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The Impact of Biochar on Soil Functioning in Two Contrasting Climates

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DECLARATION

I declare that this thesis is my own work and contains no material which has been submitted in substantially the same form for the award of a higher degree elsewhere. Any sections of this thesis containing material published and or written by other persons have been referenced.

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ABSTRACT

Previous research has demonstrated that biochar addition to soil improves the soil's physical and chemical characteristics, reduces nutrients leaching, increases crop yield and enhances microbial activity in the soil. This has attracted significant research interest into the effects of biochar application on soil in recent years. However, the literature on tropical soils following biochar addition is scarce. Even though more biochar studies were conducted in temperate soil, the physical and chemical characteristics of temperate soils vary widely, and may respond differently upon biochar addition. Moreover, to date, tropical and temperate soils studies are conducted separately. Therefore, this thesis investigates the effect of biochar amendment on the soil's physical, chemical and biological properties in two different climates at the same time. The aims of this study were to determine the effect of biochar ageing on tropical and temperate soil characteristics and also to assess the effect of different particle sizes and application rates on temperate soil properties. The present study comprised of two sets. The first set involved incubation of soils and biochar for up to 360 days (tropical soil), 300 days (temperate soil part 1) and 30 days (temperate soil part 2). The soils were kept and incubated in sealed jars. The soil's biological, chemical and physical properties were tested as to whether they were enhanced by the addition of biochar. The second set was a nutrients leaching study. In this part of the study, the soils and biochars were packed into glass and PVC columns. Ammonium, nitrate and phosphate leaching were measured to assess whether biochar application reduces nutrients leaching from the soil columns.

The results from the tropical and temperate soils revealed that at the 2% application rate, the addition of biochar increased the soil's carbon and pH ($P<0.05$), had a limited effect on the mineralization of ^{14}C glucose and water retention, a marginal effect on the cation exchange capacity, and no effect on the microbial biomass, total nitrogen, inorganic phosphorus and aggregate stability ($P>0.05$). Biochar also reduced the concentration of ammonium leaching ($P<0.05$) and showed an unclear pattern on the sorption of nitrate and phosphate of biochar in the soil's leachates ($P>0.05$). At a higher application rate (5%), biochar increased the temperate soil's carbon and pH ($P<0.05$), increased microbial activity, especially when using the finest particle size (0.1mm) ($P<0.05$), increased microbial growth ($P<0.05$) and reduced nitrate leaching in unfertilized temperate soil ($P<0.05$). These results were drawn from a small-scale study (laboratory study). The effects of biochar on a larger scale, for example in a long-term field study, must be investigated further to examine whether similar results can be obtained in a real condition. This is important to assist and provide farmers with information about the use of biochar before applying biochar in a vast agricultural area.

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LIST OF ABBREVIATIONS

Al – Aluminium

AM – Arbuscular mycorrhizal

BC – Black carbon

BRIS – Beach ridges interspersed with swales

C – Carbon

CEC – Cation exchange capacity

$CHCl_3$ - Chloroform

CH_4 – Methane

CO – Carbon monoxide

CO_2 – Carbon dioxide

CS – Coconut shell

Cu – Copper

d – Day

EC – Electrical conductivity

EL – Elementar vario

Fe – Iron

FW – Fast wetting

GBP – British Pound

GHG – Greenhouse gas

H^+ - Hydrogen

H_2SO_4 – Sulfuric acid

HW – Hardwood

IMS – Methylated spirit

K_2SO_4 – Potassium sulphate

M – Mechanical

Mag - Magnitude

Mn - Manganese

MWD – Mean weight diameter

N – Nitrogen

Na – Sodium

ND – Not detected

NM – Not measured

N_2 – Nitrogen gas

NH_4^+ - Ammonium

NO – Nitrous oxide

NO_2 – Nitrous dioxide

NO_2^- - Nitrite

NO_3- - Nitrate

P – Phosphorus

PAH – Polycyclic aromatic hydrocarbon

PO_4^{3-} - Phosphate

POME – Palm oil mill effluents

PVC – Polyvinyl chloride

RH - Rice husk

RHF – Rotary husk furnace

SEM – Scanning electron microscopy

Si – Silica

SIR – Substrate induce respiration

SOM – Soil organic matter

SW – Slow wetting

TC – Total carbon

TN – Total nitrogen

UK – United Kingdom

Zn - Zinc

CHAPTER 1

Introduction

1.1 Biochar

Biochar can be made from a range of organic materials, for instance crop residues, manure or wood, that have been heated in a closed vessel with very limited or zero oxygen (Lehmann and Joseph, 2009). This process is called pyrolysis or gasification and it produces charred materials. Biochar applications to soil began thousands of years ago in the Amazonian Basin, where fertile soil called Terra Preta (dark earth) was created by the indigenous people (Lehmann, 2003; Glaser and Woods, 2004). Biochar has many benefits as a soil additive and has been proposed as a soil amendment for sequestering carbon and improving soil properties. The effect of the addition of biochar varies based on its characteristics, production and feedstocks, as well as the soil and crop types, land management and climate (Verheijen *et al.*, 2010; Scott *et al.*, 2014).

The addition of biochar to soils can have positive or negative effects on soil and crops. The mechanisms of these phenomena are unclear. These have created significant research interest into the effects of biochar application on soil in recent years (Barrow, 2012; Ameloot *et al.*, 2013; Semple *et al.*, 2013; Kloss *et al.*, 2014; Jay *et al.*, 2015). A study by Angst *et al.* (2013) reported that sandy loam soil amended with hardwood biochar not only exhibited reduced nitrous oxide emissions, but also decreased N leaching. In addition, the application of biochar improves water holding capacity and

saturated hydraulic conductivity (Asai *et al.*, 2009; Karhu *et al.*, 2011) due to large numbers of small pores.

Adding biochar to soil can also influence soil organisms, as a result of the chemical alteration after the addition of biochar to soil (Cornelissen *et al.*, 2013). For instance, the release or sorption of organic compounds from biochar may in some cases be responsible for a decrease or increase in microbial abundance and activity (Lehmann *et al.*, 2011).

In spite of the positive effects, adding biochar to soil can also have negative effects. For example, Quilliam *et al.* (2013a) found that the application of biochar to soil in the short-term did not provide a habitat in which microbes could live. Other studies have revealed that biochar has no effect on microbial activity and biomass (Bruun *et al.*, 2008; Dempster *et al.*, 2010; Zhang *et al.*, 2014a); it decreases the cation exchange capacity (CEC) of the soil (Novak *et al.*, 2009; Méndez *et al.*, 2012; Karer *et al.*, 2013; Kloss *et al.*, 2014); and it does not increase the pH in temperate soil (McCormack, 2015; Qayyum *et al.*, 2015).

With regard to both the positive and negative effects of biochar addition to soil, however, little research has been conducted on the ageing effect after biochar application to soil, or increasing the rate of biochar loading to soil. Most of the studies have been carried out over less than 12 months (Hamer *et al.*, 2004; Bruun *et al.*, 2008; Ding *et al.*, 2010). Furthermore, most of the biochar experiments have focused

on degraded soils, such as highly weathered tropical soils (Glaser *et al.*, 2002; Blackwell *et al.*, 2009; Sohi *et al.*, 2010) under a tropical climate and nutrient poor soils under a temperate climate (Jones *et al.*, 2012; Quilliam *et al.*, 2012; Kloss *et al.*, 2014). However, these soils do not represent fertile and highly managed agricultural soils. Also, most studies of biochar in tropical (Masulili *et al.*, 2010; Peng *et al.*, 2011; Alling *et al.*, 2014; Kollah *et al.*, 2015) and temperate (Jones *et al.*, 2012; Quilliam *et al.*, 2013a; Jay *et al.*, 2015) climates have been conducted separately.

In addition, the particle size of biochar is one of the important characteristics that should be investigated further. To date, there is limited information regarding the effect of the particle size of biochar on the soil quality and its degradation by soil biota. Therefore the mechanisms by which particle size significantly influences the rate of mineralization of biochar and the stability of biochar in the soils remain poorly investigated (Sigua *et al.*, 2014). This is because when biochar reacts with soil particles (Laird *et al.*, 2009), the resistance of biochar to microbial attack varies depending on the particle size of the biochar (Manyà, 2012). For example, Kollah *et al.* (2015) found that finer particle sizes of biochar accelerated CH_4 consumption compared to larger sizes. They speculate that the colonization of microbes increased (Thies and Rillig, 2009) due to the increased surface area when using smaller particle sizes of biochar as a soil amendment. Additionally, a huge surface area of biochar particles enables a greater number of sites for contaminants, such as heavy metals or pesticides in the water and soils, to bind (Cernansky, 2015).

Thus, in this study the impact of long-term biochar amendment on particular physical, chemical and biological properties, for example, aggregate stability, water retention, pH, CEC, leaching, nutrient content, microbial activity and growth is investigated in both tropical and temperate climates. Also, the effects of different particles sizes and rates of biochar on two contrasting soils' fertility in the temperate climate are explored, to identify whether the biochar addition has an impact on the biological and chemical properties of the soils. Thus, the aims of this study are: 1) to investigate the influence of ageing biochar and soil mixtures on soil properties in both climates under gradients of soil degradation and different soil types; and 2) to explore the influence of different sizes and rates of biochar application on the properties of two temperate soils with contrasting nutrients status. With adequate information in these areas, it is hoped that biochar amendment could improve soil properties particularly in degraded land.

1.2 Thesis objectives

The objectives of this thesis are as follows:

1. To examine whether an addition of biochar on tropical and temperate soils can enhance the biological properties of the soils over time
2. To determine the effect of biochar on tropical and temperate soils' chemical properties and leaching of nutrients over time
3. To assess the impact of biochar on soil structural changes over time for tropical and temperate soils with different gradients of soil degradation
4. To investigate the effects of different particle sizes and application rates of biochar on two soils with contrasting nutrient availability in a temperate climate

1.3 Thesis structure

The thesis focuses on the effect of organic soil amendment (biochar) on the physical, chemical and biological aspects of soil. The experiments involved materials from tropical and temperate climates. For the tropical climate, three Malaysian Spodosols soils (forest, intensive farming and non-intensive farming soils) based on different gradients of degradation and two types of biochar (coconut shell and rice husk) were chosen to investigate whether the addition of biochar would have an impact and enhances the soil properties over time (ageing period).

The temperate study is divided into two parts. For the first part of the study, Brown Earth soils (grassland, arable loam and arable sandy); and biochar (hardwood) from the UK were used (temperate climate). In this study, the effect of ageing biochar for approximately 10 months was compared with biochar that had been freshly added to the soil. The physical, chemical and biological properties of the soils after adding biochar were compared with an aged soil amended with hardwood biochar. For the second part of the study, the soils were chosen based on different levels of soil management, for example highly managed soil (fertilized) and unmanaged soil (unfertilized). Different application rates (2% and 5%) and sizes (2mm, 1mm, 0.5mm and 0.1mm) of biochar were tested to examine whether the biochar addition would improve the soil's biological and chemical properties.

The thesis begins with an introduction in Chapter 1, which is followed by a literature review in Chapter 2 on the use and effects of biochar application in agricultural land. Shorter targeted reviews are also included in each chapter. Chapter 3 describes the

materials and methods used to characterize the biochars and soils in this study and details the methods used to carry out the experiments in the study.

Chapter 4 measures the effectiveness of biochar application in tropical climates. The physical, chemical and biological aspects of the soils after amending them with biochars are evaluated in this chapter. Meanwhile, Chapter 5 assesses the impact of hardwood biochar addition to three contrasting temperate soils. In this chapter the ability of biochar to enhance the soil's physical, chemical and biological characteristics is examined.

The effects of biochar in temperate climates with different levels of soil management are further discussed in Chapter 6. Different particle sizes of biochar, as well as different application rates were tested to see whether both factors improved and enhanced the quality of the soils studied. Only biological aspects and some chemical characteristics of the soils were examined. These parameters were chosen based on the some positive results obtained from the previous chapters.

Chapter 7 describes the details of both findings and the differences and similarities in the results achieved from the tropical and temperate regions. Further discussed in this chapter are the benefits of biochars in these experiments or, in other words, the positive effects of biochars after adding them to soils, as well as economic benefits from the use of biochar in both regions. These indirectly show that biochars could potentially be used in Malaysia and in the UK. The conclusion, in Chapter 8, gives a

general summary of the findings of this thesis. Finally, further works and research gaps that need to be filled are also presented at the end of this chapter.

CHAPTER 2

Literature Review

2.1 Biochar characteristics and potential agricultural use

Feedstock is a type of biomass, for example, rice husk, woody, crop residues, manures and grasses that is pyrolysed to generate biochar (Verheijen *et al.,* 2010). Different types of feedstocks influence biochar characteristics such as density, porosity and hardness (Spokas *et al.*, 2012). Biochar yield from the same feedstock depends on the conditions of pyrolysis temperature, heating rate, time and particle size (Shafizadeh, 1982; Williams and Besler, 1996; Demirbas and Arin, 2002; Uzun *et al.*, 2006; Tsai *et al.*, 2007). Biomass with high lignin contents, for instance olive husk, produces the highest biochar yields, showing the resistance of lignin to thermal degradation (Demirbas, 2004).

Woody feedstocks produce small amounts of ash (<1% by weight), whereas biomass with high mineral contents, such as grass, grain husks and straw residues, produce high ash biochar (Demirbas, 2004). Rice husk (Amonette and Joseph, 2009) and rice hull (Antal and Gronli, 2003) may produce 24% to 41% ash by weight, respectively. Ash can also be hydrophobic, thus if this material is added to soil, it can reduce soil water retention and enhance runoff. This causes erosion to occur and results in poorer crop production due to nutrient loss (Renner, 2007). Therefore, when adding high ash-containing biochar to soil, prevention measures for soil erosion must be taken into consideration. In addition, biochar produced at high temperature also results in increases in ash content (Kloss *et al.*, 2012) as shown in Table 2.1 below.

Table 2.1 Basic characterization (pH, electrical conductivity (EC), ash content, cation exchange capacity (CEC), polycyclic aromatic hydrocarbon (PAHs) and yield) of the studied feedstocks and biochars pyrolyzed at 250, 400, 460, 525 and 650^{O}C, from (Demirbas, 2004; Peng *et al.*, 2011; Kloss *et al.*, 2012).

Feedstock	**Pyrolysis Temperature**	**pH**	**EC (mScm^{-1})**	**Ash Content (%)**	**CEC (mmol kg^{-1})**	**PAHs (mg kg^{-1})**	**Yield (%)**
Straw	250^{O}C	4.2	-	-	-	-	54
	400^{O}C	9.1	1.0	9.7	161.6	5.2	-
	460^{O}C	8.7	4.9	12.0	117.0	10.7	-
	525^{O}C	9.2	4.4	12.7	97.7	33.7	-
Spruce	400^{O}C	6.9	0.4	1.9	73.5	30.7	-
	460^{O}C	8.7	1.8	3.0	54.7	5.8	-
	525^{O}C	8.6	0.7	4.7	52.2	1.8	-
Poplar	400^{O}C	9.0	1.0	3.5	144.0	4.3	-
	460^{O}C	9.2	0.7	5.7	128.3	17.9	-
	525^{O}C	8.7	0.9	6.8	107.6	2.0	-
Oliver husk	650^{O}C	-	-	-	-	-	43

Kloss *et al.* (2012), suggested that the biochar surface area increased with pyrolysis temperature. But, Antal and Gronli (2003) argued that high temperature pyrolysis resulted in a greater condensation of aromatic structures, hence less surface area and fewer surface functional groups to be oxidized (Novak *et al.*, 2009). Because of these contrasting findings, biochar is a heterogeneous material; the results of a biochar product depend on the feedstock used, as well as the methods used to produce it. In addition, the effects of the particle size of the biochar also influence its stability in the soils. For example, the smaller particle size of biochar increased CO_2-C evolution more than the larger particle sizes (281 mg kg^{-1} and 226 mg kg $^{-1}$) (Sigua *et al.*, 2014). Sigua *et al.* (2014) reported that the greater surface area of the finer particles accelerates the decomposition process by microbes, resulting in an increase in the CO_2-C evolution (Table 2.2). They also suggested that different particle sizes of

biochar affect its stability, whereby the finer particle sizes can improve the fertility of soils, whereas the coarser particles can sequester C in the soil for longer due to their resistance to microbial attack. Other studies have found that larger biochar particles remain in forest wildfire soil after thousands of years (Gouveia and Aravena, 2001; Gavin *et al.*, 2003), but smaller particle sizes of biochar have greater mobility in the soil (Wang *et al.*, 2013). A recent study on the effect of the particle size also showed that amending soil with the smaller size of biochar (<0.25mm) increased the consumption of methane in arable land and thus reduced the emission of greenhouse gases (Table 2.2) (Kollah *et al.*, 2015).

On top of that, other researchers found that biochar yield decreases with increasing temperature and the relationship between yield and temperature varies with different feedstocks (Guha *et al.*, 1986; Horne and Williams, 1996; Williams and Besler, 1996; Tsai *et al.*, 2006). Furthermore, the pyrolysis temperature affects the polycyclic aromatic hydrocarbon (PAH) content of biochar (Table 2.1). This is because, during incomplete combustion, potentially toxic aromatic hydrocarbons are formed (Kloss *et al.*, 2012). The authors also state that polycyclic aromatic hydrocarbons which have two or more condensed rings show different toxicity levels. In a study related to PAHs associated with biochar, Rogovska *et al.* (2012) investigated the impact of biochars on seedling growth. Using corn for the bioassay, results showed that shoot and radical length decreased in the presence of biochars produced at high temperature. The authors speculated that the decrease was probably due to the presence of PAHs in the biochar.

To date, field studies which have measured the characteristics of biochar relevant to soil improvement, soil C sequestration and soil management systems are also scarce. Such studies are urgently needed to identify and quantify the biochar characteristics before applying to agricultural soil. More research evidence will provide information on the biochar that is best suited to a particular agricultural site depending on soil type, hydrology, climate and soil contaminants.

2.2 Effects of biochar on soil properties

Adding biochar to soil may affect texture, structure, pore size distribution and density, as well as soil aeration and water holding capacity (Downie *et al.*, 2009). Karhu *et al.* (2011) hypothesized that the incorporation of biochar into soil would increase soil water holding capacity due to biochar's ability to retain water because of its high porosity and large number of small pores. This combined with a high surface area gives biochar the capability to absorb nutrients and water which are held by capillary forces in soil micropores (Rhodes *et al.*, 2008; Major *et al.*, 2009). This statement supports the results obtained in their study where adding biochar in soil increased soil water holding capacity by 11%. Moreover, in sandy soils biochar can increase the water holding capacity, and thus alleviate water stress on plants (NGI, 2012).

Besides improved water holding capacity, the application of biochar also improves the saturated hydraulic conductivity of the soil (Table 2.2) (Asai *et al.*, 2009). In their study, Asai *et al.* (2009) revealed that high biochar contents in soil not only enhanced soil water permeability, but also soil water holding capacity, and indirectly, water availability to plants. On the other hand, the smallest particle size fraction of biochar

potentially blocked the soil pores and may reduce conductivity, as a consequence, water infiltration is decreased (Verheijen *et al.*, 2010). The particle size distribution of biochar is a key parameter for determining its effect on soil hydraulic properties and also varies depending on the feedstock and the pyrolysis condition used to manufacture it (IBI, 2012b). More research is needed to further understand how it effects soil hydrological functions and processes.

The application of biochar which had a lower bulk density than in soils can reduce the bulk density of soil. Nevertheless, increases in bulk density of soil after adding biochar may be possible. For example, if the biochar that is applied to soil has a low mechanical strength, it will easily break into small fractions and fill soil pores and eventually, the soil bulk density will increase (Verheijen *et al.*, 2010). The potential susceptibility of biochar particles to bind or clog the soil might also result in greater runoff and lower infiltration rates. Experimental evidence of these effects are scarce, in fact no papers could be found reporting studies in this areas, and therefore further studies of the effect of biochar on soil compaction is needed.

Biochar is also brittle and often made up of small particles (< 0.60mm to 4.75mm); however, the particle size of biochar is different based on the feedstocks used and pyrolysis process (Downie *et al.*, 2009; IBI, 2012a). Hamer *et al.* (2004) claimed that when biochar is applied to soil, processes such as freeze-thaw cycles, rain and wind may not only enhance its degradation rate, but also make it susceptible to wind and water erosion. Rumpel *et al.* (2006) studied the erosion of black carbon (BC) on steep slopes with slash and burn agriculture, and found that soil erosion resulted in large

amounts of BC being easily transport from hillslope to the watershed (Table 2.2). They also speculated that the BC eroded from the soil may be buried in marine sediments, consequently leading to loss of carbon from the terrestrial carbon cycle. Major *et al.* (2010b) as displayed in (Table 2.2) also observed significant losses of biochar incorporated into flat terrains, in an area where intense rainfall events occur. In addition, Schnell *et al.* (2012) found that soluble P and K nutrients in biochar are the major nonpoint source runoff. In contrast, Beck *et al.* (2011) reported incorporation of biochar with green roof soil improved runoff water quality and water retention. Inevitably, results from these studies require a best management practice to address erosion problem in addition to biochar application. Studies on the methods used for biochar incorporation to minimize erosion losses are very limited and more work is required to quantify this. To date, biochar loss and mobility through the soil profile and into the water resources, has been scarcely quantified and transportation mechanisms remain unclear.

2.3 Effects of biochar on crop productivity

One of the potential benefits of adding biochar into the soil is increasing crop yield and the production of crops depend on the rates of biochar and the types of soil that is used. Because of this, a number of studies have been conducted to evaluate the response of crops to biochar application. Results from various studies have shown that adding biochar itself into soil increases crop productivity (Baum and Weitner, 2006; Chan *et al.*, 2008b). Some studies have found positive results when biochar is applied with fertilizers (Steiner *et al.*, 2007), but some found a negative effect on crop yields when using biochar solely as a soil amendments (Wisnubroto *et al.*, 2010; Jeffery *et*

al., 2011). The impacts are also different depending on the interaction of the various factors, including the type of biochar, crop and soil (Galinato *et al.*, 2011).

Compared to unamended soil, Collins (2008) reported a decline from 12.3 to 8.6 g in the root and 10.3 to 9.1 g in the shoot of wheat in sand soil amended at 39 mg ha^{-1} of softwood bark biochar. In contrast, an increase in the root biomass from 10.1 to 12.9 g and in the shoot biomass from 7.3 to 11.6 g of wheat were found in the Hale silt loam soil amended with softwood bark biochar at 19.5 mg ha^{-1}. The study also found that N in biochar is not available to plants (Galinato *et al.* 2011). Therefore reducing chemical fertilizer inputs after biochar applications cannot be assumed. The reason for this may be due to biochar's highly porous structure, leading biochar to retaining nutrients and making them unavailable to plants. As a result, more fertilizer may have to be applied in order to supply enough nutrients for plants growth. Another negative effect on crops was recently found by Jay *et al.* (2015) who studied the effect of short-term biochar application on barley, potato and strawberry crops. The findings from their research showed that the addition of biochar to soil had no significant effect on the growth and yield in any of those crops. The authors suggest that the effect cannot be seen in a short-term study, and also the limited effect was due to the fertile soils used in their temperate study (Table 2.2).

On the other hand, there is evidence from other studies indicating that using biochar as a soil conditioner often gives positive crop productivity in some situations and conditions. For example, Kammann *et al.* (2012) studied the effect of adding peanut hull biochar to a German Luvisol (soil) with ryegrass crop. The authors noticed a

significant crop yield in comparison with control. The cause of the increase is unclear and they speculated that due to reduced denitrification, N loss was reduced; therefore, N uptake by plants was greater in presence of biochar. Another significant effect on crop growth was observed by Lin *et al.* (2015) as displayed in Table 2.2. The researchers found that, at 16t ha^{-1} biochar application increased the growth of wheat plants by 27.7%.

Biochar increases soil quality by reducing soil acidity due to its alkalinity and acts like a lime (Galinato *et al.*, 2011; NGI, 2012). For example, Rondon *et al.* (2007) observed an improvement in bean yield due to an increase in soil pH from 5.04 in soil without biochar to 5.41 in soil with 90 g kg^{-1} biochar. Furthermore, increases in soil nutrients were also observe in their study as a consequence of using biochar. Inal *et al.* (2015) (Table 2.2) found that biochar reduced pH, but increased crop nutrients, such as P, K, Cu, Zn and Mn levels in bean and maize crops. The only exception was Fe, in which the addition of biochar decreased the availability of Fe in the soil. However, the results from their study prove that amending soil with different loads of biochar increased the growth of maize and bean crops.

Findings from the studies above clearly show that some biochars produce negative impacts on crop performance, whereas others can increase crop productivity. The knowledge gap in this area is to understand a complete mechanism of how biochars cause yield to decrease and increase. Further studies on the effect of crop performance with various types of biochars, on different soil types, climate and environmental management are urgently required.

2.4 Sequestration of pesticides and organic contaminants by biochar

During the past decade, the sorption of pesticides and organic contaminants to biochars has been studied widely due to the growing awareness of the importance of biochar to the overall sorption properties of soil (Smernik, 2009). In addition, due to its large surface area, high nanoporosity and other physiochemical properties (Cornelissen *et al.*, 2005; Lehmann, 2007b; Glaser *et al.*, 2009), sorption of pesticides and organic contaminants is the key process that controls their toxicity, transport, fate and behaviour in soil (Smernik, 2009).

A study by Wang *et al.* (2012) highlighted that amending agricultural soil with biochar produced at 850^{O}C increased pesticide sorption and at the same time reduced the pesticide uptake by earthworms. Yang *et al.* (2010) also studied the influence of two types of biochars (produced at two different temperatures – 450^{O}C and 850^{O}C) on the bioavailability of pesticide to plants. They showed that by adding biochar produced at high temperature to soil, it reduced the bioavailability of pesticide to microorganisms and plants grown in contaminated soil. Consequently, movement and plant uptake of pesticide are decreased due to the high surface area of biochar. In addition, the high microporosity of biochars produced at higher temperatures helps it to sequester pesticide in soils (Yang *et al.*, 2010).

Moreover, Sopena *et al.* (2012) found that application of biochar to soil can affect the persistence, efficacy and the fate of pesticide degradation. Their results showed that amending soil with 1% and 2% of biochar enhanced sorption, reduced desorption and reduced biodegradation of the pesticide. The study also found that after 8 days, only

10% pesticide remained in the soil without biochar, indicating that the degradation of pesticide was very fast. Whereas, in soil amended with 1% and 2% biochar, 35% and 45% pesticide remained in the soil, respectively. Therefore, biochar could provide a means of effective contaminant sequestration in agricultural soils.

Nevertheless, negative effects of biochar application also exist, such as the inactivation of pesticides in the soil preventing them controlling target organism (IBI, 2012a). Furthermore, if sorbed organic or inorganic compounds become available to organisms, they may potentially have detrimental effects on them. Therefore, future research and scientific evidence are required to verify this phenomenon. Semple *et al.* (2013) argued that the presence of black carbon in the form of biochar in agricultural soil has been shown to reduce the bioavailability of some compounds to microorganisms. However, the length of time that biochar can retain the compounds and its safety towards other organisms in terms of toxicity remain unknown. This is because the compounds may be stored temporarily within biochar's structure and they could physically remove due to physical or chemical reactions over longer period and, thus become available to other organisms (Semple *et al.*, 2004).

Other considerations such as how frequent biochar has to be applied to the soil in order to maintain its functionality must also be determined. The reason for this is biochar is no longer inert in soil (Hamer *et al.,* 2004). This is because, in natural environments, chemical and microbial breakdown and degrade biochar which resulted in alteration of the biochar surface chemistry and functional properties (Glaser *et al.*, 2002). Verheijen *et al.* (2010) also hypothesized that solubilisation, leaching and

transportation of biochar through the soil profile and into water system is expected gradually enhanced for longer time exposure in soil. Therefore, further studies are required in order to quantify how much and how often biochar has to be added to ensure the persistent of biochar in soil.

There is some potential of biochar amendment to control the toxicity and mobility of organic chemicals. Rhodes *et al.* (2008) pointed out that applications of biochar to highly contaminated areas, particularly in buffer strips, to prevent contamination of waterways would be possible. However, the applicability of such treatments would depend upon the longevity of the effect of biochar, and need to consider the potential for sorption sites to become blocked (Rhodes *et al.*, 2008).

2.5 Effects of biochar on soil biota

Adding biochar to soil can influence soil organisms. The effects are due to the chemical and physical properties of biochars and soils. The differences in physical structure between biochar and soils lead to a transformation in the soil tensile strength, bulk density, porosity, pH and water holding capacity (Lehmann *et al.*, 2011). Biochar structure may offer a similar role to soil particles, such as the retention of water and nutrients, thus creating a suitable habitat for soil microorganisms. This is due to its high porosity and large internal surface areas promote an optimum living place for microbial growth (Lehmann *et al.*, 2011). However, the porosity is influenced by many factors such as the production and application methods of biochar, the interactions of biochar with soil organic matter pools, the physical and chemical

characteristics of soils and the management practices of agriculture (Ameloot *et al.*, 2013).

Biochar is recalcitrant and this increases its resistance to oxidation, making a slow carbon cycle and therefore the associated carbon can remain in the soil for longer (Sombroek *et al.*, 2004). Despite its longevity, several studies have showed an increase in soil respiration when adding biochar to soil. For example, the mineralization rates of smaller oak biochar is approximately 10 mg C g^{-1} char in sterilized incubation compared to that inoculated with microorganisms where the biochar mineralization rate is 20 mg C g^{-1} char (Zimmerman, 2010). This study clearly indicates that the role of soil microorganisms in the degradation of biochar is essential. Moreover, biochar can not only enhance mineralization, but it can also reduce the amount of humus in organic pristine soils (Wardle *et al.*, 2008). This is due to adsorption of organic compounds in charcoal particles along with microbial activity and growth; stimulating decomposition and resulted in humus loss (Zackrisson *et al.*, 1996; Pietikainen, 2000).

According to Steinbeiss *et al.* (2009), biochar enhances soil health by stimulation of microbial activity response, however at the same time decreases soil organic C. Durenkamp *et al.* (2010) suggested that biochar could affect soil microbial biomass. This is because a fraction of the organic C, approximately 0.2 – 0.3%, may be sorbed by biochar (Bruun *et al.*, 2008; Kuzyakov *et al.*, 2009). Another study revealed that, the proportion of ^{14}C labelled biochar produced from rye grass biochar which was incorporated into microbial biomass for 624 days ranged between 1.5 and 2.6%

(Kuzyakov *et al.*, 2009). This clearly demonstrates that even with a longer period of incubation only a small proportion of the biochar was assimilated by microorganisms (Ameloot *et al.*, 2013).

Studies on the effects of biochar on soil fauna are very limited. Most of the studies on soil fauna in relation to agricultural biochar use have been devoted to earthworms. Studies have found that earthworms can digest biochar particles grinding and mixing them with soil. Interestingly, some species of the earthworms (*Pontoscolex corethrurus*) prefer living in soil with biochar than soil without biochar (Topoliantz and Ponge, 2003; Topoliantz and Ponge, 2005). The authors speculated that this may be due to the fact that they used to consume charred material (Ponge *et al.*, 2006).

Recently, there have been a few studies examining soil fauna, such as protozoa, collembolan, nematodes, microarthropods and termites (Lehmann *et al.*, 2011; Ameloot *et al.*, 2013; McCormack *et al.*, 2013). These soil fauna and invertebrates may also play an important role in the degradation and dissemination of biochar into the soil profile alongside earthworms (Ameloot *et al.*, 2013). In an observational study at the landscape scale conducted by (Matlack, 2001) no relationship between nematode populations and charred materials in the soil was observed. Furthermore, the bioavailability of pollutants, for example polychlorinated biphenyls, polyaromatic hydrocarbons and other organic agrochemical such as herbicide and pesticide to soil fauna, may be reduced due to strong sorption to biochars (Smernik, 2009). However, there is no evidence of this from biochar amended soil (Lehmann *et al.*, 2011). Therefore, there is an urgent need to elucidate the influence of soil fauna, other than

earthworms and the microbial community, after adding biochar to soils including the interaction between soil fauna and other microorganisms, biodiversity and bioturbation (Johnson *et al.*, 2005; Wilkinson *et al.*, 2009; Nielsen *et al.*, 2011).

2.6 Impacts of biochar in the Tropical Climate

Under a humid tropical climate, many soils are infertile and face difficulties in improving crop productivity. This is due to highly weathered soil with acidic pH, low organic matter content and cation exchange capacity (CEC) (Van, 1992; Zech *et al.*, 1997). In Southeast Asia, highly weathered acid soils, namely Ultisols and Oxisols, are very common. They encompass 82% of Thailand, 72% of Malaysia, and 43% of Indonesia (Ishak and Jusop, 2010). In such conditions, nutrients and mineral fertilizers are easily leached through the soil profile enhanced by intensive rainfall (Cahn *et al.*, 1993; Glaser *et al.*, 2001).

In order to solve the acidity and CEC problems, liming is used to increase pH and CEC of soils. However, in some locations such as in Indonesia, the source of lime materials is limited and far from the agricultural fields where it is needed (Masulili *et al.*, 2010). Moreover, liming effects on soil are temporary and, therefore, it has to be applied regularly, making it costly for poorer farmers to adopt (Shamshuddin *et al.*, 1998; Masulili *et al.*, 2010). Instead of using lime, studies have showed that biochar could replace the role of liming in soil. Masulili *et al.* (2010) found that application of rice husk biochar in acid sulphate soils significantly increase soil pH, improving rice growth (Table 2.2).

In addition, adding 10 L m^{-2} bark charcoal to acidic soils not only increase pH from 4.1 to 5.4 and CEC from 8.54 to 12.38 $cmol_c$ kg^{-1}, but also increases by almost 90% in the amount of root and colonization of arbuscular mycorrhizal (AM) fungi (Yamato *et al.*, 2006) as displayed in Table 2.2. Amending soil with biochar, also increase the activity of arbuscular mycorrhizal (AM) fungi and bacteria and the amount of macrofauna, such as earthworms (Barrow, 2012). Additionally, amending tropical soil with biochar can also reduce the emission of CH_4 gas. A recent study by Kollah *et al.* (2015) revealed that biochar, when applied with poultry manure in a tropical Vertisol, had the highest consumption rate (0.24) when compared with vermicompost (0.11), farmyard manure (0.09) and poultry manure (0.07). The authors speculated that increases in soil properties, such as pH, cation exchange capacity, water holding capacity and microbial community after biochar application stimulate the consumption of CH_4, thus reducing the emission of the gas into the atmosphere.

Furthermore, application of organic amendments improves soil fertility, but in hot and humid weather, it decomposes readily and must be added gradually (Malisa *et al.*, 2011) in Table 2.2. As biochar is more recalcitrant carbon remains in the soil for longer (Lehmann *et al.*, 2006). A study conducted by Major *et al.* (2010b) as shown in Table 2.2 found that only a single application of biochar into an infertile acidic tropical soil enhanced crop productivity for four years. This makes us question how long the biochar effect persists. However, there are no longer term studies and further work is therefore needed.

Another common problem in the tropics is the presence of sandy soils. For example, in Malaysia, the BRIS (Beach Ridges Interspersed with Swales) soil type consists of more than 90% sand and is poorly structured and leachable (Malisa *et al.*, 2012). It covers about 155,400 ha area in the east coast of Peninsular Malaysia (Aminah *et al.*, 2006). The BRIS soil has low cation exchange capacity (CEC), contains little organic matter, few nutrients, as well as having a limited water holding capacity (Khan *et al.*, 2008). Biochar, has the potential to ameliorate this type of soil. This statement is supported by Malisa *et al.* (2011). From Table 2.2 the authors studied the response of kenaf plants to charcoal amendment on a BRIS soil and results showed that charcoal application into soil, significantly increases CEC and the yield of kenaf plants. Moreover, another study found that adding biochar on sandy soil of Lombok Indonesia improved soil organic C, CEC and macronutrients, as well as nutrients uptake and yield of maize (Sukartono *et al.*, 2011) as shown in Table 2.2.

Table 2.2 Summary of studies on biochar in the tropical and temperate climates.

Study	Location	Design	Duration	Main findings	Authors
Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferrasol amended with compost and charcoal	Amazon	Field experiment	Short-term study	Amending soil with charcoal resulted in higher retention N in the soil and increased plant nutrient uptake.	Steiner *et al.* (2008)
Biochar amendment techniques for upland rice production in Northern Laos 1. Soil physical properties, leaf SPAD and grain yield	Laos	Field experiment	Short-term study	Amending soil with biochar improved saturated hydraulic conductivity. However, the effect of biochar application depends on soil fertility and management practices.	Asai *et al.* (2009)
Fate of soil-applied black carbon: downward migration, leaching and soil respiration	Colombia	Field experiment	> 2 years	Two years after application, 2.2% of black carbon was lost by respiration. The major of black carbon movement was assumed by water erosion during high rainfall events.	Major *et al.* (2010a)

Table 2.2 continued

Study	Location	Design	Duration	Main findings	Authors
Rice husk biochar for rice based cropping system in acid soil. 1. The characteristics of rice husk biochar and its influence on the properties of acid sulphate soils and rice growth in West Kalimantan, Indonesia	Indonesia	Glasshouse experiment	Short-term study	Rice husk biochar increased soil pH, organic matter, total P, CEC, exchangeable K and Ca and decreased bulk density, soil strength, exchangeable Al and Fe.	Masulili *et al.* (2010)
Influence of biochars on plant uptake and dissipation of two pesticides in an agricultural soil	China	Laboratory experiment	Short-term study	Biochar in contaminated soil reduced the bioavailability of pesticides to soil microorganisms and plants and decreased dissipation and plant uptake of the pesticides in soil. Biochar produced at high temperature ($850^{O}C$) was more effective than at low temperatures.	Yang *et al.* (2010)

Table 2.2 continued

Study	Location	Design	Duration	Main findings	Authors
Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity-results from a pilot field study	Finland	Field experiment	Short-term study	Amending soil with biochar improved soil aeration and water holding capacity, and mitigated CH_4 emissions.	Karhu *et al.* (2011)
Yield response of kenaf (*Hibiscus cannabinus* L.) to different rates of charcoal and nitrogen fertilizer on BRIS soils in Malaysia	Malaysia	Field experiment	Short-term study	Charcoal application had significant effects on CEC and exchangeable cations and crop yield	Malisa *et al.* (2011)
Soil fertility status, nutrient uptake and maize (*Zea mays* L.) yield following biochar and cattle manure application on sandy soils of Lombok, Indonesia	Indonesia	Field experiment	Short-term study	Biochar improved soil organic carbon, CEC, available P, exchangeable K, Ca and Mg, increased nutrient uptake and crop yield.	Sukartono *et al.* (2011)

Table 2.2 continued

Study	Location	Design	Duration	Main findings	Authors
Comparison of kiln-derived and gasifier-derived biochars as soil amendments in the humid tropics	Uganda	Greenhouse experiment	Short-term study	Amending soil with gasifier produced biochar had higher yields then kiln produced biochar. Soluble ash content of biochar also influenced productivity of acid soil in Uganda.	Deal *et al.* (2012)
Effect of rice husk (RH) biochar on growth and quality of kang kung (*Ipomeareptans*)	Malaysia	Field experiment	Short-term study	RH biochar enable plants to be harvested earlier and the yield was increased by 68-89%.	Hui Ling *et al.* (2012)
Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil	Spain	Laboratory experiment	Short-term study	Soil amended biochar, reduced metal plant availability, increased available water and field capacity of soil.	Méndez *et al.* (2012)

Table 2.2 continued

Study	Location	Design	Duration	Main findings	Authors
Biochar as a soil amendment to improve crop yield, soil health and carbon sequestration for climate change mitigation	Malaysia	Field experiment	Short-term study	Application of RH and empty oil palm fruit bunch (EFB) biochars showed significant improvement of dry matter weight of sweet corn and soil pH, total carbon and CEC.	Rosenani *et al.* (2012a)
Effect of oil palm empty fruit bunch biochar soil amendment on nutrient leaching and plant growth of sweet corn (*Zea mays*)	Malaysia	Field experiment	Short-term study	EFB biochar reduced nutrient leaching and improved soil quality but had no effect on crop performance.	Rosenani *et al.* (2012b)
Capacity of biochar application to maintain energy crop productivity: soil chemistry, Sorghum Growth and runoff water quality effects	-	Greenhouse experiment	Short-term study	After two rain events, excessive runoff resulted in 20% loss of total phosphorus and K in top dressed biochar.	Schnell *et al.* (2012)

Table 2.2 continued

Study	Location	Design	Duration	Main findings	Authors
Assessing the chemical and biological accessibility of the herbicide isoproturon in soil amended biochar	United Kingdom	Laboratory experiment	Short-term study	Desorption process in biochar was related to pesticide biodegradation	Sopena *et al.* (2012)
Effect of biochar on growth development of three *LabisiapumilaBenth.* Varieties	Malaysia	Glasshouse experiment	Short-term study	EFB biochar amendment showed positive crop performance when applied at 20t ha^{-1} but had negative effect when applied at 60t ha^{-1}.	Siti Norayu *et al.* (2012)
Transport of biochar particles in saturated granular media: effects of pyrolysis temperature and particle size	-	Laboratory experiment	Short-term study	Biochar produced at high temperature increased C sequestration and had lower mobility in sandy soil. Smaller particle sizes of biochar had a greater mobility in the soil.	Wang *et al.* (2013)
Carbon mineralization in two ultisols amended with different sources and particle sizes of pyrolyzed biochar	United States	Laboratory experiment	Short-term study	Larger size of biochar had lower C mineralization than the smaller particle sizes	Sigua *et al.* (2014)

Table 2.2 continued

Study	Location	Design	Duration	Main findings	Authors
Effect of biochar on soil microbial biomass after four years of consecutive application in the North China plain	China	Field experiment	4 years	Long-term application of biochar increased microbial biomass carbon in comparison with control treatment.	Zhang *et al.* (2014b)
Impacts of biochar and processed poultry manure, applied to a calcerous soil, on the growth of bean and maize	Turkey	Greenhouse experiment	Short-term	Biochar decreased soil pH, but increased plant nutrients in the soil, as well as plants growth.	Inal *et al.* (2015)
Why short-term biochar application has no yield benefits: evidence from three field-grown crops	United Kingdom	Field experiment	Short-term	Biochar increased pH and nutrients in the soil, but had no significant effects on the crops due to high fertility of soils used in their study.	Jay *et al.* (2015)
Effects of biochar application in greenhouse gas emissions, carbon sequestration and crop growth in coastal saline soil	China	Microcosm experiment	Short-term	Biochar addition, decreased greenhouse gas emissions, enhanced soil quality, increased crop yield and carbon storage in the soil	Lin *et al.* (2015)

This chapter has illustrated overall effects of biochar on soil properties and its use in agricultural purposes, but highlights the need for further investigations into the effects of biochar on soil including the physical aspects of soils (water retention and aggregate stability), chemical (pH, cation exchange capacity, carbon and nutrients leaching) and biological (microbial activity and biomass) in the humid tropics and the cold regions. The key studies on biochar in temperate and tropical climates are summarised in Table 2.2.

CHAPTER 3

Materials and Methods

3.1 Introduction

In this study, the series of experiments is divided into two phases. In the first phase, the soils and biochars used were from Malaysia (tropical climate), whereas in the second phase the soils and biochar were from the United Kingdom (temperate climate).

3.2 Materials

3.2.1 Tropical biochar

For the tropical climate, two types of biochars (rice husk and coconut shell) were used in this study. Both biochars have a high content of carbon and nutrients. The production methods used for both biochars are different. The rice husk (RH) biochar was produced by a rotary husk furnace (RHF) in Tanjung Karang, Selangor Malaysia. This method of production resulted in a higher biochar yield at (900^{O}C to 1000^{O}C) for only a few minutes.

Coconut shell (CS) biochar production used the slow pyrolysis technique involving drums. The method is also used by farmers in Hilir Perak, Malaysia. The biochar was obtained from the local farmer in Hilir Perak. The production of biochar involves burning coconut shell in a drum for approximately 6 to 8 hours. During the burning process, the temperature in the area immediately surrounding the drum can reach to

400^{O}C. Both biochars were then crushed and sieved using 2mm mesh. The properties of both biochars used in this study are shown in Table 3.1.

3.2.2 Temperate biochar

The temperate biochar (Bodfari Environmental, St. Asaph, UK) used in this experiment was hardwood biochar (HW). This type of biochar has a very high content of carbon. The production method used to produce is by slow pyrolysis (24 hours) in a ring-kiln at 400^{O}C. Some properties of this biochar are illustrated in Table 3.2.

3.2.3 Tropical soils

Three tropical soils (Spodosols) which are acidic, with a pH < 5.5, and a high accumulation of Al and Fe in the subsoils were used in the first phase of experiments. The soils were selected based on different gradients of degradation: secondary forest soil; non-intensive farming soil; and intensive farming soil. For several years, logging activity has occurred in a secondary forest. The non-intensive farming soil consists of the soil which has been cleared for agricultural purpose only once, whereas the intensive farming soil was cultivated with various crops under rain shelter for several years. The sampling locations for all soils are at latitudes 4.47, 4.16, 4.96; longitudes 101.39, 101.37 and 101.34 respectively.

All the soils were sampled from the Cameron Highlands. Cameron Highlands is located in Pahang District, which is in central Peninsular Malaysia (Figure 3.1). Generally, it is a mountainous area with 10 – 35^{O} slopes at an altitude of 1070 –

1830m above sea level. The Cameron Highlands has mild temperatures ranging between 14 to 24^{O}C throughout the year with 2660mm average annual rainfall (Abdullah *et al.*, 2001). The Cameron Highlands is suitable for agricultural farming and is one of the major areas for intensive vegetable cultivation in Malaysia.

A composite soil was collected from 5 random samples within a 27.5m^{2} at each sampling location. The soils were sampled with an auger at 10 – 15cm depth. The soils were kept at field moist in a cold room at 9^{O}C before being shipped to the United Kingdom for analyses. In the laboratory, all of the samples were sieved through a 5mm mesh. The samples were mixed with and without 2% of CS and RH biochars by weight. The particles size of biochars used in this study was 2mm. The non-mixture soils acted as a control treatment. Subsequently, the mixture and non-mixture soils were incubated for 0, 60, 120, 240 and 360 days at a constant temperature (21^{O}C) with 45% moisture content, close to the original temperature of the environment from which the soils were collected. The samples were then kept in amber jars. Prior to analysis the soils were dried and sieved again using a 2mm mesh to provide soil aggregates suitable for the soil analyses (Kandeler, 2007). The physical and chemical properties of the soils are demonstrated in Table 3.1.

Figure 3.1 Soil sampling location (Pahang State, 2015).

Figure 3.2 Tropical soils study in a) Forest b) Non-intensive farming and c) Intensive farming soils

Table 3.1 Physical and chemical properties of soils and biochars used in Malaysia

Soils and biochars	Forest Soil	Intensive Farming Soil	Non – Intensive Farming Soil	CS Biochar	RH Biochar
% Clay	28.63	35.06	33.89	-	-
% Silt	11.01	18.09	18.08	-	-
% Sand	60.36	46.86	48.03	-	-
Texture	Sandy Clay Loam	Sandy Clay	Sandy Clay	-	-
% Carbon	3.42	1.64	1.08	72.95	38.64
% Nitrogen	0.18	0.22	0.07	0.53	0.53
C/N Ratio	19.05	7.38	14.99	139.71	72.59
CEC (meq 100 g^{-1})	9.7	9.4	5.8	31.17	43.28
pH	4.62	5.03	5.52	8.33	8.46
Inorganic P (mg g^{-1})	0.06	1.85	0.2	0.39	1.75

3.2.4 Temperate soils

There are two parts of studies using the temperate soils. For the part one study, three Brown Earth soils (grassland, arable loam and arable sandy) from Dundee, United Kingdom were used. The soils were chosen due to an aged soil that has been amended with biochar approximately 10 months by a former PhD student. All the soils were collected from the pots using a 15cm cylindrical core. A composite soil was collected from 4 random samples in each pot. In the laboratory, the samples were mixed with and without 2% of fresh HW biochar by weight. The particles size of biochar used in this study was < 5mm. The non-mixture soils acted as a control treatment. Subsequently, the mixture and non-mixture soils; and an aged soils amended with biochar were incubated for 0, 60, 180, and 300 days. The samples were then kept in containers. Prior to analysis the soils were dried and sieved using a 2mm to provide soil aggregates suitable for the soil analyses (Kandeler, 2007). Detailed characteristics of soils were determined and are presented in Table 3.2.

Figure 3.3 The soils for the temperate study (part 1) were collected from the pots in Edinburgh

Table 3.2 Physical and chemical properties of soils and biochars used in the UK.

Soils and Biochar	**Grassland**	**Arable Loam**	**Arable Sandy**	**HW Biochar**
% Clay	34.90	34.41	27.54	-
% Silt	17.44	16.06	6.36	-
% Sand	47.67	49.53	66.10	-
Texture	Sandy Clay	Sandy Clay	Sandy Clay Loam	-
% Carbon	2.39	3.79	2.06	71.38
% Nitrogen	0.14	0.21	0.16	0.45
C/N Ratio	16.45	17.75	12.68	158.68
CEC (meq 100 g^{-1})	13.49	13.86	9.24	34.36
pH	6.08	6.53	5.81	9.05
Inorganic P (mg g^{-1})	1.15	1.57	1.15	0.41

For the part two temperate study, two Brown Earth soils from Penrith, Cumbria were used which were chosen to represent different levels of management. The first soil was from an area of agriculture that contains an oil seed rape crop, which was well managed (fertilized) and fertile. The second soil used in this study was taken from extensive grassland, which was unmanaged, unfertilized (for at least 50 years) and is

also known as nutrient poor soil. Both soils had a same texture which were sandy clay loam.

The soils were collected from the field at approximately 10 – 15cm depth. In the laboratory, the soils were sieved through a 5mm mesh, and mixed with 2% and 5% of HW biochar by weight. Biochar particle sizes used were 2mm, 1mm, 0.5mm and 0.1mm. The soils without biochar addition acted as a control. All samples were kept in jars and were incubated for 30 days. Finally, prior to analysis, the soils were dried and sieved using a 2mm mesh to provide soil aggregates suitable for the soil analyses (Kandeler, 2007). The physical and chemical properties of the soils and biochars used are shown in Table 3.3 below.

Table 3.3 Physical and chemical properties of two soils and biochar used in the UK (part 2).

Soils	**Fertilized**	**Unfertilized**	**HW Biochar**
Clay	28.29	28.12	-
% Silt	11.96	9.00	-
% Sand	59.75	62.88	-
Texture	Sandy Clay Loam	Sandy Clay Loam	-
% Carbon	2.14	3.40	71.38
% Nitrogen	0.19	0.19	0.45
C/N Ratio	11.26	17.89	158.68
pH	6.16	6.15	9.05

Figure 3.4 The types of temperate soils study (part 2) a) Fertilized and b) Unfertilized soils were collected in Penrith.

3.3 Methods

The incubation study and leaching experiment were conducted separately. The incubation study consists of soil only (control) and a mixture of soils and biochar. These were incubated in sealed jars for up to 360 days for the tropical study and for up to 300 days for temperate part 1, as well as for up to 30 days in temperate part 2 study. In contrast, the leaching experiment was carried out separately in glass columns (tropical and temperate part 2 studies) and PVC columns (temperate part 1 study) containing soils and biochar mixture and control (soil only).

The moisture levels in the sealed jars were determined in the beginning of every incubation time (days 0, 60, 120, 240 and 360 – tropical study); (days 0, 60, 180 and 300 – temperate part 1) and (days 0 and 30 – temperate part 2). In the leaching experiment, all of the treatment columns (soils and biochar mixture, as well as soil only) were subjected to 5 wetted-dried cycles over 360 days (tropical study), 4 wetted-dried cycles over 300 days (temperate part 1 study) and 2 wetted-dried cycles over 30 days (temperate part two study).

3.3.1 Soil moisture content

2g of moist soil was added into a pre-weighed crucible. The sample was placed in an oven at 105^{O}C. After 24 hours, the sample was removed and allowed to cool in a desiccator before re-weighed. The moisture content was then determined using the following calculation (Gardner and Klute, 1986):

$$\%\ \text{moisture content} = \frac{(\text{wet weight} - \text{dry weight})}{\text{dry weight}}\ \text{x}\ 100\ \%$$

3.3.2 Particle size analysis

Elimination of organic matter in soil sample was needed before particle size analysis was carried out. To remove organic matter in the soil firstly, the soil was dried and sieved through a 2mm mesh. The soil was weighed 20g and in the fume cupboard, the sample was placed in a 1000ml beaker. Subsequently, 50ml of hydrogen peroxide was added. The sample was stirred and hydrogen peroxide added until any reaction ceased. The beaker was stand overnight at room temperature to enable oxidation to take place, before being placed on the hot plate to complete the process. The sample

was then heated at 70^{O}C and hydrogen peroxide was added until frothing ceased. To decompose any excess hydrogen peroxide, the sample was heated to more than 70^{O}C.

The suspension was transferred to a mixer with 100ml of Calgon solution (10%) and 100ml of distilled water. The mixture was blended at low speed for 30 seconds. Subsequently, the suspension was transferred to a 1 litre measuring cylinder and marked up to volume with water. The cylinder was left to reach equilibrium at room temperature. The suspension was then stirred thoroughly with a plunger; at the end of the last stroke, timing is begun and a hydrometer was gently lowered into the suspension. A density reading was taken in g/cm^3 at 32 seconds. The timing or density reading was repeated after 8 hours for all cylinders without stirring.

After the last hydrometer reading, the supernatant clay suspension was carefully poured off and again the soil sludge was marked up to 1 litre volume with water. The soil sludge was stirred and allowed to settle for 32 seconds. The suspension was then decanted off. The process was repeated three times to remove all the silt and clay particles, leaving the sand in the cylinder. Then, the sand was transferred into a pre-weighed 200ml beaker, oven dried (105^{O}C for two days) and weighed. From this the soil fractions were calculated according to Klute (1986) as follows:

Silt: Density reading at 32 seconds (g/cm^3) – density reading at 8 hours (g/cm^3)

Clay: Density reading at 8 hours (g/cm^3)

Sand: Weighed

The soil fractions were then converted to a soil texture using a soil triangle that was generated by the Soil Survey of England and Wales classification.

3.3.3 Aggregate stability

Several methods have been used to determine aggregate stability. Pojasok and Kay (1990) used wet sieving, whereas (Low (1967); Young (1984); Farres (1987)) and (Loch (1994)) used raindrops and rainfall treatments. Furthermore, Emerson (1967) applied immersion treatment, and, Kemper and Chepil (1965) practised the dry sieving method. All the methods from these authors only measured one mechanism of aggregate breakdown.

An aggregate stability test should be easy to conduct and the results from the test should represent multiple stressors that affect the surface soil and also be relevant for various types of soils. Therefore, in this study the aggregate stability test using the Le Bissonnais method was chosen because it considers three main mechanisms of aggregate breakdown: fast wetting (FW) due to slaking breakdown by compression of trapped air; slow wetting (SW) due to breakdown by differential swelling (microcracking); and mechanical breakdown (M) due to raindrop impact (Bissonnais, 1996).

Before running the experiment, the air-dried soil was sieved through 2mm and 5mm mesh sieves (5mm mesh sieve was placed on top of 2mm mesh sieve) and 4g of 2 – 5mm aggregates were selected for the tests. The samples were placed in the oven at

$40^{O}C$ overnight to create constant moisture. The FW treatment, immerses samples in water to examine the stability of the soils. It mimics a fast wetting condition, such as intense rainfall. The SW treatment is equivalent to wetting under gentle rain. However, for the M method, the aggregates must be shaken after pre-wetting, representing the effect of raindrop impact (Bissonnais, 1996).

For the FW treatment, aggregates were immersed in deionised water for 10 minutes. The water was then sucked off with a pipette and the sample was transferred to a 50µm sieve, which was previously immersed in ethanol. For the SW method, aggregates were put on a filter paper on a tension table with -3 kPa water suction for 30 minutes. Aggregates were then transferred to a 50µm sieve, which was previously immersed in ethanol. For the M method, the aggregates were immersed with $50cm^3$ ethanol for 10 minutes. The ethanol was removed with a pipette and the samples were transferred to an Erlenmeyer flask containing $50cm^3$ of deionised water; the water was then marked up to $200cm^3$. The flask was screwed and agitated end over end 20 times. After 30 minutes, the water in the flask was removed with a pipette and finally the samples were also transferred to a 50µm sieve which was previously immersed in ethanol.

All of the samples from the three methods were then wet sieved by immersing them in ethanol and moved up and down 8 times to separate fragments < 50µm from those > 50µm. Ethanol was used to avoid further breakdown of aggregates. In the second stage, the aggregates remained on the > 50µm sieve were oven dried at $105^{O}C$ overnight. The samples were then gently dry sieved by hand on a column of six sieves

which are 2000, 1000, 500, 200, 100 and 50µm. The results from each breakdown mechanism were quantified by calculating the mean weight diameter (MWD) which is the sum of the mass fraction of soil remaining on each sieve (the set of 6 sieves) and multiplied by the mean aperture of the adjacent mesh (Bissonnais, 1996).

3.3.4 Water retention

Adding biochar to soil can improve water retention. Due to its high surface area, it is hypothesized that biochar can retain water and indirectly may increase soil water retention properties. To test that hypothesis, the biochars were mixed with soils and their water retention properties were measured using pressure plate equipment. The control and biochar treatments were tested at 0.3, 1, 2, 3 and 4 bars pressure.

The soil samples were put in small soil cores, approximately 4cm diameter and 1cm height in triplicate. The samples were saturated overnight on a pre-saturated plate until a thin film of water could be seen on the surface of the samples. Before running the pressure plate, the bottom of the vessel was covered with water to create a saturated atmosphere. To apply the pressure, water from the porous plate was removed using a syringe and the outflow tube was connected with the plate. Then, the vessel was closed and the pressure was applied.

All samples were then allowed to reach its equilibrium state when no outflow occurs. When that happened, the air pressure was released and all the samples were removed and weighed immediately. The samples again were then applied at different pressures

by removing and reweighing the core at equilibrium, re-inserting it and re-setting the pressure. The ceramic plate was moistened with a fine spray each time before applying the new pressure to re-establish hydraulic contact. When the last equilibrium took place, all samples were oven-dried at 105^{O}C and weighed (Wilke, 2005).

The volumetric water content was calculated using this formula (ISO11274, 1998):

$$\theta(\rho m) = \frac{m(\rho m) - md}{\rho w \text{ X V}}$$

Where,

$\theta(pm)$ = Water content at matric pressure ρm, expressed as volume fraction

m(pm) = mass of wet soil in grams

md = mass of the oven dried soil in grams

ρw = density of water, in grams per cubic centimetre

V = Volume of the core, in cubic centimetre

3.3.5 Carbon and nitrogen in soil

Soil samples were dried in the constant room temperature at 21^{O}C. After two to three days, the soil samples were ground using a pestle and mortar. Sub-samples were used to determine total C and N using an Elementar Vario EL elemental analyser. Samples of approximately 30mg were weighed into tin cups, which were subsequently loaded into an auto-sampler, which dropped the sample into a combustion column maintained at 950^{O}C. The sample and cup were flash combusted in a temporarily enriched

atmosphere of oxygen. The combustion products were carried by a carrier gas, (helium), and passed over an oxidation catalyst of copper oxide kept at $950^{O}C$ inside the combustion column.

The combustion products such as CO_2, CO, N, NO and water passed through a reduction reactor in which hot metallic copper at a temperature of $550^{O}C$ that removed excess oxygen and reduced N oxides to N_2. These gases, together with CO_2 and water, were next passed through sicapent to remove water then through a chromatographic column to a thermal conductivity detector. The detector generated an electrical signal proportional to the concentration of N or C present. This signal was graphed on a built in recorder and ported to a computer, which integrated the area under each curve and converted it to concentrations after each sample was run. The results were given in percentages.

3.3.6 Phosphorus in soil

Using the method of Allen (1989) total phosphorus was measured using ground soil. A digest reagent was prepared by adding 350ml of hydrogen peroxide to a plastic beaker (2L) containing 0.42g of selenium and 14g of lithium sulphate. Concentrated sulphuric acid (420ml) was then added with care in a fume cupboard. The mixture was stirred and then allowed to cool. Samples (0.2g) of the soil were weighed into digest tubes and 4.4ml of the digest reagent was added. A few anti-bumping granules were added to minimize the reaction of the digest reagent during heating.

The samples and digest reagent were gently heated in a heating block until the initial vigorous reaction had subsided, then the temperature was increased to 350^{O}C in approximately 100^{O}C increments and kept at 350^{O}C until the digest had cleared. This step took approximately two hours. The digests were then allowed to heat for a further 30 minutes after becoming clear then left to cool. The digests were filtered through Whatman number 44 filter papers into 100ml volumetric flasks, making up the volumes to 100ml with deionised water. The resultant samples were diluted four times with deionised water prior to analysis to give an acid content of 1% v/v (Allen, 1989). Each digest sample was then analysed for orthophosphate which was determined colourimetrically after formation of the molybdenum blue complex measured at 660nm in a reaction using a blend of acid-with antimony potassium tartrate used as a catalyst in a continuous flow stream using a Bran + Luebbe autoanalyser 3.

3.3.7 Leaching experiment

For tropical experiment, the experiment used two types of biochar (CS and RH) and three soils (forest, intensive farming and non-intensive farming). Meanwhile for temperate study, the experiment was divided into two parts. The biochar used at the first part of experiment was an aged HW biochar which was incorporated approximately 10 months in three soils (grassland, arable loam and arable sandy) and freshly added HW biochar was also amended in the same soils. The second part of temperate study was also used the same biochar with different particle sizes (2mm, 1mm, 0.5mm and 0.1mm) and was applied at different rates of biochars (2% and 5%). Two different soils were used in part two study (fertilized and unfertilized soils). For each set of experiment, all of the samples were in triplicate making a total of 27

leaching soil columns. The only exception was in temperate experiment for part 2, which was conducted in duplicate due to insufficient tools (soil columns). Soils with and without biochar with approximate 1.2 g cm^{-3} of bulk density were packed into a glass soil column (tropical and part 2 temperate experiments) and a PVC column (for part 1 temperate experiment) of 5cm diameter and 20cm long. For the tropical and part 2 temperate leaching experiments, a layer of glass wool was inserted at the bottom of the funnel to prevent blocking with soil particles. Another layer of glass wool was placed on the soil surface to reduce the impact of water drops during the leaching process.

For the temperate leaching experiment (part 1), 40g of sand was placed at the bottom of PVC column to trap the clay particles from loss during the leaching process. At the end of each column, two layers of nylon mesh were lined and secured with a cable ties. The leaching was started by pouring 100ml of deionised water slowly in the glass and the PVC columns. The leachate was collected in an Erlenmeyer flask and then kept in the fridge $4^{O}C$ prior to analysis for two to three days. Ammonia, nitrate and phosphate from the leachates were then determined using a Bran + Luebbe Autoanalyzer 3.

3.3.8 Phosphorus in soil leachates

The concentration of phosphorus in soil leachate was determined using a Bran + Luebbe Autoanalyser 3 (Revision2, 2000) in a continuous flow stream as described previously.

3.3.9 Ammonium and nitrate in soil leachates

The concentration of NH_4^+ and NO_3^- / NO_2^- in the soil leachates, blank samples and the standard samples were determined in a continuous flow stream using a Bran + Luebbe Autoanalyser 3 (Revision1, 1999; Revision3, 2000). The concentration of NH_4^+ in the leachates was calculated after reaction with salicylate and dichloroisocyanuric acid to form a blue compound with nitroprusside as a catalyst and measured at 660 nm (ISO11732, 1997).

The concentration of N was determined after the reduction of NO_3^- to NO_2^- by hydrazine in alkaline solution, followed by the reaction with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride to form a pink compound measured at 550 nm with copper used as a catalyst (ISO13395, 1996).

3.3.10 pH

To measure the soil pH, 25ml of deionised water from a measuring cylinder was added to 10gm of air-dried and sieved soil into a 50ml polyethylene beaker. The contents were stirred every so often and allowed to stand for an hour. The pH values were read with a lab pH meter model PHM 220 calibrated using buffers pH 7.0 and 4.0 (Radojevic and Baskin, 1999).

3.3.11 pH in soil leachates

The pH of soil leachates was determined using a lab pH meter model PHM 220 calibrated using buffers pH 7.0 and 4.0. The pH probe was placed in the soil leachate directly then the reading of pH was recorded.

3.3.12 Cation Exchange Capacity (CEC)

Cation exchange capacity (CEC) is a quantitative technique to determine the ability of soil to hold cations, for instance Na, Ca, Mg and K. It is known as the mass of exchangeable cation sites per unit weight of dry soil (Miller *et al.*, 1998) and can also be used to determine the fertility of soil. To carry out this measurement, the soil is saturated with a suitable cation and in this study cation such as Na in the form of sodium acetate is used. When the soil is saturated with Na, it is assumed that all the exchange sites of soil will be occupied by the Na which can be later leached off, analysed and used to calculate CEC. However, there is residual Na which is unattached that needs to be washed by leaching using methylated spirit. Therefore, leaving only attached Na for subsequent removal and quantification purpose. Ammonium acetate is then used to flush off the attached Na (Miller et al., 1998). Total Na is then measured by using flame photometry technique.

To determine the CEC, 4g of dried soil were transferred in a centrifuge tube and then saturated with 30ml of sodium acetate at pH 8.2. The sample was shaken with overend shaker for 5 minutes and centrifuged at 4600 rpm. After 5 minutes centrifuged, the supernatant was filtered and the filter paper containing the sample (biochar and soil)

were filled back in the centrifuge tube to avoid the loss of biochar due to its hydrophobicity characteristic. The residue is then discarded. The sample was then added with 30ml of 30% methylated spirit (IMS) to the residue in the centrifuge tube, shaked, centrifuged and filtered. The residue of IMS was also discarded into the solvent waste bottle provided in the fume cupboard. Subsequently, the sample was added with 30ml of ammonium acetate at pH 7. The sample again was shaken, centrifuged and filtered. The supernatant was collected into a 100ml flask. All of the steps that are mentioned above were repeated twice. At the last step in this method, the sample (biochar and soil) that was left at the filter paper, was rinsed using a small amount of 1 M ammonium acetate**.** Finally, the flasks contents were marked up with 1 M ammonium acetate up to 100 ml and the solutions used to determine the CEC using flame photometry (Chapman, 1965).

3.3.13 Substrate induce respiration

The substrate induced respiration (SIR) technique measures microbial primary respiration after adding a substrate. Indirectly, this method can also be used to evaluate the amount of carbon (C) content in living microorganisms bodies (Anderson and Domsch, 1978). In this case, glucose is added to airtight containers which contain soil sample. CO_2 emission is monitored for several hours and the initial respiration by the microbes is the relative amount of microbial C from the soil samples (Kandeler, 2007). The respired CO_2 is then measured by using an alkali trap and this is followed by adding a liquid scintillation cocktail which is for counting purposes using a liquid scintillation counting machine (Hamer and Marschner, 2002; Kandeler, 2007).

In this study, 20g of soil sample and 10ml glucose solution (3 mM) were added to a respiratory bottle which consisted of a 250 ml Schott bottle with a Teflon-lined screw lid. The centre of the lid was drilled, and a stainless steel studding was inserted to connect a crocodile clip. The clip was used to hold an open 7ml glass scintillation vial containing 1 M Sodium Hydroxide (1ml). The vial position was in the middle of the respiratory bottle and above the soil slurry (see Figure 3.5). Any $^{14}CO_2$ evolved through microbial catabolism is determined in the sodium hydroxide trap. After spiking the soil with ^{14}C glucose, the samples were shaken on an orbital shaker at 100 rpm. Then, the mineralization rate was measured every hour for four hours and every two hours for another four hours during a total of 24 hours. The mineralization rate was also measured once on a daily basis for up to 5 days. During the sampling, the vial containing sodium hydroxide was removed and wiped using acetone which was wetted on to blue roll tissue. This aimed at removing any ^{14}C activity. 5ml of liquid scintillant cocktail were then added into the vial and subsequently, the vial was incubated in a dark cupboard overnight before measuring the ^{14}C activity using liquid scintillation counting, model Canberra Packard Tri-Carb 2250A liquid scintillation analyser.

Figure 3.5 Respirometry bottle containing soil slurry

3.3.14 Fumigation and non-fumigation extraction

In principle, soils are fumigated with chloroform, incubated for 24 hours, and extracted. To correct for non-biomass organic matter of soil, the non-fumigated soil is also extracted using 0.5 M K_2SO_4. During the fumigation process, there is an increase in the amount and variety of organic and inorganic components variety that is lysed from the cells of soil microorganisms (Powlson and Jenkinson, 1976). A large part of the soil microbial biomass can be extracted from fumigated soil after 24 hours (incubation period to allow autolysis process).

To carry out these experiments, 4g of soil slurry from the 250ml Schott bottle was weighed and added in a small 10ml beaker for fumigation extraction and another 4g in a plastic tube for non-fumigation extraction. The soil slurry was took at the last day sampling of the substrate induces respiration experiment. The non-fumigation sample

was extracted with 20ml of 0.5 M K_2SO_4. The sample was then shaked with an orbital shaker for 30 minutes (100 rpm). The supernatant was filtered and 5ml of supernatant was then added into a 20ml vial. 15ml of liquid scintillant cocktail was also added into the vial and the sample were kept in the cupboard overnight before counting using liquid scintillation counting model Canberra Packard Tri-Carb 2250A liquid scintillation analyser. At the same time, the other sample was fumigated in a desiccator lined with a wet blue roll. 75ml ethanol-free chloroform ($CHCl_3$) was placed at the centre of the desiccator. A few anti-bumping granules were then added into the $CHCl_3$. The desiccator was evacuated until the $CHCl_3$ has boiled vigorously for 2 minutes. After 24 hours, the residual $CHCl_3$ vapour was removed by repeated five or six fold evacuation. Then, the soil was extracted and counting similar as non-fumigation extraction method.

3.3.15 Sample oxidation

The amounts of ^{14}C remaining in the soil treatments were determined through dry combustion of approximately 1g of dry soil plus 200µl combust aid at three minutes on a Sample Oxidiser (Packard, Model 307). Prior to analysis approximately 10ml of Permafluor-E was acted as a liquid scintillation cocktail and 10ml of Carbosorb-E was used to trap evolved $^{14}CO_2$ during combustion (trapping efficiency more than 90%) (Towell *et al.*, 2011). The samples were then, kept in the dark cupboard for at least 12 hours to reduce the effects of chemi-luminescence. Finally, solution which containing ^{14}C activity was determined by liquid scintillation counting model LSC, Canberra Packard Tri-Carb 2250A with standard calibration and quench correction techniques.

3.3.16 Statistical Analysis

The mean values of maximum rate, ^{14}C mineralization, ^{14}C biomass, total carbon, total nitrogen, phosphate and nutrients concentration in the leachate between the treatments, sorted by incubation day, were tested using a one-way analysis of variance (ANOVA) with a $P<0.05$ level of significance. The soil water retention was tested at each matric potential between the treatments in a similar way. Multiple mean comparisons were carried out using a Holm-Sidek procedure at $P<0.05$. For values that were not normally distributed, a non-parametric statistical test (Kruskal-Wallis), which is based on ranks, was used. In addition, the Tukey test was applied to determine the significant differences between the treatments for non-distributed values at the $P<0.05$ level. A two-way analysis of variance (ANOVA) was performed to test the significant difference for all of the parameters over time and between the soil treatments. All of the statistical tests were performed using the SigmaStat v3.5 (Systat Software Inc), except for two-way analysis of variance (ANOVA), which was performed using Microsoft Excel.

CHAPTER 4

Impact of Biochar Amendment of Selected Tropical Soils

4.1 Introduction

Malaysia is a tropical South East Asian country with high temperature, rainfall, as well as high humidity. With this type of climate, the soils are infertile and have very low pH and CEC (Ishak and Jusop, 2010). The nutrients that have been applied are often lost through surface runoff and leaching to the groundwater, due to high rainfall events and intensity. As a result, a large amount of fertilizer is applied to improve the fertility of the soils in order to obtain a high crop yield. In addition, high temperature and humidity associated to this climate create a favourable condition for microbes to mineralize organic matter rapidly, thus resulting in inadequate amount of organic C in the soil (Peng *et al.*, 2011). To overcome this problem, biochar has been suggested as one way to improve the quality of the soils. Biochars have a liming effect that can increase soil pH and ultimately increase CEC (Masulili *et al.*, 2010; Sukartono *et al.*, 2011). With a high CEC, biochar can prevent nutrients from leaching, consequently reducing the surface and river water pollution. Moreover, the recalcitrance of biochar C resulting from pyrolysis helps biochar resist degradation by microbes (Sukartono *et al.*, 2011). Biochar can sequester C in the soils and has the potential to increase the productivity of typical infertile Malaysian soils. In this study, it was hypothesized that adding biochars to tropical soils could enhance microbial activity, reduce nutrients leaching, and improve the physical and chemical properties of the soil. The objectives of this chapter are:

1. To measure the microbial activity and microbial biomass in the soil with biochar amendments
2. To quantify the impact of biochar amendments on nutrient leaching from soil
3. To investigate the effects of biochars on the soil's chemical properties (C, N, P, CEC and pH) and physical properties (aggregate stability).

4.2 Materials and Methods

Three Spodosols soils with different gradients of degradation (forest, non-intensive farming and intensive farming soils) from Cameron Highlands, Malaysia were used in this study. Spodosols soils are acidic (pH < 5.5) and contain a high accumulation of Al and Fe in subsoils. The biochar types used in this study were coconut shell (CS) biochar and rice husk (RH) biochar. The former is produced by slow pyrolysis, while the latter was produced by fast pyrolysis. Details of the materials (soils and biochars) used in this study can be found in the previous chapter (Section 3.2.1 and Section 3.2.3).

The experiments are divided into three parts, which are biological, chemical and physical aspects of soils. Details for all the methodology can be found in Sections 3.3.1-15. For the biological properties, the methodology employed to carry out the experiments were substrate induced respiration, where 3 mM of glucose solution (10ml) was added into soil samples, which had a radioactivity of 733 Bq on days 0, 60 and 120 (incubation time); and 1086 Bq on days 240 and 360 (incubation time). For fumigation and non-fumigation extraction 0.5 M potassium sulphate was used. C-14

glucose associated activity remaining in soil was determined via combustion (3 minutes) on a sample oxidiser (Packard, Model 307).

For the chemical properties, the total carbon (C) and nitrogen (N) were determined by dry combustion and measured with an elemental analyser (Elementar Vario EL), phosphorus digestion with hydrochloric acid and hydrogen peroxide; the concentration of P in the soil was then measured with Bran + Luebbe autoanalyser 3, as well as phosphate, ammonium and nitrate concentrations in the soil leachate. pH was measured using a pH meter model PHM 220 calibrated using buffers pH 7.0 and 4.0 and CEC was determined using 1 M ammonium acetate. The measurement of Na attached to soils was obtained by using flame photometry. For the physical characteristics, soil moisture content was determined through oven drying at $105^{O}C$ for 24h and, particle size analysis determined by the hydrometer method and aggregate stability using the Le Bissonnais method (Bissonnais, 1996).

For the statistical analyses, the mean values of maximum rate, ^{14}C mineralization, ^{14}C biomass, total carbon, total nitrogen, phosphate and nutrients concentration in the leachate between the treatments, sorted by incubation day, were tested using a one-way analysis of variance (ANOVA) with a $P<0.05$ level of significance. Multiple mean comparisons were carried out using a Holm-Sidek procedure at $P<0.05$. For values that were not normally distributed, a non-parametric statistical test (Kruskal-Wallis), which is based on ranks, was used. In addition, the Tukey test was applied to determine the significant differences between the treatments for non-distributed values at the $P<0.05$ level. A two-way analysis of variance (ANOVA) was performed to test the significant difference for all of the parameters over time. All of the statistical tests

were performed using the SigmaStat v3.5 (Systat Software Inc), except for the two-way analysis of variance (ANOVA), which was carried out in Microsoft Excel.

4.3 Results and discussion

4.3.1 Mineralization of ^{14}C glucose to $^{14}CO_2$ and uptake of ^{14}C glucose into microbial biomass

The extent of mineralization of ^{14}C glucose in three different soils were not constant over time. Overall, the mineralization of ^{14}C glucose was highest ($P<0.05$) at the end of incubation time in all soils and treatments (Tables 4.1 to 4.3). Further, adding CS biochar and RH biochar to the soils also did not lead to much significant change during the period of the study. Only after 120 d was the mineralization in RH biochar amended soils in forest soil (62.22 ± 6.40) significantly higher ($P<0.05$) than in the CS biochar amended soils (45.01 ± 6.05) (Table 4.1). The maximum rates in forest soils amended with RH biochar were significantly higher ($P<0.05$) than the CS and control treatments at all times (days 0 to 360) (Table 4.1). On the other hand, there were no significant difference of the maximum rates between the treatments in the other two soils (non-intensively farmed and intensively-farmed) at any time ($P>0.05$) (Tables 4.2 and 4.3). Generally, the incorporation of ^{14}C glucose into the microbial biomass showed no consistent pattern (Tables 4.1 to 4.3).

Table 4.1 Maximum rate (% h^{-1}), ^{14}C extent mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for forest soil, over 360 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extent mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	1.79 ± 0.11	29.02 ± 2.69	14.19 ± 1.03	56.78 ± 3.66
	60	2.15 ± 0.19	49.41 ± 5.49	24.77 ± 3.61	25.81 ± 7.41
	120	2.03 ± 0.25	37.10 ± 2.11	ND	ND
	240	1.02 ± 0.08	48.55 ± 1.73	27.59 ± 6.22	23.86 ± 7.83
	360	1.03 ± 0.30	**59.37 ± 12.23**	21.73 ± 2.31	18.90 ± 10.14
CS	0	2.06 ± 0.19	29.40 ± 1.44	14.53 ± 2.32	56.07 ± 2.04
Biochar	60	2.23 ± 0.13	44.71 ± 3.84	24.33 ± 6.45	30.95 ± 6.09
	120	2.33 ± 0.48	45.01 ± 6.05	29.44 ± 3.80	25.54 ± 4.27
	240	1.54 ± 0.19	44.91 ± 1.74	26.49 ± 3.45	28.59 ± 3.62
	360	2.35 ± 0.20	**83.50 ± 8.92**	15.08 ± 2.61	1.41 ± 6.57
RH	0	**2.99 ± 0.16**	35.68 ± 2.22	12.96 ± 4.11	51.35 ± 2.87
Biochar	60	**3.22 ± 0.19**	58.60 ± 4.89	22.81 ± 3.79	18.58 ± 1.10
	120	**4.23 ± 0.35**	**62.22 ± 6.40**	**14.31 ± 2.33**	23.47 ± 8.62
	240	**1.79 ± 0.08**	52.41 ± 6.16	28.81 ± 5.13	18.78 ± 1.56
	360	**4.47 ± 0.23**	**88.79 ± 6.86**	12.00 ± 1.83	0.00 ± 0.00

ND = Not Determined

Values in bold font indicate significance at $P<0.05$

Table 4.2 Maximum rate (% h^{-1}), ^{14}C extent mineralization (%), ^{14}C biomass uptake (%) and ^{14}C remaining (%) for non-intensive farming soil, over 360 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extent mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	3.34 ± 0.52	50.88 ± 2.64	5.11 ± 0.57	44.00 ± 2.09
	60	1.51 ± 0.54	29.57 ± 10.24	11.49 ± 5.09	58.94 ± 15.07
	120	2.15 ± 0.31	45.70 ± 4.07	15.43 ± 2.94	38.87 ± 5.06
	240	1.96 ± 0.10	39.85 ± 1.47	15.63 ± 0.96	44.52 ± 1.83
	360	2.34 ± 0.70	**48.37 ± 7.56**	18.76 ± 2.13	32.87 ± 6.18
CS	0	3.49 ± 0.50	50.28 ± 4.83	8.67 ± 0.76	41.05 ± 4.09
Biochar	60	2.00 ± 0.79	34.65 ± 13.32	6.21 ± 1.82	59.14 ± 11.65
	120	1.89 ± 0.16	40.66 ± 1.92	16.87 ± 1.01	21.07 ± 1.08
	240	2.47 ± 0.37	43.90 ± 4.47	14.52 ± 2.80	41.59 ± 4.06
	360	2.51 ± 0.33	**54.57 ± 6.17**	12.24 ± 1.94	33.19 ± 5.33
RH	0	4.21 ± 0.58	47.35 ± 3.22	5.36 ± 0.94	47.29 ± 2.29
Biochar	60	1.46 ± 0.29	30.19 ± 3.80	3.06 ± 0.95	66.75 ± 4.74
	120	2.13 ± 0.20	44.73 ± 3.46	9.17 ± 3.61	46.10 ± 6.84
	240	2.71 ± 0.29	48.82 ± 3.20	13.87 ± 1.10	37.30 ± 2.65
	360	4.17 ± 0.91	**72.77 ± 9.37**	7.88 ± 1.29	19.35 ± 8.12

Values in bold font indicate significance at $P<0.05$

Table 4.3 Maximum rate (% h^{-1}), ^{14}C extent mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for intensive farming soil, over 360 d. Error bars are SEM (n=3).

Treatment	Day	Maximum rate (% h^{-1})	^{14}C extent mineralization (%)	^{14}C biomass uptake (%) fixed k_{EC}	^{14}C activity remaining in soil (%)
Control	0	2.83 ± 0.33	44.1 ± 1.36	16.29 ± 3.67	39.61 ± 3.24
	60	1.65 ± 0.17	42.7 ± 2.94	17.82 ± 4.07	39.48 ± 5.70
	120	2.12 ± 0.32	43.91 ± 4.76	14.60 ± 1.14	41.49 ± 5.51
	240	1.79 ± 0.08	36.81 ± 1.48	15.44 ± 2.31	47.75 ± 3.60
	360	3.70 ± 0.61	**69.80 ± 3.51**	14.41 ± 1.67	15.78 ± 1.88
CS	0	3.27 ± 0.36	45.79 ± 2.51	14.47 ± 2.99	39.73 ± 2.92
Biochar	60	1.83 ± 0.07	42.00 ± 1.70	12.75 ± 3.64	45.24 ± 4.89
	120	1.79 ± 0.25	40.47 ± 3.16	17.13 ± 2.24	42.39 ± 2.61
	240	2.05 ± 0.22	41.49 ± 2.97	15.62 ± 5.80	42.89 ± 8.44
	360	2.81 ± 0.77	**56.58 ± 4.95**	9.67 ± 1.82	23.75 ± 12.60
RH	0	2.98 ± 0.72	51.90 ± 3.78	9.76 ± 3.47	40.24 ± 2.15
Biochar	60	1.58 ± 0.29	41.23 ± 2.75	18.00 ± 3.58	40.77 ± 1.21
	120	1.57 ± 0.19	38.42 ± 3.93	19.48 ± 3.57	42.10 ± 1.41
	240	1.54 ± 0.07	31.92 ± 1.90	15.26 ± 1.15	52.82 ± 2.76
	360	2.40 ± 0.58	**51.77 ± 6.45**	13.51 ± 1.06	34.72 ± 5.41

Values in bold font indicate significance at $P<0.05$

The addition of CS and RH biochar had no effect on the mineralization and uptake of ^{14}C glucose. However, on day 120 RH biochar and forest soil showed an increase of ^{14}C glucose mineralization and a decrease in ^{14}C uptake into microbial biomass ($P<0.05$). The maximum rates in forest soil amended with RH biochar significantly increased ($P<0.05$) at all times, as compared to control and CS biochar treatments. In contrast, the maximum rates in the other two soils (non-intensively farmed and intensively farmed soils) did not increase significantly ($P>0.05$) between biochar treatments and control treatment at any time. This is possibly due to the fertilizer that was supplied in these two soils as compared to the forest soil, which is a background soil not treated with any fertilizer. The biochar showed an effect in the soil with lower

nutrients. The higher effect of biochar for soil with low nutrients content is in line with the study on rice crop in the tropics and with the study on the effect of fertilizers on plant yield (Haefele *et al.*, 2011; Jeffery *et al.*, 2011). According to Haefele *et al.* (2011), adding RH biochar had no effect on crop yield in fertile soil, but increased crop yield from 16% to 35% in nutrient poor soil. Cornelissen *et al.* (2013) also found that there was a prominent effect of biochar on crop yield in the soil with the lowest fertility compared to soil with higher fertility. In addition, in this study mineralization of ^{14}C glucose increased due to increasing carbon content in the soil amended with RH biochar, as compared to unamended soil, resulted in a decrease of ^{14}C uptake by microorganisms. Adding CS and RH biochars to soil might affect the amount of carbon in the soils, and this may influence the microbial activity.

Unlike forest soil, the intensively farmed soil and non-intensively farmed soil showed the opposite pattern of mineralization and biomass uptake. At the beginning of the incubation period, the mineralization of ^{14}C glucose in the intensive farming soil was higher than in forest soil (44.1% and 29.02% respectively) (Tables 4.3 and 4.1). The application of CS and RH biochars also increased the mineralization rate in this soil but not in forest soil. However, the trend was opposite towards the end of the incubation time. The mineralization of ^{14}C glucose in intensive farming soil amended with RH biochar (51.77%) (Table 4.3) was lower than in forest soil added with the same biochar (88.79%) (Table 4.1). The ^{14}C glucose uptake by the microbial population decreased in forest soil, as compared to intensive farming soil (12% and 13.51% respectively). The results indicated that amending intensive farming soil with biochars had no effect on microbial activity because low carbon content and organic materials in intensive farming soil. In comparison the higher carbon and it organic

matter content in the forest soil resulted in higher mineralization of ^{14}C glucose to CO_2 by microbes. It is hypothesized that, with a very limited content of carbon in the soil, the microbes may store added glucose in their cells rather than respired (Bremer and Kuikma, 1994; Nguyen and Guckert, 2001), thus promoting a greater biomass uptake than mineralized into CO_2 (Boucard *et al.*, 2008).

4.3.2 Ammonium leaching in forest, non-intensive farming and intensive farming soils

The concentration of ammonium decreased over time in all soils (Figure 4.1). Adding biochar to soil exhibited various trends of ammonium leaching. For example, in forest soil biochar decreased the concentration of ammonium in the leachate and reduced the concentration of ammonium in non-intensive farming soil at the beginning of the leaching process from 0.03 (control) to 0.00067 (CS) and 0.003 mg/L (RH) (Figure 4.1) ($P<0.05$). While in intensive farming soil biochar had no effect on ammonium leaching see Figure 4.1. The concentration of ammonium was higher in unamended forest soils than in biochar treatments. Biochar treatments adsorbed ammonium in the forest soil over time ($P<0.05$). This is probably due to the negative charge on the biochars surface (Baldock and Smernik, 2002; Glaser *et al.*, 2002; Chen *et al.*, 2008; Novak *et al.*, 2009; Mukherjee *et al.*, 2011) which enables positively charge cation, for example ammonium, to be attached (Alling *et al.*, 2014). Therefore, less ammonium was leached in the soils treated with both CS and RH biochars.

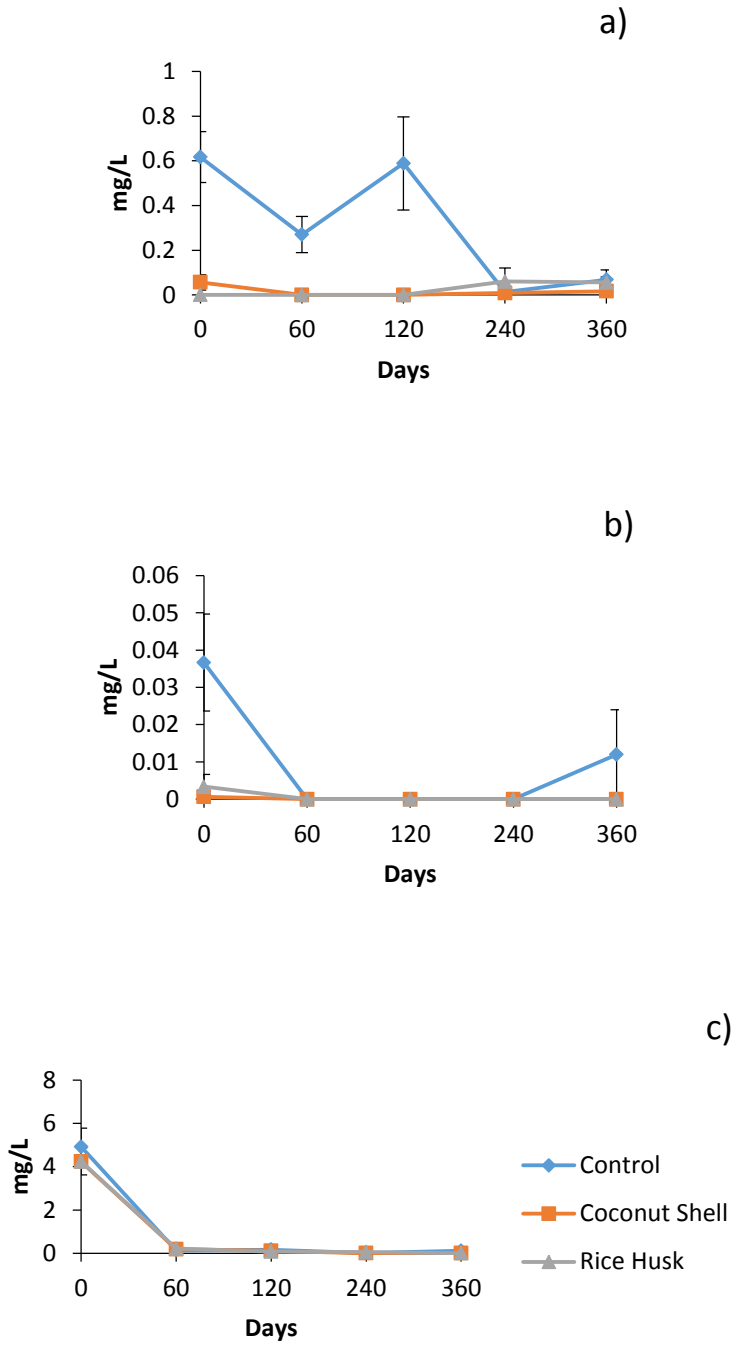

Figure 4.1 Amount of ammonium in the leachate of three soils a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

4.3.3 Nitrate leaching in forest, non-intensive farming and intensive farming soils

The pattern of nitrate leaching was different among the three types of soils studied. Unlike ammonium, the concentration of nitrate in forest soil leachate increased over time ($P<0.05$), whereas in non-intensive and intensive farming soils nitrate leaching decreased over time ($P<0.05$) (Figure 4.2). Amending soils with biochar did not show a clear pattern, and also the differences were generally insignificant ($P>0.05$) see Figure 4.2.

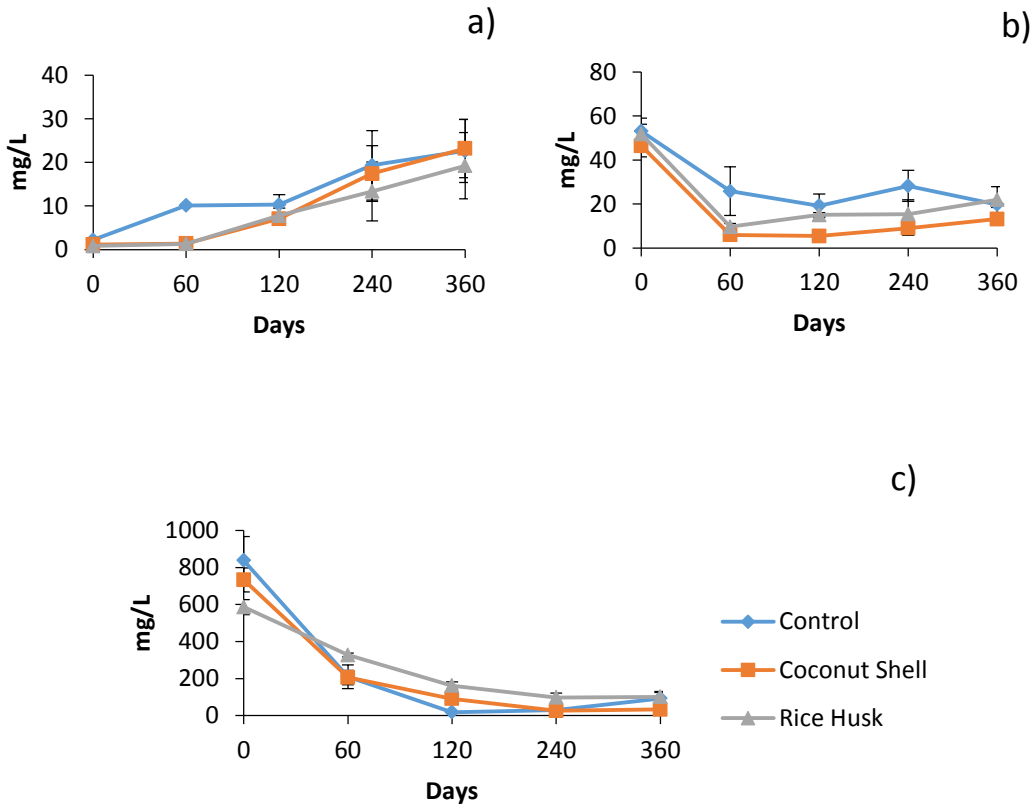

Figure 4.2 Amount of nitrate in the leachate of three soils a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

In forest soil the concentration of nitrate in the leachate increased and this suggests that nitrification process occurred in the soil. The results were in line with Alling *et al.* (2014), where the nitrate concentrations in the soil leachate increased after addition of 5 and 10% of biochar. They speculated that the biochar itself may contribute to the release of nitrate and also enhance the nitrification process in the soil. On the other hand, the concentration of nitrate in non-intensive and intensive farming soils decreased over time. Less organic matter in these two soils resulted in slow microbial activity in the soils, hence the nitrification process by microbes was also affected.

4.3.4 Phosphate leaching in forest, non-intensive farming and intensive farming soils

Phosphate leaching exhibited a different pattern in each of the soils. Phosphate leaching in forest soil fluctuated over time, whereas in other two soils phosphate leaching decreased over time and slightly increased at the end of the leaching process (Figure 4.3). Amending forest soil with biochars decreased phosphate leaching, but the decrease was not significantly different ($P>0.05$). In non-intensive farming soil RH biochar increased phosphate leaching ($P<0.05$), especially at the beginning of the leaching process (Figure 4.3). There was no any different on the leaching of phosphate in the intensive farming soil ($P>0.05$) (Figure 4.3).

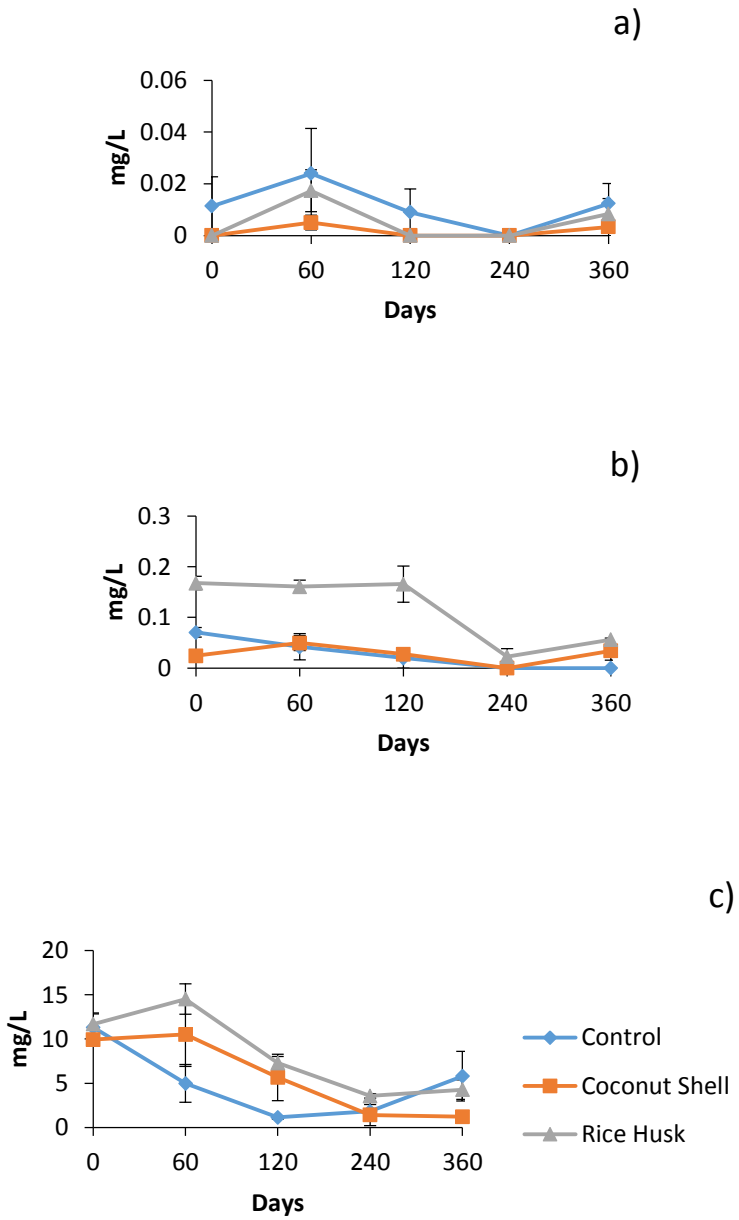

Figure 4.3 Amount of phosphate in the leachate of three soils a) forest b) non-intensive and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

The decreasing level of phosphate leaching in the forest soil leachate treated with biochars was in agreement with the findings yielded in Alling *et al.* (2014) where addition of 5% of biochar increased the sorption of phosphate in a Lampung, tropical

soil in Indonesia. The reduction of phosphate content in the soil leachate was not only because of biochar alone, but could also be attributed to the other soil properties, such as mineral and clay content that might affect the CEC and the sorption of phosphate in the soils (Staunton and Leprince, 1996). Furthermore, with low anion exchange capacity, biochars can only adsorb a very small amount of phosphate in the soils (Singh *et al.*, 2010). Because of this, they are likely to absorb only a small amount of phosphate and nitrate concentrations in intensive and non-intensive farming soil leaching.

Yao *et al.* (2012), studied nutrient sorption on thirteen biochars in sandy soils and revealed that only five biochars can absorb phosphate, while the remaining biochars released phosphate in the leachate. In addition, more than 2% phosphate was released from three types of bamboo biochars, and a hydrothermally produced biochar released the highest concentration of nitrate and phosphate in the soil leachates. In the present study, the greater amount of phosphate in the leachate from soils treated with RH biochar may be due to the high phosphate content in the biochar itself (1.75mg g^{-1}) (Table 3.1). Additionally, the phosphate content in intensive farming soil was also high because of the massive application of fertilizer to the soil.

4.3.5 Leachate and soil pH

Amending soils with 2% of CS and RH biochars by weight had a small effect on the pH of the leachate. The biochar treatments significantly increased leachate pH in forest and non-intensive farming soils, but in intensive farming soil there was no clear pattern (Figure 4.4).

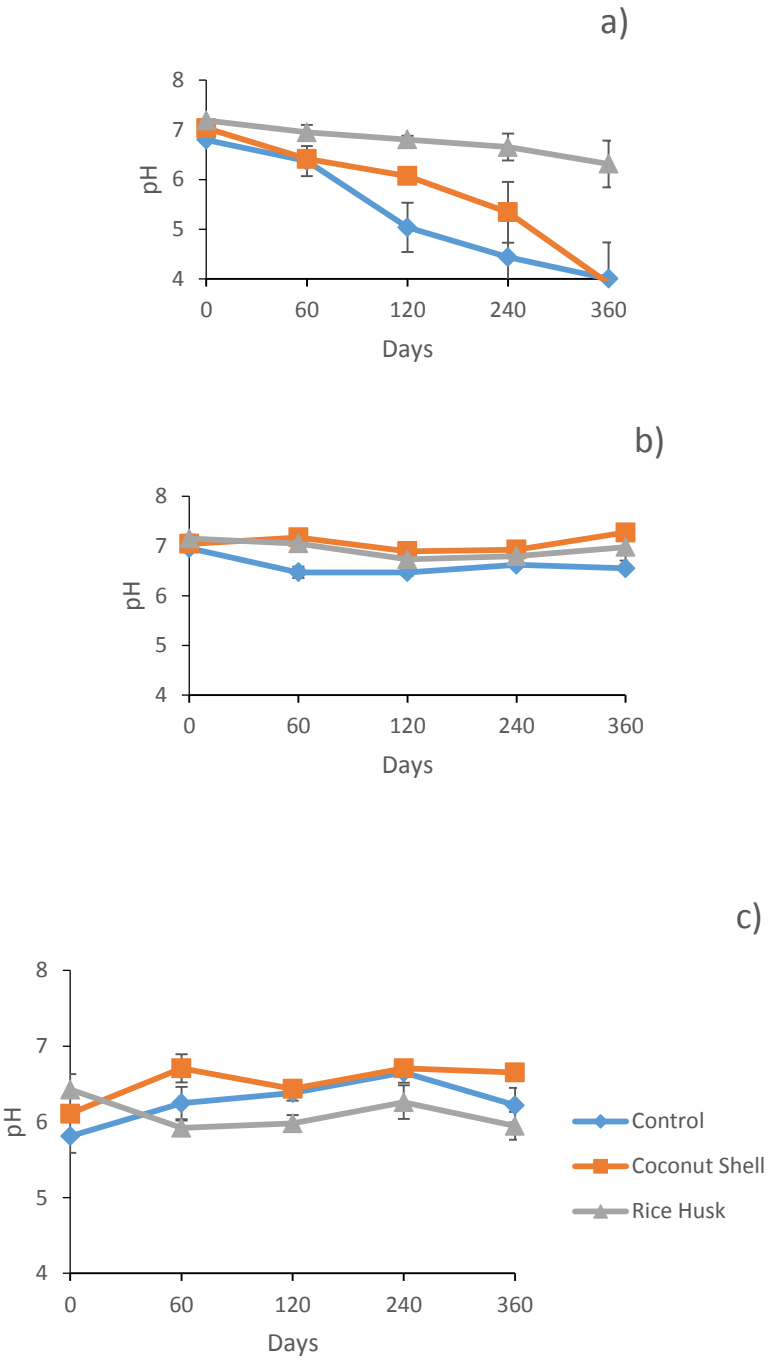

Figure 4.4 pH in the leachate of three soils a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

The application of biochars affects soil pH. Adding 2% of CS and RH biochars by weight to soils increased significantly ($P<0.05$) their pH (Figure 4.5). Although the application of biochar to soil increased soil pH, it subsequently declined over time ($P<0.05$). Figure 4.6 shows the relationship between soil pH and time, which was also strong. For instance, the R^2 values of forest soil pH in control, CS and RH treatments were 0.88, 0.94 and 0.85 respectively. Meanwhile, the R^2 values for intensive farming were 0.91 (control), 0.86 (CS), 0.98 (RH) and non-intensive farming soils was 0.85 (control), 0.89 (CS) and 0.83 (RH) respectively (Figure 4.6).

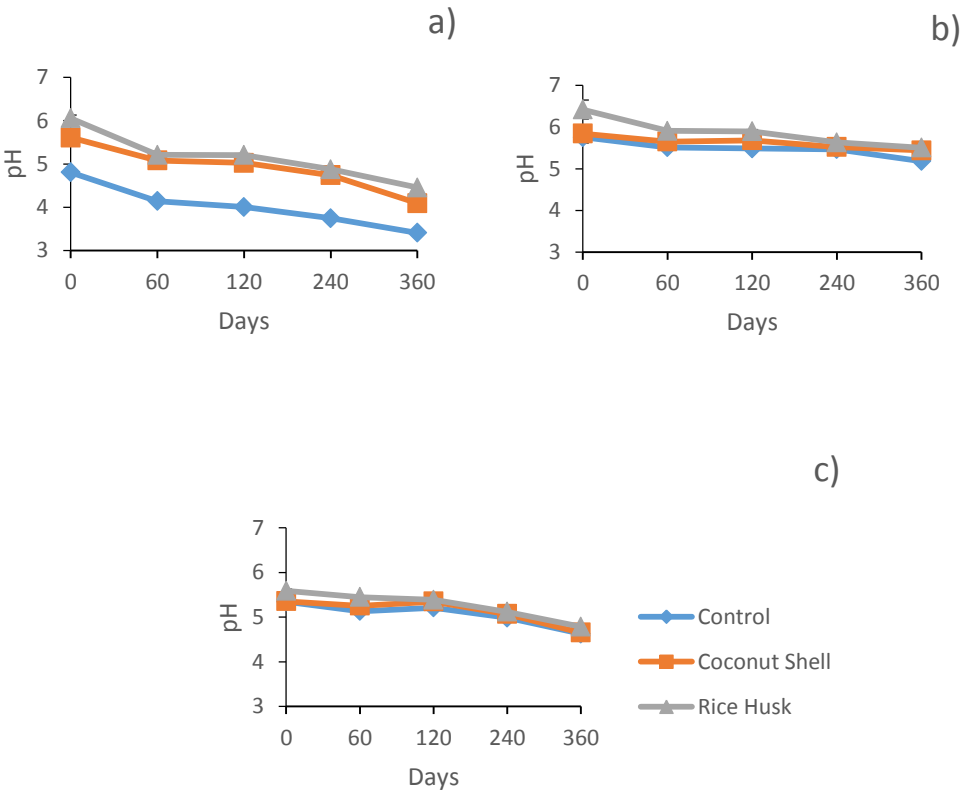

Figure 4.5 pH in a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

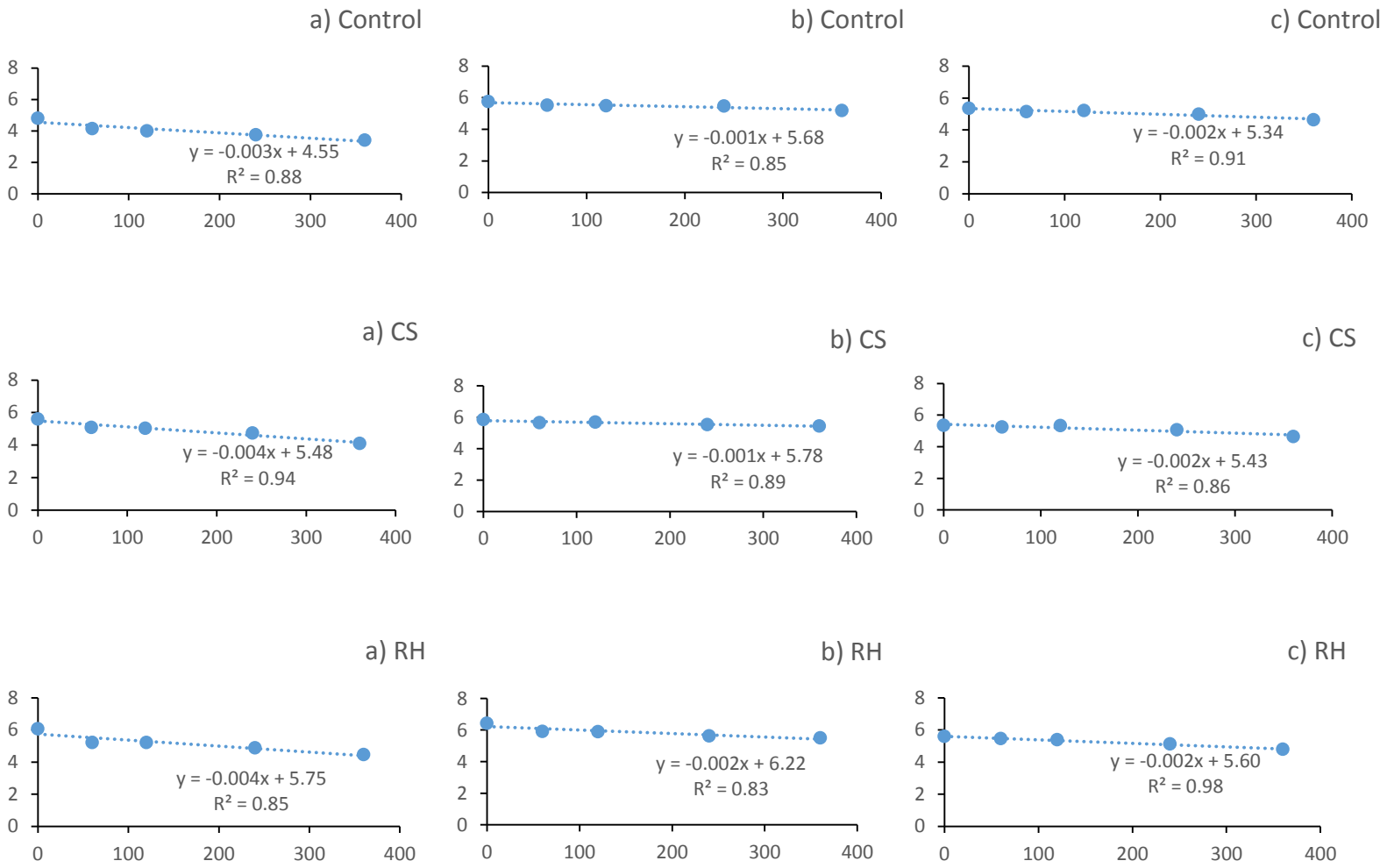

Figure 4.6 Correlation between soil pH with days in three different soils a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar.

In this study, generally amending the soils with 2% CS and RH biochars by weight increased leachate and soils pH. CS and RH biochars had high pH (8.33 and 8.46 respectively) (Table 3.1) and this suggests that biochar could be used to increase soil pH and ameliorate an acidic soil. The results of this study were also in line with the findings of previous research (Novak *et al.*, 2009; Masulili *et al.*, 2010; Peng *et al.*, 2011).

Although biochars can increase the soil pH and leachate due to their alkalinity, the pH values decreased over time, especially in forest soil (Figure 4.5 and Figure 4.6). This could be attributed to the oxidation of ammonium ions in the forest soil by microbes, also known as nitrification, leading to a decrease in the soil pH (Fageria and Baligar, 2008). With high organic matter in the soil and the addition of organic matter from the biochar itself, the nitrification process in the soil could be enhanced, hence increasing soil acidity. This explanation can also be considered in connection with the leaching results, where the nitrate content in forest soil leachate increased over time. Another possibility is the leaching of basic cations from the soil (Schulz and Glaser, 2012; McCormack, 2015).

4.3.6 Cation exchange capacity (CEC)

Adding biochar to soils in this study had little effect on the CEC. The results from Figure 4.7 show that adding RH biochar to soils marginally increased the CEC in forest soil and non-intensive farming soils (Figure 4.7). None of the biochars had an impact on CEC of the intensive farming soil, while CS biochar did not affect the CEC in any of the soils.

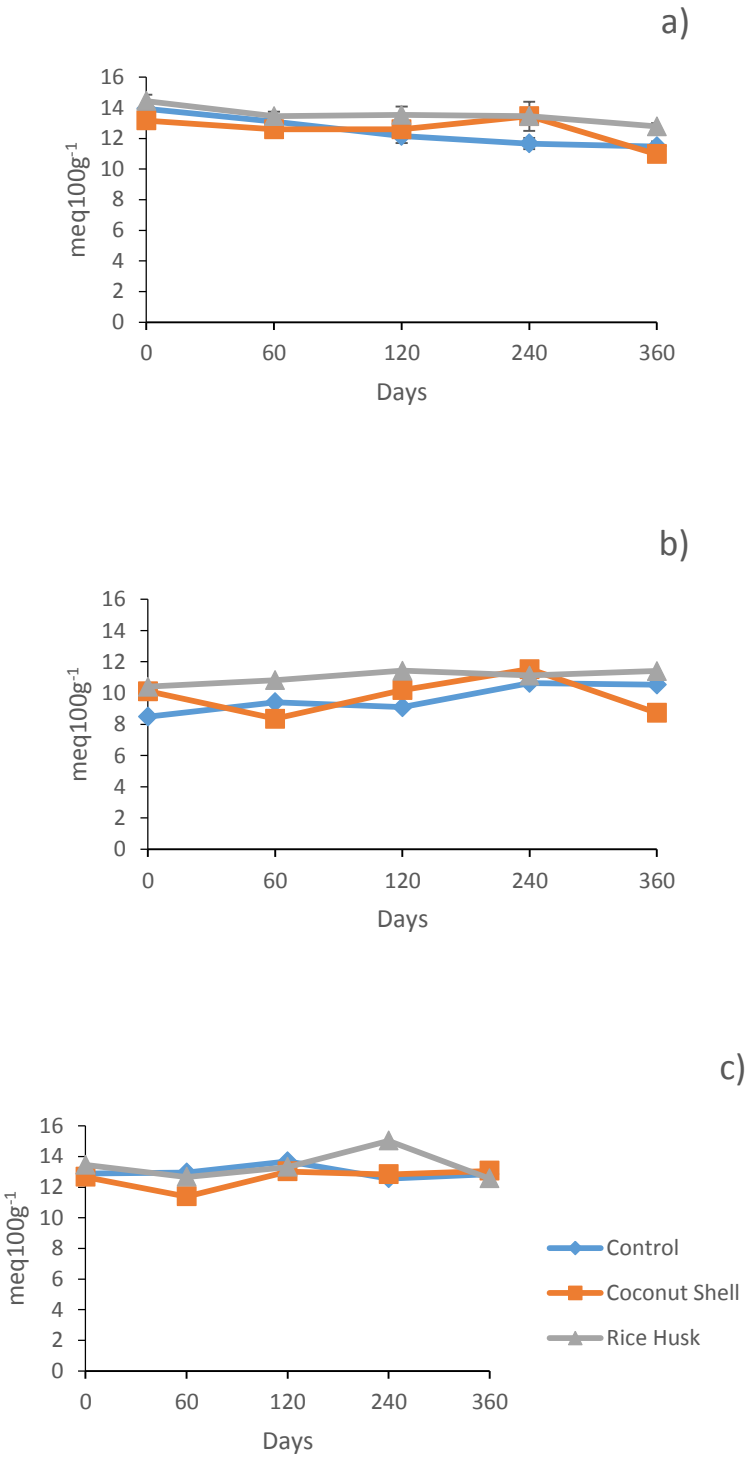

Figure 4.7 CEC in a) forest b) non-intensive and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

The CEC of the soils amended with CS and RH biochar exhibited no significant difference (P>0.05). Most of the studies have found that application of biochars increased CEC (Liang *et al.*, 2006; Van Zwieten *et al.*, 2010; Masulili *et al.*, 2010; Peng *et al.*, 2011). However, the present results were consistent with Novak *et al.* (2009), in which the addition of 0.5, 1.0 and 2.0% pecan shell biochar by weight did not change the CEC of agricultural soils significantly. They stated that the weak effect of biochar in increasing the CEC of the soils was due to the production of biochars at high temperature.

Biochar produced in high temperature has low surface negative charge, which resulted from the low oxidation of carboxylic and phenolic groups on the outer surface of biochar particles will possibly decrease the CEC of soils (Novak *et al.*, 2009). The biochars used in this study were also produced in high temperature (the temperature ranged from 400 – 1000^{O}C). Therefore, the lack of a significant biochar impact on the CEC in this experiment may be due to the high temperature during biochar production.

4.3.7 Carbon, nitrogen and phosphate in soils

Adding biochar increases the carbon content of all soils. The relative increase was greatest in the forest soil amending with CS biochar (8.2%) (Appendix 1). However, the results showed a decline in carbon content over time. This was also reported by (Sukartono *et al.*, 2011). The researchers found that the organic C content in the soil amended with CS biochar and cattle dung biochar decreased over time from 1.15 to 1.13 mg kg^{-1} and from 1.14 to 1.11 mg kg^{-1}. Besides, they noticed that the reduction of

organic C in the soil amended with cattle manure was higher than in the soil amended with biochars. They speculated that the slow reduction of C in the soil was due to the resistance of biochars aromatic C structure, which could slower the decomposition of C. Because of this characteristic, biochars would potentially sequester C in the soil. In addition, adding CS and RH biochars to forest, intensive and non-intensive farming soils had no significant effect ($P>0.05$) at any time on the total nitrogen and phosphate (see Appendix 2 and Appendix 3).

4.3.8 Effect of biochar on aggregate stability of soils

Figures 4.8 to 4.10 show that forest soil was more stable in comparison to intensive farming and non-intensive farming soils ($P<0.05$). Forest soil was also more stable in the fast wetting (FW), slow wetting (SW) and mechanical (M) treatments compared to non-intensive farming and intensive farming soils (Figures 4.8 to 4.10). The non-intensive farming soil was only stable in the M treatment, where the mean weight diameter (MWD) values ranged between 2.98 to 3.45 mm (Figure 4.10). In contrast, intensive farming soil was the least stable in all treatments (FW, SW and M) see Figures 4.8 to 4.10. The MWD values ranged from 0.10 to 0.41 mm, 0.25 to 0.63 mm, and 0.39 to 0.67 mm respectively (Figures 4.8 to 4.10).

Amending all soils with biochar had a small effect on the stability of the soils. Biochar amendment only had an effect on the FW treatment, but had no effect on the other two treatments (SW and M) at any time in all soils.

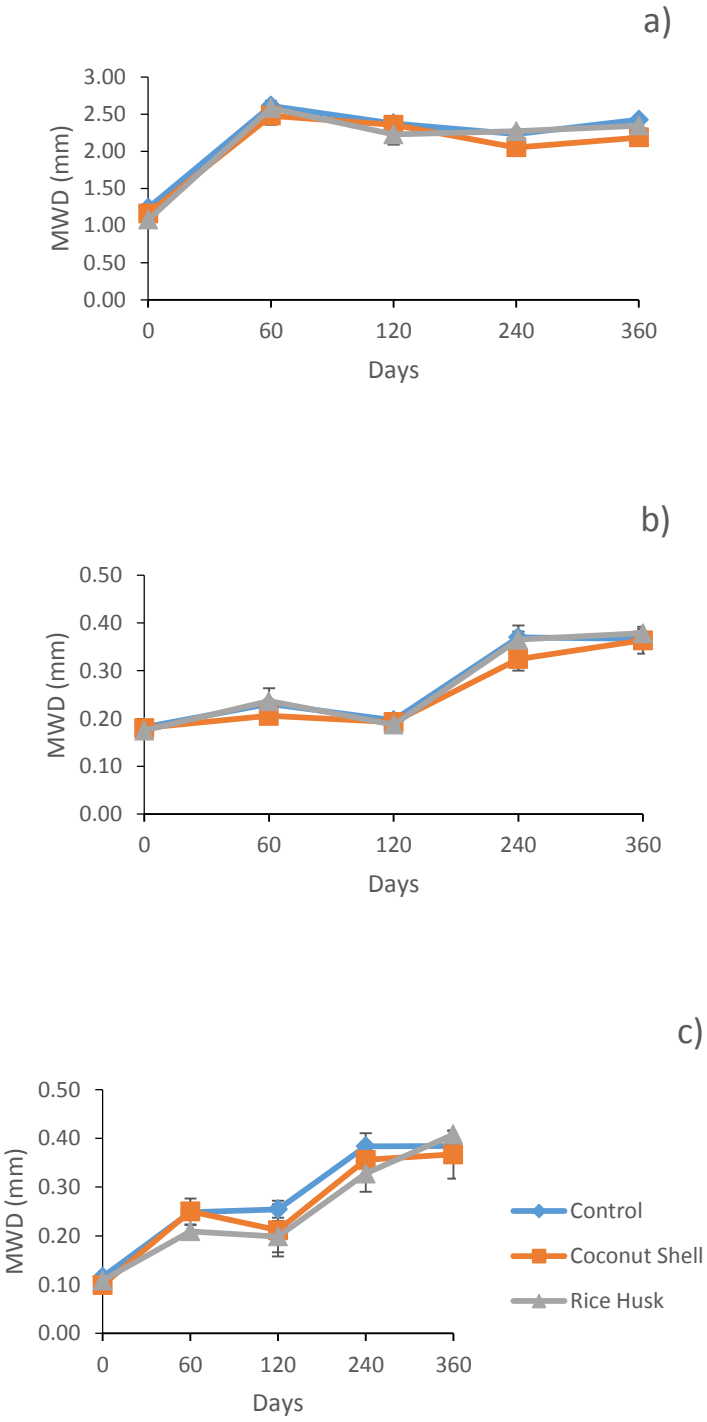

Figure 4.8 MWD values for FW treatment in a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

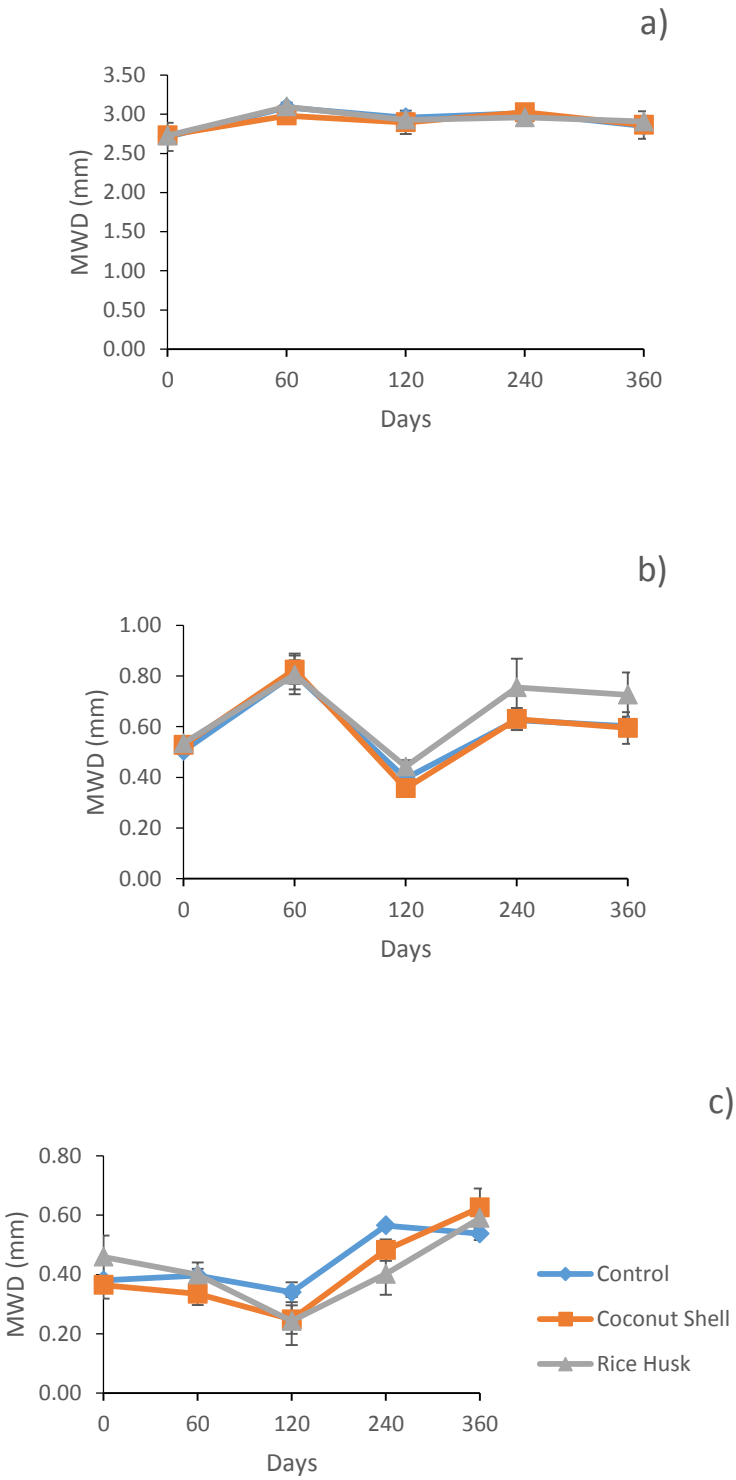

Figure 4.9 MWD values for SW treatment in a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

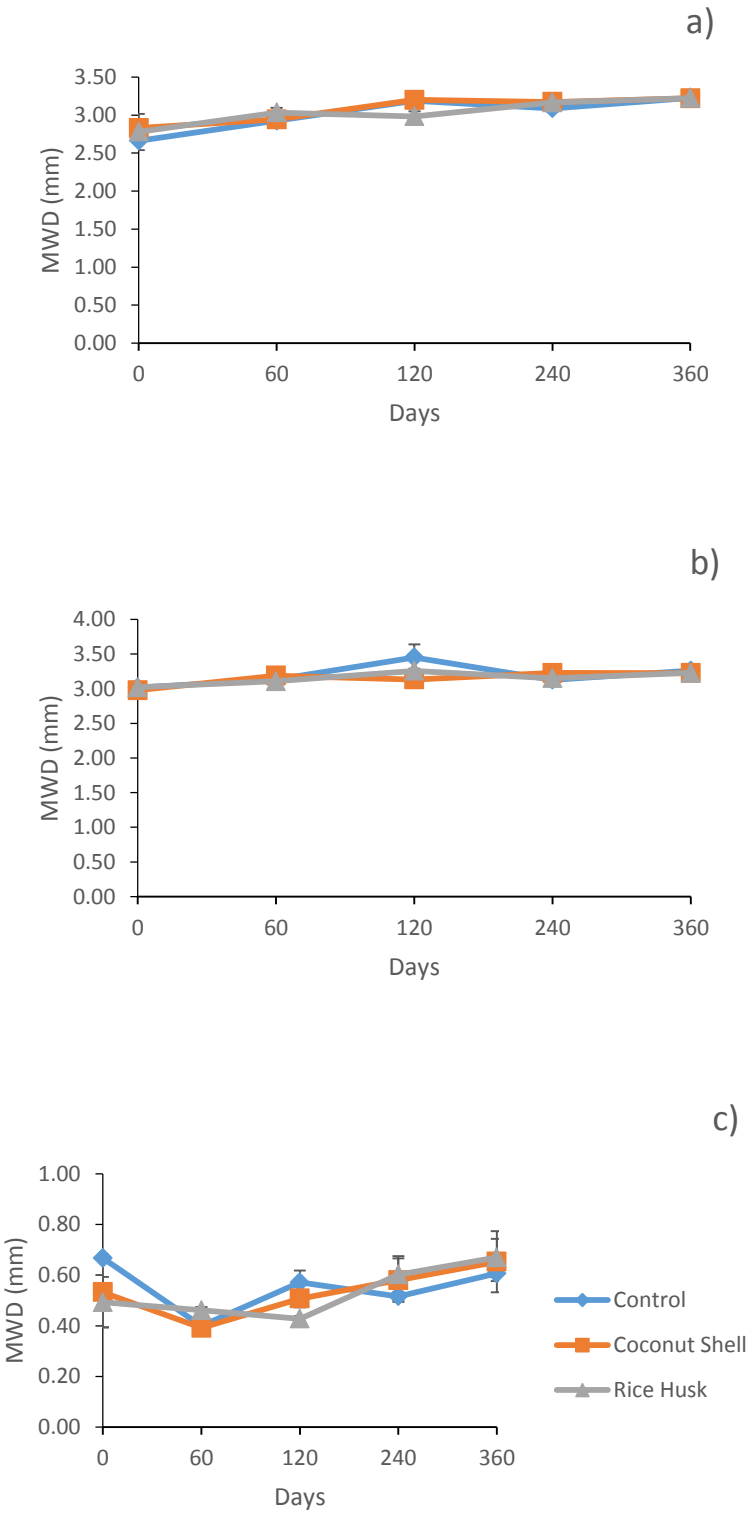

Figure 4.10 MWD values for M treatment in a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

In terms of physical properties, the forest soil had better aggregation than the other two soils, and it was also more stable in all treatments (FW, SW and M). The MWD values for the forest soil ranged from 1.24 to 2.58 mm (FW), 2.71 to 2.90 mm (SW) and 2.66 to 3.23 mm (M) respectively (Figures 4.8 to 4.10). This is due to the high organic matter, carbon content and fungal hyphae in the soil that can act as cementing agents to bind the soil particles, and eventually enhance soil aggregation (Ishak and Jusop, 2010). However, incorporation of biochars to soils had no effect on aggregate stability, which is in agreement with the observation from another study (Peng *et al.*, 2011). Peng *et al.* (2011), did not find any effect of biochar amendment on soil aggregation. The results were expected as the formation of soil aggregate by biological activity and other organic materials, including biochars are unlikely to take place immediately (Herath *et al.*, 2013), therefore the effects cannot be seen in the period of this study. In contrast to these findings, Herath *et al.* (2013) reported that the stability of soil amended with corn stover biochar was higher than that of unamended soil. They argued that the formation of water stable macro aggregate against slaking in amendment soils and the presence of fungal hyphae within the biochar pores increased soil aggregation. Apart from that, the FW treatment in the pots amended with biochar also improved aggregate stability by more than 17% in comparison with the SW and M treatments in which stability improved by only 4 to 16% (Herath *et al.*, 2013).

4.5 Conclusion

In conclusion, only RH biochar had a little effect on the mineralization of ^{14}C glucose to CO_2 in forest soil, while no significant difference was observed in intensive and non-intensive farming soils relative to biochar amendment. Forest soil had increased

nitrification due to high carbon and organic matter contents in comparison with intensive and non-intensive farming soils. The nitrate contents in forest soil leachate increased over time, whereas the nitrate in intensive and non-intensive farming soil leachates declined over time. Biochars showed unclear trend in the adsorption of nitrate and phosphate in each of the soil leachates. High pyrolysis temperatures during the production of biochar likely resulted in low CEC after amending soils with biochars. Moreover, with limited anion exchange capacity, biochars have also shown less ability to adsorb nutrients in the soils. However, amending soils with 2% of CS and RH biochars by weight increased soil C and pH. Results indicated that biochar could sequester C in the soils together with the liming effects. Biochar could also ameliorate the acidity in typical Malaysian soils. Finally, the application of both biochars to the tropical soils had no effect on soil aggregation. Longitudinal experiments are advisable in order to investigate the impact on soil aggregation.

Chapter 5

The Impact of Biochar Amendment on Soil Properties in Three Different Temperate Soils

5.1 Introduction

Numerous studies have found that the application of biochar enhances soil quality (Downie *et al.*, 2011; Uzoma *et al.*, 2011; Quilliam *et al.*, 2012; Qayyum *et al.*, 2015). Lehmann *et al.* (2011) reported that adding biochar to soil affects the soil biological community and improves soil microbial biomass (O'Neill *et al.*, 2009; Jin, 2010; Liang *et al.*, 2010). This is due to improvements in soil structure and increasing nutrient concentrations (P and Ca) (O'Neill *et al.*, 2009). Furthermore, biochar itself has a large surface area with a porous structure, which creates an ideal habitat for microorganisms (Pietikainen, 2000). These improvements in soil physico-chemical properties lead to changes in the soil microbial community (O'Neill *et al.*, 2009).

Biochar can alter soil chemical properties through changes in cation exchange capacity and pH, as well as reducing the leaching of nutrients (Scott *et al.*, 2014). In addition, biochar has also the potential to sequester soil C (Qayyum *et al.*, 2015). The recalcitrance of biochar C is due to its production; for example, biochar produced at high temperature of $400^{O}C$ or higher can lead to depolymerisation, loss of functional groups and also results in larger aromatic ring structures (Zimmerman and Gao, 2013). This structure is more likely to be resistant to biotic or non-biotic degradation processes (Zimmerman and Gao, 2013). The resistance of aromatic C structure of

biochar, may slow the decomposition of C and leading to the sequestration of C in the soil.

Furthermore, biochar can also reduce emission of greenhouse gas, and ultimately mitigate against global warming (Kammann *et al.*, 2012). The decomposition rate of biochar in soil is slow, therefore may mitigate against CO_2 emissions into the atmosphere. In addition, the alkalinity of biochar may increase the conversion of greenhouse gas, for example, N_2O to N_2 by N_2O-reductase enzyme activity (Van Zwieten *et al.*, 2009). Increasing the C/N ratio, as a result of biochar addition, would promote N immobilization, thus reducing N_2O emissions through immobilization of inorganic N (Lehmann *et al.*, 2006; Van Zwieten *et al.*, 2009; Kammann *et al.*, 2012). However, there are a number of studies showing the opposite effect in soil following the addition of biochar (Wardle *et al.*, 2008; Jones *et al.*, 2011; Lehmann *et al.*, 2011; Qayyum *et al.*, 2015). This is due to phytotoxic substances that have been found from freshly made biochar, such as polycyclic aromatic hydrocarbons (PAHs), phenolic compounds and acetic or formic acids (Bargmann *et al.*, 2013; Quilliam *et al.*, 2013b). These compounds may not only affect crops (Busch *et al.*, 2012; Rogovska *et al.*, 2012), but could also contaminate the soils (Quilliam *et al.*, 2013b).

The results of incorporating biochar into the soil vary depending on several factors, such as the types of soil and climates, the types of feed stocks used to produce biochar, as well as the temperature and duration of the pyrolysis process. For instance, biochar produced from plant materials exhibited different properties to biochar produced from animal products. Generally, plant-derived biochars contain higher C

content, lower nutrient concentrations (N, P and K) and lower CEC values. Whereas, animal-derived biochars have lower C contents, higher nutrient concentrations and higher CEC values (Scott *et al.*, 2014). Furthermore, biochars made at low temperatures have high C, N and S compared to biochars made at higher temperatures. This is because more nutrients tend to volatize as temperature increases (Scott *et al.*, 2014). This variability means that there is uncertainty regarding the effects of biochar on the properties of soil. This has created an interest in studying biochar across the globe from tropical to temperate climates (Karer *et al.*, 2013). Often, the addition of biochar to soil benefits infertile or degraded soils, but has little effect on fertile soils, thus the application of biochar to agricultural soils in temperate climates remains debatable (Quilliam *et al.*, 2012). In fact, some researchers have claimed that adding biochars to temperate soils has shown only transient effects (Jones *et al.*, 2012; Quilliam *et al.*, 2012). Some studies reported that the addition of biochar to temperate soils showed some positive effects, but this was dependant on the soil type (Kolb *et al.*, 2009; Kloss *et al.*, 2014). Even though measured effects appear to be transient, but more importantly, adding biochar to these type of soils did not show any negative effects to the plant growth and soil quality, in fact promote a little advantage on agricultural land (Jones *et al.*, 2012; Quilliam *et al.*, 2012).

In this chapter, the impact of 2% fresh and aged HW biochar on soils was investigated to determine whether biochars could stimulate microbial activity, hold nutrients in the soil, or alter the soil's physico-chemical characteristics in grassland, arable loam and arable sandy soils. The objectives of this chapter are as follows:

1. To measure the microbial activity and microbial biomass in the soil amended with biochar
2. To quantify the impact of biochar amendment on nutrient leaching from soil
3. To investigate the effects of biochars on the soil's chemical properties (C, N, P, CEC and pH) and physical properties (aggregate stability).

5.2 Materials and Methods

Three Brown Earth soils (grassland, arable loam and arable sandy) from Dundee, United Kingdom were used in this study. The soils were chosen due to an aged soil that has been amended with biochar approximately 10 months by a former PhD student. The biochar used in this experiment was hardwood (HW) biochar. The HW biochar used in this experiment was produced in a kiln. More details about the materials (soils and biochars) used in this study can be found in the previous chapter (Section 3.2.2 and Section 3.2.4).

The experiments are divided into three parts, which are biological, chemical and physical aspects of soils. Details for all the methodology can be found in Sections 3.3.1-15. For the biological properties, the methodology employed to carry out the experiments were substrate induced respiration, where 3 mM of glucose solution (10ml) was added into soil samples, which had a radioactivity of 1086 Bq on days 0 and 60 (incubation time); and 654 Bq on days 180 and 300 (incubation time). For fumigation and non-fumigation extraction 0.5 M potassium sulphate was used. C-14 glucose associated activity remaining in soil was determined via combustion (3 minutes) on a sample oxidiser (Packard, Model 307).

For the chemical properties, the total carbon (C) and nitrogen (N) were determined by dry combustion and measured with an elemental analyser (Elementar Vario EL), phosphorus digestion with hydrochloric acid and hydrogen peroxide; the concentration of P in the soil was then measured with Bran + Luebbe autoanalyser 3, as well as phosphate, ammonium and nitrate concentrations in the soil leachate. pH was measured using a pH meter model PHM 220 calibrated using buffers pH 7.0 and 4.0; and CEC was determined using 1 M ammonium acetate. The measurement of Na attached to soils was obtained by using flame photometry. For the physical characteristics, soil moisture content was determined through oven drying at $105^{O}C$ for 24h and, particle size analysis determined by the hydrometer method and aggregate stability using the Le Bissonnais method (Bissonnais, 1996).

For the statistical analyses, the mean values of maximum rate, ^{14}C mineralization, ^{14}C biomass, total carbon, total nitrogen, phosphate and nutrients concentration in the leachate between the treatments, sorted by incubation day, were tested using a one-way analysis of variance (ANOVA) with a $P<0.05$ level of significance. Multiple mean comparisons were carried out using a Holm-Sidek procedure at $P<0.05$. For values that were not normally distributed, a non-parametric statistical test (Kruskal-Wallis), which is based on ranks, was used. In addition, the Tukey test was applied to determine the significant differences between the treatments for non-distributed values at the $P<0.05$ level. A two-way analysis of variance (ANOVA) was performed to test the significant difference for all of the parameters over time. All of the statistical tests were performed using the SigmaStat v3.5 (Systat Software Inc), except for two-way analysis of variance (ANOVA), which was performed using Microsoft Excel.

5.3 Results and Discussion

5.3.1 Mineralization of ^{14}C glucose to $^{14}CO_2$ and uptake of ^{14}C glucose into microbial biomass

The extent of mineralization of ^{14}C glucose in three different soils were relatively constant over time. Further, adding fresh biochar and aged biochar to the soils also did not lead to much significant change during the period of the study. Only after 180 d incubation the extent of mineralization of the aged biochar amended soils in grassland (74.86% ± 2.07) were significantly higher ($P<0.05$) than in the fresh biochar amended soils (62.83% ± 2.24) (Table 5.1). The mineralization of ^{14}C glucose was also increased over time in all soils (see Tables 5.1 to 5.3). There was no significant difference ($P>0.05$) in the maximum rates observed in loamy and sandy soils at any time (Tables 5.2 and 5.3). The significant effect on the maximum rates can only be seen on day 180 in grassland soils with aged biochar, which was 4.46% h^{-1} ± 0.20, compared to the rate for the fresh biochar amendment (3.51% h^{-1} ± 0.20) and the rate without biochar (3.80% h^{-1} ± 0.15) in the same soil, as shown in Table 5.1. Overall, biochar amendment did not have a prominent effect on the microbial biomass in the soil. For example, only after 300 d incubation biomass uptake in grassland soil amended with the fresh biochar (25.20% ± 2.56) was observed to be significantly higher ($P<0.05$) than the aged biochar amendment (11.36% ± 2.90) as shown in Table 5.1.

Table 5.1 Maximum rate (% h^{-1}), ^{14}C extent mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for grassland soil, over 300 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extent mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	2.32 ± 0.15	42.54 ± 2.46	7.99 ± 0.54	49.47 ± 2.46
	60	3.68 ± 0.28	75.91 ± 2.61	20.28 ± 5.07	3.81 ± 4.61
	180	3.80 ± 0.15	67.18 ± 0.45	11.32 ± 0.24	21.49 ± 0.35
	300	3.39 ± 0.11	**76.39 ± 3.99**	20.77 ± 1.89	2.83 ± 4.82
Fresh	0	2.35 ± 0.24	41.37 ± 3.09	10.73 ± 2.25	47.90 ± 3.92
Biochar	60	3.43 ± 0.49	71.95 ± 5.28	25.30 ± 3.51	2.74 ± 6.26
	180	3.51 ± 0.20	62.83 ± 2.24	11.02 ± 0.90	26.15 ± 1.35
	300	2.84 ± 0.21	**71.14 ± 2.74**	**25.20 ± 2.56**	3.66 ± 4.45
Aged	0	2.17 ± 0.43	42.72 ± 5.27	12.96 ± 4.21	44.31 ± 2.67
Biochar	60	3.21 ± 0.40	75.44 ± 5.17	22.73 ± 0.06	1.83 ± 5.18
	180	**4.46 ± 0.20**	**74.86 ± 2.07**	9.77 ± 1.75	15.37 ± 1.68
	300	5.37 ± 1.30	**93.59 ± 16.09**	11.36 ± 2.90	0.00 ± 0.00

Values in bold font indicate significance at $P<0.05$

In addition, after 60 d incubation the aged biochar treatment in loamy soil significantly ($P<0.05$) increased the extent of mineralization (88.12% ± 7.15) compared to the fresh biochar amended soil (68.33% ± 0.82), and unamended soil (69.91% ± 3.25), as shown in Table 5.2. The maximum rates were observed after 300 d incubation in control (4.77 h^{-1} ± 0.77), compared to the rate for the fresh biochar amendment (3.00 h^{-1} ± 0.14) and aged biochar (3.44 h^{-1} ± 0.18) in the same soil, ($P>0.05$) (Table 5.2). Only at day 0, was the incorporation of ^{14}C-carbon into the microbial biomass in loamy soil for the aged biochar treatment significantly higher ($P<0.05$) compared to the fresh biochar treatment (12.56% ± 0.09 and 7.68% ± 0.19, respectively) (Table 5.2).

Table 5.2 Maximum rate (% h^{-1}), ^{14}C extent mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for loamy soil, over 300 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extent mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	3.08 ± 0.58	54.76 ± 7.56	11.23 ± 0.57	34.01 ± 7.23
	60	3.09 ± 0.22	69.91 ± 3.25	21.00 ± 3.03	9.09 ± 5.87
	180	4.01 ± 0.21	71.94 ± 2.75	8.72 ± 0.41	19.34 ± 2.42
	300	4.77 ± 0.77	**85.66 ± 10.72**	15.15 ± 1.68	0.00 ± 0.00
Fresh	0	2.80 ± 0.31	52.47 ± 4.24	7.68 ± 0.19	39.85 ± 4.43
Biochar	60	2.88 ± 0.32	68.33 ± 0.82	19.74 ± 2.33	11.93 ± 2.05
	180	3.39 ± 0.24	63.99 ± 3.75	10.05 ± 1.27	25.95 ± 4.95
	300	3.00 ± 0.14	**69.88 ± 0.72**	18.19 ± 1.92	11.91 ± 2.47
Aged	0	2.24 ± 0.05	46.94 ± 1.35	**12.56 ± 0.09**	40.49 ± 1.44
Biochar	60	3.79 ± 0.47	**88.12 ± 7.15**	18.61 ± 1.91	0.00 ± 0.00
	180	3.76 ± 0.12	70.29 ± 0.55	8.96 ± 0.09	20.75 ± 0.48
	300	3.44 ± 0.18	75.52 ± 2.93	15.68 ± 2.21	8.81 ± 1.53

Values in bold font indicate significance at $P<0.05$

The changes in the extent of mineralization of ^{14}C glucose had a limited effect in correspond to the fresh and aged biochars amendment. The significant effects occurred only in the middle of the study (days 60 and 180), when the aged biochar amendment increased the extent of mineralization of ^{14}C glucose in grassland and loamy soils. Sandy soil exhibited no significant difference in relation to biochar amendment at any time (Table 5.3). This may be due to the soil's low levels of nutrients and C. This finding is in agreement with Jones *et al.* (2012), who reported that the addition of biochar to soil increased microbial activity in year 2 compared to year 1 and year 3. Moreover, the authors stated that adding biochar to soil only resulted in a minor impact on the turnover of ^{14}C-labelled soil organic carbon, sugars, organic and amino acids.

Table 5.3 Maximum rate (% ha^{-1}), ^{14}C extent mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for sandy soil, over 300 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extent mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	2.08 ± 0.04	44.40 ± 1.18	8.98 ± 3.64	46.61 ± 4.15
	60	2.89 ± 0.24	77.67 ± 11.19	27.95 ± 7.73	0.00 ± 0.00
	180	2.40 ± 0.15	66.92 ± 1.31	13.21 ± 3.45	19.87 ± 4.63
	300	1.64 ± 0.06	**75.79 ± 10.41**	13.71 ± 2.54	10.50 ± 8.43
Fresh	0	2.18 ± 0.10	45.97 ± 3.66	9.20 ± 0.98	44.83 ± 3.72
Biochar	60	4.23 ± 1.03	87.26 ± 11.36	4.28 ± 1.51	0.00 ± 0.00
	180	3.37 ± 0.48	72.51 ± 6.20	6.94 ± 1.42	20.55 ± 6.25
	300	2.40 ± 0.51	**73.60 ± 7.53**	16.41 ± 3.64	9.98 ± 10.23
Aged	0	2.13 ± 0.41	42.17 ± 2.13	10.7 ± 1.16	47.13 ± 2.21
Biochar	60	3.42 ± 0.46	78.27 ± 7.43	27.29 ± 1.15	0.00 ± 0.00
	180	3.54 ± 0.55	73.83 ± 4.60	8.99 ± 1.63	17.17 ± 4.44
	300	3.23 ± 0.57	**84.30 ± 5.12**	14.65 ± 1.80	1.04 ± 6.80

Values in bold font indicate significance at $P<0.05$

Similarly, Quilliam *et al.* (2012) studied the effects of the fresh and aged biochar with different rates of application. Results from their study revealed that after three years of biochar application, there was no significant effect on microbial growth, the emergence of wheat (mycorrhizal colonisation), or soil nutrients between the control and the soil with biochar. Both studies agreed that adding biochars to highly fertile and productive soils, especially in temperate climates, may contribute to the least significant effects of biochars (Jones *et al.*, 2012; Quilliam *et al.*, 2012).

The ^{14}C glucose uptake by microbial biomass did not show many differences either. The incorporation of ^{14}C glucose into the microbial biomass in soil amended with biochar also did not show a clear trend, and differences were generally insignificant

(P>0.05). The results obtained in this study showed that ^{14}C glucose mineralization was consistently higher than that incorporated into the microbial biomass in all treatments. In this study, adding fresh and aged biochars to soil increased the amount of carbon in the soils, and this may affect the microbial activity. Therefore, mineralization of ^{14}C glucose increased after increasing carbon content in the soil amended with biochar, which resulted in a decrease of ^{14}C uptake by microorganisms.

In contrast with these findings and the previous studies (Jones *et al.*, 2012; Quilliam *et al.*, 2012), Wardle *et al.* (2008) and Kolb *et al.* (2009) found positive effects from biochar amendment, on the biological aspect of the soils. Kolb *et al.* (2009) investigated the correlation between microbial activity and biomass following the addition of charcoal to four contrasting temperate soils. The results suggested that charcoal amendment significantly increased microbial activity and biomass with an increasing application rate in all soils studied. Wardle *et al.* (2008) found that the addition of charcoal in the Boreal Forest stimulated microbial activity in the soil.

5.3.2 Ammonium leaching in grassland, loamy and sandy soils

The concentration of ammonium in all soils leachate increased over time (P<0.05) (Figure 5.1). In this study, both biochars (fresh and aged) significantly reduced ammonium leaching in all three soils. In the first leaching event, there was no significant difference (P>0.05) in the concentration of ammonium between the treatments with and without biochar amendment. In the final leaching, the concentration of ammonium in grassland soil had peaked and biochar treatments significantly (P<0.05) reduced ammonium leaching from 0.34 mg/L (control) to 0.06

mg/L (fresh biochar) and 0.14 mg/L (aged biochar) (Figure 5.1). The ammonium concentration was also reduced in loamy at the final leaching process from 0.25 mg/L to 0.09 mg/L (fresh biochar) and 0.15 mg/L (aged biochar) (Figure 5.1). Sandy soil also reduced ($P<0.05$) ammonium leaching at the end of the leaching process from 0.88 mg/L to 0.27 mg/L (aged biochar) and 0.13 mg/L (fresh biochar), (Figure 5.1).

The results are in agreement with Yao *et al.* (2012), where the ability of nine biochars studied to adsorb ammonium ranged from 1.8% to 15.7%. Singh *et al.* (2010) reported that adding poultry manure biochar reduced ammonium leaching by about 55% to 93% in an Alfisol soil and 87% to 94% in a Vertisol soil. Ding *et al.* (2010) also observed that biochar sorbed ammonium by cation exchange, and that within 70 days, by adding 0.5% biochar to soil; biochar was found to retain the vertical movement of ammonium from the soil layers.

Most of the studies found that the ability of biochar to adsorb cations is due to the negative charges on the biochar's surface (Baldock and Smernik, 2002; Glaser *et al.*, 2002; Novak *et al.*, 2009; Mukherjee *et al.*, 2011). Furthermore, Clough and Condron (2010) and Zheng *et al.* (2013) reported that acid functional groups, for example (carboxyl and hydroxyl) on the biochar's surface could hold ammonium ions through cations exchange.

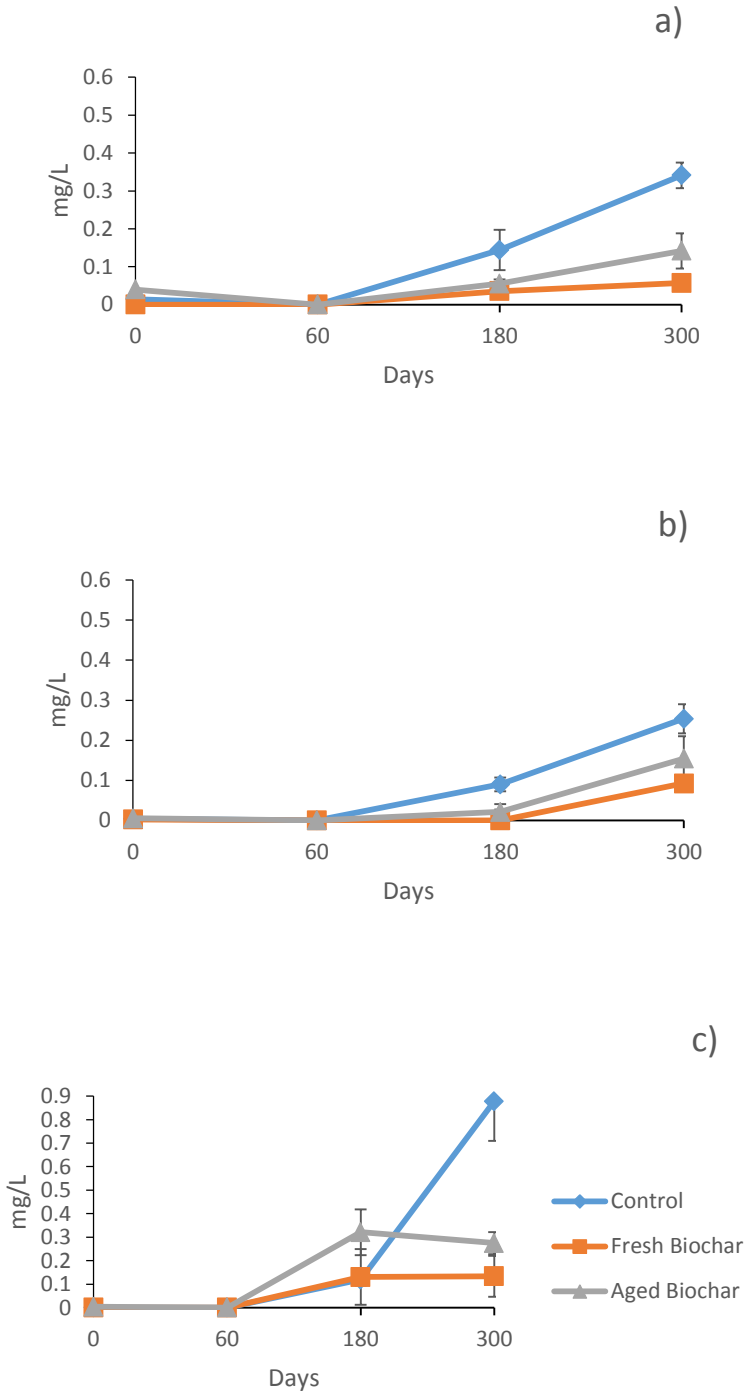

Figure 5.1 Amount of ammonium in the leachate of three soils a) grassland b) loamy and c) sandy amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

5.3.3 Nitrate leaching in grassland, loamy and sandy soils

The concentration of nitrate in the leachate fluctuated over time, and again the pattern of nitrate leaching occurred in a relatively similar manner in all soil types studied (Figure 5.2). Amending soils with biochar did not show a clear pattern, and also the differences were generally insignificant (P>0.05).

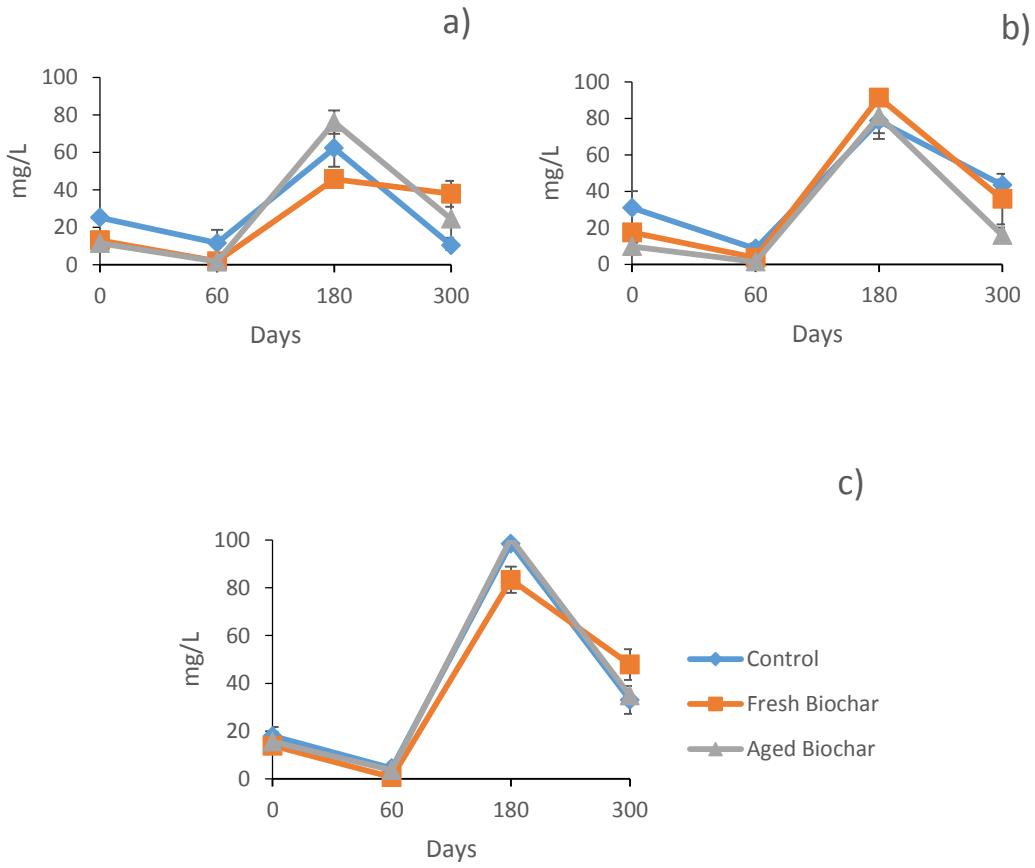

Figure 5.2 Amount of nitrate in the leachate of three soils a) grassland b) loamy and c) sandy amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

The concentration of nitrate was low in the first leaching event, then rose in the middle and finally reduced at the end of the leaching process (Figure 5.2). This suggests that nitrification occurred in the middle of the leaching event and the inhibition of nitrification took place at the end of the leaching process, though the mechanisms by which this happens are unclear. The results can also be considered in connection with the mineralization of ^{14}C glucose in the soil. The mineralization rate increased in grassland and loamy soils during the middle of the study. This is possibly due to the addition of biochar that could stimulate microbial activity in the soil, thus enhancing the soil's fertility through mineralization and nitrification by the microbes.

Biochar application had a minor effect on nitrate sorption. Biochar only reduced nitrate leaching on days 0 and 60 in grassland and loamy soil, whilst in sandy soil biochar had no effect at all (Figure 5.2). These findings were in agreement with Yao *et al.* (2012), where most of the thirteen biochars studied showed very limited, or no, ability to hold nitrate in the soil. Furthermore, the results were also in line with Alling *et al.* (2014), where the nitrate concentrations in the soil leachate increased on the addition of 5% and 10% biochar. The authors speculated that the biochar itself may contribute to the release of nitrate and also enhance the nitrification process in the soil. In contrast to these findings, a number of studies (Novak *et al.*, 2010; Knowles *et al.*, 2011; Zheng *et al.*, 2013) reported that adding biochars to soil reduced nitrate leaching. For example, Knowles *et al.* (2011) stated that amending soil with biochar plus biosolids reduced nitrate leaching to the levels of untreated soil or below. The mechanisms through which this occurred are not yet fully understood.

5.3.4 Phosphate leaching in grassland, loamy and sandy soils

Unlike ammonium and nitrate, the trend of phosphate leaching was slightly different among the three types of soils studied. The concentration of phosphate reduced over time (Figure 5.3). Biochar amendment did not show a clear trend of phosphate adsorption in the soil (see Figure 5.3). The only exception was the types of soils. For example, in grassland soil the presence of biochar made no difference to the leaching of phosphate compared to soil alone. On the other hand, loamy and sandy soils showed slightly similar patterns (Figure 5.3). The only exception was in the types of biochar amendment used: in loamy soil, the aged biochar increased the concentration of phosphate leachate in the middle of the study, whereas in sandy soil the concentration of phosphate in the aged biochar amendment decreased over time. The fresh biochar amendment led to increased phosphate leaching in sandy soil at all times especially at time 0 (Figure 5.3).

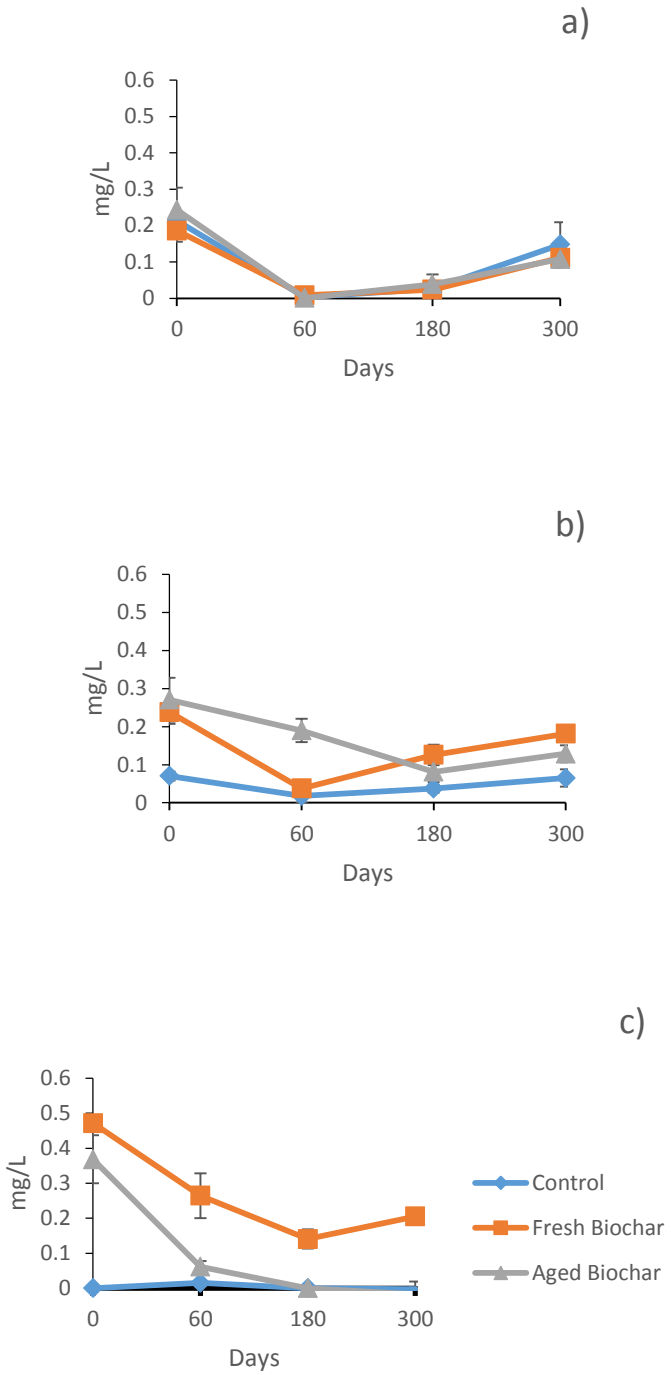

Figure 5.3 Amount of phosphate in the leachate of three soils a) grassland b) loamy and c) sandy amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

These results were in line with the findings in Yao *et al.* (2012), where eight of the biochars studied released phosphate into the solution, of which three released more phosphate into the leachate. In addition, Alling *et al.* (2014) found that adding 5% and 10% of biochar to soil did not significantly change the sorption of phosphate in comparison with the absence of biochar in the soil. The authors speculated that, because the high pH of the biochar resulted in a decrease in its positively-charged surface, the availability of phosphate in the solution increased.

The high pH in biochar is due to the alkaline substances that exist in biochar, which ultimately would increase the soil's pH. In acidic soil, phosphate can be attached with other substances and become unavailable to crops. Biochar which act as a lime can reduce iron and aluminium that was previously attached with phosphate, thereby phosphate becomes available in the soil and increase in the presence of biochar (Cui *et al.*, 2011; Biederman and Harpole, 2013). In the current study, the greater amount of phosphate leachate in soils treated with the fresh and the aged biochars may be due to the high pH content in the biochar itself (pH 9.05) (Table 3.2) that might contribute to the high phosphate in the leachate. On top of that, other researchers also claimed that biochars can only adsorb a very small amount of phosphate in the soils due to the low anion exchange capacity (Singh *et al.*, 2010).

5.3.5 Leachate and soil pH

In this study, amending soil with 2% of fresh and aged HW biochars by weight had a small effect on the pH of the leachate. Biochar treatment significantly increased leachate pH in grassland and loamy soils, but not in sandy soil (Figure 5.4). Leachate

pH also decreased over time ($P<0.05$) see Figure 5.4. The reduction of pH was due to the oxidation process of biochar which released acid functional groups for example, carboxyl and hydroxyl and which consequently reduced the pH (Liu and Zhang, 2012). The decrease in leachate pH could also be connected with the leaching results, where the concentration of nitrate was increased especially on day 180 see Figure 5.2. The increase in nitrate suggested that a nitrification process occurred, which could reduce the pH due to microbial activity (Fageria and Baligar, 2008). This finding reflected Inal *et al.* (2015), where the authors found that the addition of poultry manure biochar significantly reduced the pH from 7.8 to 7.6. Karer *et al.* (2013) also reported that adding biochar to Chernozem did not increase the pH, but also reduced pH significantly when increasing the rate of biochar application plus N fertilizer.

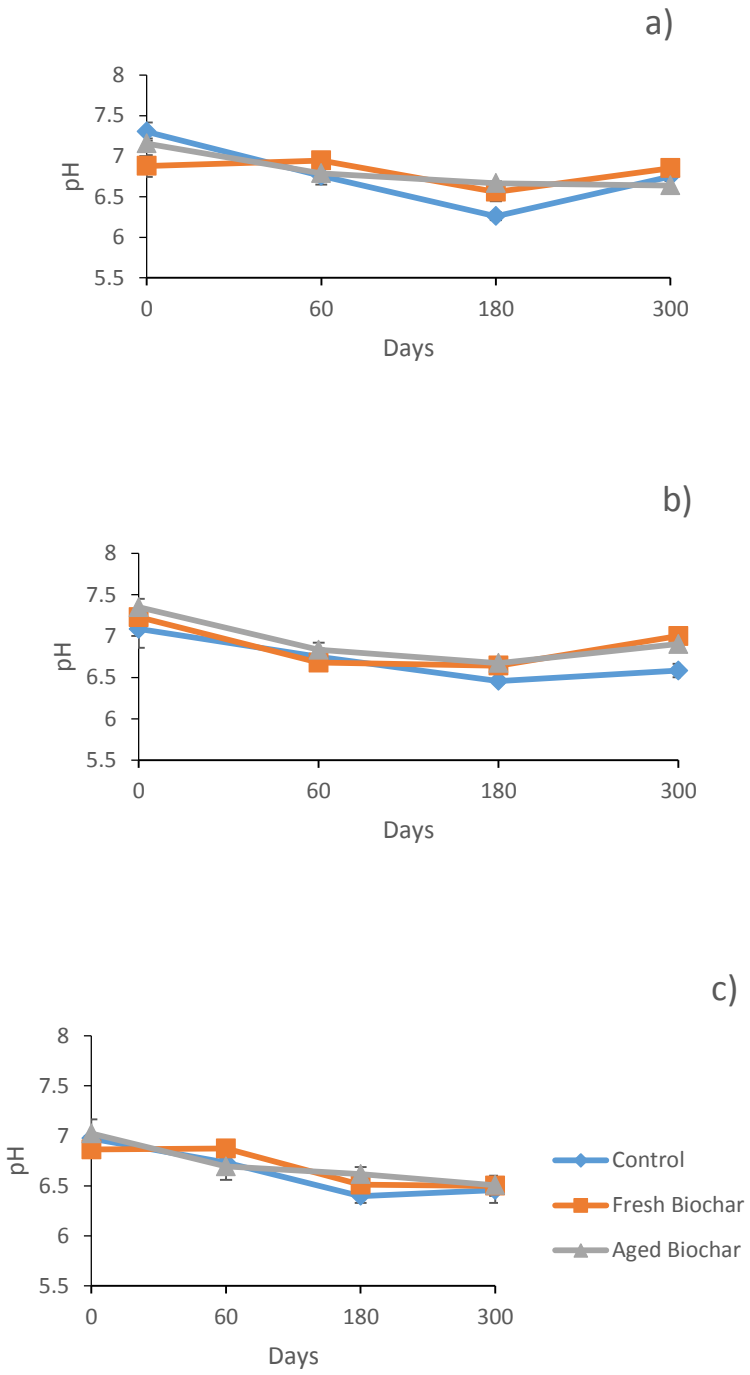

Figure 5.4 pH in the leachate of three soils a) grassland b) loamy and c) sandy amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

Application of fresh and aged biochars significantly (P<0.05) increased soil pH over time. Figure 5.5 shows that all soils pH without biochar treatments decreased over time. This is possibly due to the high pH of biochar used in this study see Table 3.2. The increasing of soil pH after adding biochar was also in line with the findings from previous research (Novak *et al.*, 2009; Masulili *et al.*, 2010; Peng *et al.*, 2011). Therefore, the biochar had a liming effect, and the potential to increase soil pH and alleviate acidic soil.

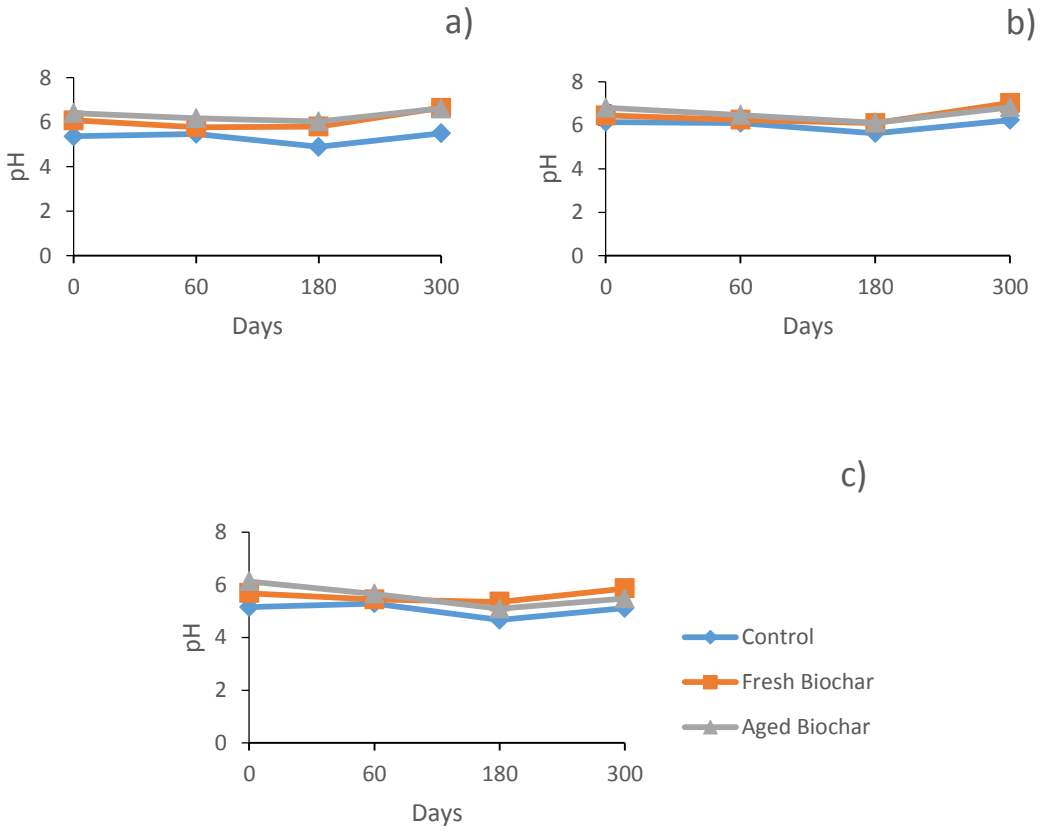

Figure 5.5 pH in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

5.3.6 Cation exchange capacity

Although most of the studies showed positive results for the CEC (Liang *et al.*, 2006; Masulili *et al.*, 2010; Van Zwieten *et al.*, 2010; Hale *et al.*, 2011; Peng *et al.*, 2011), amending soil with biochar in this study had little effect on the CEC. Results showed no significant difference ($P>0.05$) between biochar treatments and soil alone (without biochar) on days 0, 60 and 180, although the aged biochar significantly ($P<0.05$) increased CEC at the end of the study in grassland soil (Figure 5.6). CEC also increased at the end of the incubation time in the loamy and sandy soils. However, the increase was not significant ($P>0.05$) (Figure 5.6). The results can also be considered in connection with the ammonium leaching, where both biochars significantly ($P<0.05$) reduced ammonium leaching in all soils, especially after 300 d.

The finding that biochar amendment had minimal effect matched that of other research, such as Karer *et al.* (2013), who found that adding biochar to soils did not alter the CEC of the soil. The mechanisms of how CEC decreased, however, were not clearly stated in their study. The findings are also consistent with those of Novak *et al.* (2009) and Kloss *et al.* (2014) who reported that the addition of biochars to soil did not affect the CEC of the soils. Both studies agreed that the small effect of the biochar was due to the way in which biochar is produced. For example, high temperature biochar production reduced the CEC of the soil, because biochar produced at high temperatures has low surface negative charges. This resulted in the low oxidation of carboxylic and phenolic groups, which would ultimately reduce the soils' CEC. Therefore, the lack of a significant biochar impact on the CEC, especially at the

beginning of this experiment, may be due to the temperature during biochar production.

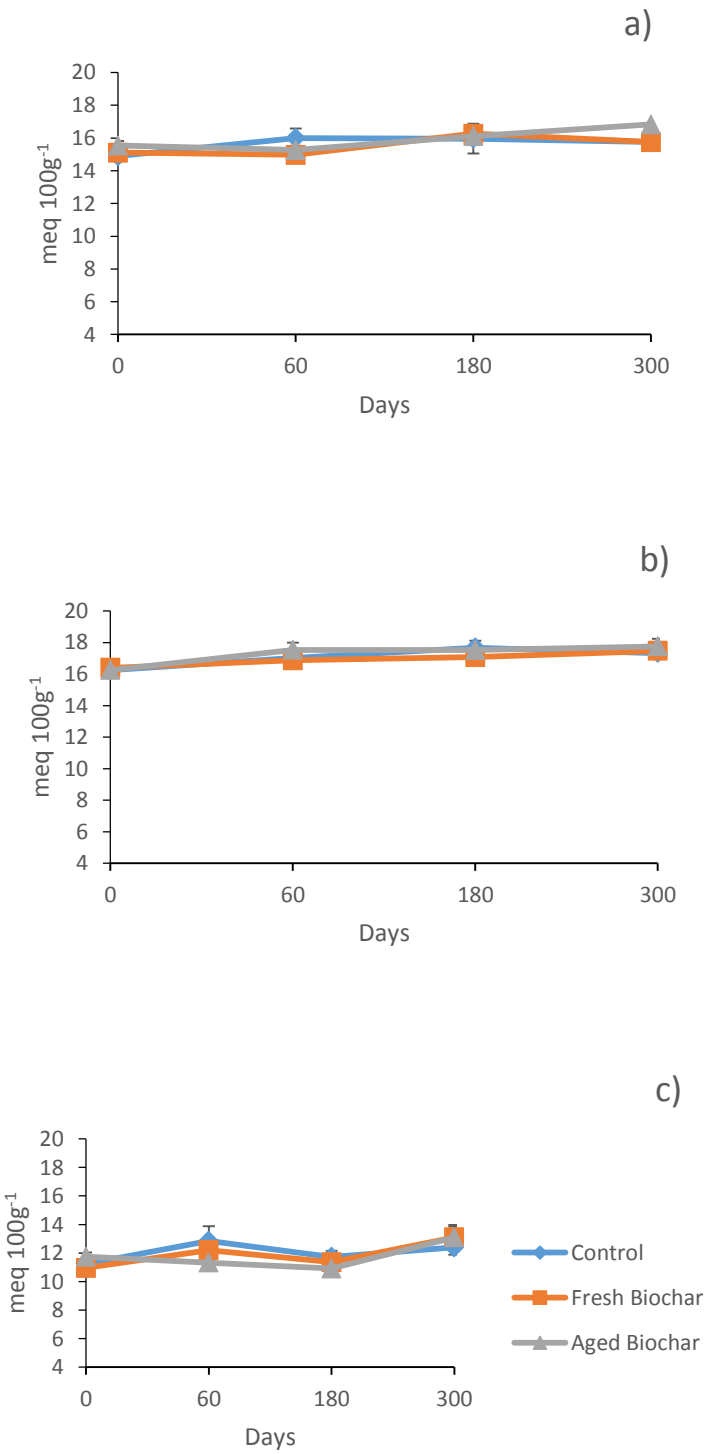

Figure 5.6 CEC in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

5.3.7 Carbon, nitrogen and phosphate in soils

Adding fresh and aged HW biochar to grassland, arable loam and arable sandy soils increased the carbon content. With high carbon content in the biochar, the incorporation of biochar into soil increased the carbon content in each of the soils. For example, nearly twice as much additional carbon was found in grassland, loamy and sandy soils treated with the fresh and aged HW biochar (Figure 5.7). Sandy soil showed the lowest carbon content in the soil without biochar amendment, in comparison with the grassland and loamy soils (Figure 5.7). The biochars used in this study had a high carbon content (72.14%) and low nitrogen content (0.24%), as shown in Table 3.2 page 38. This indicated that the value of the C/N ratio was very high. The results can also be considered alongside the results of nitrate leaching, where the nitrification process decreased especially at the end of the leaching event, suggesting that N immobilization may have occurred at this stage.

Although biochar increased the carbon content in all soils at all the incubation times, the C content in the soil also decreased over time (Figure 5.7). The reduction of C content was due to decomposition of organic C in the soil and biochar. Reapplication of biochar could enable the effects of C sequestration in the soil to last longer. Decrease in organic C following soil organic amendment has also been reported by Sukartono *et al.* (2011). The researchers found that the organic C content in the soil amended with cattle dung reduced 18% faster than in the soil amended with biochar. They speculated that the slow reduction of C in the soil was due to the resistance of biochar's aromatic C structure, which could slow the decomposition of C. Because of this characteristic, biochar would potentially sequester C in the soil.

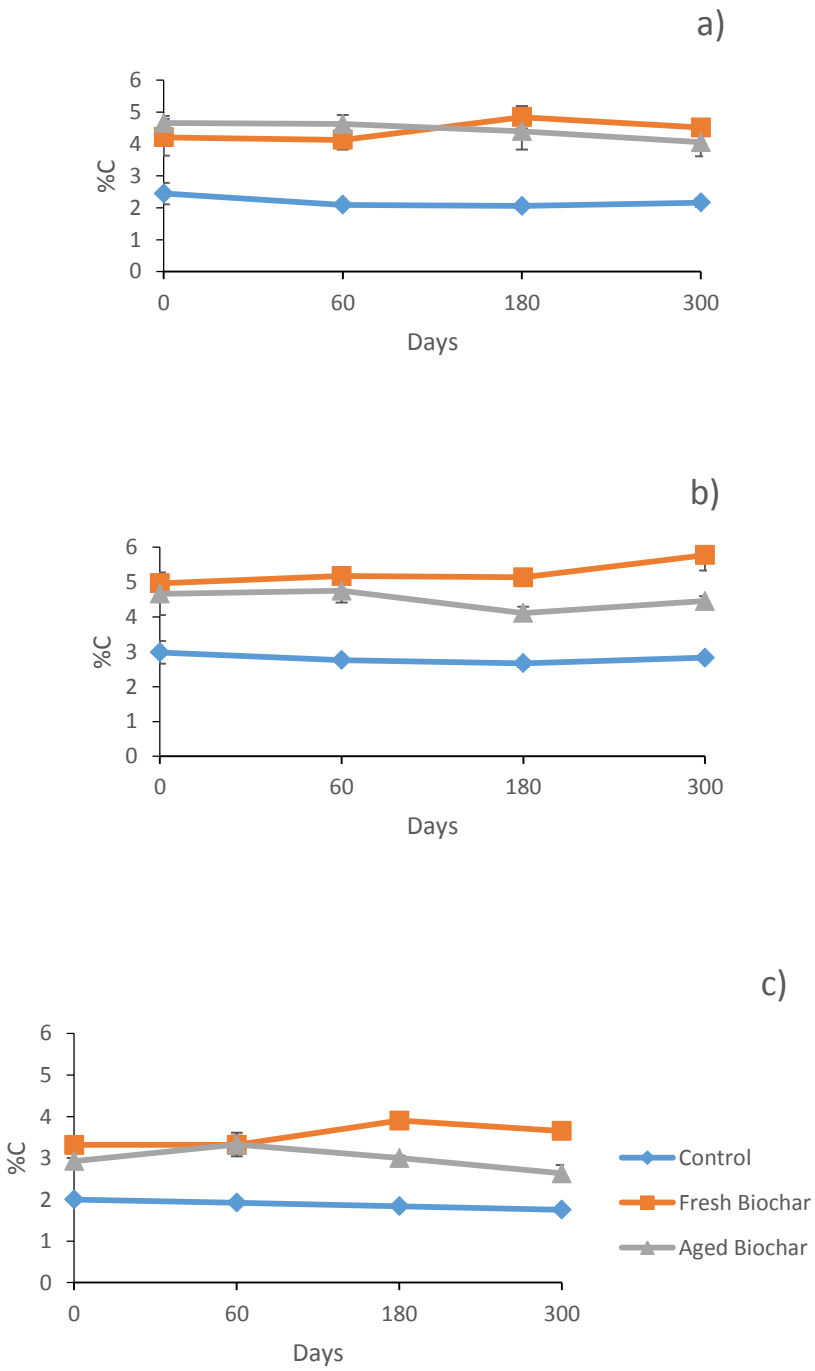

Figure 5.7 Carbon content in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

Little effect could be seen upon nitrogen when adding biochar to soils. Only on day 60, biochar treatments increased N significantly ($P<0.05$) in grassland and loamy soils (Figure 5.8). Even though biochar treatments in sandy soil increased N from 0.16% (control) to 0.23% and 0.24% (fresh and aged biochar) on the same day, but these changes were not significant ($P>0.05$).

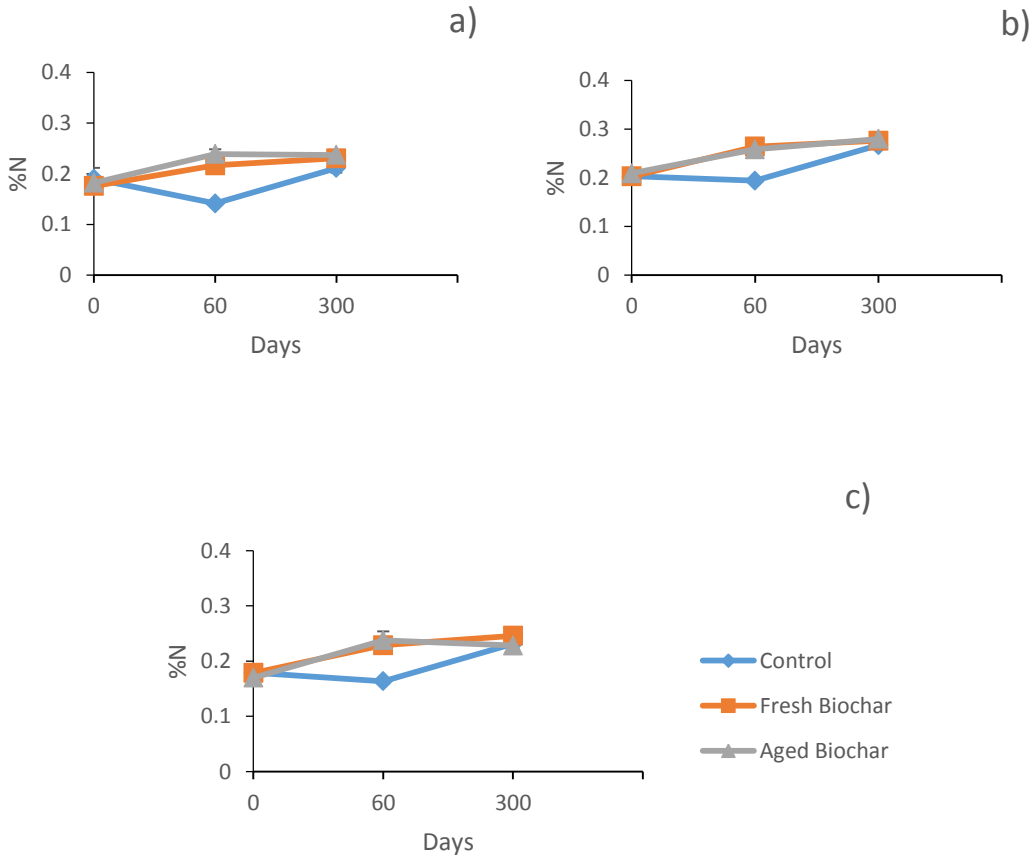

Figure 5.8 Nitrogen content in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

Overall, on days 0 and 300, the phosphate content in loamy soil was higher than that in grassland and sandy soils (Table 5.4). Results from Table 5.4 also show that there was no significant effect ($P>0.05$) when adding fresh and aged biochar to grassland, loamy and sandy soils at any time.

Table 5.4 Phosphate content (Mg g^{-1}) in grassland, loamy and sandy soils amended with and without fresh and aged biochar (0 and 300 d). Error bars are SEM (n=3).

Soil	Treatment	Day 0	Day 300
Grassland	Control	1.48 ± 0.15	0.92 ± 0.03
	Fresh Biochar	1.40 ± 0.13	0.92 ± 0.02
	Aged Biochar	1.22 ± 0.04	0.93 ± 0.03
Loamy	Control	1.74 ± 0.05	1.47 ± 0.02
	Fresh Biochar	1.76 ± 0.06	1.44 ± 0.04
	Aged Biochar	1.77 ± 0.03	1.66 ± 0.13
Sandy	Control	1.28 ± 0.01	0.99 ± 0.03
	Fresh Biochar	1.23 ± 0.03	0.97 ± 0.03
	Aged Biochar	1.32 ± 0.03	0.93 ± 0.01

However, other findings show that the addition of biochar significantly increased P in the soil (Glaser *et al.*, 2002; Chan *et al.*, 2008a; Gaskin *et al.*, 2010; Hossain *et al.*, 2010; Kloss *et al.*, 2014). However, the mechanisms behind this process are poorly understood. The P content measured in the leachate samples was opposite to that found in the soil, whilst the concentration of phosphate leaching was higher in the biochar treatments than in the control. Nevertheless, biochar amendment had no effect on the soil P content at any time in any of the soils. According to Kloss *et al.* (2012), biochars may contribute soluble nutrients such as P, K and S, thus more P may be lost via the leaching. Other researchers also found that the incorporation of biochar in soils increased the concentration of soluble P (Liu *et al.*, 2012), as well as increased plant-available P, Zn, Cu and Mn concentrations (Inal *et al.*, 2015).

5.3.8 Effect of biochar on aggregate stability of soils

Figures 5.9 to 5.11 show three different aggregate stability treatments - fast wetting (FM), slow wetting (SW) and mechanical (M) - in three types of soil, with and without biochar amendment over time. In the FW treatment, sandy soil was more stable than the other two soils. Sandy soil with no biochar was stable over time, but with biochar amendment the mean weight diameter (MWD) values fluctuated (Figure 5.9). On the other hand, grassland and loamy soils were less stable than sandy soil, and over time there were no changes. Biochar amendment in all the types of soil had no effect on the FW treatment at all.

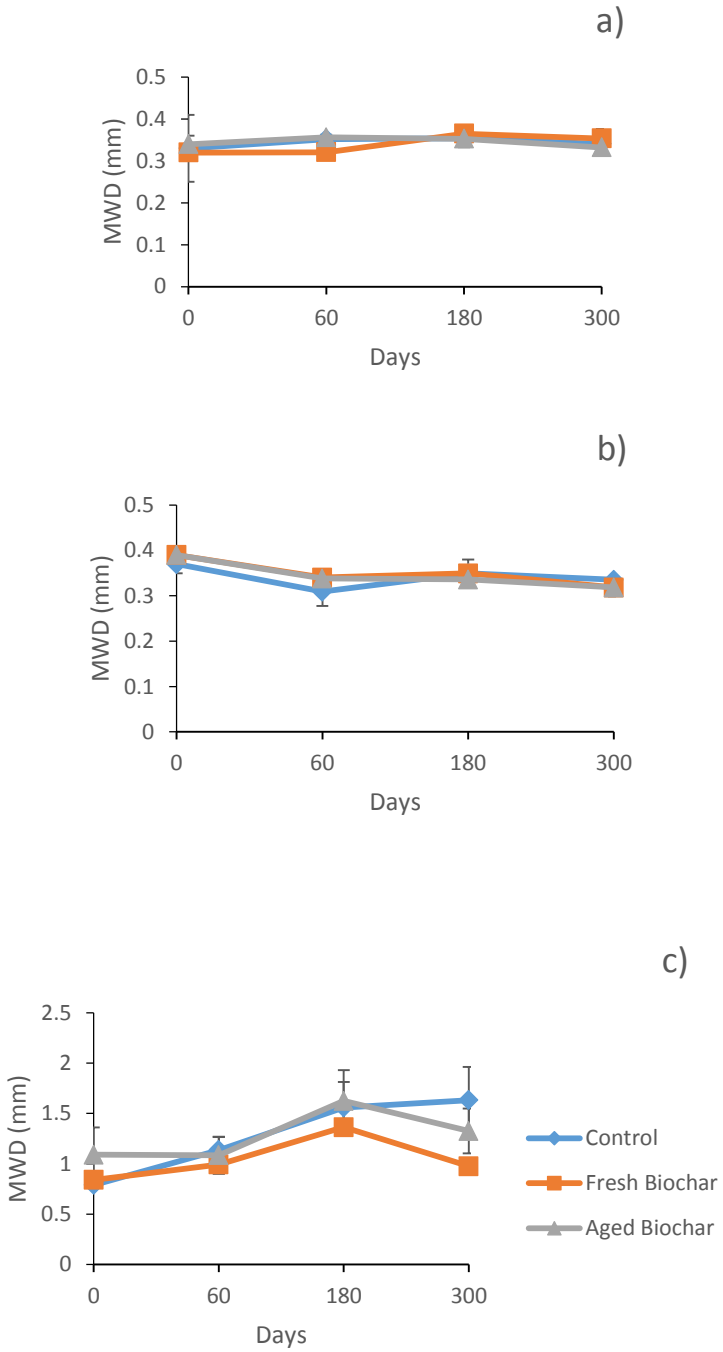

Figure 5.9 MWD values for FW treatment in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

For the SW treatment, the stability of the three types of soil studied was quite similar. Biochar treatments had a little effect on the stability of the soils, see Figure 5.10.

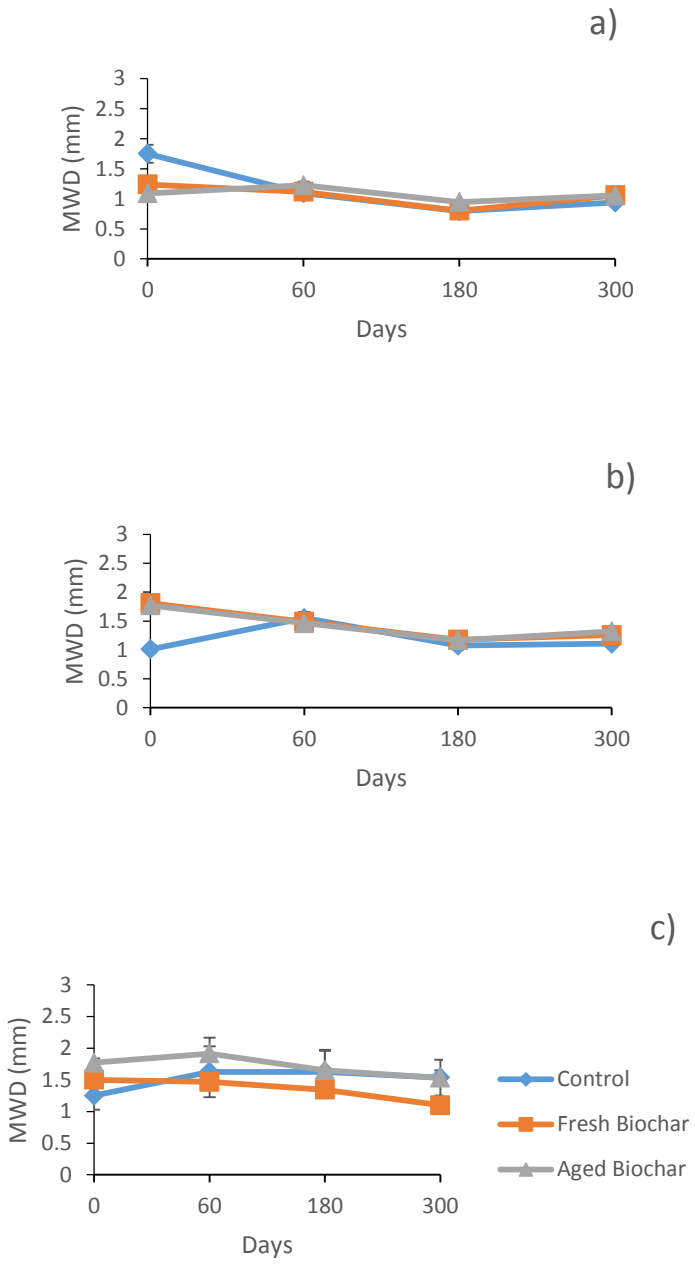

Figure 5.10 MWD values for SW treatment in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

Unlike FW and SW treatments, M treatment indicated a different pattern in the aggregation of the soils. In this treatment, grassland and loamy soils were more stable than sandy soil. In fact, as time went by the stability of the soils increased under most of the treatments, except the fresh biochar treatment in grassland and sandy soils (see Figure 5.11). In terms of biochar effect, only on day 0 did the fresh biochar amendment in loamy soil (2.50 mm) have a significantly different effect ($P<0.05$) than the aged biochar amendment (2.23 mm).

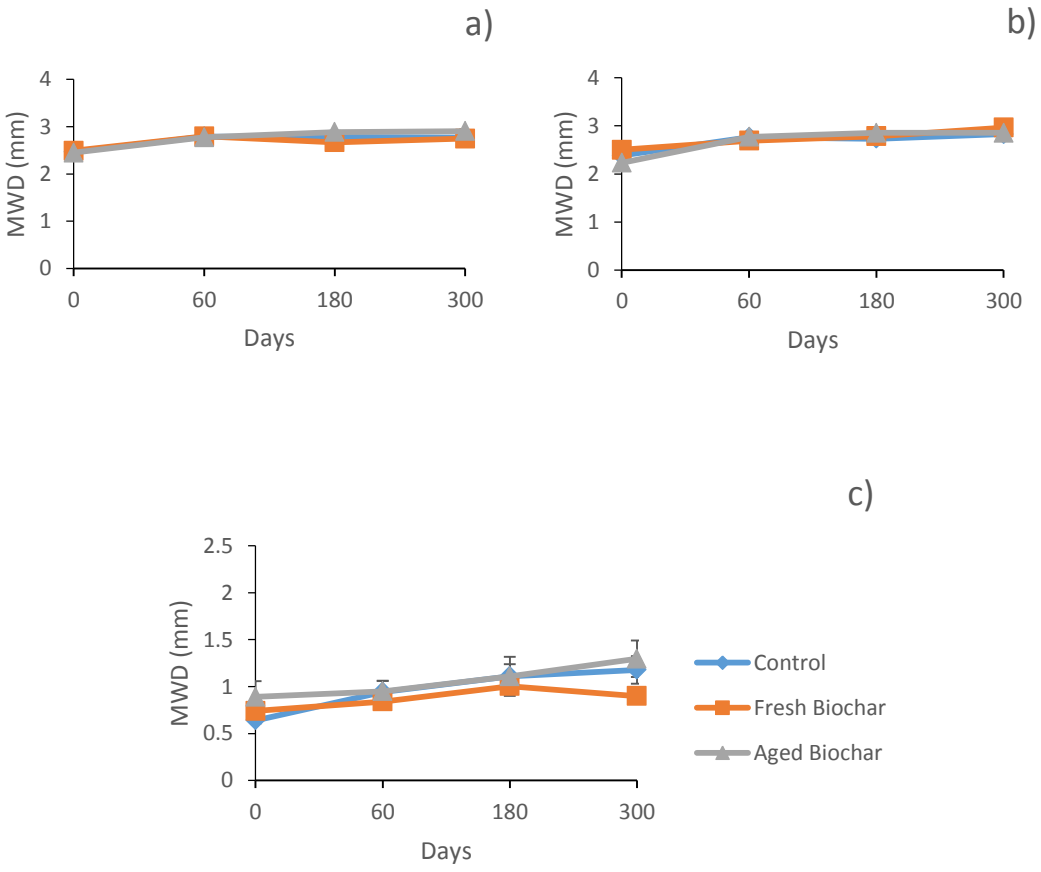

Figure 5.11 MWD values for M treatment in a) grassland b) loamy and c) sandy soils amended with and without fresh and aged biochar, over 300 d. Error bars are SEM (n=3).

As regards the soils' physical properties, results showed a varied pattern of soil aggregation in the three types of soil studied. For example, sandy soil was more stable than grassland and loamy soils in the FW treatment (Figure 5.9). For the SW treatment, the pattern of the soils' aggregation showed a similar trend (Figure 5.10). Nevertheless, grassland and loamy soils had a better aggregation than sandy soil under the M treatment (Figure 5.11). This is due to the comparatively high level of organic matter and carbon content in both soils, that can act as cementing agents to bind the soil particles, and eventually enhance soil aggregation (Ishak and Jusop, 2010).

Application of biochars to soils was observed to have a very limited effect on aggregate stability, where an aged biochar in grassland was more stable than the control on day 180 and biochar treatments was more stable in loamy soils on day 300 under the SW treatment. Only fresh biochar increased the stability of loamy soil under the M treatment at the beginning of the study, whereas there were no effects under the FW treatment at any time. The limited effects of the biochar amendment may have been due to the formation of the soil's structure from organic materials that usually take longer. Brodowski *et al.* (2006), reported that a long-term study could establish that the soils were achieving stability through the formation of micro-aggregates. The researchers speculated that black carbon could be acting as a cementing agent, improving the stability of the soil through the formation of micro-aggregates. This is because a larger amount of black carbon was found in the < 53 µm soil fraction than the > 2 mm one, in a field experiment lasting approximately 25 to 85 years.

Furthermore, results from the current study were also in line with the findings from another study (Peng *et al.*, 2011). The researchers did not find any effect of biochar amendment on soil aggregation. However, Herath *et al.* (2013) reported that adding biochar to an Alfisol and an Andisol significantly increased the stability of soils. They argued that the soil aggregation improved due to the increase in polysaccharides produced by fungi that could bind the soils' aggregates. The results also indicated that biochar produced in high temperatures in the Typic Fragiaqualf was more stable than biochar produced in low temperatures in the Typic Hapludand. However, the mechanisms of how different temperatures affect aggregate stability remained unravelled.

5.5 Conclusion

The following conclusions can be drawn from this chapter:

- Amending soil with 2% of fresh and aged biochars showed some small positive effects on the soils studied.
- The effects vary depending on the biochars and the soil types.
- The aged biochar amendment had a minor effect on the mineralization of ^{14}C glucose to CO_2 in grassland and loamy soils.
- No significant difference was observed in sandy soil in relation to biochar amendment. Due to the low content of the initial C in sandy soil, might contribute to the least effects of the mineralization of ^{14}C glucose and ^{14}C biomass uptake.
- Adding biochars to soil significantly increased carbon in all soils studied.

- The leaching results showed that biochars could adsorb nutrients depending on the types of nutrients. Biochars can hold slightly better ammonium than nitrate and phosphate, but even released more nitrate and phosphate in the solution.
- Biochar treatments increased soil pH and had some effect on the CEC, whilst only the aged biochar increased the CEC of soils at certain time points.
- Biochar had limited effect on soil aggregate stability.

CHAPTER 6

The Effect of Biochar Particle Size and Application Rate on Soil Functioning in Two Soils of Contrasting Fertility

6.1 Introduction

Biochar has been viewed by many authors as a soil conditioner. As well as improving soil properties, such as water retention (Karer *et al.*, 2013), cation exchange capacity (Liang *et al.*, 2006) and soil carbon and pH (Uzoma *et al.*, 2011; Cornelissen *et al.*, 2013), it also reduces nutrient leaching (Angst *et al.*, 2013). The addition of biochar to soil affects the biological aspects of the soils. For example, increases and decreases in the abundance and activity of microorganisms are dependent on the release or sorption of organic molecules from the biochar (Lehmann *et al.*, 2011). In addition, biochar can also influence the abundance of mycorrhizal, thus enhancing the uptake of nutrients by plants (Warnock *et al.*, 2007). However, biochar in this study (Chapters 4 and 5) and others (Jones *et al.*, 2012; Quilliam *et al.*, 2012; Karer *et al.*, 2013) has not been as effective. A number of reasons for the ineffectiveness of biochar have been suggested: 1) the presence of organic contaminants and heavy metals in biochar (Bridle and Pritchard, 2004; Chan and Xu, 2009; Wisnubroto *et al.*, 2011), 2) the recalcitrant C in biochar resists microbial decomposition (Quilliam *et al.*, 2013a), 3) the different feedstock and production of biochar, and 4) the types of soil. There is also some evidence that biochar has a greater effect in less fertile soils (Kolb *et al.*, 2009; Jones *et al.*, 2012; Anders *et al.*, 2013).

The results from the previous chapters (Chapters 4 and 5) show that adding 2% of biochar by weight in the tropical and temperate soils increased the soil carbon and pH and reduced ammonium leaching. However, biochar had little effect on cation exchange capacity (Novak *et al.*, 2009; Méndez *et al.*, 2012; Kloss *et al.*, 2014), inconsistent effect on nitrate and phosphate leaching (Alling *et al.*, 2014); and had no effect on aggregate stability (Peng *et al.*, 2011), or nitrogen (Jones *et al.*, 2012; Wang *et al.*, 2015a) and phosphate content in the soil. Nor did it affect the microbial biomass (Bruun *et al.*, 2008; Dempster *et al.*, 2010; Zhang *et al.*, 2014a) or ^{14}C glucose mineralization (Zhang *et al.*, 2014a). Possible explanations are that the particle size of biochar used was too coarse (Sigua *et al.*, 2014) (< 2mm and < 5mm) and the application rate was low (2%) (Quilliam *et al.*, 2012). Insufficient surface area and lower application rate may explain the lack of positive results obtained from the previous chapters. Therefore, to explore this further in this chapter, the effects of particle size and application rates of biochar on intensive arable and extensive grassland soils are considered further by looking at various particle sizes (2mm, 1mm, 0.5mm and 0.1mm) and application rates (2% and 5%). The parameters used to examine the effectiveness of biochar are based on the most significant results from the previous findings, for example soil pH, carbon and nutrient leaching, as mentioned earlier. Although the findings from Chapters 4 and 5 also indicate that the biochar had a very limited effect on the biological properties, other authors did find an effect (Kolb *et al.*, 2009; Lehmann *et al.*, 2011; Zhang *et al.*, 2014b). Therefore, in this chapter the effect of biochar on the biological properties was tested again to examine whether the addition of biochar with larger surface areas would give a different result for the soils.

This chapter tests the hypotheses that: 1) biochar will make a greater improvement to the biological and chemical properties of the nutrient poor soil than the nutrient rich soil, 2) biochar with a smaller particles size will retain more nutrients than the larger particles size, and 3) a higher application rate of biochar will improve the fertility of both soils than a lower application rate.

It is expected that biochar addition will benefit nutrient poor soil, because Cornelissen *et al.* (2013) found that biochar increased the nutrient and water retention in the soil with the lowest fertility. In addition, Jay *et al.* (2015) found that adding biochar to fertile soil had no effect on the growth of three different crops. The authors speculated that well managed fertile soil supplied enough nutrients for the crops. Jones *et al.* (2012) and Quilliam *et al.* (2012) also stated that biochar often benefits poor quality soil. Furthermore, it is expected that ammonium leaching will decrease after the addition of finer particles of biochar to the soil. This is because finer biochar particles will have large surface areas.

6.2 Materials and methods

In this study, two Brown Earths soils with same classification, as well as parent material from Penrith, Cumbria were used. They were chosen to represent different levels of management and contrasting nutrient status. The first soil was from an area of agriculture that contained an oil seed rape crop, which was well managed and fertile. The second soil used in this study was taken from extensive grassland, which was unmanaged and unfertilized (for at least 50 years). This soil is also known to be nutrient poor soil. Both soils had the same texture, which was sandy clay loam.

The soils were collected from the field at a depth of approximately 10 – 15cm. In the laboratory, the soils were sieved through a 5mm mesh, and mixed with 2% and 5% of HW biochar by weight. The HW biochar particle sizes used were 2mm, 1mm, 0.5mm and 0.1mm. Soils without HW biochar addition acted as a control. All of the samples were kept in jars and incubated for 30 days. Finally, prior to the analysis, the soils were dried out and sieved using a 2mm mesh to provide soil aggregate suitable for the soil analysis (Kandeler, 2007). The physical and chemical properties of the soils are displayed in Table 6.1.

Table 6.1 Physical and chemical properties of two soils used in the study.

Soils	**Fertilized**	**Unfertilized**
Clay	28.29	28.12
% Silt	11.96	9.00
% Sand	59.75	62.88
Texture	Sandy Clay Loam	Sandy Clay Loam
% Carbon	2.14	3.40
% Nitrogen	0.19	0.19
C/N Ratio	11.26	17.89
pH	6.16	6.15

The experiments were divided into two parts, in order to examine the biological and chemical properties of the soils. Details of all of the methodologies can be found in Sections 3.3.1-15. With regard to the biological effects, the methodology employed to carry out the experiments was substrate induced respiration, whereby 3 mM of glucose solution (10ml) was added to the soil samples, which had a radioactivity of 1051 Bq on days 0 and 30 (incubation time). For fumigation and non-fumigation extraction 0.5 M potassium sulphate was used. C-14 glucose associated activity

remaining in soil was determined via combustion (3 minutes) on a sample oxidiser, (Packard, Model 307).

With regard to the chemical properties, the total carbon (C) and nitrogen (N) were determined by dry combustion and measured with an elemental analyser (Elementar Vario EL). The ammonium, phosphate and nitrate concentration in the soil leachate were measured with a Bran + Luebbe autoanalyser 3. pH was measured using a pH meter, model PHM 220, calibrated using buffers pH 7.0 and 4.0. The soil moisture content was determined through oven drying at $105^{O}C$ for 24h and particle size analysis was determined by the hydrometer method.

For the statistical analyses, the mean values of maximum rate, ^{14}C mineralization, ^{14}C biomass, total carbon, total nitrogen, phosphate and nutrients concentration in the leachate between the treatments, sorted by incubation day, were tested using a one-way analysis of variance (ANOVA) with a $P<0.05$ level of significance. Multiple mean comparisons were carried out using a Holm-Sidek procedure at $P<0.05$. For values that were not normally distributed, a non-parametric statistical test (Kruskal-Wallis) based on ranks was used. In addition, the Tukey test was applied to determine the significant differences between the treatments for non-distributed values at the $P<0.05$ level. A two-way analysis of variance (ANOVA) was performed to test the significant difference for all the parameters over time and between the soils treatments. All of the statistical tests were performed using the SigmaStat v3.5 (Systat Software Inc), apart from the two-way analysis of variance (ANOVA), which was conducted in Microsoft Excel.

6.3 Results and Discussion

6.3.1 Mineralization of ^{14}C glucose to $^{14}CO_2$ and uptake of ^{14}C glucose into microbial biomass

The extents of mineralization of ^{14}C glucose in the fertilized soil was low over the 30 day incubation period (Table 6.2). This results contrasted with the findings of the previous chapters (Chapters 4 and 5) where the extents of mineralization of ^{14}C glucose was higher during the study period. The maximum rate of mineralization did not show a consistent trend at any time. Overall, the greatest value of the maximum rate of mineralization was observed on day 0 with a 5% application rate and at 1mm particle size of biochar (1.20% h^{-1} ± 0.19) ($P<0.05$), as displayed in Table 6.2. The lowest value of the maximum rate of mineralization was observed on day 0 with the same application rates and size of biochar (5%, 0.1mm) (Table 6.2). The maximum rates in control, 2mm and 1mm treatments at 5% application rate were also significantly higher ($P<0.05$) than the 0.1mm particle size of biochar at similar rate (Table 6.2) on day 0. No significant difference in the maximum rates was found on day 30 ($P>0.05$).

Amending the fertilized soil with 2% of HW biochar in different particle sizes also had no significant effect ($P>0.05$) compared with the higher application rate of HW biochar at any time. However, the finest particle size (0.1mm) significantly increased ($P<0.05$) the mineralization of ^{14}C glucose at the 5% application rate on the last day of incubation, as shown in Table 6.2. For the ^{14}C uptake into the microbial biomass, the results show that at both application rates (2% and 5%) biochar decreased the microbial biomass ($P<0.05$) compared with the untreated soil at all times (Table 6.2).

Even though the biomass at 2mm size on day 30 higher than other treatments, however it was not significantly different (P>0.05).

Table 6.2 Maximum rate (% h^{-1}), ^{14}C extent of mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for the fertilized soil, over 30 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extents mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	**1.01 ± 0.10**	12.02 ± 1.23	93.22 ± 11.34	0.00 ± 0.00
	30	0.76 ± 0.12	10.34 ± 0.84	47.67 ± 5.45	37.78 ± 7.78
2% (2mm)	0	1.05 ± 0.32	11.30 ± 1.18	**72.25 ± 6.55**	11.61 ± 11.60
	30	1.10 ± 0.07	12.89 ± 0.96	61.61 ± 12.30	20.33 ± 15.47
2% (1mm)	0	0.82 ± 0.16	9.80 ± 0.40	**73.49 ± 11.18**	13.08 ± 13.58
	30	0.73 ± 0.03	12.78 ± 0.64	**39.63 ± 2.23**	42.75 ± 6.79
2% (0.5mm)	0	1.19 ± 0.11	9.66 ± 0.22	**45.65 ± 0.86**	41.28 ± 3.39
	30	1.07 ± 0.27	13.77 ± 1.72	**46.90 ± 6.51**	33.17 ± 11.27
2% (0.1mm)	0	1.07 ± 0.13	9.68 ± 0.24	**48.92 ± 4.70**	37.96 ± 7.72
	30	1.02 ± 0.10	13.79 ± 1.41	**43.48 ± 7.70**	36.84 ± 8.81
5% (2mm)	0	**0.99 ± 0.08**	9.84 ± 0.47	**23.54 ± 1.57**	62.91 ± 2.33
	30	0.97 ± 0.18	12.31 ± 1.15	**29.04 ± 3.06**	53.50 ± 6.80
5% (1mm)	0	**1.20 ± 0.19**	9.66 ± 0.18	**27.17 ± 4.80**	59.80 ± 3.11
	30	0.86 ± 0.08	13.09 ± 1.13	**41.19 ± 2.21**	40.33 ± 2.92
5% (0.5mm)	0	0.76 ± 0.03	8.08 ± 0.22	**31.65 ± 7.49**	57.39 ± 8.45
	30	0.82 ± 0.12	13.32 ± 0.5	**37.08 ± 4.50**	44.70 ± 7.56
5% (0.1mm)	0	0.47 ± 0.02	7.97 ± 0.94	**30.05 ± 2.53**	58.47 ± 3.37
	30	0.85 ± 0.09	**16.15 ± 0.52**	**42.65 ± 2.05**	35.35 ± 7.59

Values in bold font indicate significance at P<0.05

The unfertilized soil also exhibited a similar trend to the fertilized soil for the mineralization of ^{14}C glucose (Table 6.3). In this soil, the percentage of the extent of mineralization of ^{14}C glucose was generally lower than the ^{14}C biomass uptake. The results from Table 6.3 also show that the finest particle size (0.1mm) increased the ^{14}C mineralization of glucose (P<0.05) after 30 d at the higher application rate.

The maximum rate of mineralization in the unfertilized soil showed a consistent trend where the maximum rate increased over time. For example, the maximum rate of mineralization on days 0 and 30 ranged from 0.36 to 0.95 (% h^{-1}) and 1.26 to 2.12 (% h^{-1}), respectively (Table 6.3). Also, the maximum rate in control treatment on day 0 was significantly higher ($P<0.05$) than at 5% application rate with 0.1mm particle size of biochar (Table 6.3). As in the fertilized soil, the biomass uptake of ^{14}C glucose also decreased at the 5% application rate of biochar in comparison with the untreated soil ($P<0.05$). No significant effect of the biomass uptake was observed at the lower application rate at any time ($P>0.05$).

Table 6.3 Maximum rate (% h^{-1}), ^{14}C extent of mineralization (%), ^{14}C biomass uptake (%) and ^{14}C activity remaining (%) for the unfertilized soil, over 30 d. Error bars are SEM (n=3).

Treatment	**Day**	**Maximum rate (% h^{-1})**	**^{14}C extents mineralization (%)**	**^{14}C biomass uptake (%) fixed k_{EC}**	**^{14}C activity remaining in soil (%)**
Control	0	**0.94 ± 0.15**	11.55 ± 0.39	41.20 ± 5.40	43.04 ± 7.20
	30	1.36 ± 0.08	19.09 ± 1.22	38.31 ± 4.13	35.12 ± 11.47
2% (2mm)	0	0.79 ± 0.07	10.87 ± 0.59	**11.62 ± 2.88**	73.35 ± 2.26
	30	1.26 ± 0.17	22.26 ± 2.06	**30.31 ± 3.85**	38.14 ± 7.82
2% (1mm)	0	0.81 ± 0.09	11.65 ± 1.12	**13.40 ± 0.97**	70.04 ± 5.82
	30	1.31 ± 0.20	26.04 ± 3.44	**24.12 ± 5.61**	38.03 ± 17.72
2% (0.5mm)	0	0.56 ± 0.05	13.39 ± 0.59	**14.45 ± 0.27**	67.15 ± 5.44
	30	1.43 ± 0.17	23.43 ± 1.49	**36.02 ± 2.44**	31.37 ± 11.68
2% (0.1mm)	0	0.95 ± 0.01	11.98 ± 1.16	**13.67 ± 1.43**	69.30 ± 4.36
	30	1.86 ± 0.35	29.75 ± 2.80	**23.34 ± 3.45**	34.44 ± 15.64
5% (2mm)	0	0.88 ± 0.06	10.27 ± 1.15	**6.05 ± 0.89**	79.21 ± 5.73
	30	2.12 ± 0.01	24.20 ± 0.17	**26.80 ± 1.90**	40.78 ± 6.83
5% (1mm)	0	0.74 ± 0.15	12.19 ± 0.51	**9.15 ± 4.21**	74.13 ± 4.71
	30	1.95 ± 0.30	22.26 ± 1.01	**31.28 ± 2.84**	38.12 ± 10.72
5% (0.5mm)	0	0.63 ± 0.05	11.83 ± 1.49	**4.37 ± 1.39**	78.49 ± 3.38
	30	1.86 ± 0.28	25.37 ± 1.26	**31.59 ± 1.89**	33.44 ± 10.71
5% (0.1mm)	0	0.36 ± 0.03	11.96 ± 0.09	**8.17 ± 1.56**	75.79 ± 4.47
	30	1.51 ± 0.05	**30.92 ± 0.73**	**21.40 ± 1.97**	36.70 ± 8.71

Values in bold font indicate significance at $P<0.05$

The results from this study show that, generally, the mineralization and biomass uptake of ^{14}C glucose in both the fertilized and unfertilized soil indicated no significant effect at the 2% biochar application rate, or for the different particle sizes of biochar. The results support the previous findings, as reported in Chapter 5 (temperate study) and also those of other researchers, such as Jones *et al.* (2012) and Quilliam *et al.* (2012). However, there was a significant effect in mineralization of ^{14}C glucose at higher application rates of biochar, and also a significant effect of mineralization of ^{14}C glucose increased over time in two soils studied ($P<0.05$). These results suggest that the effect of biochar amendment showed an increase in microbial activity when a higher loading of biochar was applied. Also the effect was observed after 30 d of incubation time. The increased carbon mineralization suggests that there was a positive priming and degradation of labile carbon fractions of biochar after adding biochar to soil (Hamer *et al.*, 2004). Similarly, Quilliam *et al.* (2012) found that after three years of biochar application, there was a significant effect on soil quality and microbial growth in the treatments that had received double rates of biochar (25+25t ha^{-1} and 50+50t ha^{-1}) compared to the treatments that had received only a single rate (25t ha^{-1} and 50t ha^{-1}) of biochar. The authors demonstrate that higher rate biochar applications increase soil nutrients (dissolved organic carbon and basic cations), as well as enhancing the soil structure, thereby creating a suitable habitat for microbes to grow.

In addition, the findings of this study show that finer particle sizes mineralized more ^{14}C glucose than the larger sizes in both soils. The effect was apparent in both soils; the extents of mineralization increased significantly in the treatment amended with the finest particle size. The results agree with the findings reported by Sigua *et al.* (2014).

In their study, they found that powdered-sized biochar (<0.42mm) increased the mineralization rate and amount of CO_2 evolution in comparison to the coarser-sized biochar (>2mm). The researchers claimed that the huge surface areas of the finer particle size accelerated the carbon mineralization (smaller particle sizes are easier for the microbes to degrade).

There was a greater increase in the biomass uptake of ^{14}C glucose over time ($P<0.05$) in the unfertilized soil. This suggests that biochar amendment with different particle sizes at different rates stimulates microbial growth in the unfertilized soil. These results are supported by Anders *et al.* (2013). In their research, the authors observed a positive correlation between nutrients and microorganisms especially after adding biochar to nutrient poor soils. They suggest that biochar enhances soil quality, thus affecting the microbial community in less fertile soil. The authors also highlighted that biochar acted as a carbon sink rather than improving the nutrient status in nutrient rich soil.

Furthermore, the results revealed that the biomass uptake in the fertilized soil was higher than in the unfertilized soil ($P<0.01$). Different nutrient status between the two soils might affect the biomass in these soils. For example, high nutrient content in the fertilized soil increased the microbial growth, and this resulted in more biomass uptake than mineralization. More nutrients can be derived from the crop and additional nutrients (fertilizer supply) in the fertilized soil are among the reasons for microbial growth in this soil. Conversely, low nutrient content in unfertilized soil is subject to decreased microbial biomass uptake and a higher mineralization rate compared with fertilized soil. In unfertilized soil, the limited source of nutrients (the

soil has not received any fertilizer for at least 50 years) restricts the microbial growth in the soil. The differences between the biomass uptakes was due to the nutrient availability, which affects the biomass in these two soil systems. This explanation is supported by Zhang *et al.* (2014b), who report that a limited nutrient supply and available C content in a coarse-textured soil create an unfavourable environment for microbial growth.

6.3.2 Ammonium, nitrate and phosphate leaching in fertilized and unfertilized soils

The concentration of ammonium in the fertilized soil leachate was very low (0.00 to 0.07mg/L). During the first leaching event (day 0), the concentration of ammonium in the fertilized soil leachate for the control treatment was high. However, the concentration of ammonium during the second leaching event (day 30) was reduced in all of the treatments. No significant difference was observed among the treatments (Table 6.4).

In the unfertilized soil, the concentration of ammonium leaching was low. The effect of particle size on the leaching could be observed only in the 1mm and 0.1mm particle sizes of biochar. At these sizes, the incorporation of biochar into soil decreased the ammonium leaching ($P<0.05$) compared to the 2mm particle size at the 2% application rate from 0.03 ± 0.003mg/L to nil (day 0) (Table 6.4). For the second leaching event (day 30), the leaching of ammonium was very low and no significant difference was observed either in the application rate or in the particle sizes (Table 6.4).

The concentration of nitrate was higher than ammonium in the leachate of the fertilized soil (Table 6.5). The incorporation of biochar into fertilized soil affected the nitrate leaching only at the beginning of the leaching process. For example, the concentration of nitrate at the 5% application rate with the finest particle size (0.1mm) significantly increased was on day 0, but at the end of the leaching process (day 30) biochar had no effect on the nitrate leaching in this soil (Table 6.5). Moreover, soil amended with 2% and 5% HW biochar and with different particle sizes also had no effect on the phosphate leaching at any time (Table 6.6).

For the unfertilized soil, there was also a significant difference in nitrate leaching between the soils at the 5% application rate of biochar (Table 6.5). The smallest particle size (0.1mm) increased the concentration of nitrate ($P<0.05$) in the soil leachate, whereas the larger particle sizes had no effect on the nitrate leaching in this soil during the first leaching event (Table 6.5). However, during the second leaching event, soil amended with (2mm, 1mm and 0.5mm) biochar decreased the concentration of nitrate at the 5% application rate compared to the control treatment (Table 6.5).

Phosphate leaching in unfertilized soil exhibited the same trend as in the fertilized soil, where biochar had no effect either on the application rates or the particle sizes at any time (Table 6.6).

Table 6.4 Ammonium leaching amount, over 30 d in fertilized and unfertilized soils. Error bars are SEM (n=2).

Variable	Treatments	Fertilized Day 0 (mg/L)	Fertilized Day 30 (mg/L)	Difference	Unfertilized Day 0 (mg/L)	Unfertilized Day 30 (mg/L)	Difference
Ammonium Leaching	Control	0.07 ± 0.05	0.00 ± 0.00	0.07	0.008 ± 0.008	0.00 ± 0.00	0.008
	2% (2mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	0.03 ± 0.003	0.01 ± 0.01	0.02
	2% (1mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	**0.00 ± 0.00**	0.00 ± 0.00	0.00
	2% (0.5mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	0.003 ± 0.003	0.00 ± 0.00	0.003
	2% (0.1mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	**0.00 ± 0.00**	0.00 ± 0.00	0.00
Variable	**Treatments**	**Fertilized Day 0 (mg/L)**	**Fertilized Day 30 (mg/L)**	**Difference**	**Unfertilized Day 0 (mg/L)**	**Unfertilized Day 30 (mg/L)**	**Difference**
Ammonium Leaching	Control	0.07 ± 0.05	0.00 ± 0.00	0.07	0.008 ± 0.008	0.00 ± 0.00	0.008
	5% (2mm)	0.00 ± 0.00	0.001 ± 0.00	-0.001	0.00 ± 0.00	0.01 ± 0.01	-0.01
	5% (1mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
	5% (0.5mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	0.006 ± 0.006	0.00 ± 0.00	0.006
	5% (0.1mm)	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00

Values in bold font indicate significance at $P<0.05$

Table 6.5 Nitrate leaching amount, over 30 d in fertilized and unfertilized soils. Error bars are SEM (n=2).

Variable	Treatments	Fertilized Day 0 (mg/L)	Fertilized Day 30 (mg/L)	Difference	Unfertilized Day 0 (mg/L)	Unfertilized Day 30 (mg/L)	Difference
Nitrate Leaching	Control	19.70 ± 0.45	15.92 ± 2.42	3.78	11.48 ± 0.63	15.04 ± 0.92	-3.56
	2% (2mm)	16.60 ± 3.50	15.81 ± 2.54	0.79	12.08 ± 0.88	7.04 ± 4.58	5.04
	2% (1mm)	16.28 ± 0.33	9.72 ± 4.36	6.56	10.45 ± 0.30	9.38 ± 0.11	1.07
	2% (0.5mm)	17.70 ± 0.55	5.29 ± 2.87	12.41	10.48 ± 0.63	12.11 ± 2.93	-1.63
	2% (0.1mm)	18.83 ± 0.88	4.47 ± 0.76	14.36	10.90 ± 0.05	8.03 ± 0.54	2.87
Variable	**Treatments**	**Fertilized Day 0 (mg/L)**	**Fertilized Day 30 (mg/L)**	**Difference**	**Unfertilized Day 0 (mg/L)**	**Unfertilized Day 30 (mg/L)**	**Difference**
Nitrate Leaching	Control	19.70 ± 0.45	15.92 ± 2.42	3.78	11.48 ± 0.63	15.04 ± 0.92	-3.56
	5% (2mm)	17.53 ± 2.63	17.11 ± 6.01	0.42	10.83 ± 0.08	**4.88 ± 0.20**	5.95
	5% (1mm)	11.55 ± 1.60	22.08 ± 3.64	-10.53	9.28 ± 0.43	**3.66 ± 0.62**	5.62
	5% (0.5mm)	15.30 ± 1.15	14.15 ± 2.34	1.15	9.63 ± 0.73	**2.18 ± 0.12**	7.45
	5% (0.1mm)	**22.30 ± 1.75**	6.00 ± 3.47	16.3	**12.53 ± 0.13**	**5.25 ± 0.37**	7.28

Values in bold font indicate significance at $P<0.05$

Table 6.6 Phosphate leaching amount over 30 d in fertilised and unfertilised soils. Error bars are SEM (n=2).

Variable	Treatments	Fertilized Day 0 (mg/L)	Fertilized Day 30 (mg/L)	Difference	Unfertilized Day 0 (mg/L)	Unfertilized Day 30 (mg/L)	Difference
Phosphate Leaching	Control	0.36 ± 0.07	0.30 ± 0.02	0.06	0.04 ± 0.02	0.23 ± 0.03	-0.19
	2% (2mm)	0.26 ± 0.03	0.29 ± 0.005	-0.03	0.05 ± 0.004	0.20 ± 0.005	-0.15
	2% (1mm)	0.29 ± 0.01	0.29 ± 0.001	0.00	0.05 ± 0.007	0.20 ± 0.003	-0.15
	2% (0.5mm)	0.29 ± 0.05	0.35 ± 0.06	-0.06	0.04 ± 0.006	0.21 ± 0.01	-0.17
	2% (0.1mm)	0.28 ± 0.001	0.31 ± 0.01	-0.03	0.07 ± 0.002	0.21 ± 0.003	-0.14
Variable	**Treatments**	**Fertilized Day 0 (mg/L)**	**Fertilized Day 30 (mg/L)**	**Difference**	**Unfertilized Day 0 (mg/L)**	**Unfertilized Day 30 (mg/L)**	**Difference**
Phosphate Leaching	Control	0.36 ± 0.07	0.30 ± 0.02	0.06	0.04 ± 0.02	0.23 ± 0.03	-0.19
	5% (2mm)	0.28 ± 0.04	0.29 ± 0.01	-0.01	0.04 ± 0.02	0.20 ± 0.001	-0.16
	5% (1mm)	0.27 ± 0.02	0.31 ± 0.01	-0.04	0.03 ± 0.03	0.20 ± 0.001	-0.17
	5% (0.5mm)	0.22 ± 0.02	0.35 ± 0.03	-0.13	0.04 ± 0.004	0.21 ± 0.002	-0.17
	5% (0.1mm)	0.19 ± 0.03	0.32 ± 0.001	-0.13	0.13 ± 0.04	0.21 ± 0.004	-0.08

The leaching process reduced the amount of ammonium in the leachate in all of the soils studied. The results were in agreement with the leaching data from the previous chapters (Chapters 4 and 5) and also from other studies (Singh *et al.* (2010); Yao *et al.*, 2012). In the present study, the concentration of ammonium in the control treatment was higher than in the biochar treatments. In the fertilized soil, for example, the concentration of ammonium in the control was 0.07 mg/L and nil for the other treatments (Table 6.4). In the unfertilized soil, adding 1mm and 0.1mm particle sizes at the 2% application rate decreased the ammonium significantly ($P<0.05$) (Table 6.4). This suggests that biochar can retain ammonium ion in the soil columns. The presence of acid functional groups on the biochar's surface enables positively charged cations to attach to its surfaces (Ding *et al.*, 2010; Zheng *et al.*, 2013; Alling *et al.*, 2014). During the final leaching process (day 30) the concentration of ammonium was also low in all of the treatments and soils. This suggests that either biochar still holds the ammonium ion or a nitrification process may have occurred; therefore less ammonium was leached from the soil columns.

However, the addition of biochar to soil had a minimum effect on the nitrate leaching in both of the soils (Table 6.5). The pattern of leaching was also different depending on the soil types and particle sizes. For example, in both the fertilized and unfertilized soils, the incorporation of 0.1mm biochar at the 5% application rate increased nitrate leaching. But the concentration of nitrate in the leachate of the unfertilized soil was significantly decreased ($P<0.05$) during the second leaching event with the particle sizes of 2mm, 1mm and 0.5mm at the 5% application rate (Table 6.5).

The increase in nitrate in the leachate may be because of the nitrification process that occurred in the soil. This is because the concentration of ammonium in the soil's leachate reduced considerably, and on the other hand the nitrate in the leachate was increased as shown in Tables 6.4 and 6.5. The increasing of nitrate concentration in the solutions suggests that ammonium is converted to nitrate. Adding biochar to soil enhances microbial activity, and accelerates nitrification in soil (DeLuca *et al.*, 2006; Warnock *et al.*, 2007; Laird *et al.*, 2009). In addition, the ability of biochar adsorb organic compounds is also influences the nitrification process (DeLuca *et al.*, 2002; Berglund *et al.*, 2004). Biochar may reduce the presence of elements that inhibit nitrification by adsorbing organic compounds, such as phenolic (White, 1994; DeLuca *et al.*, 2006; Warnock *et al.*, 2007), as well as reducing the presence of C compounds that might stimulate immobilization (Fierer *et al.*, 2001; Castells *et al.*, 2003).

This finding is in line with Alling *et al.* (2014), who claim that the nitrate concentration increased at the 5% and 10% application rates because of the nitrification process that took place in the soil, as well as the release of nutrients from the biochar itself. An additional reason for the increase in nitrate in the leachate may be due to the repellence of negative charges sites of biochar therefore, resulted in an increase of nitrate concentration in the leachate. However, biochar reduced nitrate leaching in the unfertilized soil during the final leaching process and this result was in agreement with other researchers (Knowles *et al.*, 2011; Zheng *et al.*, 2013). In their studies, the authors did not identify the mechanisms of how the biochar reduced nitrate leaching. The effect of biochar was also not consistent between the two soils, it reduced nitrate leaching in unfertilized soil, but had no effect on the fertilized soil. The reason biochar increased and decreased nitrate leaching in different type of soils

is unclear. One possible explanation on the ability of biochar reduce nitrate leaching is that the adsorption of nitrate to basic functional groups of biochar (Scott *et al.*, 2014), such as chromenes and pyrenes (Amonette and Joseph, 2009). Moreover, biochar used in this study was produced at high temperature ($400^{O}C$). According to Guo and Rockstraw (2007) the loss of acid functional groups starts at $400^{O}C$ and basic functional groups increase as the pyrolysis temperature increases (Chun *et al.*, 2004). Thus, the ability of biochar adsorb of nitrate in the soil's leachate may be related with the temperature used to produce biochar.

There was no effect on the leaching of phosphate in any of the soils studied (Table 6.6). Amending soils with different particle sizes of biochar and at different rates exhibited no differences among the treatments (see Table 6.6). This result is supported by Alling *et al.* (2014), who found that amending soil with biochar made no difference compared with soil alone. In contrast with these findings, Yao *et al.* (2012) found that adding biochar to soil increased the phosphate in the leachate. The limited effect of biochar in reducing phosphate leaching is possibly because of the low anion exchange capacity (Singh *et al.*, 2010).

6.3.4 Soil pH, total carbon and total nitrogen in fertilized soil and unfertilized soils

Amending soils with 2% and 5% HW biochar with different particle sizes increased the pH significantly ($P<0.05$) in all of the soils at all times (Table 6.7). The finest particle sizes (0.5 and 0.1mm) had the highest soil pH ($P<0.05$) compared to the coarser sizes (2mm and 1mm) and the control (Table 6.7).

The C content in both of the soils showed a similar pattern when adding 2% and 5% biochar to soil. In terms of the effects of the particle sizes in both soils, the smaller particle sizes (1mm, 0.5mm and 0.1mm) increased the C significantly ($P<0.05$) compared to the larger particle sizes (2mm), as well as the control treatment (Table 6.8). The contrasting C content between the smaller and larger particle sizes of biochar when adding the same amount of C to soil was probably due to the sampling error (unrepresentative sample while measuring C from the larger particle sizes of biochar).

Unlike pH and C, N content in the fertilized and unfertilized soils showed a different trend. For example, the N content in the unfertilized soil was significantly higher ($P<0.05$) than in the fertilized soil. Also, the N content in both soils reduced significantly ($P<0.05$) after 30 d incubation time (Table 6.9).

Table 6.7 Soil pH in fertilized and unfertilized soils, over 30 d. Error bars are SEM (n=3)

Variable	Treatments	Fertilized Day 0	Fertilized Day 30	Difference	Unfertilized Day 0	Unfertilized Day 30	Difference
Soil pH	Control	6.16 ± 0.02	5.92 ± 0.04	0.24	6.15 ± 0.02	5.91 ± 0.01	0.24
	2% (2mm)	**6.22 ± 0.01**	**6.11 ± 0.02**	0.11	**6.19 ± 0.02**	**6.03 ± 0.02**	0.16
	2% (1mm)	**6.25 ± 0.04**	**6.28 ± 0.04**	-0.03	**6.53 ± 0.02**	**6.29 ± 0.04**	0.24
	2% (0.5mm)	**6.57 ± 0.02**	**6.51 ± 0.01**	0.06	**6.80 ± 0.03**	**6.39 ± 0.01**	0.41
	2% (0.1mm)	**6.89 ± 0.02**	**6.71 ± 0.03**	0.18	**6.98 ± 0.04**	**6.60 ± 0.01**	0.38
Variable	**Treatments**	**Fertilized Day 0**	**Fertilized Day 30**	**Difference**	**Unfertilized Day 0**	**Unfertilized Day 30**	**Difference**
Soil pH	Control	6.16 ± 0.02	5.92 ± 0.04	0.24	6.15 ± 0.02	5.91 ± 0.01	0.24
	5% (2mm)	**6.31 ± 0.03**	**6.34 ± 0.03**	-0.03	**6.31 ± 0.02**	**6.36 ± 0.05**	-0.05
	5% (1mm)	**6.64 ± 0.05**	**6.66 ± 0.02**	-0.02	**6.86 ± 0.04**	**6.57 ± 0.01**	0.29
	5% (0.5mm)	**6.95 ± 0.01**	**7.06 ± 0.02**	-0.11	**7.16 ± 0.05**	**6.99 ± 0.02**	0.17
	5% (0.1mm)	**7.35 ± 0.03**	**7.52 ± 0.08**	-0.17	**7.48 ± 0.06**	**7.44 ± 0.04**	0.04

Values in bold font indicate significance at $P<0.05$

Table 6.8 Total carbon in fertilized and unfertilized soils, over 30 d. Error bars are SEM (n=2)

Variable	Treatments	Fertilized Day 0	Fertilized Day 30	Difference	Unfertilized Day 0	Unfertilized Day 30	Difference
Total C (%)	Control	2.14 ± 0.03	1.94 ± 0.03	0.2	3.40 ± 0.03	3.25 ± 0.10	0.15
	2% (2mm)	2.65 ± 0.14	2.12 ± 0.06	0.53	4.34 ± 0.24	3.89 ± 0.15	0.45
	2% (1mm)	**4.20 ± 0.24**	**3.50 ± 0.35**	0.7	**5.34 ± 0.61**	**4.29 ± 0.29**	1.05
	2% (0.5mm)	**4.37 ± 0.09**	**3.44 ± 0.14**	0.93	**5.54 ± 0.22**	**4.70 ± 0.20**	0.84
	2% (0.1mm)	**3.79 ± 0.29**	**4.06 ± 0.22**	-0.27	**4.92 ± 0.11**	**5.14 ± 0.14**	-0.22
Variable	**Treatments**	**Fertilized Day 0**	**Fertilized Day 30**	**Difference**	**Unfertilized Day 0**	**Unfertilized Day 30**	**Difference**
Total C (%)	Control	2.14 ± 0.03	1.94 ± 0.03	0.2	3.40 ± 0.03	3.25 ± 0.10	0.15
	5% (2mm)	2.73 ± 0.07	2.45 ± 0.16	0.28	5.73 ± 0.15	4.23 ± 0.09	1.50
	5% (1mm)	**7.05 ± 0.87**	**5.09 ± 0.24**	1.96	**7.72 ± 0.71**	**6.37 ± 0.57**	1.35
	5% (0.5mm)	**6.64 ± 0.17**	**6.60 ± 0.10**	0.04	**7.47 ± 0.39**	**7.61 ± 0.49**	-0.14
	5% (0.1mm)	**5.77 ± 0.46**	**6.71 ± 0.41**	-0.94	**7.10 ± 0.40**	**7.87 ± 0.36**	-0.77

Values in bold font indicate significance at $P<0.05$

Table 6.9 Total nitrogen in fertilized and unfertilized soils, over 30 d. Error bars are SEM (n=3).

Variable	Treatments	Fertilized Day 0	Fertilized Day 30	Difference	Unfertilized Day 0	Unfertilized Day 30	Difference
Total N (%)	Control	0.19 ± 0.002	**0.17 ± 0.02**	0.02	0.19 ± 0.003	0.17 ± 0.021	0.02
	2% (2mm)	0.19 ± 0.010	**0.13 ± 0.005**	0.06	**0.23 ± 0.012**	**0.19 ± 0.012**	0.04
	2% (1mm)	0.17 ± 0.001	**0.12 ± 0.001**	0.05	**0.21 ± 0.012**	**0.18 ± 0.022**	0.03
	2% (0.5mm)	0.17 ± 0.012	**0.10 ± 0.06**	0.07	**0.21 ± 0.006**	**0.19 ± 0.012**	0.02
	2% (0.1mm)	0.13 ± 0.008	**0.08 ± 0.01**	0.05	**0.22 ± 0.003**	**0.19 ± 0.001**	0.03
Variable	**Treatments**	**Fertilized Day 0**	**Fertilized Day 30**	**Difference**	**Unfertilized Day 0**	**Unfertilized Day 30**	**Difference**
Total N (%)	Control	0.19 ± 0.002	**0.17 ± 0.02**	0.02	0.19 ± 0.003	0.17 ± 0.021	0.02
	5% (2mm)	0.12 ± 0.006	**0.07 ± 0.01**	0.05	**0.28 ± 0.024**	**0.19 ± 0.006**	0.09
	5% (1mm)	0.15 ± 0.005	**0.07 ± 0.01**	0.08	**0.29 ± 0.001**	**0.22 ± 0.026**	0.07
	5% (0.5mm)	0.14 ± 0.009	**0.10 ± 0.03**	0.04	**0.29 ± 0.021**	**0.19 ± 0.011**	0.10
	5% (0.1mm)	0.13 ± 0.008	**0.09 ± 0.001**	0.04	**0.26 ± 0.008**	**0.17 ± 0.008**	0.09

Values in bold font indicate significance at $P<0.05$

The biochar used in this study had a high pH (9.05). Therefore the addition of biochar to the fertilized and unfertilized soils increased the soil pH significantly ($P<0.05$). The results also showed that finer particles sizes with a greater application rate had the highest pH compared with other treatments ($P<0.05$). The finer particle size has a huge surface area, and thus there is more contact between the biochar particles and the soil solution, which increased the pH more than when using the coarser sizes. The pH in the fertilized soil increased after 30 days of incubation, especially in the treatments with the smaller sizes of biochar. Even though biochar increased the pH, on day 30 the pH reduced, especially in the unfertilized soil. This result agrees with the previous study, where the highest rate of biochar decreased the soil pH from 7.8 to 7.6 (Inal *et al.*, 2015). The authors claimed that the oxidation process of biochar releases acid functional groups, therefore reducing the soil pH.

Similar trends were also observed for the C content in the soil. Amending soils with biochar to the fertilized and unfertilized soils increased the C. However on the last incubation day (day 30), the C content decreased. Several studies reported considerable losses of biochar C in soils only a few years after the biochar application (Tagoe *et al.*, 2008; Lehmann *et al.*, 2009). This is due to the slow abiotic oxidation in soil and biochar will eventually be degraded (Cheng *et al.*, 2008). An increase in C after biochar addition was also reported in Wang *et al.* (2015a). According to the authors, biochar increased the C compared to the control treatment. The recalcitrant of C in the biochar is one of the characteristics of biochar, which can sequester C in the soil longer (Kuzyakov *et al.*, 2009; Wang *et al.*, 2015b).

The results from the current study also showed that, the C and N content in the unfertilized soil was higher than in the fertilized soil (Tables 6.8 and 6.9). Legume plants that were found while sampling the soil might have contributed the organic C and N in that soil. This findings supported by De Deyn *et al.* (2009) who reported that the presence of legume species in grassland (*L. corniculatus* and *T. repens*) increased carbon and nitrogen storage in the soil. Moreover, massive earthworms were also found in the unfertilized soil during the soil sampling. The presence of living things may add organic material to the soil, thus increasing the C and N content. In contrast with this finding, Jones *et al.* (2012) and Wang *et al.* (2015a) observed that biochar application made no significant difference to the total N in the soil. Furthermore, adding biochar to the soil decreased the total N in both soils (Table 6.9). The reduction of total N in the soil may be attributed to the denitrification process that occurred in the soils during the incubation time. The soil samples were kept in sealed jars, and this could have perhaps created an anaerobic environment, possibly reducing nitrate to nitrogen gas.

6.4 Conclusion

Overall, amending soils with biochar had a significant effect on the microbial activity in both the fertilized and unfertilized soils. In this study, finer biochar stimulated the mineralization of ^{14}C glucose at a high application rate in both soils after 30 d of incubation. Furthermore, biochar adsorbed ammonium in the soil leachate, but only the 5% application rate of the biochar reduced nitrate leaching at the second leaching event in the unfertilized soil. No significant effects were observed with regard to the phosphate leaching either of the soils at any time. Finer particle sizes were also shown to increase the pH of the soil in both the fertilized and unfertilized soils. At higher

application rates of biochar, the pH and C increased compared to the lower application rates. The results supported the hypotheses that finer particles sizes, as well as higher application rates, give a more prominent effect than coarser particle sizes and the lower application rate of biochar. In terms of the differences in the nutrient status of the soils, biochar application had a different impact on the soils study, for example, the contrasting effects that could be seen when the microbial growth in the unfertilized soil increased over time, compared to the lack of effect of microbial growth in fertilized soil.

CHAPTER 7

Biochar in Amended Soils: A Comparison between the Tropics and the Temperate Regions

7.1 Introduction

Previous research has shown that the effectiveness of biochar is dependent on various factors (Downie *et al.*, 2009; Scott *et al.*, 2014), which include: 1) the feedstock and method used to produce the biochar 2) the soil type used to apply the biochar and 3) the climate. The method used, for instance slow or fast pyrolysis (Scott *et al.*, 2014), as well as the temperature used to produce the biochar also influence the end product. In addition, soils have their own contrasting characteristics, in which all of these factors are dependent on the association of the mineral and the organic matter (Brady and Weil, 2008). According to Kolb *et al.* (2009) and Kloss *et al.* (2014) biochar demonstrates positive effects on soil, but the effects are dependent on the type of the soil.

Tropical soils are old, dominated with 1:1 clay minerals, have more variable charge, are reddish in colour and highly weathered (Ishak and Jusop, 2010). High rainfall and temperature accelerate the weathering process in the tropics, and also enhance mineralization of organic matter in the soils (Tiessen *et al.*, 1994; Hashim and Wan Abdullah, 2001; Haruna *et al.*, 2012). Because of these characteristics, tropical soils are often infertile and less productive. Furthermore, typical tropical soils are acidic, have lower CEC and lower bases due to the process of weathering; as a result, Al and Fe are released into the soil solution (Cornelissen *et al.*, 2013; Alling *et al.*, 2014;

Kloss *et al.*, 2014). With low pH, the exchangeable Al and Fe are high and can cause Al toxicity in the plants. Unlike tropical soils, temperate soils are younger and dominated with 2:1 clay minerals, are less variable in charge, and are more resistant to physical and chemical weathering (Ishak and Jusop, 2010). On the other hand, temperate soils are fertile and Al toxicity is unlikely to occur in this soil. This is because temperate soils have higher pH and higher soil organic matter content (Kloss *et al.*, 2014). The different characteristics between tropical and temperate soils may result in a different respond upon biochar addition to both tropical and temperate soils.

In addition, often biochar application benefits degraded soils, such as soils in the tropics that are highly weathered, have a low CEC or increased nutrient leaching and are acidic, as mentioned above (Ishak and Jusop, 2010; Alling *et al.*, 2014). Meanwhile, biochar addition to temperate soils that are more fertile than soils in the tropics show less effects or only a minor advantage. In this chapter, the long-term effects of biochar on various soil types from different geographical regions and climates are highlighted. The similarities and differences in the physical, chemical and biological properties of soils after biochar addition are compared. In sections 7.5, 7.6 and 7.7, the potential use of biochar, as well as economic benefits of using biochar in these two different regions are further discussed. A summary of the findings from the previous chapters (Chapters 4, 5 and 6) is displayed in Table 7.1 below:

Table 7.1 Summary of the findings from the tropical study (Chapter 4), temperate part 1 (Chapter 5) and temperate part 2 (Chapter 6).

Variable	Tropical Chapter 4	Temperate (Part 1) Chapter 5	Temperate (Part 2) Chapter 6
Microbial activity	+	+	++
Microbial biomass	O	O	++
Ammonium leaching	++	+++	+++
Nitrate leaching	+	O	+-
Phosphate leaching	+	+	O
CEC	+	+	NM
pH	+++	+++	+++
Total Carbon	+++	+++	+++
Total Nitrogen	+-	+-	+-
Soil Phosphate	O	O	NM
Aggregate stability	O	O	NM

+++ = Significant effect

++ = Some significant effect

+ = Limited effect

+- = Trend is not clear (increase and decrease)

O = No effect

NM = Not measured

7.2 The effects of biochar on the biological properties of soils in Malaysian and the UK

Generally, the results from the biochar experiment on both Malaysian and UK soils (Chapters 4 and 5) exhibited similar effects in terms of the microbial activity and growth at a 2% application rate. For example, amending soil with RH biochar and an aged HW biochar only gave a minimal effect on the microbial activity in the soil. The limited effects of biochar in these two soils may be because of the lower application rate of biochar applied to the soil and the particle size of biochar, which was too coarse. However, amending soil at a 5% application rate of 0.1mm biochar increased the microbial activity in the temperate soil (Chapter 6). A higher application rate and finer particle size accelerated the mineralization of ^{14}C in the soil.

Furthermore, the soil with a limited amount of carbon reduced the mineralization of ^{14}C carbon in the soil. The results from the previous chapters (Chapters 4 and 5) show that the initial carbon content in the forest, grassland and loamy soils was higher than in the non-intensively farmed, intensively farmed and sandy soils. As a consequence, amending soil with RH biochar, and an aged HW biochar increased the extent of mineralization of ^{14}C glucose in forest, grassland and loamy soils more than in the non-intensively and intensively farmed soil, as well as in the sandy soils.

The results also showed no effects on the biomass uptake in the tropical and UK soils (part 1) (Chapters 4 and 5). These results are consistent with the findings of Bruun *et al.* (2008). They did not find any microbial assimilation even after 20 days of incubation. Nevertheless, biochar increased microbial growth over time in the

unfertilized temperate soil more than in the fertilized temperate soil (part 2) (Chapter 6). Biochar increased the growth of microbes in the unfertilized soil for various reasons: the biochar itself can serve as a food source due to labile fraction C on the biochar (Bruun *et al.*, 2012); and its surfaces contain nutrients (Cheng *et al.*, 2008). Additionally, the sorption of organic C and the ability of the biochar to hold nutrients (Lehmann *et al.*, 2011), including the pores that provide a habitat for microbes (Pietikainen, 2000), enhance the microbial growth in the unfertilized soil.

The scanning electron microscopy (SEM) images below indicate the pores in all of the biochar types used in this study (Figure 7.1). The presence of pores in the biochar, which can provide a habitat for microorganisms, may be the reason for the increase in microbial activity and growth in the soil. Chenu *et al.* (2001) found that the amount of microorganisms on clayey soil surfaces increased and the number of microbes increased both inside and on the surface of sandy soils after the addition of glucose. Ascough *et al.* (2010) observed that fungi colonized on the surface and in the pores of charcoal. The authors claimed that the physical structure of the biochar and the available nutrients on the surface of the charcoal were the reasons for the fungal colonization.

Even though the biomass in the fertilized soil was higher than in the unfertilized soil, over time there was no effect on the microbial biomass after the addition of biochar in this soil (Chapter 6). A limited effect was observed in the fertilized soil, which was attributed to the high fertility of the soil. This is because the microorganisms in the fertilized soil had already received enough nutrients (Anders *et al.*, 2013). The

findings are in line with Kolb *et al.* (2009) and also Anders *et al.* (2013), who reported that the microbial biomass in sandy soil, which has a low organic matter content, increased significantly more when compared with unamended soil. The former authors argued that the increase was due to the increase of available C content and charcoal also providing a habitat for the microbes.

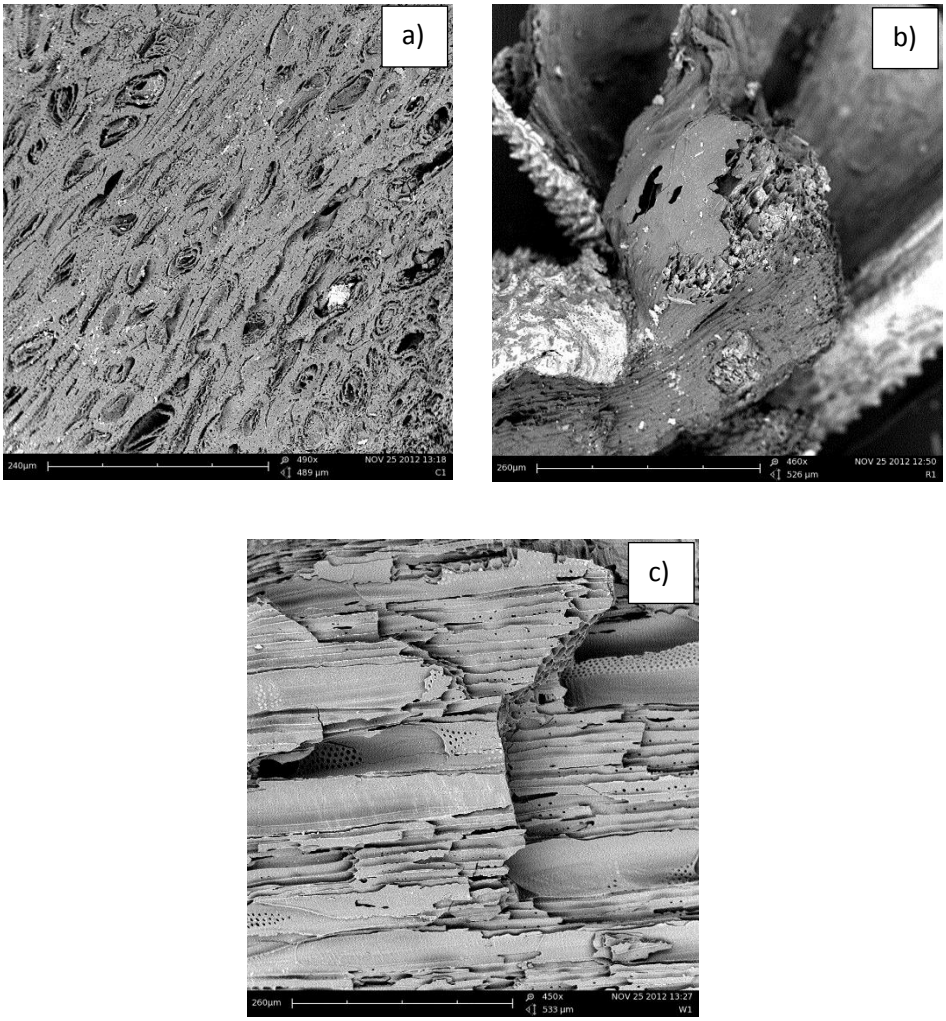

Figure 7.1 Scanning electron microscopy (SEM) pictures of biochars made of a) CS (Mag: 490x), b) RH (Mag: 460x), and c) HW biochars (Mag: 450x).

7.3 The effects of biochars on the tropical and temperate soil leaching

Biochar application to soil has different effects, based on the types of nutrients, as well as the types of biochar. For example, amending soil with biochar decreased the ammonium leaching in the soil leachate (Chapters 4, 5 and 6). The reason for the decrease in ammonium leaching in the tropical and temperate soils is due to the sorption of the ammonium ion to the acidic biochar functional groups (Clough and Condron, 2010; Zheng *et al.*, 2013; Scott *et al.*, 2014). Furthermore, the aged biochar in the temperate soils adsorbed more ammonium than the fresh biochar amendment (Chapter 5). Aged biochar has a high CEC (Cheng *et al.*, 2008), and therefore can hold greater amounts of nutrients (Major *et al.*, 2009). According to Singh *et al.* (2010), as biochar ages, the effectiveness of its ammonium adsorption capacity increases because of the oxidation on the biochar's surfaces, which can also increase the CEC of the soils.

However, nitrate and phosphate exhibited an inconsistent trend on the soil's leaching in both the tropical and temperate studies (Chapters 4, 5 and 6). In terms of the status of different nutrients in the temperate study (Chapter 6), biochar reduced nitrate leaching in the unfertilized soil and no effect was observed in the fertilized soil. But, at the beginning of the leaching process, the finer particles increased nitrate leaching in both of the soils. Finer particles are porous and lighter than coarser particles. The smaller particles are more likely to move through the soil and accelerate nutrient transportation in the soil (Major *et al.*, 2009).

Additionally, the minimal effect of nitrate and phosphate leaching in both soils was due to the negatively charged (NO_3^- and PO_4^{3-}) anions being repelled by the negative charges on the biochar (Hale *et al.*, 2013). However, the ability of biochar to adsorb nitrate in this study (Chapters 5 and 6); and previous studies, such as those by Knowles *et al.* (2011), Yao *et al.* (2012) and Gronwald *et al.* (2015) is not fully understood. A possible explanation may be because of the N immobilization by microbes in the soil that can lead to a decrease of nitrate concentration in the soil leachate (Novak *et al.*, 2010). More studies are needed in order to elucidate this mechanism.

Unlike nitrate, which is susceptible to leaching, phosphate can form ligand bonding (Mukherjee *et al.*, 2011) with other cations or metals and alter its anion characteristics (Ashman and Puri, 2013). As a consequence, phosphate leaching decreased, possibly due to ligand bonding. Furthermore, the difference in the temperatures of biochar production may affect the effectiveness of the sorption of phosphate in the soils. In this study, the RH biochar was produced at 900^{O}C, whereas the CS biochar was produced at 400^{O}C. Results showed that the RH biochar treatment increased the phosphate leaching more than the CS biochar (Chapter 4). Consistent with this finding, Lentz and Ippolito (2012) found that switchgrass biochar produced at 250^{O}C reduces phosphate leaching 2-3 times better than biochars produced at 500^{O}C. Therefore, the difference temperatures during biochar production may affect the ability of phosphate sorption in the soils.

In addition, the leaching experiment was conducted in a different moisture content and this is subject to wet and dry cycles in every leaching event. Unlike the soil-biochar incubation study, the soils were kept in sealed jars, which are likely to have become anaerobic relatively quickly. For the leaching study, the variation in moisture levels during the leaching experiments might have affected the results. Therefore, it is difficult to assess and understand what happens to nitrification over time and comparisons of ammonium concentrations in leachate over time are difficult to interpret.

7.4 The effects of chemical and physical properties of tropical and temperate soils amended with biochar

There were no prominent effects in terms of the CEC in the tropical and temperate soil studies (Chapters 4 and 5). For example, the CEC of the soil only increased at the end of the incubation time in the forest soil amended with the RH biochar (Chapter 4) and the grassland soil amended with the aged HW biochar (Chapter 5). The effects of CEC were not consistent in the soil amended with CS biochar and fresh HW biochar. The less significant effect of the biochar in the tropical and temperate soils is possibly due to the temperature during the production of the biochar. The temperature used to produce the biochar in this study was high: 400^{O}C for the CS and HW; and 900^{O}C for the RH biochar. High temperature biochar has a low negative surface charge due to the loss of functional groups (hydroxyl and carboxyl); ultimately this decreases the CEC of the soils (Novak *et al.*, 2009). According to Gaskin *et al.* (2008), Novak *et al.* (2009), Singh *et al.* (2010) and Kloss *et al.* (2014), increasing the pyrolysis temperature significantly decreases the CEC of biochars. Guo and Rockstraw (2007) reported that the loss of acid functional groups starts at 400^{O}C and basic functional

groups increase as the pyrolysis temperature increases (Chun *et al.*, 2004). In addition, the marginal effect of CEC that could be seen in the temperate soils is possibly due to the ageing process of the biochar. A recent study by Scott *et al.* (2014) suggests that the oxidation reaction on the biochar's surface increases the CEC of the soil as the biochar ages in the soil. Therefore, in the present study, amending soils with the aged HW biochar increased the CEC in grassland soils, but fresh HW biochar amendment had no effect on the CEC of the soils.

Biochar application significantly increased the pH of the tropical soil (Chapter 4). This is due to the alkalinity of the biochar. Gaskin *et al.* (2010) and Uzoma *et al.* (2011) suggest that the pyrolysis process during the production of biochar leads to the accumulation of alkaline substances in the biochar. Thus, in the current study, amending soil with the RH and CS biochars caused a liming effect (Van Zwieten *et al.*, 2007) where the pH of the acidic soil rose. Generally, a low soil pH, especially in the tropics, increases the level of available Al toxicity (Cornelissen *et al.*, 2013; Kloss *et al.*, 2014). Other studies have found that a higher pH is associated with a lower Al level in the soil (Van Zwieten *et al.*, 2007; Kuka *et al.*, 2013; Yang *et al.*, 2013). Therefore, adding biochar to this soil not only increases the soil pH, but may also reduce the Al toxicity in the soil. However, the Al level was not measured in this current study.

Adding biochar to soil can not only decrease the acidity of tropical soil, but can also increase the pH of temperate soils (Chapters 5 and 6). An increasing soil pH in relation to biochar application has been found in other studies (Uzoma *et al.*, 2011;

Cornelissen *et al.*, 2013; Kloss *et al.*, 2014). However, biochar application does not always increase temperate soil pH; it can have the opposite effect. For example, Karer *et al.* (2013) reported that the addition of biochar decreased the pH of Chernozem soil. Qayyum *et al.* (2015) found that the application of 1% biochar to an alkaline soil did not affect the pH significantly. The smaller effect on the pH of the temperate soils was attributed to the high initial pH of the soil (Karer *et al.*, 2013; Qayyum *et al.*, 2015). Qayyum *et al.* (2015) recommended that biochar can be used as a soil amendment in temperate soil when applied at a lower rate. Furthermore, McCormack (2015) recently reported that incorporation of biochar to temperate soil had no effect on the soil's pH. The author speculated that an absent of pH effect is due to the low rate of biochar application and also the leaching of basic cations after biochar addition to soil.

The results from this thesis also indicated that the pH in the leachate of temperate soils decreased, whereas the pH in the soil increased over time (Chapter 5). The pH declined in the soil leachate has been previously attributed to the leaching of basic cations (Schulz and Glaser, 2012) and the biological process that took place in the soil (Fageria and Baligar, 2008). During leaching, CO_2 from microbial respiration is combined with water and carbonic acid is formed, leading to a reduced soil pH (Ashman and Puri, 2013). Furthermore, according to Singh *et al.* (2010), nitrification increases with soil moisture, thereby reduces the pH in the soil's leachate. In contrast with the pH of the soil, the soil samples are drier (kept in the container during incubation time), there is less biological activity and less carbonic acid is released from the soil, thus resulting in a higher pH compared with the soil leachate.

In contrast with the temperate soils study, tropical soils study exhibited an opposite trend, for example both the leachate and soil pH decreased over time (Chapter 4). The reduction of soil pH in the leachate was possibly due to the biological activity and the leaching of basic cations during the leaching process. However, it is unclear how the pH in the soil declined with time. The pH reduced over time may be related to the biochar application rate (McCormack, 2015), particle sizes or oxidation process of biochar (Inal *et al.*, 2015). Lower biochar doses (2%) and coarser particle sizes (<2mm) used in the tropical study (Chapter 4) may be the reasons of the pH reduction in the soil. Additionally, the oxidation process of biochar releases acid functional groups, therefore reducing the soil pH (Inal *et al.*, 2015). Unlike tropical soils, temperate soils study amended with higher dosage of biochar (5%) and finer particle size (0.1mm), increased the pH in the fertilized soil through time (Chapter 6). Nevertheless, further research is needed to determine whether these factors (application rate, particle sizes or oxidation of biochar's surfaces) influence the pH in both the tropical and temperate soils.

Biochar addition increased the carbon content in all of the soils studied (Chapters 4, 5 and 6). Although, biochar increased carbon content in both tropical and temperate soils, but it decreased over time. The loss of C may be attributed to the degradation of labile C in biochar. McCormack (2015) noticed a reduction of C content in the soil treated with biochar. The author speculated that, the decrease in C may be due to the loss of biochar via leaching and wind erosion. In contrast with these findings, Cross and Sohi (2011) revealed that several soils in their study exhibited a negative priming effect after the addition of biochar, demonstrating that labile soil C is stable in those soils. Despite higher C values following biochar addition, the N values in biochar are

low. Results from this thesis showed that biochar application had no effect on the total N content in the tropical soil or in the temperate study.

In terms of the soil's physical properties, both the tropical and temperate soil studies showed little effect on the aggregate stability (Chapters 4 and 5). Biochar application in the tropical and temperate soils study did not show much difference in the soil aggregation. There is some debate in the literature over the role of biochar in promoting soils structure. For example, Peng *et al.* (2011), found that amending soil with biochar decreased the aggregate stability by 1-17%, but contrast with that of Lehmann *et al.* (2008) who showed biochar could enhance soil aggregates through the formation of the soil's structure; for instance, biochar particles were found to be attached to microaggregates of soil.

As well as soil aggregation, Figures 7.2 and 7.3 show the water release characteristics of the tropical and temperate soils studied amended with different types of biochars. Generally, the biochar had a minor impact on the water retention in the tropical soils, but no effect in the temperate study. For example, in the tropical study, the results from Figure 7.2 indicate that CS increased the water retention significantly ($P<0.05$) more than the RH biochar and control at higher tension. No significant difference was observed in the soils amended with biochars at the lower tensions (<0.3) bars (Figure 7.2). CS biochar held more water possibly because the volume of smaller pores present in the CS was greater than in the RH biochar, as illustrated in Figure 7.1. RH has less pores; therefore less water can be retained in the soils at any matric potential. Conversely, Masulili et al. (2010) found that RH biochar application increased water

retention by 15.47% in the soil compared with the control treatment (11.34%). The authors suggested that this was or due to the high soil porosity (more than 50%) in the treated soil caused by the addition of organic amendment.

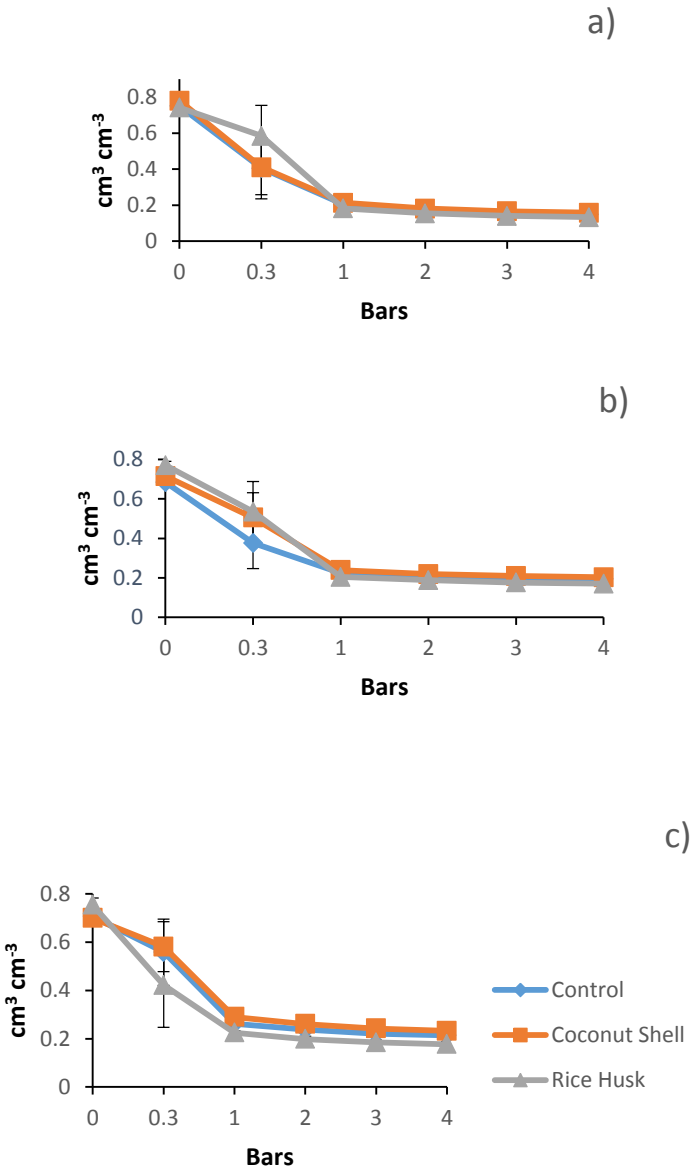

Figure 7.2 Water release curves of a) forest b) non-intensive farming and c) intensive farming soils amended with and without CS and RH biochar at different tension bars. Error bars are SEM (n=3).

For the temperate study, amending three soils with 2% of fresh and aged HW biochars showed a similar pattern and the changes were generally insignificant at all tensions ($P>0.05$). The results also indicated that as the tensions increased, the volumetric water content declined considerably (Figure 7.3). In contrast with this finding, Herath et al. (2013) reported that biochar application increased the available water content in an Alfisol by 22% and in an Andisol by 19-33% compared to untreated soil at a specific matric potential. The effects of biochar on water retention are not only due to the different types of soils as suggested by Herath et al. (2013), but also the temperatures used to produce the biochar. The same authors revealed that biochar produced at higher temperatures retained more water than that produced at lower temperatures. This is because at permanent wilting point the former biochar retained more water due to more micropores than the latter.

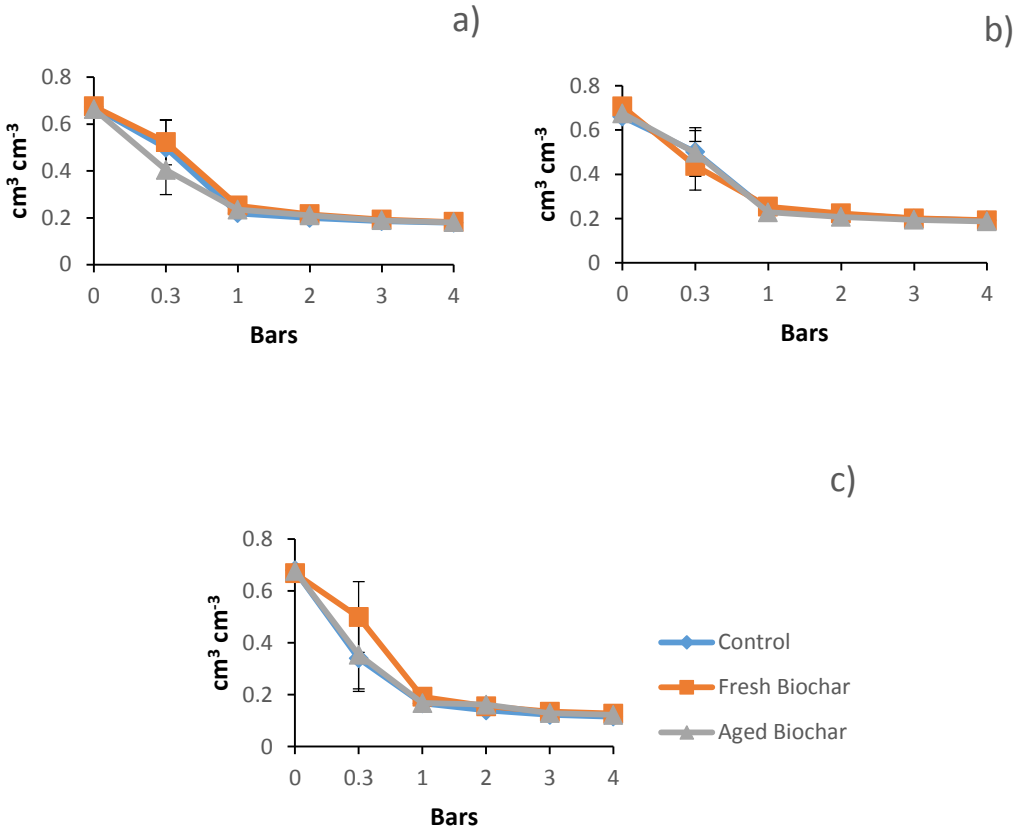

Figure 7.3 Water release curves of a) grassland b) loamy and c) sandy soils amended with and without fresh and aged HW biochar at different tension bars. Error bars are SEM (n=3).

A number of studies have also suggested that biochar improves soil's water holding capacity (Chan *et al.*, 2008a; Karer *et al.*, 2013). Moreover, biochar not only increases the water holding capacity, but also decreases the bulk density by 9% and increases the total porosity from 45.7% to 50.6% (Oguntunde *et al.*, 2008). With a high infiltration rate and low bulk density, the soil is less susceptible to compaction and erosion when biochar is incorporated into it (Karer *et al.*, 2013).

7.5 The potential use of biochar amendment in Malaysia

In Malaysia, the Cameron Highlands is a mountainous area, with a low temperature (14-24^{O}C) and high rainfall. The average annual rainfall is about 2660mm (Abdullah *et al.*, 2001). The climate is ideal for the cultivation of subtropical and temperate vegetables (Salama and Kookana, 2001). Approximately 5251 ha of land is used for agriculture and 47% of that is cultivated with vegetables (Abdullah *et al.*, 2001). The crops are planted on the subsoils. The top soils are lost because of the high intensity of rainfall. In addition, most of the top soils are no longer pristine due to changes in the soil management, such as the application of fertilizers or liming to sustain crop productivity (Aminuddin *et al.*, 2005). The subsoils in the Cameron Highlands are also infertile; they have a lower CEC and pH.

The tropical soils used in this study were spodosols, which are acidic, with a pH < 5.5, and a high accumulation of Al and Fe in the subsoils. To ameliorate these soils, the addition of biochar can increase the pH of the soils. In this study, the pH of the soils increased as a result of adding the biochar to them. Biochar has a high pH due to the alkaline minerals that exist within it. The pyrolysis process during the biochar production leads to an accumulation of these alkaline minerals (Gaskin *et al.*, 2010). In acidic soils, the amount of exchangeable aluminium is also moderately high (Ishak and Jusop, 2010). To overcome the aluminium levels in the soil, liming is introduced to the soils. In this case, biochar can act as a liming agent due to its alkalinity and high pH. Petter *et al.* (2012) reported that the pH increased with an increasing rate of biochar addition. The authors also observed a reduction in acidity (H + Al) of approximately 20% when 32 Mg ha^{-1} biochar was applied compared to the soil alone. Similar findings were also reported by Mbagwu and Piccolo (1997), Topoliantz *et al.*

(2005) and Masulili *et al.* (2010), in which consistent with the high pH, the levels of (H+ and Al) decreased when charcoal was incorporated into the soil.

The leaching experiment showed that the use of CS and RH biochars reduced ammonium leaching in forest soil considerably throughout the study. However, there was no consistent trend in terms of the biochar reducing nitrate and phosphate. This suggests that biochar retains ammonium better than nitrate and phosphate in the soils. The CEC of the soils after adding biochar was no different. However, the CEC of the soils was low after biochar addition; a reduction in the ammonium concentration in the soil's leachate is possible as demonstrated in the findings. According to Lehmann *et al.* (2003), decreasing ammonium leaching is likely for a biochar with a low CEC (Rajkovich *et al.*, 2012). Therefore, the addition of biochar to tropical soils is expected to decrease ammonium loss through leaching. Moreover, nutrients leaching has been identified is a problem because many Malaysian soils carry a positive charge, it is likely to ammonium leaching occurs in this soil. With the addition of biochar, the reduction of ammonium leaching is possible and; may decrease the demand for ammonium fertilizer, ultimately, reduce the eutrophication problem in lakes and rivers.

Another common problem associated with the tropics is soil erosion and the loss of organic matter. Soil loss in vegetable farms is estimated to be as high as 82 t ha^{-1} yr^{-1} (Aminuddin *et al.*, 2001), whereas nutrient loss is reported to be approximately 470 kg N ha^{-1} yr^{-1} (Aminuddin *et al.*, 2005). Cerri *et al.* (2007) and Lal *et al.* (2007) have reported that about 20 to 80 t C ha^{-1} of agricultural soils is lost in the tropics and

released into the atmosphere. The high temperature and humidity create suitable conditions for organisms to decompose the organic matter. Less organic matter, as a result of the rapid turnover rates of organic matter, reduces the level of carbon in the soil, and over time the soil becomes degraded and infertile (Mekuria and Noble, 2013). Biochar has the potential to sequester carbon in the soil for longer. This is due to its aromatic structure and long mean residence time in the soil. Therefore, the addition of biochar may alleviate the loss of organic carbon and organic matter in tropical soils. The findings from this study revealed that amending soil with 2% of CS and RH biochar increased the C in soil (Chapter 4). Nearly twice as much as C was found after adding biochar to all of the tropical soils studied (forest, non-intensive and intensively farmed soils). In addition, a study by Rosenani *et al.* (2012a) found that there was an increase of total C even after the second crop cycle when adding empty oil palm fruit bunch (EFB) biochar to Malaysian soil compared to control treatment from 1.06 to 1.92%. Also, the increase was significant with increasing biochar application rate (1.49% at 10t ha^{-1} and 1.79% at 15t ha^{-1} respectively). EFB biochar application also increased the amount of total C in acid sulphate soil from 4.45 to 5.21% (Rosenani *et al.*, 2013). Other studies in the tropics (Indonesia), for example Sukartono *et al.* (2011), found that biochar addition increased carbon in sandy soil from 0.9% to 1.2% carbon. The C in soil amended with biochar was also more stable compared to the soil treated with cattle manure, suggesting that biochar C remained in the soil longer. Another study also conducted in Indonesia and reported by Islami *et al.* (2011) found that C in soil treated with biochar remained high in the soil even after the second year of cassava crop harvesting compared to the soil alone ranged from (20.3 to 25.8 g kg^{-1} and 10.3 to 11.2 g kg^{-1} respectively). With this evidence, therefore,

biochar may sequester C and could improve the fertility of degraded tropical soils over time.

7.6 The potential use of biochar in the UK

Some studies have stated that adding biochar only benefits poor soils, and that incorporating biochar into productive soil, especially temperate soils, does not make much difference (Jones *et al.*, 2012; Karer *et al.*, 2013; Kloss *et al.*, 2014; Quilliam *et al.*, 2012). This is because fertile soils are always associated with a high pH, CEC, soil organic matter and nutrients (Kloss *et al.*, 2014). However, fertile soils, which are found widely in temperate climates, demonstrate huge variability in their physico-chemical properties. Therefore, they may respond differently to biochar amendment (Kloss *et al.*, 2014). In the current study, the application of the fresh and the aged HW biochar to temperate soils exhibited some positive effects. Furthermore, the addition of different particle sizes of biochar with a higher application rate to the fertile and less fertile soils showed some prominent effects on the soil quality. Therefore, biochar could potentially be used as a soil amendment in temperate soils.

One of the positive effects of biochar is that it can enhance microbial activity in both nutrient rich and nutrient poor soils. The results indicate that the finest particles of biochar increased the activity of microbes in those soils at a higher loading of biochar (5%). This is because the smaller size biochar has a greater surface area and can react faster when mixed with the soil, ultimately enhancing the mineralization of C in the soils (Sigua *et al.*, 2014). Furthermore, biochar addition also stimulates microbial

growth in unfertilized temperate soil. Biochar provides a habitat and serves as a food source for microorganisms, thus increasing the biomass in the nutrient poor soil.

Another significant effect of biochar is that it can increase C in temperate soils. Similarly, previous studies have reported increased C in soils (Kloss *et al.*, 2014; Tammeorg *et al.*, 2013; Uzoma *et al.*, 2011) after the addition of biochar. Moreover, biochar is recalcitrant, because the biomass C in the biochar is in pyrogenic form, where the C in biochar is hard to mineralize (Zimmerman and Gao, 2013). According to Atkinson *et al.* (2010), during the pyrolysis process, about 50% of the carbon in the feedstocks can be retained in the biochar. Moreover, the low mean annual temperature of temperate soils may promote long-term biochar stability. A lower temperature can slow down the degradation of labile C fractions (Haruna *et al.*, 2012) and thus biochar has the potential to provide a stable C sink in temperate soils.

Moreover, adding biochar to temperate soils significantly ($P<0.05$) increased the soil pH. An increase in soil pH has also been reported in other studies, such as those by Liang *et al.* (2006), Warnock *et al.* (2007) and Tammeorg *et al.* (2013). In terms of nutrient leaching, amending soils with biochar reduced the ammonium concentration in the leachate. Although there was little effect on the CEC of these soils, there is a possibility that ammonium leaching could be reduced in temperate soils. In the temperate study, the biochars exhibited greater ability to absorb ammonium than nitrate and phosphate. A reduction in ammonium leaching may decrease the demand for ammonium fertilizer for crop growth. However, nitrate and phosphate nutrients showed an inconsistent trend in terms of leaching when biochar was added to the

soils. More studies using different types of biochars and soils should be conducted in the future in order to understand the mechanisms of the nitrate and phosphate sorption of biochar.

7.7 Economic benefits of biochar in both tropical and temperate regions

The previous chapters demonstrated that biochar has a significant effect on pH and carbon and could also impact on nutrients leaching and biological properties of the soils at 5% biochar dosage. These effects are important particularly in Malaysia because Malaysian soils face problems with acidity and lower CEC because they are deeply weathered. Lower organic matter is always associated with the soils in the tropics as a result of rapid mineralization and decomposition. Controlling acidity in Malaysia is also an issue due to inadequate sources and higher price of agricultural lime to consumer, as compared to biochar. In contrast, UK soils tend not to be acidic, from the only exception being the Western UK. Also, the liming cost in the UK is far cheaper than in Malaysia due to the abundant sources of limestones. Table 7.2 shows the available feedstock and cost of biochar and agricultural lime in Malaysia and in the UK. The liming rate shown in Table 7.2 (2t ha^{-1}) is the sufficient rate for liming requirement in Malaysia. The effect of liming at this rate is reported to last over 4 years (Ishak and Jusop, 2010).

Table 7.2 The available feedstock and cost of biochar and agricultural lime in Malaysia and the UK

Malaysia	**Available feedstock (tonne)**	**Production (tonne)**	**Cost of production**	**Cost to consumer (2t ha^{-1})**
CS Biochar	88,000 (ha)	30,000 (shell)	GBP 10	GBP 200
RH Biochar	-	32,000	GBP 4,200	GBP 500
EFB Biochar	188 Million	4.3 Million	-	GBP 150
Agricultural Lime	-	-	-	GBP 350
UK	**Available feedstock (tonne)**	**Production (tonne)**	**Cost of production**	**Cost to consumer (2t ha^{-1})**
Biochar	48 Million	203,000	GBP 148 to 389 t^{-1}	GBP 3500
Agricultural Lime	-	-	-	GBP 1120

Sources: Shackley *et al.* (2010), Shackley *et al.* (2011), Anem (2015) and Biochar Malaysia (2015).

According to Table 7.2, it can be seen that biochar prices vary widely; for example, 2t ha^{-1} of EFB biochar in Malaysia is approximately GBP 150, compared to GBP 3500 in the UK. Therefore, biochar in Malaysia is great because it is cheaper than to agricultural lime. This contrasts with the situation in the UK, where agricultural lime is much cheaper than biochar. This is due to the massive limestones that can be found to produce agricultural lime in the UK and to the fact that biochar is not widely available. For example, the available feedstock in the UK is 48 million tonnes, compared to 188 million tonnes of EFB biochar in Malaysia (Table 7.2). The cost of agricultural lime to consumer in the UK is cheap, GBP 1120, compared to the cost of using biochar in the UK, which is GBP 3500 at 2t ha^{-1} (Table 7.2). The cheaper cost of biochar in Malaysia is attributed to the abundant sources of agricultural waste, for

example RH, CS and EFB from oil palm crop. In Malaysia, the most commonly available biochars are produced from RH and EFB (Rosenani *et al.*, 2012b). RH is available at rice mills as a by-product of burning to produce heat for drying rice, whereas EFB is produced from oil palm extraction and has high potential to be converted to biochar because Malaysia produces a large quantity of EFB from the oil palm industry (Rosenani *et al.*, 2012b). Additionally, to the best of my knowledge, in some parts of the East Coast of Malaysia, farmers can even obtain RH biochar for free. Although the cost of biochar to consumer is cheap in Malaysia, other issues must be taken into consideration, such as whether the sources used to produce biochar are sufficient enough to supply the demand if that increases, and the logistics cost (transportation for biomass collection in order to produce biochar or biochar collection from one place to another).

According to Table 7.2, biochar is potentially used in Malaysia due to the cheaper source as a soil amendment. In contrast, in the UK, the cost of biochar is far more expensive than using other liming materials, such as agricultural lime. Even though the cost of biochar is high in the UK, biochar is potentially used for other purposes, such as carbon sequestering. Biochar can lock up the carbon in soils due to its recalcitrance and resistance to degradation, ultimately reducing the emission of carbon in the atmosphere and eventually, reducing global warming. More research is needed to find alternative feedstocks, as well as low cost and sustainable technology to produce biochar, thus, the price of biochar, especially in the UK market can be reduced. It is possible that the economic returns from using biochar may be higher than that from using agricultural lime after considering other non-pH benefits to the growers (Galinato *et al.*, 2011).

Building of soil carbon content is important for a number of reasons, including promotion of soil structure stability, increase of water retention and infiltration, carbon fixation, and reduction of soil erosion (Victoria *et al.*, 2012). In Malaysia, organic matter is low (1 to 2%), and therefore, not sufficient for crop growth. Consequently, organic amendment is needed to enhance soil quality and productivity (Ishak and Jusop, 2010). Unlike soils in the tropics, UK soils are more fertile and have higher organic matter content (Kloss *et al.*, 2014). However, different soil properties from one place to another may exhibit various effects, thus, detailed investigation regarding the use of organic amendment to the temperate soil is essential to better understand whether the effects are beneficial or detrimental. Besides biochar, there are a number of organic materials (carbon sources) available to farmers. Other organic amendments that have been used in agricultural lands include agricultural wastes, such as manure and compost. Table 7.3 shows the differences of organic matter sources and highlights the advantages and disadvantages of these materials to the farmers in Malaysia and the UK.

Table 7.3 The advantages and disadvantages of various organic amendment sources in Malaysia and the UK

Organic Amendment Source (Malaysia)	Advantages	Disadvantages	Source
Biochar	• Low cost • Recalcitrance • Increases pH • Increases CEC • Reduces nutrients leaching • Adsorbs water, nutrients and contaminants • Reduces greenhouse gases emission	• Competition in the use of feedstock for animal feeding • Deforestation • Risk of contamination (PAH, heavy metals) • Occupational health and fire hazard	Masulili *et al.* (2010) Sukartono *et al.* (2011) Rosenani *et al.* (2012b) Rosenani *et al.* (2013) Alling *et al.* (2014)

Table 7.3 continued

Organic Amendment Source (Malaysia)	Advantages	Disadvantages	Source
Rice husk compost	• Increases pH • Alleviates Al toxicity • Increases CEC	• Higher decomposition rate • Increases greenhouse gases emission • Reduces crop growth at high application rate (80t ha^{-1}) • Production of humic substances from composting reduces micronutrients at high rate	Ishak and Jusop (2010)
Chicken manure	• High nutrients (N, P and K) sources	• Accumulation of heavy metals	Ishak and Jusop (2010)
EFB (Empty fruit bunch)	• Improves soil structure • High nutrients (N, P, K, Ca and Mg)	• Short-lived	Ishak and Jusop (2010)

Table 7.3 continued

Organic Amendment Source (UK)	Advantages	Disadvantages	Source
Biochar	• Recalcitrance • Improves hydraulic conductivity • Increases pH, total C and N, Olsen-P and CEC • Reduces nutrients leaching • Adsorbs water, nutrients and contaminants • Reduces greenhouse gases emission • Increases microbial activity and biomass	• High cost • Risk of contamination (PAH, heavy metals) • Deforestation • Occupational health and fire hazard	Verheijen *et al.* (2010) Uzoma *et al.* (2011) Karer *et al.* (2013) Zhang *et al.* (2014b) Gronwald *et al.* (2015)
Cow manure	• High nutrients (N and P)	• Short-lived • Ammonia volatilazation • Dangerous pathogens	Verheijen *et al.* (2010) Uzoma *et al.* (2011)
Sewage sludge	• Improves soil structure • Increases infiltration rate and water holding capacity	• High metals • Dangerous pathogens • Leaching of contaminants from the soils	Verheijen *et al.* (2010) Méndez *et al.* (2012)

The main limitation of using organic materials as a soil amendment rather than biochar is the fact that organic materials are short-lived because they can be easily decomposed, compared to biochar. Therefore, the application of these materials has to be done repeatedly in every crop cycle and every year (Masulili *et al.*, 2010). In addition, there is a competition in the use of fresh organic materials for animal feeding, as well as energy resources. Also, rapid mineralization and decomposition of

these materials contribute to the emission of greenhouse gases, thus resulting in global warming (Rondon *et al.*, 2007).

Furthermore, Table 7.3 illustrates that organic amendment sources from manure and sewage sludge may contain heavy metals and other contaminants, such as PAH. Research showed that biochar can absorb these contaminants due to large surface areas, as well as macro and micro pores which are present in biochar (Cornelissen *et al.*, 2005; Lehmann, 2007a). Other studies also found that biochar can adsorb not only contaminants, but pesticides too (Yang *et al.*, 2010; Sopena *et al.*, 2012; Wang *et al.*, 2012). However, the ability of biochar to retain pesticides may prevent them from controlling target organism (IBI, 2012b). Moreover, if sorbed organic or inorganic compounds become available to organisms, they may potentially have detrimental effects on them. The presence of black carbon in the form of biochar in agricultural soil has been shown to reduce the bioavailability of some compounds to microorganisms. However, the length of time that biochar can retain the compounds and its safety towards other organisms in terms of toxicity remain unknown (Semple *et al.*, 2013). In addition, biochar itself may contain heavy metals and other organic pollutants, especially when poultry manure is used to produce biochar. However, this depends on the types of feedstocks, because the physico-chemical properties of biochar depend on the feedstock used to produce biochar. Moreover, during slow pyrolysis process at $500^{O}C$, heavy metals and PAH accumulate in the biochar (Painter, 1998; Verheijen *et al.*, 2010). Brown (2009) reported that several chars produced at > $500^{O}C$, the concentration of PAH ranged between 3-16 µg g^{-1} compared to that of 28 µg g^{-1} in char from burned pine forest. Therefore, full risk assessment for such

contaminants is required to understand the safety of biochar use before its application in vast agricultural areas.

7.8 Conclusion

In conclusion, biochars could potentially be used in both tropical and temperate climates as mentioned earlier in the previous sections. For example, biochar increased carbon mineralization when using finer particle sizes at a higher dosage of biochar and it improved the biomass in the unfertilized temperate soil (Chapter 6). Furthermore, biochar increased the pH and carbon content, as well as adsorbing ammonium leaching from both tropical and temperate soils (Chapters 4, 5 and 6). Although tropical and temperate soils' chemical and physical properties are not similar, however, not much different could be seen in terms of the effects of biochar application in the tropical and temperate climates at 2% application rate (Chapters 4 and 5). Higher rates of application maybe needed to make a real and prominent effect following biochar addition to these two soil systems.

In addition, other issues should be taken into consideration when applying biochar in both climates, especially in huge agricultural areas. For instance, the cost of biochar production is one of the issues because most of the technologies for biochar production require high investment cost (Kong *et al.*, 2014; Cernansky, 2015). The production of finer particle sizes of biochar would increase the cost even more (Kollah *et al.*, 2015). Another issue regarding biochar production is that if the demand for biochar increases, the feedstock used would need to be produced on a massive scale. Using HW biochar, for example, may lead to deforestation (Cernansky, 2015) and

create other environmental problems, such as increasing GHG gas emissions, reducing biodiversity and accelerating soil erosion. Safety issue regarding organic contaminants on biochar is also a matter of concern. More research is needed in this area to ensure its safety, particularly when applying biochar at higher application rates. Therefore, the use of biochar needs further assessment before making a recommendation.

CHAPTER 8

Conclusions

8.1 General conclusions

Overall, this thesis has investigated the effects of biochar amendment on soil properties in two different geographical regions. The biochars used were coconut shell and rice husk biochars (tropical study) and hardwood biochar (temperate study). The mixtures of biochar and soil were evaluated to see whether the biochar improved the soil's biological and chemical properties, including nutrient leaching, and physical properties over time.

The major findings of this study were that the addition of biochar at 2% and 5% application rates increased the soil's carbon content and pH in all of the soils studied. This indicates that biochar addition has the potential to benefit the environment by sequestering carbon in the soil and ameliorating acidic soils. Another finding from this research is that biochar has the ability to absorb ammonium better than nitrate and phosphate. Ammonium absorption by biochar may reduce the demand for ammonium fertilizer, as well as reducing the loss through leaching, and indirectly minimizing eutrophication by nitrogen.

For the biological properties experiment, a higher application rate of biochar at 5% enhanced the mineralization of ^{14}C glucose in the soil. The results also showed that finer particles with a higher loading of biochar mineralized more carbon than other sizes. Biochar addition also benefits nutrient poor soil more than nutrient rich soil in

terms of the growth of microbes. The results from this study show that the effects of biochar amendment on the biological properties depend on a higher application rate and smaller particle size of biochar, as well as the different nutrient status of the soils.

Based on the findings from this thesis, the results provide important information regarding the use of biochar in two different climates. For example, the effects of biochar in this thesis are dependent on various factors, such as the types of soils and biochars, the application rates and the particle sizes of biochar. All of these factors exhibit different responds. For instance, some of the positive effects were increased carbon and pH; some of the unclear effects were nitrate and phosphate leaching; and no effects or limited effects was seen in terms of the physical properties, as well as cation exchange capacity as a results of the biochar addition. Therefore, this reflects the impacts of biochar application, which need to be further assessed and examined before applying biochar on a wider scale to agricultural land can be suggested.

8.2 Recommendations for future research

The long-term effects of biochar amendment should be explored further in order to identify how long the effect of biochar persists. The current study has also examined the long-term effect (based on the laboratory incubation); however the effects were unclear and some of them could not be seen. Therefore, longer effects of biochar (more than 3 years) should be investigated especially the physical properties of the soil, such as aggregate stability. This is because the formation of aggregate in the soil after organic amendment usually takes a long time.

There is limited research on the particle sizes of biochar and its stability in the soil that should be explored. This is because biochar is porous, lighter than soil particles and hydrophobic. Research showed that finer size of biochar may adsorb better contaminants, such as PAH and pesticides compared to larger size (Bucheli and Gustafsson, 2001; Hiller *et al.*, 2007). The finer particles of biochar, for instance, are also subject to travelling further and loss through leaching. Additionally, smaller particle sizes of biochar may degrade faster than the larger sizes. Thus the effectiveness of biochar as a carbon sink is reduced due to both effects: loss via leaching and degradation by microbes. However, the information on the breakdown of biochar's particle size is limited. It is unclear whether different particle sizes affect the mineralization rate and biochar stability. Thus, these areas must be investigated and documented. In addition, in this thesis (Chapter 4-tropical soils study) only measures the effects of biochar at lower application rate with coarser particle sizes. Finer particle sizes and higher application rate should be investigated further using the biochar and soils in the tropics. This is because, different types of biochar, methods to produce biochar (fast or slow pyrolysis) and temperatures used to produce biochar may exhibit various effects of biochar to the soils. Therefore, more research are needed to explore these areas.

Furthermore, biochar application in this study increased soil pH. Therefore, the effects of soil pH should be explored on a larger scale, such as in a field trial to assess whether the effects exist in a real condition after it is shown that there is a liming effect of biochar in a small scale study (laboratories study) (Chapters 4, 5 and 6). Moreover, the long-term effect of biochar on pH should be investigated further. This is important to observe how long the effect of pH in the soil can last after biochar

addition. Ideally, biochar should have a long residence time in soil, but more work is needed to quantify this. The comparison of biochar with other liming materials, such as dolomitic lime or a combination of both, is required to observe the long term effect, particularly in the tropics. This is because agricultural lime application in Malaysia can only ameliorate the topsoil, the subsoil is not ameliorated enough for better root growth (Ishak and Jusop, 2010). Therefore, combining lime and biochar may solve this problem and may decrease the demand of feedstock used to produce biochar. Also, the use of biochar might reduce the cost of liming, because biochar is a cheaper liming material than dolomitic lime.

For the leaching experiment, biochar had the ability to retard ammonium better in the soil than nitrate and phosphate. But biochar can also retain nitrate in the soil. However the mechanism is poorly understood. Thus, more studies in this area should be conducted to understand the mechanisms through which biochar can retain anions, such as nitrate in the soil. Moreover, as well as the study on the carbon mineralization, a study on nitrogen mineralization is also required. The reduction of nitrate leaching may be possible due to N immobilization, and this mechanism is related to N mineralization and the C/N ratio.

In addition, there are few studies on the use of radioactive isotope (^{14}C) labelling techniques in biochar. To the best of my knowledge, until now, there have been no studies on biochar using this technique in the tropics. The technique is beneficial and needed to better understand the fate of biochar and its interaction in the ecosystem (Ladygina and Rineau, 2013). Furthermore, the study in this thesis was conducted on a

small scale (laboratory study). The effects of biochar in a large-scale study should be investigated, especially in actual conditions. For example, the effectiveness of biochar in field situations should be assessed along with crops. This is because the crops may respond differently with different biochars, as well as in different soils. Moreover, more research regarding the application techniques of biochar in the field is warranted. This is to avoid the loss of biochar via water and wind erosion, which can also affect humans through inhalation during the application. Finally, the effect of organic contaminants in the biochar, such as PAHs, heavy metals and pathogens must be taken into consideration. More research are urgently needed, particularly in this area to ensure the safety of biochar amendment, especially when applied at higher dosage.

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APPENDIX 1

Table A1 Carbon content (%) in forest, non-intensive and intensive farming soils amended with and without CS and RH biochar, over 360 d. Error bars are SEM (n=3).

Treatment	Day	Forest	Non-intensive farming	Intensive farming
Control	0	3.63 ± 0.17	1.09 ± 0.04	1.69 ± 0.31
	60	3.61 ± 0.16	0.96 ± 0.02	1.52 ± 0.17
	120	4.30 ± 0.13	1.00 ± 0.05	1.47 ± 0.05
	240	2.97 ± 0.21	0.78 ± 0.02	1.12 ± 0.06
	360	3.01 ± 0.06	0.94 ± 0.05	1.23 ± 0.03
CS Biochar	0	8.15 ± 0.43	3.71 ± 0.25	2.50 ± 0.05
	60	7.06 ± 0.52	4.38 ± 0.46	2.98 ± 0.30
	120	6.96 ± 0.70	3.86 ± 0.51	2.80 ± 0.17
	240	6.25 ± 0.35	3.65 ± 0.50	3.47 ± 0.42
	360	6.35 ± 0.24	3.68 ± 0.07	3.47 ± 0.33
RH Biochar	0	5.98 ± 0.28	3.13 ± 0.04	2.67 ± 0.13
	60	6.61 ± 1.01	3.13 ± 0.22	3.01 ± 0.40
	120	4.82 ± 0.21	2.38 ± 0.03	2.28 ± 0.03
	240	4.33 ± 0.03	2.30 ± 0.06	2.18 ± 0.11
	360	4.22 ± 0.22	2.44 ± 0.18	2.28 ± 0.07

APPENDIX 2

Table A2 Nitrogen content (%) in forest, non-intensive and intensive farming soils amended with and without CS and RH biochar, over 240 d. Error bars are SEM (n=3).

Treatment	Day	Forest	Non-intensive farming	Intensive farming
Control	0	0.19 ± 0.01	0.06 ± 0.01	0.25 ± 0.04
	60	0.18 ± 0.02	0.06 ± 0.001	0.22 ± 0.02
	120	0.23 ± 0.01	0.06 ± 0.001	0.23 ± 0.002
	240	0.15 ± 0.01	0.05 ± 0.002	0.16 ± 0.01
CS Biochar	0	0.22 ± 0.02	0.08 ± 0.01	0.22 ± 0.01
	60	0.23 ± 0.02	0.07 ± 0.01	0.20 ± 0.01
	120	0.22 ± 0.03	0.07 ± 0.002	0.20 ± 0.004
	240	0.16 ± 0.01	0.06 ± 0.004	0.18 ± 0.01
RH Biochar	0	0.22 ± 0.01	0.10 ± 0.001	0.22 ± 0.01
	60	0.25 ± 0.03	0.09 ± 0.01	0.23 ± 0.02
	120	0.19 ± 0.01	0.08 ± 0.001	0.20 ± 0.003
	240	0.16 ± 0.003	0.07 ± 0.003	0.19 ± 0.01

APPENDIX 3

Table A3 Phosphate content (Mg g^{-1}) in forest, non-intensive and intensive farming soils amended with and without CS and RH biochar (0 and 360 d). Error bars are SEM (n=3).

Soil	Treatments	Day 0	Day 360
Forest	Control	0.21 ± 0.06	0.31 ± 0.13
	CS Biochar	0.13 ± 0.001	0.25 ± 0.01
	RH Biochar	0.24 ± 0.01	0.33 ± 0.06
Non-Intensive	Control	0.51 ± 0.04	0.68 ± 0.02
Farming	CS Biochar	0.50 ± 0.05	0.72 ± 0.05
	RH Biochar	0.52 ± 0.03	0.84 ± 0.05
Intensive	Control	2.20 ± 0.08	3.19 ± 0.21
Farming	CS Biochar	2.29 ± 0.10	3.19 ± 0.11
	RH Biochar	2.42 ± 0.03	3.27 ± 0.08