL	2.13 (0.1) g	2.33 (0.1) e	1.53 (0.1) g	1.10 (0.1) f	10.8 (0.4) f	12.1 (0.4) e	8.3 (0.3) bc	9.5 (0.3) def	19.1 (1.5) d	18.1 (2.1) de	8.3 (1.4) d	8.0 (1.3) e
LP-BC	5.40 (0.1) d	5.38 (0.2) c	1.24 (0.1) g	4.17 (0.1) c	20.5 (1.5) b	41.2 (2.1) a	16.2 (0.9) ab	30.2 (0.9) a	15.8 (1.1) g	13.1 (1.5) g	8.3 (0.7) d	8.2 (1.3) e
HP-BC	7.72 (0.2) b	8.48 (0.5) a	3.20 (0.1) de	5.00 (0.2) b	29.0 (0.1) a	25.2 (0.3) b	21.4 (2.2) a	14.0 (2.2) cd	17.4 (1.7) ef	16.0 (1.5) ef	8.9 (2.2) d	8.9 (1.1) e
LP-CM	3.57 (0.1) f	1.53 (0.2) f	2.63 (0.9) ef	1.17 (0.3) f	13.5 (0.3) e	15.4 (0.1) d	7.5 (3.2) bc	18.4 (5.1) bc	23.9 (2.3) c	22.7 (2.1) c	14.8 (1.4) b	15.6 (1.8) cd
HP-CM	2.42 (0.1) g	1.61 (0.2) f	1.57 (0.1) g	1.20 (0.1) f	12.6 (0.2) e	9.7 (0.2) f	10.9 (1.5) bc	8.5 (1.5) ef	28.6 (2.4) b	26.1 (1.4) b	15.7 (2.3) b	16.6 (1.7) bc
LP-CG	3.95 (0.4) f	0.90 (0.1) g	3.58 (0.2) d	2.19 (0.1) e	10.5 (0.9) f	17.7 (0.3) c	7.1 (0.6) c	14.0 (1.3) cd	18.8 (2.1) de	19.7 (2.4) d	9.1 (0.8) c	19.6 (1.5) e
HP-CG	2.19 (0.3) g	1.45 (0.1) f	1.85 (0.7) fg	1.30 (0.1) f	18.1 (0.5) c	24.5 (0.5) b	11.8 (1.4) bc	21.0 (1.4) b	19.4 (1.2) d	22.0 (1.0) c	15.1 (0.4) b	13.0 (2.5) d
Two way ANOVA (Significance)												
Plant	***		***		***		***		***		***	
Treatment	***		***		***		***		***		***	
Plant x Treatment	***		***		***		***		***		***	

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Treatments are CK = control, BS = biosolids, BL = broiler litter, BC = green waste biochar, CM = compost mixed and CG = compost green waste; Treatments marked with LP are low P (50 kg P ha⁻¹) and marked with HP are high P (100 kg P ha⁻¹). P fractions are: BPo = NaHCO₃-Po; NPo = NaOH-Po; Res-P = residual (non-extracted) P.

Table 5.9 Mean values of soil total inorganic and organic P fractions (mean, mg P kg⁻¹) as affected by amendments of different recycled organics (RO) in sandy loam (SL) and loam (L) soils and P values from analysis of variance (ANOVA) on effects of plant types, RO treatments and their interactions. The mean values followed by different letters indicate significant differences from each other. Treatments are CK = control, BS = biosolids, BL = broiler litter, CG = compost green waste, CM = compost mixed, BC = green waste biochar. Data in brackets are the standard deviation of the mean.

RO Treatment	TPi				TPo				TP			
	Bulk		Rhizosphere		Bulk		Rhizosphere		Bulk		Rhizosphere	
	R	W	R	W	R	W	R	W	R	W	R	W
CK	12 (1.3) f	8 (0.6) e	8 (0.5) e	5 (0.5) g	27 (1.6) b	15 (0.2) fg	23 (0.2) a	12 (4.9) de	53 (2.4) f	36 (3.9) i	36 (1.1) d	21 (2.8) f
LP-BS	37 (2.6) d	25 (1.4) c	28 (0.6) b	22 (0.8) b	26 (3.3) b	24 (0.2) d	21 (2.7) ab	16 (5.6) cd	88 (2.8) b	75 (2.6) cd	67 (2.4) b	55 (2.7) b
HP-BS	69 (3.4) a	49 (2.8) a	43 (1.7) a	30 (2.0) a	23 (3.5) c	19 (0.3) e	20 (1.6) abc	16 (2.1) cd	118 (1.9) a	97 (4.3) a	78 (1.2) a	64 (2.6) a
LP-BL	26 (1.7) e	23 (1.4) c	22 (1.2) c	16 (0.9) d	15 (2.2) f	14 (0.1) g	10 (2.1) d	16 (3.3) cd	57 (2.5) ef	52 (2.6) h	39 (2.8) cd	38 (1.7) cd
HP-BL	50 (0.6) b	38 (3.2) b	42 (2.6) a	21 (1.5) bc	13 (2.1) g	14 (2.1) g	10 (0.9) d	31 (3.5) a	82 (1.7) c	71 (3.3) ef	60 (4.7) b	60 (2.1) ab
LP-BC	14 (0.4) f	13 (0.6) de	10 (1.2) de	7 (0.8) fg	26 (1.9) b	47 (0.9) a	17 (0.8) abcd	16 (1.9) cd	60 (2.4) f	72 (2.5) de	39 (4.3) d	31 (3.4) e
HP-BC	17 (1.1) f	20 (1.1) d	12 (0.8) d	12 (0.6) ef	37 (1.1) a	34 (0.4) b	25 (0.3) a	15 (1.1) cde	76 (10.6) d	65 (3.4) fg	51 (1.9) bc	33 (2.6) de
LP-CM	34 (0.8) d	21 (0.9) c	24 (0.6) c	18 (0.9) cd	17 (3.5) e	17 (0.4) ef	10 (0.4) d	10 (1.0) e	74 (2.7) d	61 (2.9) g	50 (3.3) bc	43 (3.2) c
HP-CM	47 (1.5) bc	39 (2.7) b	43 (2.3) a	28 (0.5) a	15 (5.1) f	11 (0.2) h	12 (0.6) bcd	20 (0.8) cd	89 (4.1) b	76 (2.8) c	71 (4.1) a	63 (3.1) a
LP-CG	28 (2.3) e	24 (1.5) c	21 (1.8) c	11 (1.3) e	14 (1.3) fg	19 (0.4) e	11 (0.5) cd	23 (1.3) b	61 (3.3) e	62 (3.6) g	44 (1.3) bc	43 (1.9) c
HP-CG	45 (2.1) c	42 (3.6) b	42 (2.1) a	27 (2.1) a	20 (2.5) e	26 (0.4) c	14 (5) bcd	16 (0.5) cd	87 (2.6) c	90 (2.2) b	72 (3.1) a	56 (2.5) b
Two way ANOVA (Significance)												
Plant	***		***		***		***		***		***	
Treatment	***		***		***		***		***		***	
Plant x Treatment	***		***		***		***		***		***	

TPi (total extractable Pi) = APi + BPi + N₁Pi + HPi + N₂Pi; TPo (total extractable Po) = BPo + N₁Po + N₂Po; TP (total soil P) = TPi + TPo + Res-P.

5.4.3 Soil microbial biomass C and P in bulk and in rhizosphere soils

Soil microbial biomass results are presented in Figure 5.4. The RO amendments significantly increased the concentrations of soil microbial biomass (MBC) in both rhizosphere and bulk soil under both ryegrass and wheat plants ($P < 0.001$). The extent of the effects of RO on MBC in the rhizosphere and bulk soil was similar. The concentrations of MBC in the rhizosphere soil under ryegrass and wheat were significantly greater than those in the bulk soil in all treatments ($P < 0.001$). The MBC concentration under ryegrass was highest in the HP-CM amendment with mean values of 322 mg kg^{-1} and 222 mg kg^{-1} in the rhizosphere and in bulk soils, respectively, compared to the corresponding values of 223 mg kg^{-1} and 128 mg kg^{-1} in the control. Meanwhile in soil under wheat, the HP-BL treatment greatly increased MBC in the bulk soil (215 mg kg^{-1}), while HP-CM increased MBC in the bulk soil (362 mg kg^{-1}) compared to the control (228 mg kg^{-1} and 131 mg kg^{-1} in the rhizosphere and in bulk soils respectively). However, compared to the control, it was observed that the BS treatments (HP and LP) did not significantly increase MBC in bulk and rhizosphere soils under both plants.

Based on ANOVA analysis, there were significant treatment differences in microbial biomass P (MBP) concentration ($P < 0.001$) in soil under both plants (Figure 5.4). Under ryegrass, the MBP ranged from 6.22 to 33.31 mg kg^{-1} in rhizosphere soil, while in the bulk soil, the MBP ranged from 4.73 to 17.25 mg kg^{-1} . In soil under ryegrass, the HP-CM treatment had the highest MBP concentration among all treatments with mean values of 33.31 mg kg^{-1} and 17.25 mg kg^{-1} in rhizosphere and bulk soil respectively, compared to the control (6.22 mg kg^{-1} and 4.73 mg kg^{-1} , respectively). The LP-BS and BC treatments (HP and LP) were observed not to have any significant effect on MBP (Figure 5.4). Meanwhile in soil under wheat, the MBP concentration in the rhizosphere was the highest in the HP-CM with a mean value of 40.91 mg kg^{-1} , while the highest MBP concentration in the bulk soil was found in the HP-BL with a mean value of 18.49 mg kg^{-1} .

¹. It was observed that the BS treatments (HP and LP) had the lowest impact on the MBP in the bulk soil, while the HP-BC has the lowest impact on the MBP in the rhizosphere soil under wheat.

Microbial C to P ratios varied among the treatments as well as with plant species (Figure 5.4). Except for the BC (both LP and HP) treatments, all other RO treatments decreased microbial C: P ratios in both rhizosphere and bulk soils under ryegrass (Figure 5.4). The extents were smaller in the CG, CM, BL and BS treatments (Figure 5.4). In the rhizosphere soils, the lowest microbial C: P ratio was observed in the CM treatments (HP and LP), while in the bulk rhizosphere soils the lowest microbial C: P ratios were observed in the LP-CM and HP-BL treatments under ryegrass. Under wheat plant, all of the treatments decreased microbial C: P in both rhizosphere and bulk soils in relation to the control. Among all treatments, the microbial C: P ratios were lowest in the HP-CG and HP-BL treatments in rhizosphere and bulk rhizosphere soils respectively, and the microbial C: P ratios were highest in the HP-BC and HP-BS in the rhizosphere and bulk soil respectively (Figure 5.4).

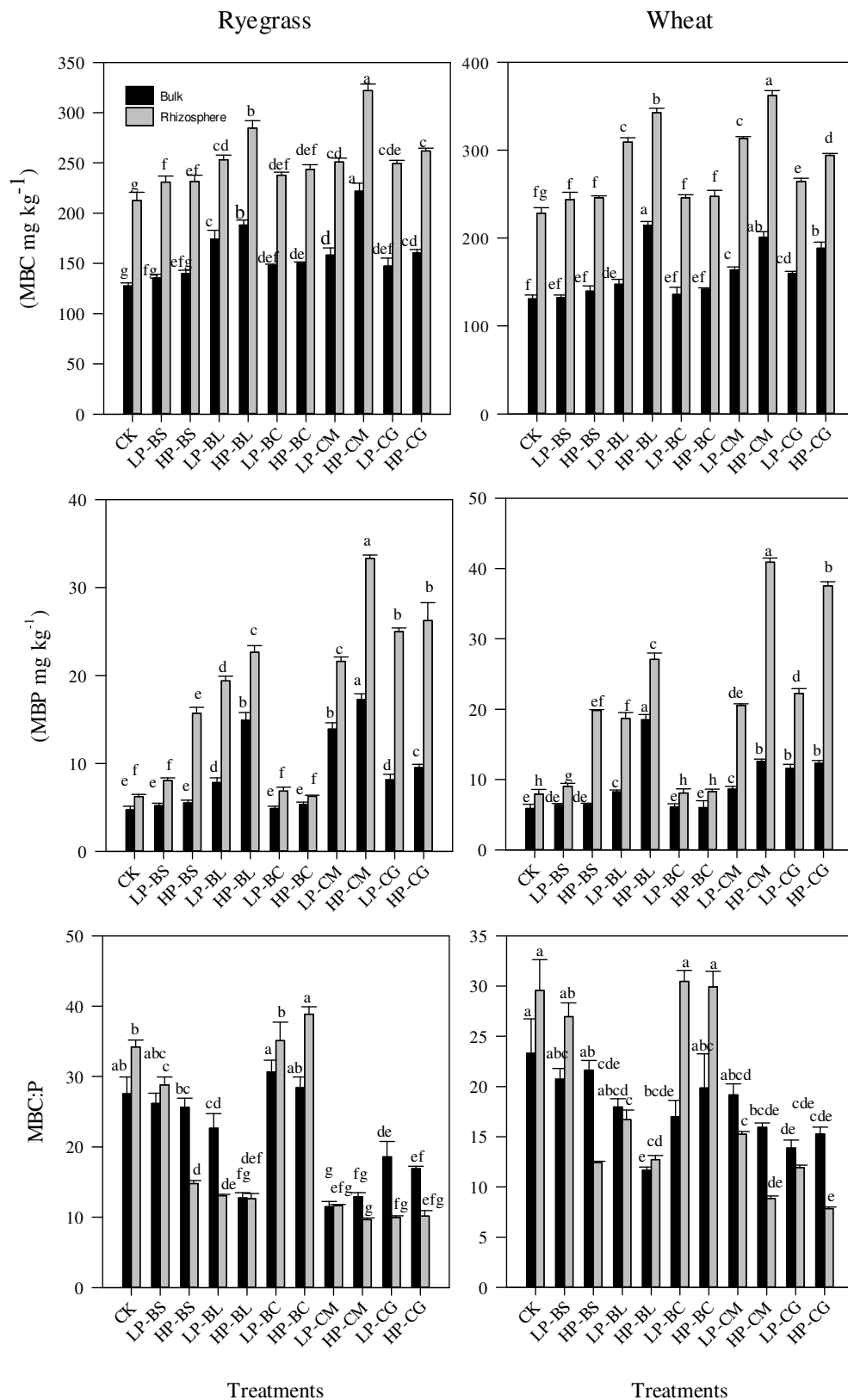


Figure 5.4 MBC, MBP and MBC:P Ratio for all treatments in rhizosphere and bulk soils following RO and P amendments under ryegrass and wheat after 12-weeks of growth. Treatments are CK= control, BS= biosolids, BL= broiler litter, BC= green waste biochar, CM= compost mixed and CG= compost green waste. Treatments marked with LP are low P (50 kg P ha⁻¹) and marked with HP are high P (100 kg P ha⁻¹).

5.5 Discussions

5.5.1 Effects of the RO on soil P fractions and availability in the rhizosphere

In this study, it was found that the concentrations of Pi pools (e.g. NH_4Cl -Pi, NaHCO_3 -Pi, NaOH -Pi and HCl -Pi) increased, particularly in RO-amended soils with high P rate, after 12 weeks of pot trials (Table 5.6). Generally, in most of the P pools, it was found that P in rhizosphere soil was lower than the bulk soil. The difference in P concentration between the rhizosphere and the bulk soil would be due to the differences in the microenvironments of the rhizosphere and bulk soil such as higher plant absorption and acquisition in the rhizosphere compared to the bulk soil as reported by Hinsinger et al. (2011) and Sinegani et al. (2011). In this study, depletion of P fractions in the rhizosphere varies with different RO characteristics and different plant types. The P depletion mechanisms by plants absorption have been attributed to differences in root morphology such as root density, root surface area, root length, root-induced biochemical changes as well as soil-root interactions in the rhizosphere (Hinsinger et al. 2006; Marschner et al. 2011; Mat Hassan et al. 2012). In the current study, the concentrations of labile Pi increased in the rhizosphere in all of the RO under both plants in comparison with the control ($P < 0.01$). The labile Pi concentrations in the rhizosphere were in the sequence of $\text{BL} > \text{CM} > \text{CG} > \text{BS} > \text{BC}$ compared to the control (Table 5.6). The increase of labile Pi in RO-amended soils showed the same trend under different plant species but with different magnitude. It was found that concentrations of labile Pi and moderately labile Pi fractions in the HP-BL treatment were highest compared with other treatments under both plants. The results were not expected as at the beginning of this study, the HP-BS was expected to increase the labile Pi concentration the most as the properties of this treatment was high in available P (Colwell P) (Table 5.2). In addition, it was found to increase labile Pi the most in the previous experiment (Chapter 4). There are a few possible explanations for the finding: (i) the labile P of HP-BS must have been used up in the beginning of the

experiment, and as the HP-BS was low in the microbial biomass C and P compared to the HP-BL, it could not continuously provide nutrients to plants and microbes; (ii) the different results would be due to root existence as the release of root exudates as well as rhizosphere microbial processes stimulated by the additions of substrates from the BL amendments. This might have led to the increase of the available P as well as increase the high C and N content. The extents of the effects of the CM treatments and the CG treatments (HP and LP) on labile Pi were similar but lesser than the HP-BL. They were found to be low in the BPo fraction indicating the occurrence of mineralisation in these treatments in order to meet plant demand. In comparison to the other RO, the lowest labile Pi in rhizosphere was found in the LP-BC under both ryegrass and wheat. However, it was surprising to see that the BC, could significantly increase soil labile Pi under both plants compared to the control. The increase of labile Pi in the BC treatments was probably attributed to the potential of BC to act as refuge to rhizosphere microorganisms increased the microbial population and the increase in the labile Pi might have resulted from the microbial turn over.

The NaOH-Pi (NPi) pool represents Pi strongly associated with Fe and Al (Hedley et al. 1982, Tiessen and Moir, 1993). There are reports on depletion of NPi fraction in the rhizosphere soil. For example, a study done by Chen et al. (2002) observed the depletion on NPi from the root surface. In this study, the NPi was higher in the HP-BS treatment in the rhizosphere under both plants compared to the control despite the low microbial biomass. This indicates that it was not mediated by rhizosphere microorganisms but may have been formed from plant root origin. It was observed that the NPi was low in the BL treatments indicating that this moderately labile fraction has been mineralised into more labile fractions to meet plants and microbial demands. Low NPi was also observed in the CM and CG treatments indicating that this fraction also acts as a source of labile Pi for plants. The lowest NPi found in the rhizosphere soil amended with the BC treatments

(compared to the control) under both plants indicating that root excreted carboxylates/organic acid as exudates that release P by anion exchange or Al and Fe solubilisation in the rhizosphere in order to make sure that the charge balance in the rhizosphere is in equilibrium (Alvarez et al. 2012).

The recalcitrant-Pi (HPi) fraction which represents Ca-bound P was low in most of the treatments and there were not much differences in this P fraction between the rhizosphere and bulk soils (Table 5.7). The study done by Waldrip et al. (2011) in soil amended with poultry manure also found that there was no increase in this fraction at week 16 compared to un-amended soil. However, in this study, the HP-BS treatment was highest in the HPi in the rhizosphere soil under both plants. This could be due to the high Ca content and also indicating that this treatment is high in stable P and is expected to be less vulnerable to P loss in runoff. Other RO treatments showed low HPi content indicating that plants have utilised this fraction after 12 weeks.

5.5.2 Effects of RO on microbial biomass C and P in the rhizosphere under ryegrass and wheat

The soil microbial biomass is defined as the living component of the soil organic matter which acts as an agent for the transformation of organic matter in soil and a source and sink of the nutrients in soil (Jenkinson and Ladd, 1981). In this study, the soil microbial biomass MBC and MBP were greater in the rhizosphere relative to the bulk soil in all treatments under both ryegrass and wheat plants (Figure 5.4). The release of root exudates in rhizosphere soils might play an important role in the increase of microbial biomass as it could increase microbial density and metabolic activity in rhizosphere compared to the bulk soil (Marschner et al. 2011). It was interesting to see significant increase of MBC concentrations ($P < 0.05$) in all RO-amended soil compared to the control as the MBC in the amendments were expected to be depleted after 12 weeks especially in the biologically

inert amendment such as the BC. One of the reason for the increase in microbial growth such as MBC even after 12 weeks is partly caused by rhizodeposition of C compounds in soil which is commonly known as the 'rhizosphere effect' (Paterson, 2003). The rhizodeposition of C compounds would have supplied the soil microorganisms with available C source aiding the microbial growth and activities. Other explanation would be partly due to the fact that the surface of BC could act as micro-pore habitat providing protection and shelter for soil microbes thus increasing microbial population growth (Pietikainen et al. 2000). The significant increase in the MBC in the highly acidic amendments such as the BC indicates that soil microorganisms could tolerate low pH condition which might have resulted from root-induced pH change process in the rhizosphere. On the other hand, the increase in BS treatment in MBC in both rhizosphere and bulk soil even after 12 weeks compared to the control was also not expected. It was because the BS is expected to suffer from phytotoxicity of Cu as found in the previous incubation experiment (Chapter 4). The explanation behind this is that the root-induced changes the rhizosphere pH to be more alkaline thus reducing the solubility of Cu in acidic soil and decrease the bioavailability of soil Cu of the plant and finally reduce the risk of Cu phytotoxicity (Chaignon et al. 2002). In the beginning, it was expected that the HP-BL treatment would show highest increase in the MBC after 12 weeks of amendment due to the high amount of C added in the amendment. However, it was found that the greatest increase of MBC was in the HP-CM-amended soils under both ryegrass and wheat. The explanation for the higher increase in MBC in the HP-CM treatment would be partly due to high readily available organic C content in the CM amendment compared to the others. This finding is supported by a short-term study done by Zhang et al. (2014). They found that the high MBC in soil amended with compost was derived from high activity and growth of microorganisms in the rhizosphere through the high content of readily available organic C materials including rhizosphere C mineralisation. The release of higher easily

degradable compounds and substrate from roots such as organic acids and amino acids may have also been partly responsible for the higher MBC in the HP-CM-amended soil.

The concentration of MBP can change according to the composition of the microbial community, growth stage of microorganisms, addition of organic matter and other environmental conditions (Tang et al. 2014). In this pot trial, it was found that the MBP was lowest in the BC treatments (HP-BC and LP-BC) and highest in the CM treatments (HP-CM and LP-CM). Generally, in comparison with the control, the concentration of MBP at week 12 was in the sequence of CM>CG>BL>BS>BC. The trend was the same under both plants but with a different magnitude. The MBP in the rhizosphere of the BL treatments were lower compared to CM and CG treatments but were found to be higher in labile Pi. This could be due to greater competition for available P by plants and microbes in soil or might be due to high amount of P released from microbial biomass during microbial turnover in order to meet high demand. The higher concentration of the MBP in the CM and CG-amended soils compared to the BL-amended soil indicating that these treatments could promote the P storage in the microbial biomass and contain high easily available C in the rhizosphere (Marschner et al 2006). The MBP concentrations in the BC treatments were low and not significantly different with the control under ryegrass and the same was true in soil under wheat. The finding was in contradiction with that in the incubated soils amended with BC as discussed in the previous chapter (Chapter 4) where the HP-BC treatment increased the MBP concentration. This could be due to the turn-over of the microbial P in order to provide P to meet plant demand in this pot trial or P released from dead microbial cells was converted to Po as the concentration of Po fractions in the BC treatments were high (Table 5.8).

5.5.3 Effects of RO on plant P uptake and plant biomass yield

The plant P uptake from soil occurs predominantly in the form of Pi (Damon et al., 2014) therefore the soil Pi pool represents soil P forms that are potentially available for uptake by plants. Most of the Pi taken up by roots is transported into the plant xylem and finally will be transferred into shoots (Schachtman et al. 1998). The increases of P uptake by plants are common following the addition of organic amendments such as RO (Butler et al. 2007; Khan and Joergensen 2012). However, most studies have been focusing on P contents in shoot (Waldrip et al. 2011; Malik et al. 2013; Jin et al. 2014) and there was very little study on P in plant roots although there were numerous studies on plant response to amendment additions (Brechtin and McDonald 1994; O'Connor et al. 2004; McBride et al. 2013). It is believed that the current work is one of the first studies on shoot and root P uptake as well as P content in ryegrass and wheat grown in soils amended with a wide range of RO. In this study, it was observed that all RO treatments significantly promoted root and shoot P uptake ($P < 0.001$) under both plants except for the BC treatments (HP-BC and LP-BC) under ryegrass. The results could be due to the enhanced P availability in the rhizosphere by the inputs of P in the RO compared to the control. The HP-BL treatment increased both root and shoot P uptake the most under both plants indicating that this treatment have promoted the transformation and mineralisation of less labile Pi and Po into labile P in the rhizosphere (as shown in the Table 5.7). Other RO treatments such as the CG treatments (HP-CG and LP-CG) also generally increased nutrient root and shoot P uptake but to a lesser extent than the CM (HP-CM and LP-CM) and BL (HP-BL and LP-BL). This could be due to lesser available C in the former treatments. The total P concentration in the BC treatments increased significantly in high P rate in root and shoot in both plants. However, the total P uptake was not significantly different in both root and shoot in the BC treatments (LP-BC and HP-BC) under ryegrass (Table 5.5). The BC treatments did not affect P uptake under ryegrass even though labile

Pi was greater than the control. Looking at the effect of P uptake in different plant, the total P uptake was observed to be higher in wheat compared to ryegrass in all RO treated soils (Table 5.5). This implies that wheat used more P than ryegrass in order for it to grow. This could be explained by the depletion of labile Pi as illustrated in Table 5.6 as well as enhanced mineralisation of organic P in wheat compared to the ryegrass (Table 5.8). The differences in P uptake between ryegrass and wheat may be attributed to the root exudates release, larger root systems and biochemical properties of wheat compared to the ryegrass. It was observed that the wheat plant can survive in the BC-amended soils and it might be attributed to bigger root systems and higher exudates released as reflected by higher MBC concentrations data under wheat compared to ryegrass (Figure 5.4). This is supported by the study done by Zhang et al. (2014) which showed positive correlation between biomass yield (root growth) and MBC.

In terms of the RO effects on total biomass yield (plant growth), the effect was similar to the plant P uptake where only the BC treatments (LP-BC and HP-BC) did not show significant effect towards the ryegrass. However, the BC treatments significantly affected wheat growth. In other studies, biochar-amended soil has shown to promote plant productivity and yield through several mechanisms. One of the mechanisms responsible for the significant increase in wheat yield under BC treated soils might be due to the soil exploration by root production that contributes to nutrient acquisition (Lambers et al. 2008). The insignificant yield of the ryegrass in the BC treatments compared to the control might also indicate that the ryegrass has become stressed in acid soils as a result of toxic concentrations of Al similar to Mosquera-Losada and González-Rodríguez (1999) findings. In their study, they found that the ryegrass was adversely affected by soil acidity. The results also indicate that wheat has the ability to adapt to such conditions and is able to grow in very acidic soils as they have developed mechanisms for tolerating such acid conditions. The wheat plant might have undergone root-induced modifications of the

rhizosphere, such as changes in the cation-anion uptake ratio as well as exudation of Al chelators as exudation amount and composition vary among plant species (Alvarez et al. 2012). Among other RO, the HP-BL treatment stimulated total biomass yield (root and shoot) the most followed by HP-CM and HP-CG under both plants (Table 5.4). The high root biomass in the HP-BL treatment also explained the depletion of large amount of labile Pi compared to the other amendments (Table 5.6).

Throughout 12 weeks of experiment, there were no apparent signs of nutrient deficiency observed across all treatment soils although no additional nutrients were added apart from RO at the beginning of the experimental period (Figure 5.1 and 5.2). The results were influenced by the composition of RO amendment and the plant type. The difference in plant growth measured by biomass dry weight for both ryegrass and wheat under the same environmental conditions reflects the differences in plant genetics even though the growth among all RO showed the same trends under both plants (Chen et al. 2003). The differences are governed not only by plant genus, species or even genotypes of the same species, but plants also vary in their ability to take up nutrients and their interaction with soil microorganisms (Gahoonia et al. 1999, Marschner et al. 2011). The differences in plant growth was also due to factors such as root size, root length, root surface area and distribution, root exudation, mycorrhizal infection and transpiration rate (Marschner et al. 2011). In the present study, it could be concluded that the plant species with contrasting nutrient requirement and root characteristics had the similar pattern of rhizosphere effects on P uptake and biomass yield although the magnitude of rhizosphere effects varied considerably among different RO treatments and P rates.

5.6 Conclusions

This study showed that RO, particularly at high P application rates, could stimulate microbial biomass growth and plant available P in soil with low available P. This showed

that RO could promote continuing supply of P to meet the plant demand and could promote P cycling and storage for microbial biomass in soil. Greater P availability and plant growth were not only attributed to higher easily available substrates in these amendments but also induced and accompanied by root processes and microbial processes. Among RO tested, the BL treatment was found to be the most efficient P supplier to both plants tested and presents the largest fraction of labile P, followed by the CM and CG treatments. The BS and BC treatments were found to be less efficient of P suppliers to plants due to its properties leading to a very little influence on soil microbial biomass. A precise assessment on P availability and microbial biomass for individual RO amendment prior to soil incorporation could therefore be carried out in the future in order to reduce the use of chemical fertilisers. This study showed that the rhizosphere is responsible for the changes in the effects of the RO-amended soils on P availability, microbial biomass and plant growth. The findings therefore highlight the importance of examining the rhizosphere soil of each RO-amended soil in order to understand the effect of RO in soil. The current findings add substantially to our understanding of the effects of RO amendment on soil P availability and microbial biomass in the rhizosphere. The finding also provide important information on the soil P cycling as well as provide effective approach to improve P usage efficiency through the manipulation of RO and reduce the over-dependence on the conventional chemical fertiliser.

5.7 References

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Chapter 6 General Discussion, Conclusion and Future Research

6.1 Summary

Low P availability in soil is considered a worldwide constraint to crop production. Recycled organic (RO) could be used as an alternative to conventional fertilisers, but a clear understanding of the relationship among type of P amendment, microbial activity and changes in soil P fractions is required to optimise their use. This study was conducted based on the gaps in knowledge about the utilisation of RO as a source of P following soil amendment. The lack of information on the effects of applications of RO on P availability and fractions as well as on the associated microbial properties has been addressed in this study. Therefore, the following three important knowledge gaps in the effects of RO-amended soils on P fractions and microbial properties were identified to be studied:

- 1) To date, most studies have evaluated N in RO-amended soil but knowledge on the repeated and continuous RO amendment on P availability and pools in agricultural soil is scarce.
- 2) In terms of P dynamics, there is limited assessment on the relationship of P and microbial changes that attribute to a limited understanding of the mechanisms of P behaviour and fate in soil amended with RO.
- 3) With regard to the RO amendment in soil, there is limited knowledge on the P dynamics and microbial changes in the rhizosphere soil and its effects on plant P uptake and plant biomass (shoot and roots).

The overall objective of the work described in this thesis was to investigate the effects of different RO on P availability by looking at the P fractions of different forms and

processes/ mechanisms associated with soil microbial biomass and activity. The specific objectives of this study were to: (i) to conduct experiments on the effects of RO amendment on the soil P availability and their impacts on chemical and biological properties in an agricultural soil (Chapter 3); (ii) to investigate the biochemical and microbial mineralisation processes of RO-amended soil and its effects on the availability of P in incubated soils (Chapter 4); (iii) to examine the effect of RO-amended soils on P pools associated with microbial biomass in rhizosphere soil (Chapter 5). A combination of field, pot and incubations experiments were used to achieve these aims.

At the start of this research, 22 different RO from wide ranging sources all over Australia were collected and characterised for their basic properties (Chapter 2). Properties comprised of pH, EC, total carbon (C), total nitrogen (N) total P and available P (Colwell P), C:N ratio and C:P ratio. From these 22 RO, five RO were selected based on their C: P ratios and their effects as a soil amendments were selected for further experiments. These RO were biosolids (BS), broiler litter (BL), green waste biochar (BC), CM (mixed compost) and CG (green waste compost). Subsequently, three set of experiments were carried out to investigate variables such as P availability and microbial biomass in soils amended with the selected RO to answer the main objectives. The P sequential fractionation method was used to investigate P availability and sizes of different soil inorganic and organic P fractions, as described in Chapter 2. The soil microbial biomass was determined using the fumigated and un-fumigated method and biological properties such as microbial biomass carbon (MBC) and phosphorus (MBP) and soil respiration (Cumulative CO₂-C) as described in Chapter 2.

The first experiment was conducted on soil collected from a vegetable production field from Centre of Recycled Organics (CROA) in New South Wales, Australia. Seven treatments were applied with low and high P input rates of compost, half-compost, conventional practice and a control. The experiment investigated the effects of repeated

organic green waste compost amendments on soil properties and P fractions and its availability to vegetable crops. It was expected that RO used in this study will provide sufficient P supply for crop with less risk of P runoff in water ways compared to the conventional treatments. The results showed that the compost treatment significantly increased labile Pi, hot water extractable carbon (HWEOC), MBC, MBP and soil respiration compared to conventional and half-compost treatments. The results in the compost treatment indicated the occurrence of rapid mineralisation and the accumulation of available P from the compost after repeated application. The higher microbial biomass and activity in compost treatments were due to the increased organic labile C (as shown in the HWEOC data). The result indicated that repeated compost application with high P input has no significant differences from the conventional treatments which may pose risks of P runoff in water bodies.

In the second experiment, five selected RO using two different P-based application rates (50 kg P ha⁻¹ and 100 kg P ha⁻¹) were used. The five RO used includes biosolids (BS), broiler litter (BL), green waste biochar (BC), mixed compost (CM) and green waste compost (CG) were amended to two different soils (sandy loam and loam soil) and were incubated at 22°C for 20 weeks. Results showed that the effects of RO application on soil P dynamics and P availability varied with both RO and soil types. The extent of the effects of RO application on soil microbial properties also varied with the RO types, soil types and P application rates. The different effects of RO between the two soil types were found to be related to their different physical and chemical characteristics such as the Fe and Al contents, pH, readily available organic C and P contents as well as their clay contents. The BS treatment increased labile Pi fraction the most in both soils, but had minimal effect on the microbial properties due to the high proportion of heavy metals. The BC unexpectedly increase microbial properties in the sandy loam soil indicating the potential of this treatment in enhancing the P and C cycling in such soil.

Overall, the CM treatment showed the greatest influence on P fractions and availability as well as microbial properties, followed by BL and CG treatments. Importantly, this study has highlighted that it is vital to understand the mechanisms involved in P dynamics associated with microbial properties following RO addition in maximising the potential benefits of RO in enhancing nutrient cycling in soil.

In the third experiment, a pot trial was carried out with soil amended with five selected RO; biosolids (BS), broiler litter (BL), green waste biochar (BC), mixed compost (CM) and green waste compost (CG) using two different P-based application rates; low P rate, LP (50 kg P ha⁻¹) and high P rate, HP (100 kg P ha⁻¹). Ryegrass and wheat grass were grown in a greenhouse pot experiment over 12 weeks, to determine the effects of the RO on plant P availability, plant growth, plant P uptake, MBC and MBP in the rhizosphere soil. All P fractions measured were higher in the bulk soil compared to the rhizosphere soil. Among RO tested, the BL treatment was found to be the most efficient P supplier to both plants tested and presents the largest fraction of labile P, followed by the CM and CG treatments. The BS and BC treatments were found to be less efficient as P suppliers to plants due to their chemical properties leading to a very little influence on soil microbial biomass. This experiment showed that the greater P availability and plant growth were not only attributed to higher easily available substrates in these amendments but also induced and accompanied by root processes and rhizosphere microbial processes.

6.2 General Discussion and Conclusion

It is recognised that there were various effects on P availability and microbial properties of different types of RO although the same amount of P was added. The detailed studies showed that the composition of the RO used and soil physical and chemical properties had strong effects on the microbial biomass responses and activities which consequently influenced the P availability and P pools in amended soils. From the occurrence of

mineralisation and immobilisation processes observed in the experiments, it was found that the biochemical mechanisms responsible for the P availability and transformation of P pools is mainly depending on the needs for the available P and available organic C for the growth of the soil microbial biomass. The RO with higher easily available substrates such as available organic carbon stimulates the microbial biomass growth and thus, increase the P availability in soil. The finding provides important information in maximising the potential benefits of RO such as the use of green waste amendments (CG and BC) as effective fertiliser practices, thus improving agricultural management particularly in acidic soils.

In conclusion, the use of RO in agro-ecosystems can increase soil organic matter and nutrient contents and help to solve environmental and economic problems related to the disposal of these waste materials. This study also highlighted the potential role of the RO amendment in increasing soil fertility in soil. This quantitative information on P fractions and the effects of RO application will be valuable for the development of economic management strategies for minimizing inorganic fertiliser use and maximising the benefits of biological cycling of P in agricultural practices in Australia.

6.3 Future research

The three experiments reported in this thesis provide further information about the P pools and associated microbial response in the soils amended with RO comprising a wide range of C: P ratios. Also, the findings add substantially to our understanding of the mechanisms involved in the transformation of soil P availability in bulk and rhizosphere soils. However, there were limitations identified in this study. These limitations and the recommendations for future work are listed below:

(1) The Incubation experiments (Chapter 4) and the Pot trials (Chapter 5) of this research work were all short-term and conducted under controlled conditions. The used RO needs to be verified under longer term and in field conditions with varying soil types and plant species. It would be important to observe plant P uptake in the RO-amended soils over a longer period of time in order to assess long-term effects of RO on plant growth. Also, repeated applications of RO used in the Chapter 4 and 5 should be conducted in the future to determine the accumulation effects of repeated RO on soil using P based application rates.

(2) The microbial biomass response determined in all chapters indicated the size of the whole microbial community in the soil, but not specific microbial functional group (e.g. Alkaline phosphatase gene (*alp*)). In order to further understand the effects of RO on the composition of soil microbial communities, the composition of microbial community should be analysed using techniques such as 454 pyrosequencing in future studies. At the same time, quantitative-PCR (q-PCR) can be used for detecting the levels of functional genes (e.g. *alp* gene). These will greatly improve our understanding of soil microbial community as affected by the amendment of RO.

3) Information on the chemical composition of soil Po is fundamental in order to further understand plant nutrition and soil biogeochemical cycles. The analysis using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy to characterise Po compositions could be employed in future studies.

4) In rhizosphere study, it is suggested that acid phosphatase and phytase need to be studied to further understand the mobilisation of Po in the rhizosphere soil, as other root exudates such as phosphatase enzymes hydrolyse Po compounds and contribute to P acquisition.

5) The results of this study suggest that there are possibilities of combining different types of RO to manipulate different benefits and improve the utilisation of the organic

wastes. Therefore, it would be interesting to assess the dynamics and transformations of P as well as the soil microbial response from combined RO amendments.

6) Economic analysis on the amounts of RO needed to produce sufficient P concentration for acceptable crop yields/crop quality needs to be conducted. A study of the cost associated with RO processing and transporting from source to application site would be useful to determine the economic benefits of RO in crop production. Also, for the purpose of field application, modelling the effects of different RO on soil P dynamics would be very useful.

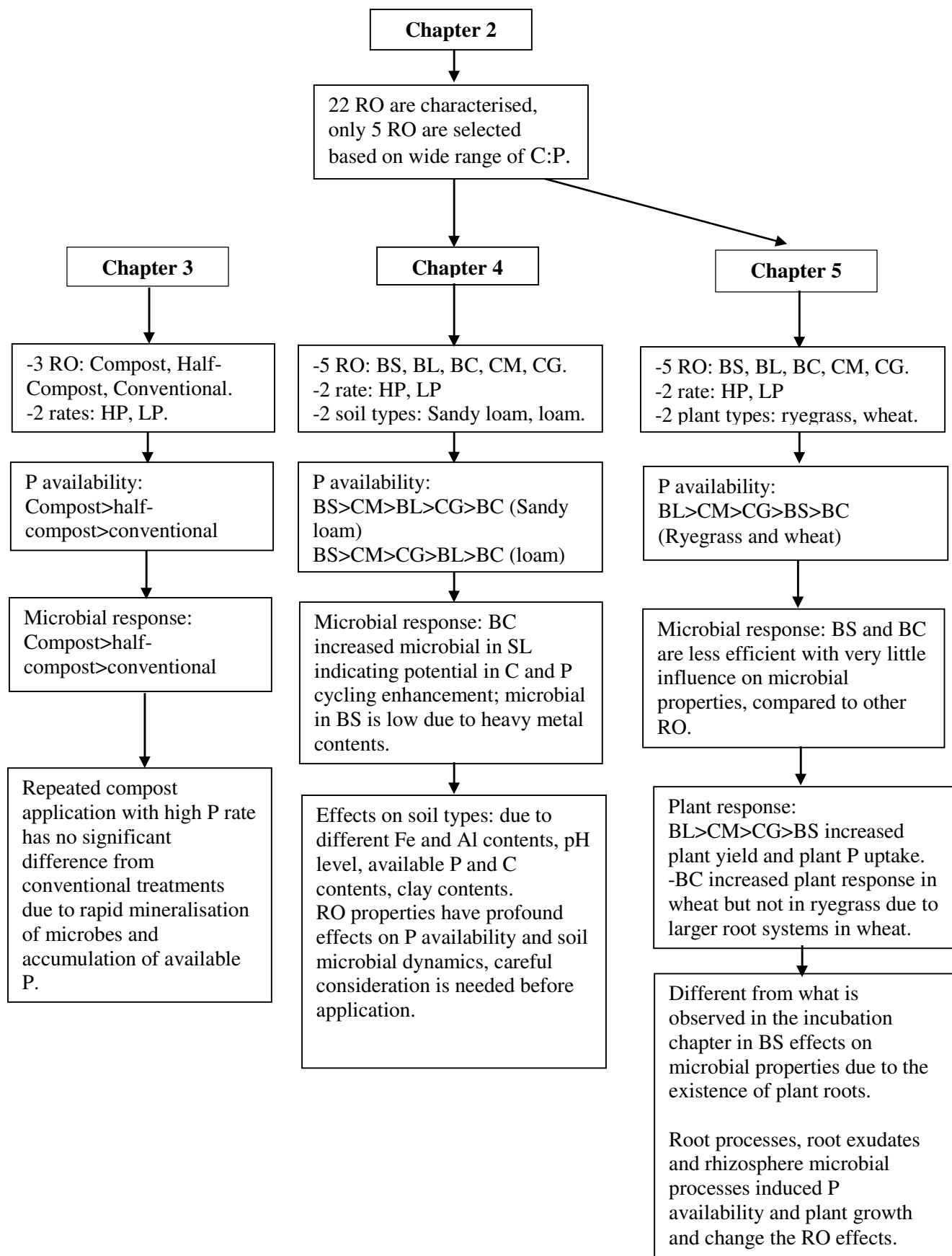


Figure 6.1 Conceptual diagram on the general conclusion of the research.