THE EFFECT OF COMPOSTED BIOCHAR ON COMPOST PROPERTIES AND MINERALISATION

By

Ockert Guillaume Botha

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Supervisor: **Dr Ailsa Hardie**
Department of Soil Science
Faculty of AgriSciences

Co-supervisor: **Dr Andrei Rozanov**
Department of Soil Science
Faculty of AgriSciences

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DECLARATION

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ABSTRACT

Pyrolized carbon, also known as biochar, is a widely used soil conditioner recognized for its adsorption, C sequestration and agricultural qualities. This led to the investigation into the possible use thereof by small-scale sustainable farmers as a filter for agricultural olive or wine effluent, where after the spent biochar can be incorporated into composts to sterilize it from toxins and pathogens before being used as soil amendment. However, before these used biochar filters can be applied to compost, research is required to assess the affect that biochar could have on the composting process. This research project was therefore initiated to investigate the feasibility of adding biochar to composts, specifically focusing on the effect of type and amount of biochar on the composting process and mineralisation of the composts in soils. The final aim was to construct a method for quantifying biochar content in compost and soil that can be used to assess the stability of biochar in soils. Furthermore, none of this research has previously been done in South Africa or on two locally produced biochars.

The first experiment was constructed to evaluate the effect of two contrasting commercial biochars on composting; a relatively low-cost, crude, pine wood biochar produced using a low-tech slow pyrolysis technique at 450°C, and a significantly more expensive, refined eucalyptus biochar produced using a high-tech slow pyrolysis technique at 900°C. The biochars were applied at two application rates (10% and 20% dry weight) to a mixture of green and animal waste. The effect was measured through composting indices such as temperature, C/N ratio, pH and EC, and microbial activity. Results showed that the robust, low temperature pine biochar applied at 10% (d/w) is the most suitable for composting due to higher composting temperatures measured, lower C/N ratios in the final product and higher cumulative microbial activity relative to the other biochar treatments. However, all biochar and control composts were all classified as successfully matured and stabilized according to the indices used, indicating that both types of biochar and application rates can be used to produce compost.

The second experiment was aimed at comparing the carbon (C), nitrogen (N) and phosphorus (P) mineralisation of the composted biochar in relation to compost with biochar and biochar only under ideal laboratory conditions. The incorporation of these treatments into the soil showed that the composting process increased the composted biochars degradability with 7.6 – 11.7% more carbon dioxide (CO_2) being respired than compost with biochar of the same quantity. Biochar type and quantity influenced the mineralisation as eucalyptus char in general, and all treatments containing 20% biochar proved to be least degradable by microbes. Nitrogen mineralisation

results showed that regardless of biochar type, quantity or composting, all biochar containing treatments caused net N immobilization and reduced nitrification. Phosphorus availability was found to be improved for both biochars through composting and the addition of compost, especially for eucalyptus biochar of which the amount of available P surpassed that of pine biochar although pine biochar only applications released more P. A 6-month field trial experiment was also constructed to further evaluate the five composts' C mineralisation under natural conditions. In this experiment there was found that all biochar containing compost produced 7.6 – 20.1% less CO_2 than the control compost, of which eucalyptus biochar showed the least amount of respiration. Loss on ignition results also revealed that composted eucalyptus biochar was the least degradable composts as only 7.4% and 7.8% of the total SOM was lost. Density fractionation further illustrated that composted biochar remains in the soil in particulate form longer than conventional compost and is slower to transform into the mineral fraction. No discernable difference in biochar content within the composts could be seen after field application at 50 t/ha.

The final aim of developing a rapid and cost-effective quantification method with the use of near-infrared spectroscopy (NIRS), was completed by constructing a calibration range of soils and compost from both types of biochar. The spectra acquired was then used to create regression models that were used to predict biochar content in the final mature composts and field trial soils. The results showed that NIRS can be used to quantify biochar, to within the same order of magnitude, in both composts and soil mixtures, which is of great importance for C stock audits and assessing biochar decay over time.

Selecting the type of biochar for water filtration, composting and soil conditioning, would be dependent on the purpose of the application. Both biochars show the ability to be successfully composted and used as soil amendment with good C sequestration capabilities. However, pine biochar is more suitable for the composting process and sterilization as it results in higher temperatures and increased microbial activity. Eucalyptus biochar however, would be the best option for phosphorus mineralisation and building soil carbon stocks.

OPSOMMING

Biochar is 'n grond verbeteringsmiddel wat wydbekend is vir adsorpsie, koolstof vaslegging en verskeie ander landboukundige gebruike. Hierdie eienskappe het na die ondersoek vir die moontlike gebruik daarvan deur kleinskaalse, boere gelei. Die doel is om dit as `n volhoubare filter vir olyf- of wynuitvloeisel te gebruik, waarna dit in kompos toegedien kan word om dit van gifstowwe en patogene te steriliseer. Voordat die gebruikte biochar filters egter toegevoeg kan word in komposhope, is navorsing nodig om te bepaal wat die invloed van biochar op die komposproses en komposkwaliteit sal wees. Hierdie navorsingsprojek was dus tot stand gebring om die haalbaarheid van gekomposteerde biochar te ondersoek met spesifieke fokus op die effek wat die tipe en hoeveelheid biochar op die afbreek en mineralisasie van die kompos in die grond sal hê. Die finale doel was ook om 'n metode te skep vir die kwantifisering van biochar in kompos en grond sodat die stabiliteit van biochar beoordeel kan word.

Die eerste eksperiment was opgestel om die effek van twee kontrasterende, kommersiële biochars op kompos te evalueer. Die een biochar is relatief goedkoop, ru en geproduseer uit dennehout deur middel van stadige pirolise by 450°C, terwyl die ander een aansienlik duurder, meer verfynd en uit bloekomhout teen 900°C geproduseer is. Die biochars was in twee toedieningshoeveelhede (10% en 20% droë gewig) in 'n mengsel van groen materiaal en beesmis toegedien. Die effek van die biochar op die kompos is deur middel van verskeie kompos indekse soos temperatuur, C/N verhouding, pH, EG, en mikrobiese aktiwiteit gemeet. Resultate het getoon dat die ru, lae temperatuur dennehout biochar, toegedien teen 10% (D / w), die mees geskikte is vir kompos aangesien hoër kompos temperature gemeet is, laer C/N verhoudings in die finale produk was en hoër kumulatiewe mikrobiese aktiwiteit in vergelyking met die ander biocharbehandelings gevind is. Al die biochar-ryke komposhope was egter geklassifiseer as volledig gestabiliseer wat daarop dui dat beide tipes biochar en die toedieningshoeveelhede gebruik kan word om suksesvolle kompos te vervaardig.

Die tweede eksperiment was daarop gemik om die mineralisasie van koolstof (C), stikstof (N) en fosfor (P) onder ideale laboratoriumtoestande van die gekomposteerde biochar met kompos saam met biochar, en slegs biochar te vergelyk. Die toediening van hierdie behandelings in die grond het getoon dat gekomposteeerdebiochar tussen 7,6-11,7% meer koolstofdioksied (CO_2) in vergelyking met kompos met dieselfde hoeveelheid biochar vrystel. Die hoeveelheid en tipe biochar het ook 'n invloed gehad aangesien mineralisasie van bloekombiochar in die algemeen laer was, terwyl behandelings met 20% biochar die minste afbreekbaar was. Stikstof mineralisasie

resultate het getoon dat, ongeag van die tipe of hoeveelheid biochar in die kompos, alle biochar-ryke behandelings netto immobilisasie van N veroorsaak. Fosforbeskikbaarheid het verbeter deur kompostering en die byvoeging van kompos, veral vir bloekomhoutbiochar waarvan die hoeveelheid beskikbare P dennehoutbiochar se hoeveelheid oortref het. 'n Ses maande veld-eksperiment is ook opgestel om koolstofmineralisasie van die vyf komposte onder natuurlike omstandighede verder te evalueer. In hierdie eksperiment is daar bevind dat alle gekomposteerde biochar behandelings 7,6-20,1% minder CO_2 as die kompos beheerdes geproduseer het, waarvan bloekombiochar die minste gerespireer het. Verlies op ontstekingsresultate het ook getoon dat gekomposteerde bloekombiochar die minste afbreekbaar was aangesien net 7,4% en 7,8% van die totale OM verlore gegaan het. Digtheidsfraksionering het ook verder getoon dat gekomposteerde biochar langer in die grond bly as konvensionele kompos.

Die finale doel was om 'n vinnige en koste-effektiewe kwantifiseringsmetode te skep deur gebruik te maak van naby infrarooi spektroskopie (NIRS). Dit voeris uitgevoer deur 'n kalibrasie reeks te konstrueer met 'n verskeidenheid van biochar hoeveelhede in beide grond en kompos. Die spektra wat verkry is, was daarna gebruik om 'n regressiemodel te skep, wat dan gebruik was om biocharinhoud te voorspel in die finale kompos en veldgronde. Die resultate het getoon dat NIRS wel gebruik kan word om biochar te kwantifiseer binne dieselfde orde grootte in beide kompos en grondmengsels. Hierdie resultate is van groot belang vir koolstofvoorraad oudits en die beoordeling van biochar verval met verloop van tyd.

Die selektering van 'n tipe biochar vir waterfiltrasie en kompos- en grondkondisionering is gevind om afhanklik te wees van die wyse van toediening. Beide biochar's het die vermoë om suksesvol gekomposteer te word en as grondwysiging met 'n goeie K sekwestrasie vermoë gebruik te kan word. Dennebiochar blyk meer geskik te wees vir die komposterings proses in terme van sterilisasie, aangesien dit tot hoër temperature en verhoogde mikrobiese aktiwiteit lei. Bloekombiochar sou egter die beste opsie wees vir fosformineralisasie en die bou van grondkoolstof

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CHAPTER 1 – GENERAL INTRODUCTION AND RESEARCH AIMS

The National Research Foundation's (NRF) Centre of Excellence in Food Security is currently investigating the continuum between water availability and quality, soil health, plant health, food safety and nutrient pathways to consumer well-being. It was established in the context of changing food systems facing ecological, social, economic and physical challenges with its focus on the generation of energy and knowledge to improve access to sustainable and sufficient amounts of nutritious food for poor, vulnerable and marginal populations. In the light of improving food production in a sustainable manner that can overcome physical challenges, a range of agronomic interventions can be proposed. However, typical socio-economic factors that small-holding producers from poor and marginal populations are faced with such as poverty, high family dependency, lack of sufficient land, security of tenure, and lack of financial options (Mdlalo 2008) makes it difficult to find amendments that are environmentally sustainable, agriculturally beneficial, and economically viable. Biochar could be one of the only organic amendments that has environmental benefits in terms of long-term C sequestration, being easily available or producible by small-holding farmers, and also suitable for assimilation into current agronomic regimes (Lehmann 2007), thereby meeting the criteria as stipulated by the NRF.

Interest in biochar particularly lies within its C sequestration capabilities in soils that can be used as a tool for offsetting anthropogenic carbon dioxide (CO_2) emissions whilst showing potential for agronomic benefits (Clough et al. 2013). Woolf et al. (2010) showed that the implementation of a global sustainable biochar program could potentially offset 12% of the current anthropogenic CO_2–C equivalent emissions. The attention to biochar is thus based on the importance of the global C cycle (Forbes et al. 2006) and biochars potential as C sink in soils and sediments over long-periods of time. These long-term attributes are due to the apparent slow rates of microbial decomposition and chemical transformation expressed through: (i) high resistance to a range chemical oxidants, (ii) its preservation in geological records over a long period, (iii) and the existence thereof in soil depths where the residence times exceeds millennia (Kuzyakov et al. 2014). One of the other important environmental properties of biochar that sets it apart from other organic amendments, is its affinity and capacity for sorbing organic compounds (Smernik 2009). Sorption of organic pollutants by biochar or similar forms of activated carbon include compounds such as polycyclic aromatic hydrocarbons (PAHs) (Sander and Pignatello 2005), benzene (Braida et al. 2003), organochlorine insecticides (Lichtenstein et al. 1968), polychlorinated biphenyls

(Strek et al. 1981), 2,4,6-tripnitro-toluene (Vasilyeva et al. 2001) and phenanthrene (Rhodes et al. 2008).

These strong environmental qualities of biochar consequently initiated the possible use thereof for creating a low-cost mechanism to filter agricultural waste water, such as those produced by wineries and olive-mills, where after these filters can be applied to the soil as organic amendment with longer-term C sequestration potential. However, applying biochar filters directly to the soil could also introduce pathogens and organic toxins sorbed by the biochar, potentially leading to soil health problems. Composting of the spent biochar filters could help to sterilize and stabilize the adsorbed organic materials by the process of humification. Research on the application of pure biochar in agricultural soils have also shown some other fertility related challenges such as nitrogen immobilization (Lehmann et al. 2009; Nelson et al. 2011; Sika and Hardie 2014) and over-liming (Shultz et al. 2013; Sika and Hardie 2014). Composting of biochar has also been a proposed method to overcome these soil fertility constraints (Schultz et al. 2013). Compost production is a sustainable and generally inexpensive practice used by small-scale farmers to enhance soil quality utilizing local plant and animal waste materials. Composts however, can only store carbon temporarily in soil, depending on soil type, temperature and cultivation practices (Favoino and Hogg 2008). Thus the addition of biochar could also serve to enhance the longevity of compost by adding more recalcitrant carbon.

Therefore this pilot research project was initiated to investigate the feasibility of adding biochar to composts, specifically focusing on the effect of type and amount of biochar on the composting process and mineralisation of the composts. Another project running concurrently with this project is looking specifically at the sorption capacity of biochar based filters on different types of agricultural effluents. After the evaluation of these filters' effectiveness to remove organic pollutants and microbes from the waste waters, the used biochar filters would then be incorporated into compost piles. However, before this can be done, effects of biochar addition to the composting process must first be understood.

The first aim of the project was to see how the addition of biochars affected the composting process and properties (i.e., temperature, microbial activity and chemical characteristics). Compost temperature was a key parameter to monitor as high composting temperatures are critical to the sterilization of the composting materials, especially if spent biochar filters are being composted upon filtration of waters tainted with pathogens. This first aim is addressed in Chapter 3, where the effect of the addition of two contrasting pinewood and eucalyptus biochars (10 and

20% w/w) to fresh composting materials on composting process was measured by monitoring temperature, microbial activity, C/N ratio, and various chemical properties.

The second aim of the project was to examine the effect of the composted biochars on C, N and P mineralisation and soil C functional pools. This aim is addressed in Chapter 4 where a two-month laboratory incubation study and a 6-month field study were conducted, which also focused on determining how composting may have altered the biological inertness of the biochar itself, which could then influence the long-term C sequestration capabilities thereof.

The third aim of the project was to develop a near-infrared reflectance spectroscopy (NIRS) method to cheaply and rapidly quantify the amount of biochar in composts and in soil amended with the composted biochar. This aim is addressed in Chapter 5, whereby a NIRS-based method was developed and used to estimate the biochar contents in both the final compost products and also the field trials soils in comparison to other methods such as proximate analysis.

CHAPTER 2 – LITERATURE STUDY

2.1 Introduction

Biochar is a chemically complex organic compound that has potential as a soil conditioner, waste management system, nutrient cycler, and agent for long-term carbon sequestration. This literature review aims to look at how this product is produced, which factors determines its physical and chemical properties and how these properties affect the application thereof in soils and compost systems. Finally, it will conclude with highlighting gaps in research and future research directions.

2.2 Production and properties of biochars

2.2.1 Biomass conversion process

Biochar can be produced from any carbonaceous material through thermochemical processing. Feedstocks can vary from garden- and agricultural-, to municipal- and sewage sludge wastes. There are five different pyrolysis processes used to transform the feedstock, each following different reaction conditions, into three basic products: solid (biochar/ash), liquid (bio-oil/tar) and gas (syngas). Producers will select a pyrolysis process to optimize the quality and quantity of one or more of these products depending on their purpose. The five processes are slow pyrolysis, torrefication, fast pyrolysis, flash pyrolysis and gasification (Brewer 2012). Slow pyrolysis is the most traditional and widely used form of biochar production. This process consists of heating the feedstock to moderate or high temperatures in the absence of oxygen. In general, it would be characterized by slow heating rates over several hours or even days, depending on the feedstock/purpose specific temperature range. The ultimate goal is an amorphous biochar product that is high in carbon compounds and energy dense (Brewer 2012; Chun et al. 2004)

2.2.2 Biochar properties

All forms of biochar consist of two major fractions; a carbon (C) fraction and an inorganic ash fraction. The carbon fraction can be crudely divided into recalcitrant C and labile or leachable C (Lehmann et al. 2011). These C fractions include hydrogen, oxygen and other elements similar to any other form of organic material. However, the greatest difference between other organic material and biochar is the high proportion of fused aromatic C structures (Brewer 2012, Lehmann et al. 2011). The presence of these C structures is the main reason for biochars' stability and inertness (Kuzyakov et al. 2011; Lehmann et al. 2011). The density and amount of these aromatic carbon structures is dependent on the temperature range and process used for charring.

The ash or mineral fraction is mostly affected by the characteristics of the feedstock and not too much by the reaction conditions (Gaskin et al. 2008). During the pyrolysis process, all of the

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minerals present in the feedstock are partitioned into the ash fraction of the biochar (Laird et al. 2010). However, the incorporation of some of these minerals into the aromatic structures of the biochar may be favoured at higher temperatures (Freitas et al. 2001; Gaskin et al. 2010; Leinweber et al. 2007). The reaction conditions therefore determine the ash-to-carbon ratio, which in turn can affect the net surface charge of the biochar. Fresh biochar would typically have a low initial cation exchange capacity, and could have both a net positive and a net negative charge (Brewer 2012; Lehmann et al. 2011). Research by Nguyen and Lehmann et al. (2009) and Nguyen et al. (2007) have shown that greater pyrolysis temperatures results in greater surface area production, which causes a decrease in CEC and loss of volatile matter (Lehmann et al. 2011). High temperature biochars also become more stable due to their high amount of polycondensed aromatic structures which are less prone to decomposition (Novak et al. 2014)

Biochars physical properties could be compared to that of a soil aggregate. It consists of a large surface area, constructed from various pores. The size and arrangement of these pores are determined by the feedstock properties and pyrolysis temperature (Downie et al. 2009). The chemical inertness of the aromatic carbon compounds allows for the physical structure to remain intact and particulate over long periods of time (Skjemstad et al. 1996; Lehmann et al. 2009), which means that biochar can be applied as a long-term soil amendment and not just a temporary ameliorant.

2.2.3 Quantifying biochar in the environment

Biochars heterogeneity, chemical complexity and inherently non-reactive nature of the C compounds after pyrolysis, presents many analytical challenges to the quantification of biochars in soils. Several researchers have found methods to determine the relatable black C contents in soils. However, there are other sources such as natural coal-based minerals (Scott and Glasspool 2007) or inertinite (Senftle et al. 1993) that can make it difficult for these methods to accurately determine the quantity of biochars (Manning and Lopez-Capel 2009). The six most suitable methods available for the determination of black C as indices for biochar content are: (1) determination of solvent-extractable aromatic compounds as benzene polycarboxylicacids (Brodowski et al. 2005); (2) chemo-thermal oxidation at 375°C followed by elemental analysis of the residue (Gélinas et al. 2001); (3) chemical oxidation using acid dichromate or sodium hypochlorite, followed by elemental analysis of the residue by 13C nuclear magnetic resonance (NMR) analysis (Simpson and Hatcher 2004); (4) thermal/optical laser transmittance or reflectance (Huang et al 2006); (5) ultraviolet (UV) photo-oxidation of the sample followed by 13C-NMR analysis of the residue (Skjemstad et al. 1996); (6) thermogravimetric analysis of the sample

under flowing $He_{80}O_{20}$ (Manning and Lopez-Capel 2009). These methods however, are all expensive and time consuming.

Mid-infrared spectroscopy (MIR) has also been used successfully to measure C fractions, including biochar, in the past (Allen and Laird 2013). It is based on absorption spectra that are constructed from fundamental vibration signals of the charcoal's aromatic structures which can be distinguished from other biogenic soil organic C (Janik et al. 2007). MIR however, generally requires higher quantities of instrument setup and running costs, and is not field mobile. NIR, on the other hand, is a relatively inexpensive and potentially field-deployable technology that has been used in industry for various process applications over the last number of years (Allen and Laird 2013). This makes NIR an ideal candidate to measure biochar content in soils for C accounting purposes.

2.2.4 Effects of biochar as soil amendment

2.2.4.1 *Physiochemical*

The beneficial effects of adding biochar to soils are: increased pH, CEC, soil water retention, nutrient retention, improved soil structure, soil aeration, hydraulic conductivity and adsorption of heavy metals (Anderson et al 2011; Borchard et al. 2012; Buss et al. 2012; Case et al. 2012; Schmidt et al. 2014). When biochar is applied to soils, it undergoes a wide variety of changes due to its surface interacting with microorganisms, minerals, dissolved organic and inorganic compounds, roots, root exudates and gasses (Kammann et al. 2015). The exposure to these factors allows for the exterior and internal surfaces of the biochar to become enriched in oxidised functional groups, for example, carboxyl groups. These functional groups can explain the high CEC, liming effect and high charge density of biochar (Lehmann et al. 2011; Liang et al. 2006). The physical structure characterized by a network of micro-, meso- and macro pores allows for improved soil structure by increasing aeration, decreasing bulk density and reducing the soils tensile strength (Chan et al. 2008; Downie et al. 2009).

2.2.4.2 *Microbial*

Biochar addition can also have a large effect on the microbial activity, soil organic matter (SOM) levels (Anderson et al. 2011; Tian et al 2016), C cycling (Bolan et al. 2012), and nitrogen (N) dynamics (Lehmann et al. 2011; Nelissen et al. 2012) within the soil environment. The porous structure of the charred material seems to generate a micro-location for microorganisms. The total surface area allows for "storage space" of different nutrients and organic compounds that optimizes microbial growth (Lehmann et al. 2011). The various pore sizes serve as protection and habitat for almost all types of microorganisms such as; viz. bacteria (0.3 - 3.0 mm), fungi (2 - 80

mm), protozoa (7 - 30 mm) and with the macropores (>200 nm) being the ideal size for bacteria (Bhaduri et al. 2016). Several previous studies have demonstrated how biochar additions can cause shifts in microbial activity and community structure (Pietikainen et al. 2000; DeLuca et al. 2006; Grossman et al. 2010; Liang et al. 2006). However, recent research by Tian et al. (2016) found that the addition of biochar alone did not alter the soil microbial community structure, but did significantly increase enzyme activity. The type of feedstock and pyrolysis temperature could therefore have a large effect on the role biochar plays in the soil. These factors affect the pore sizes and surface area, which in turn can influence the size of organisms able to enter the biochar as well as the total surface area that could adsorb compounds (DeLuca et al. 2006). This is important as the adsorption of nutrients and the presence of microorganisms directly affects the C use efficiency (Lehmann et al. 2011), and the N cycle by influencing ammonification, nitrification and immobilization (Gundale and DaLuca 2006).

2.2.4.3 *Nitrogen mineralisation*

A major problem associated with biochar applications to agricultural and grassland soils has been decreases in net N mineralisation (Rondon et al. 2008) and lower N availability for crops (Lehmann et al. 2003; Anderson et al. 2011). Deenik et al. (2010) did research on this occurrence and theorized that the lower N availability can be ascribed to the N being immobilized when the labile fraction of biochar with a high C/N ratio is mineralised. They found that biochar with a greater fraction of volatile matter (mineralizable fraction) would result in greater immobilization and thus decreases in N availability (Deenik et al. 2010). Steiner et al. (2007) reported similar results and proposed that lower temperature biochars would induce net immobilization due to microbes degrading the residual bio-oils and functional groups first (DaLuca et al. 2006). Several studies have also reported on reduced ammonification as a cause for lower N availability. This is possibly caused by entrapment or the adsorption of NH_4 – N by biochar (Mizuta et al. 2004; Saleh et al. 2012), but no exact mechanism for NH_4 retention has been found. A recent study by Sigua et al. (2016) suggested that the net N immobilization and adsorption of NH_4 – N could both be linked to the high cation exchange capacity of biochar. When ammonium gets bound to the char, less nitrogen is available for nitrification, which ultimately leads to less NO_3 – N being produced (Sigua et al. 2016). The physical entrapment of NH_4^+ could, however, be linked to the pore structure of the biochar, as NH_4 – ions have a diameter of 286 pm and biochars have a wide range of pore sizes (Clough et al. 2013). Agegnehu et al. (2016) and other researchers (Castaldi et al. 2011; Anderson et al. 2011; Kammann et al. 2012) have reported on reduced N_2O and CO_2 emissions when biochar is added to the soil. Anderson et al. (2011) proposed that an increase in microbial

activity could either promote the denitrification of N_2O through to N_2, or the microbes could potentially produce NH_4^+ that becomes adsorbed by the biochar, thus altering the soil N dynamics.

2.2.4.4 *Environmental application*

Biochar is commonly applied for environmental purposes such as (i) managing pollution and eutrophication risks, (ii) re-vegetation of degraded land, and (iii) C sequestration (Blackwell et al. 2009). The leaching of nutrients from fertilizer and pollutants from pesticide applications could be of special concern for ecosystem health by altering nutrient and ecological dynamics. Biochar has shown very good adsorbing capabilities for nutrients such as ammonium and phosphate (Lehmann et al. 2007) which could lead to eutrophication, as well as the adsorption of pesticides (Blackwell et al. 2009). The use of biochar to re-establish vegetation in degraded lands, is based on the physiochemical properties of the char that can increase microbial activity, improve CEC, and also increased water holding capacity. This could promote re-establishment of seedlings and nutrient retention in otherwise barren poor quality soils (Beesley et al. 2011). The origin for using biochar to sequester C in the soil comes from an effort to prevent surface fires to further release CO_2 into the atmosphere. After pyrolysis, biochar becomes inert and resistant to degradation, which allows it to be sequestered for long periods of time in soils. Residence times of biochar in temperate climates have been estimated to be about 4000 years (Kuzyakov et al. 2014).

2.3 Composting

Compost has been recognized as an important soil amendment in marginal land and sandy soils since it is able to improve organic matter levels and sustain long-term fertility and productivity (Esse et al. 2001; Castaldi et al. 2008). Similar to biochar, several studies have shown benefits of adding compost (Bass et al. 2016) to the soils' physical and chemical structure by causing reduced bulk density, improved soil pore volume and water conductivity (Carter et al. 2004), increased water retention (Evanylo et al. 2008), reduced erosion, increased CEC and improved mineralisation (Hartz et al. 2000; Bass et al. 2016).

Composting has been described as the biological decomposition and stabilisation of organic substrates, under aerobic conditions that allow development of thermophilic temperatures as a result of biologically produced heat (Haug 1993; Stentiford and Zane 1996; Barrena 2008). The goal is to produce a final product that is stable, free of pathogens and plant seeds, has reduced fermentability and bad odors, and is mature (Haug 1993; Stentiford and Zane 1996; Sequi 1996). This process has become one of the most widely accepted technologies for the treatment and transformation of organic wastes in agriculture. It originated as a method of avoiding the drawbacks associated with direct application of raw wastes and poorly stabilized materials such

as the immobilisation of plant nutrients, phytotoxicity and pathogens (Dias et al. 2010). The process requires the participation of a wide range of microbial groups to transform (Jindo et al. 2012) part of the organic matter to humic substances, and mineralise the other part into carbon dioxide (Dias et al. 2010). Composting is therefore basically an accelerated version of the naturally occurring decomposing process of organic debris when conditions are favourable for microbial activity (Senesie and Brunetti 1996). There are three factors that control the composting process and determine the quality of the final product (Stentiford 1996).

2.3.1 Factors affecting composting process

2.3.1.1 *Aeration*

Aeration is important for providing aerobic conditions so that micro-organisms can oxidize the organic carbon. This can be achieved through the implementation of two different composting systems; agitation and forced aeration. Agitation is a method that consists of turning cycles where the organic mixture is mixed or 'turned' anywhere at regular intervals, usually every three to four days. Temperature can also be used to determine when piles are to be turned. This works on the basis of measuring the compost's temperature daily and turning the pile when the temperature reaches a certain point. Forced aeration is a system that supplies air to the composting mass with the use of a pressurised air system. These systems work either by blowing air into the mass, or by sucking the air through the composting pile. This method allows for a higher level of oxygen control, but requires constant temperature and oxygen feedback in a controlled environment.

2.3.1.2 *Temperature*

Temperature determines the rate at which the biological processes within the pile take place. The variety of ingredients, thermal properties and breakdown rates are all factors that cause temperature variations with a composting mass. The operating temperatures of a composting pile are controlled to maximise both sanitation and stabilisation of the final product. This objective is difficult to achieve without compromise as both are obtained at different operating temperatures. In process terms the operating temperatures can be categorised as follows; temperatures above 55°C are optimal for sanitation, temperatures between 45° and 55°C would maximise the biodegradation rate, and temperatures between 35° and 40°C are optimal for microbial diversity.

2.3.1.3 *Moisture Content*

The moisture content of the composting pile is important to control as it can affect the structural and thermal properties of the materials as well as the rate of biodegradation (Stentiford and Zane 1995). The initial moisture content of the composting mass is dependent on the materials used, but typically lies between 55 and 65%. Research has shown that the optimal moisture content for

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composting is between 40-60%, this allows for enough water to maintain microbial activity whilst ensuring aerobic conditions. Rapid drying to moisture levels between 30 and 35% would result in the inhibition of microbial activity and an end to the initial phase of composting. If the materials would be wet again, it would result in uncontrolled biodegradation under anaerobic conditions. Anaerobic biodegradation is undesirable as it decreases the final product's sanitation and causes odours.

2.4 Combining biochar and compost

The use of organic residues such as composts and manures as soil amendments have brought forth problems in terms of carbon sequestration due to their relative fast rates of degradation. This means that compost applications leads to the release of carbon dioxide, instead of being a sink for greenhouse gasses (Bolan et al. 2012). Both compost and biochar, as separate amendments, could therefore have beneficial and negative effects on soil health and fertility. However, with combined applications of compost and biochar, these negative effects could possibly be overcome. Biochars recalcitrant structure has proven to be a great way of storing C in the soil by reducing CO_2 emissions and increasing microbial activity (Clough et al. 2013), but has shown problems with over liming and reduced net mineralisation (Sika and Hardie 2014; Schulz et al. 2013). Compost has similar beneficial aspects as biochar, but could compensate for biochars poor mineralisation and nitrogen immobilisation. Liu et al. (2012) did studies on combined application of compost and biochar under field conditions and provided the synergistic benefits to, soil organic matter (SOM) quantity, nutrient content and soil water holding capacity (Bass et al. 2016).

An alternative method that can be used to stabilize the C in composts without impacting its quality and fertility (Bolan et al. 2012), is the incorporation of biochar as a bulking agent during the composting process. A bulking agent is an amendment made to improve composts' structural support by preventing physical compaction of the pile and allowing adequate aeration (Haug 1993). Composting of biochar may, however, be promising, as it could create bio-activated surfaces which are promoted during composting by the high temperatures, microbial activity and the sorption of organic matter. Several researchers (Forbes et al. 2006; Kuzyakov et al. 2009; Zimmerman 2010) have described the biological oxidation of biochar surfaces to oxygen-containing functional groups by microbial degradation, as well as the modification of these surfaces through the uptake or sorption of organic molecules which are rich in functional groups (Liang et al. 2006; Joseph et al. 2010; Borchard et al. 2012). If biochar is integrated at the initial stages of composting, it could affect the microbial community and activity during the composting

process and influence the performance of the process with the possibility of improving the final product (Jindo et al. 2012). In theory, this means that the increased microbial activity of the composting process could potentially increase the nutrient content of the biochar, which could lead to greater mineralisation and improved soil fertility (Lehmann et al 2011; Borchard et al. 2012) However, few studies have been done on the effects that biochar has on composting, and also how composting effects the properties of the biochar (Kammann et al. 2015). Prost et al. (2013) found that composting reduced the surface area of biochar due to compost derived products that clog the biochar pores as well as the biochar adsorbing nutrients and leachates. They also reported improved nitrogen retention by reducing N losses from ammonia volatization (Prost et al. 2013). Steiner et al. (2011) and Dias et al. (2010) both illustrated that biochar can be used as a bulking agent to adjust the C/N-ratio and increase the formation of stable humic compounds during composting. Research by Chen et al. (2011) proved that heavy metal mobility is reduced with biochar additions, which is similar to a finding from Wang et al. (2013) who reported suppressed N_2O emissions, and Jindo et al. (2016) which indicated that biochar amendments can change the microbial community structure during composting, depending on the original organic wastes used for composting.

2.5 Conclusions and gaps in knowledge

From the current literature, it can therefore be concluded that fresh biochar's chemical and physical properties are affected by the feedstock used and the pyrolysis temperature at which it was produced. These properties play a cardinal role in the way biochar reacts in the soil by affecting; (i) physical factors such as bulk density, water holding capacity and tensile strength, (ii) chemical react ability through functional group differences and degree of condensed structures, (iii) microbial activity through the provision of a suitable habitat whilst storing nutrients, and (iv) nutrient availability by altering mineralisation dynamics.   Furthermore, clear gaps in knowledge are seen in terms of how the adding of biochar to composts could alter composting dynamics such as temperature, aeration and moisture contents, along with the stability and maturity of the final product. Different types of biochar might therefore also cause dissimilar degrees of change in these compost dynamics if applied at varying rates, and especially alter C sequestration capabilities of the composts if the degradability and stability of the biochar itself is modified. Finally, research has shown that there is currently no rapid, cost effective and accurate method of measuring the quantity and stability of biochar in soils.

In the light of these findings and the goals of the Centre of Excellence in Food Security, research needs to be conducted that can compare how biochar properties (as a product of feedstock and

pyrolysis temperature) could affect long term agricultural benefits and environmental sustainability through C sequestration when composted, while being easily obtainable and usable by a small-scale farmer from a marginal population. To do this, two contrasting biochars obtained from different feedstocks and produced at different temperatures, one that is expensive and refined, and one that is cheap and robust, similar to something producible by a subsidence farmer himself, needs to be applied at different application rates at the start of a composting process. These composts should then be assessed during composting for maturity and stability indices to see the effects of these biochars. Finally, the compost products need to be evaluated for nutrient availability and changes to the biochars inertness under ideal and field conditions. Since all methods for the quantification of biochar is time consuming and expensive, another method, preferably quick and cheap, needs to be constructed to measure the stability of biochar in soils and compost over a long period of time.

CHAPTER 3 – COMPOST PRODUCTION AND CHARACTERIZATION

3.1 Introduction

The application of organic materials on agricultural soil has been a common practice for various farmers across the world. However, field application of raw or high C organic material has shown negative effects on crop growth. When fresh manure or immature composts are applied, it can interfere with plant growth by immobilizing nitrogen, causing phytotoxicity and supporting pathogen growth (Baberiz and Nappi 1996; Butler et al. 2001; Dias et al. 2010). The principal requirement for organic amendment is therefore that it is safe, convenient and efficient (Senesi and Brunetti 1996). To ensure a product that fits this description, composting systems are used to degrade the organic matter under controlled aerobic conditions to produce an amendment that is stabilized, mature and of high quality (Cesaro et al. 2014).

Composting systems consist of three parts. All three parts must be conducted correctly for the final product to be matured and stabile. The first step is pre-treatment. This involves the collection, shredding and blending of material to give an optimum nutrient balance, mass structure and moisture content. The second step is the composting process which is controlled by the principal factors described in the chapter one. And the final step is compost post-treatment, where unwanted components are removed and compost is prepared for a particular application (Sequi 1996).

Compost maturity and stability evaluation is still one of the fundamental problems faced by compost producers (Provenzano et al. 2000). Compost stability refers to the level of O_2 and CO_2 activity as a result of microbe respiration (Benito et al. 2003; Castaldi et al. 2008) and maturity refers to the level of phytotoxic organic substances degraded during the active composting phase (Wu et al. 2000; Castaldi et al. 2008). Various chemical, physiochemical and biological (microbial) methods are used in combination to characterize and evaluate these parameters (Provenzano et al. 2000). Physiochemical properties measured throughout the composting process include temperature, odor and colour (Itavaara et al. 2002; Benito et al. 2003). Chemical parameters measured are pH, CEC, C/N ratio, loss on ignition, NH_4- and NO_3-levels, organic compounds, and degree of humification. Microbial activity is determined through O_2 reductions, CO_2 produced, enzyme levels and biological diversity (Jimenez and Garcia 1992; Inbar et al. 1993; Itavaara et al. 2002; Benito et al. 2003; Jindo et al. 2016).

Biochar has been seen as an excellent long term soil amendment due to its high content of stabile C, resistance against decay and contributions to C sequestration (Jindo et al. 2016). These

properties could however bring forth challenges with regard to the composting process and how biochar will affect the stability and maturity of the compost. The aim of this study was therefore to investigate the effect of adding varying amounts of two contrasting commercial biochars to green and animal waste on the composting process and compost quality. The one biochar is a cheap, low tech and crude char produced from pine sawmill waste using slow pyrolysis at low temperatures (450°C) which is typically produced for the charcoal briquette industry. The other biochar is a more expensive, and refined, high temperature (900°C), slow pyrolysis eucalyptus wood char, produced with the aim of using it a cost-effective industrial sorbent with similar properties to activated charcoal. The low-tech, low temperature, slow pyrolysis technique used to produce the pine wood biochar is representative of what a small-scale biochar producer such as a farmer would be using. Pine and eucalyptus wood are among the most abundant woody biomass materials available in the Western Cape. The effect of these biochars on the levels of stability and maturity of the compost was measured with selected chemical, physiochemical and microbial parameters. These parameters were temperature, C/N ratio, pH, EC and dehydrogenase activity. Temperature is an important indicator as operating temperatures determine the sanitation, degradation rate and ultimately the duration of the composting process (Garcia et al. 1994; Stentiford and Zane 1995; Massiani and Domeizel 1996). The C/N ratio is one of the most widely used parameters to evaluate the level of decomposition during the composting process (Barberis and Nappi 1996; Benito et al. 2003) and measures the amount of carbon compounds transformed to CO_2 under aerobic respiration. Dehydrogenase is a group of enzymes that participates in the metabolic reactions of all microorganisms that produce energy in the form of ATP through the oxidation of organic matter (Barrena et al. 2008). Dehydrogenase activity (DA) is there for an indicator of microbiological redox systems and may be considered a good measure of microbial oxidative activities in soils and especially interesting in the composting process (Von Mersi & Schinner 1991). All of these parameters were measured across the composting period to elucidate the effect that the two contrasting biochars would have on the maturity and character of the final compost products.

3.2 Materials and Methods

3.2.1 Biochar preparation

3.2.1.1 Biochar production and preparation description

The Pine biochar used in this study is made by a small-scale commercial producer from the Eastern Cape, South Africa who submits the pinewood sawmill waste to slow pyrolysis at 450°C (Sika 2012). This biochar was then crushed and sieved (<2 mm) before being incorporated into

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the compost mixtures. The eucalyptus char was produced by Brenn-O-Kem (Pty) Ltd. in Wolseley (South Africa) by placing eucalyptus wood in a 3.5 m high pyrolysis chamber and through slow pyrolysis heating the internal pyrolysis chamber to approximately 900°C with a residence time of at least 1 hour. The final product received was already of a fine structure with a diameter of <2 mm so direct application into compost mixtures was conducted without sieving the eucalyptus biochar.

3.2.2 Biochar characterization

3.2.2.1 pH and EC determination

The pH and electrical conductivity (EC) was determined in water by mixing 10 g of air-dried biochar (sieved <2 mm) with 100 mL of water (1:10 w/v) and shaking it for 30 min (Benito et al. 2003). pH and EC was measured using a Metrohm 827 lab pH meter and a Jenway 4510 Conductivity Meter.

3.2.2.2 BET Surface area determination

The Brunauer-Emmett-Teller (BET) specific surface area (SSA) was determined on a Micometrics ASAP 2010 system using nitrogen gas.

3.2.2.3 Proximate analysis

Proximate analysis is a thermogravimetric method traditionally used for the basic determination of charcoal quality by quantifying the amount of ash, fixed C and volatile organic material in the char. The ASTM standards specify that wood charcoals (D1762-84) can be assessed according to the following parameters; moisture is the mass lost at 105°C, volatile matter is the mass lost at 900°C in an inert atmosphere, and the mass lost at 750°C in an oxic atmosphere is the fixed carbon. The remainder is the ash content (Brewer 2012).

3.2.2.4 Elemental analysis

The total C and N content of the biochar samples using dry combustion with a EuroVector Elemental Analyzer 3000 (Nelson et al. 1996). Total inorganic elemental analysis was performed with the microwave-aided acid digestion method where 0.1 g of biochar and mature compost was placed in microwave vessels and 1.75 ml of HNO_3 and 5.25 ml of HCl was added. The vessels were then left open for 20 minutes to predigest before being sealed for the microwave heating program. A MARS microwave digester was used at 1600 W and 100 % for a total of 40 minutes. The first 25 min (ramp time) was to heat the sample from 20 degrees to 185 degrees. The conditions within the microwave thereafter was 800 psi and at 200°C for another 15 minutes. Samples were then cooled down for 25 min before adding 43 g of deionized water to the

microwave vessel to make up 50 ml. The concentration of inorganic elements (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Zn) in the samples was then determined using a Thermo ICap 6300 ICP-AES.

3.2.2.5 SEM imaging

Biochar samples were prepared for scanning by placing double sided tape on analysis stubs and gently sprinkling milled and sieved biochar over it. Hereafter the stubs were slightly tapped to remove excess material and placed in an Edwards S150A Sputter Coater to be plated with 10 nm of pure metallic gold. Stubs were then removed from the coater and loaded into the Zeiss Merlin Scanning Electron Microscope with which images were taken and analyzed with Zeiss SmartSEM software.

3.2.3 Compost production

Green biomass was grown during the summer of 2014/2015 to form the base of the composts. Maize, sunflowers and lucerne were sown at the beginning of Dec. 2014 at the Welgevallen Experimental Farm, at Stellenbosch University, and the above-ground biomass was harvested at the end of Feb. 2015. The crops received fertilizer and irrigation during the growth period. Representative samples of the crops' above ground biomass were taken one week before harvest to determine each feedstock's C and N content for the compost mixture calculation (Table 3.1). After harvest, the maize, sunflowers, and lucerne above ground material was homogenized with a Flymo Pac-a-shred Garden shredder. Kikuyu grass clippings were obtained from the Stellenbosch University sports fields and fresh cow manure was obtained from the Stellenbosch University dairy at Welgevallen Experimental Farm. The base compost mixture (without biochar) was mixed from the plant materials (maize, sunflowers, lucerne and kikuyu grass) and cow manure to achieve a starting C/N ratio of 26:1 on a dry mass basis. The base material was thoroughly mixed where after five compost piles were constructed, each with a total dry weight of 10 kg (312 liters) (Table 3.1).

Table 3.1 – Carbon nitrogen ratio of fresh materials used to construct compost piles along with their respective wet bulk density (BD) and dry bulk density as well as volume of the shredded material required of each feedstock to obtain a total C/N ratio of 26:1.

	C/N	Wet BD (kg/L)	Dry BD (kg/L)	% of mixture for C/N ratio of 26:1	Wet eq. volume per 10 kg of dry compost (L)
Maize	40:1	0.143	0.024	40	165
Lucerne	12:1	0.082	0.020	10	51
Manure	15:1	0.700	0.259	20	8
Sunflower	23:1	0.195	0.035	20	57
Kikuyu	13:1	0.090	0.032	10	31

After the construction of the five base piles, four of the piles were altered to contain increasing amounts of biochar. The biochars were added to the green materials to achieve a biochar content of 10 and 20 % of pine (PB10 and PB20, resp.) and 10 and 20 % eucalyptus (EB10 and EB20, resp.) according to the dry weight of the materials. The 10 and 20% biochar concentrations were selected as it is was known that biochars contain relatively low amounts of labile C and therefore there was concern that if too much biochar was added, compost piles would not reach the necessary high temperature levels for sterilization. After the addition of the biochar, piles were thoroughly mixed to ensure uniform distribution of the biochar within the pile. Thereafter the piles were left to mature for 12 weeks (90 days) in a greenhouse tunnel to ensure optimal temperature and moisture levels. A turned-pile system of two turns per week for the first month was used to ensure a homogenized and well aerated mix that stimulated microbial activity, where after the piles were only turned once a week. The temperature of the compost was measured twice a week for the first 3 weeks in the center of each pile (approximately 30 cm from the crown) with a Digitales Thermometer. After the first 3 weeks, temperature change became less variable and was therefore only measured once a week for the remainder of the trial. Due to the small size of the piles, temperature decreased rather dramatically after the first week. To counter this, all five piles were covered with shade netting and insulated with straw to prevent excessive temperature loss. The insulation was removed at the three-week mark when the bio-oxidative phase was complete. Moisture content was maintained between 50-60% by measuring the water content and adding water to the piles once a week. Representative samples of approximately 200 g (dry weight) from each pile were taken at days 0, 14, 31, 62 and 94. These samples were air dried to ensure no further microbial activity, milled and used for further analysis.
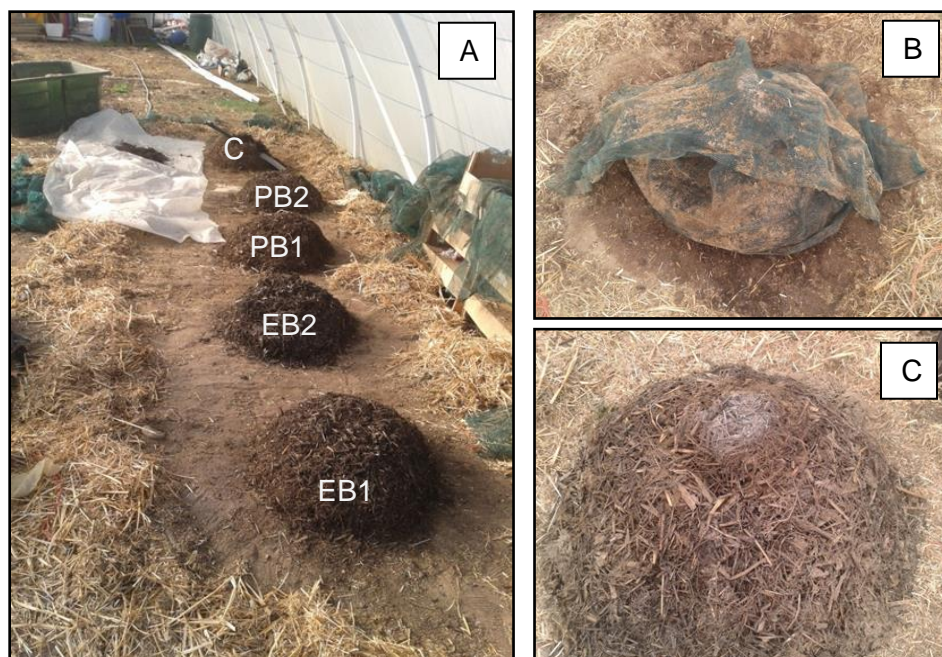
Figure 3.1 - Images of the five mature compost piles constructed inside a greenhouse tunnel (A) and the shade netting used to reduce temperature loss (B) which resulted in signs of fungi at the crown of the piles when the shade netting was removed (C).

3.2.4 Compost characterization

The following compost maturity parameters were measured on air-dried the samples taken throughout the composting process (0, 14, 31, 62 and 94 days): pH, EC, total C and N, and dehydrogenase activity. Water content of the piles were measured by drying a representative 20 g of compost at 105°C for 24 hours and calculating percentage moisture loss.

3.2.4.1 pH and EC

The pH and electrical conductivity (EC) was determined by mixing 10 g of air-dried compost (sieved < 2 mm) with 100 mL of water (1:10 w/v) and shaking it for 30 min (Benito et al. 2003).

3.2.4.2 Total C and N

The total C and N content of the compost samples was determined using dry combustion with a EuroVector Elemental Analyzer (Nelson et al. 1996).

3.2.4.3 Dehydrogenase activity

Dehydrogenase activity was determined colourimetrically with a modified version of the method described by Tabatabai (1994). An air-dried sample (< 2 mm) of 5 g was mixed with 0.05 g of $CaCO_3$ and divided into three test tubes, each containing 1 g. Hereafter, 1 mL of 3% aqueous solution of TTC and 2.5 mL of distilled water was added to each tube. The contents were mixed

with a glass rod, sealed and incubated at 37°C for 24h. The stopper was removed and 10 mL of methanol was added before sealing and shaking the tube for 1 min. The tube was opened and the suspension was filtered through a glass funnel that was plugged with absorbent cotton, into a 100-mL volumetric flask. After the filtration, the tube was washed with methanol and the compost remains were quantitatively transferred to the funnel, where after additional methanol was added in portions of 10 mL until the colour from the cotton plug disappeared. The filtrate was then diluted to 100-mL volume with methanol and the intensity of the reddish colour was measured with a spectrophotometer, using a 1-cm cuvette with methanol as a blank at a wavelength of 485nm. The amount of TPF produced was calculated with reference to a calibration graph prepared from TPF standard solutions. This graph was prepared by diluting 10 mL of TPF standard solution to 100 mL with methanol (100µg of TPF mL-1). Aliquots of this solution (5-, 10-, 15-, 20-mL) was then pipetted into a 100-mL volumetric flask (500, 1000, 1500, and 2000 µg of TPF 100 mL-1) of which the volumes were made up with methanol and mixed thoroughly. The red colour intensity was measured as described for the samples and the absorbance readings were plotted against the amount of TPF in the 100-mL standard solution.

The following parameters were only measured on the mature compost (90 days): total elemental analysis and proximate analysis (See section 2.2.2 for method description).

3.3 Results and discussion

3.3.1 Biochar characterization

The results obtained from the characterization of the pine and eucalyptus biochar show clear differences in physical and chemical properties of the biochars (Table 3.2). The pH of the pine biochar (PB) was found to be 9.88 whilst the eucalyptus biochar (EB) was 10.15. This typically indicates that the PB contained more acidic functional groups (which lower the final pH reading in water), due to it being produced at a lower temperature, thus being more oxidized (Chang et al. 2007). The EC of the PB was more than double (0.724 mS cm^{-1}) that of the EB (0.345 mS cm^{-1}), indicating that PB contained more soluble compounds which is illustrated by the large difference in nutrient content seen in Table 3.3. The PB ash content (0.49%) was higher than that of the EB (0.36%), which could explain the PB's higher EC (Table 3.2). The EB contained 3.1% more total C (85.5%) than PB (82.4%) (Table 3.2). The EB had more than 10 times the surface area (623.9 m^2 g^{-1}) than PB (59.89 m^2 g^{-1}). Lehmann et al. (2011) combined various researchers' information on biochars produced from three different feedstocks at three different temperatures. They reported that pH, total C (%), surface area and nutrient content of biochar increased with pyrolysis temperatures, which is similar to our results.

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Table 3.2 - Chemical and physical properties of pine biochar (PB) and eucalyptus biochar (EB).

	Production temperature (°C)	pH	EC (mS cm^{-1})	SSA (m^2 g^{-1})	Total C (%)	Fixed C (%)	Volatiles (%)	Ash (%)
PB	450	9.88	0.723	59.9	82.4	52.26	47.22	0.52
EB	900	10.15	0.344	623.9	85.5	79.01	20.62	0.38

From the proximate analysis it can also be seen that fixed C content was higher for EB (79.01%) than PB (52.26%), while PB had both higher volatile (47.22%) and ash (0.52%) contents. Enders et al. (2012) did a comparable study on biochar from different suppliers in which they too found that fixed C increased as pyrolysis temperature became higher. This increase in fixed C can give a relative measure of biochars stability (Keiluweit et al. 2010) along with the volatile content which has been correlated to decomposition rates (Zimmerman 2010). These results would therefore indicate that theoretically the pine biochar would be less stable as it has a larger labile fraction with more volatiles than the eucalyptus char.

Table 3.3 - Total elemental content (mg/kg) of PB and EB as determined with acid digestion.

	PB (mg/kg)	EB (mg/kg)
Ca	90026	5572
K	9891	1398
Mg	7542	1153
Na	1195	617
P	1126	426
Al	3187	542
B	31	11
Ba	311	25
Cu	20	11
Fe	3527	1210
Mn	477	488
S	2238	170
Si	1706	1330
Sr	689	37
Zn	37	10

The scanning electron microscope (SEM) allowed the opportunity to look at the physical structure and pore arrangement of the two biochars to better understand the large difference in surface area (Table 3.1). In Figure 3.2 the vast difference in pore arrangement, size and porosity can clearly be seen. The pine biochar is constructed of similar sized pores of approximately 20 µm whilst the eucalyptus has a larger variety of pore sizes with some being much greater than 20 µm and others closer to 2 µm. The combination of physical and chemical differences measured for the two biochars could have a large effect on the way it reacts during the composting process as well as the quality and maturity of the final compost product.
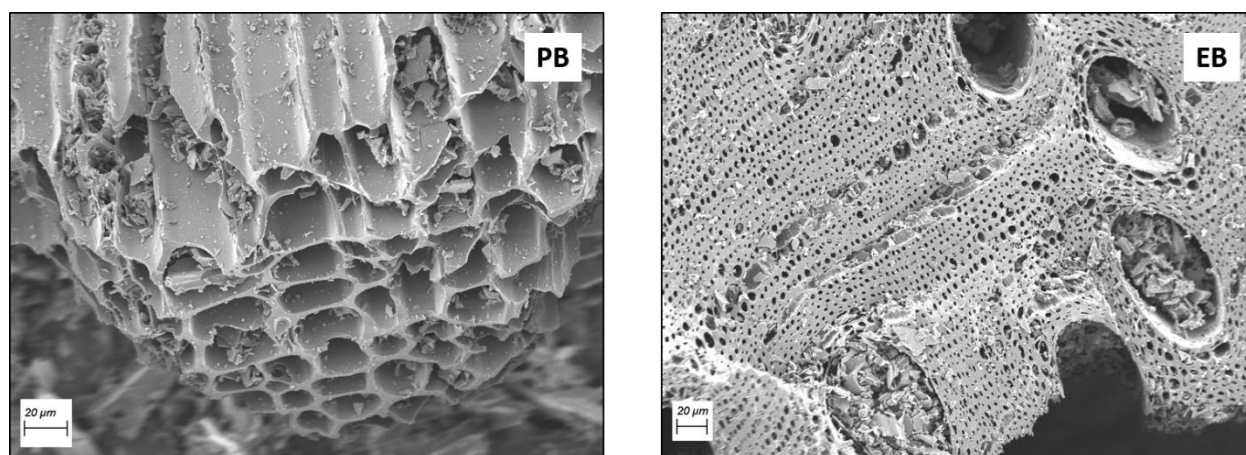
Figure 3.2 - Images of pine biochar (PB) and eucalyptus biochar (EB) taken by a Zeiss Merlin scanning electron microscope. Both scale bars represent 20µm of which images were taken at a working distance of 4.8 mm for PB and 3.7 mm for EB.

3.3.2   Compost maturity

3.3.2.1 Temperature dynamics

The compost piles all showed the typical temperature dynamics as would be expect from a composting system. The temperature graphs illustrate the rapid self-heating of the compost piles from the ambient temperature of 17.3°C to above 40°C in the first 3 days (Figure 3.3). Four of the five treatments reached maximum temperatures in the 53 - 58°C range whilst EB10 never peaked higher than 45°C. After the initial spike at day three, there was a steady decline of temperature to the 30 to 36-degree range at day 11 before spiking again towards the 38 to 42-degrees by day 14. Day 14 marked the final peak before all piles started gradually cooling towards ambient temperatures which was reached at day 77. Hereafter no further measurements were recorded as temperature remained in the ambient range for the remainder of the trial period. The compost material is considered to be matured if the declining temperature reaches ambient level (Tiquia et al. 2002).

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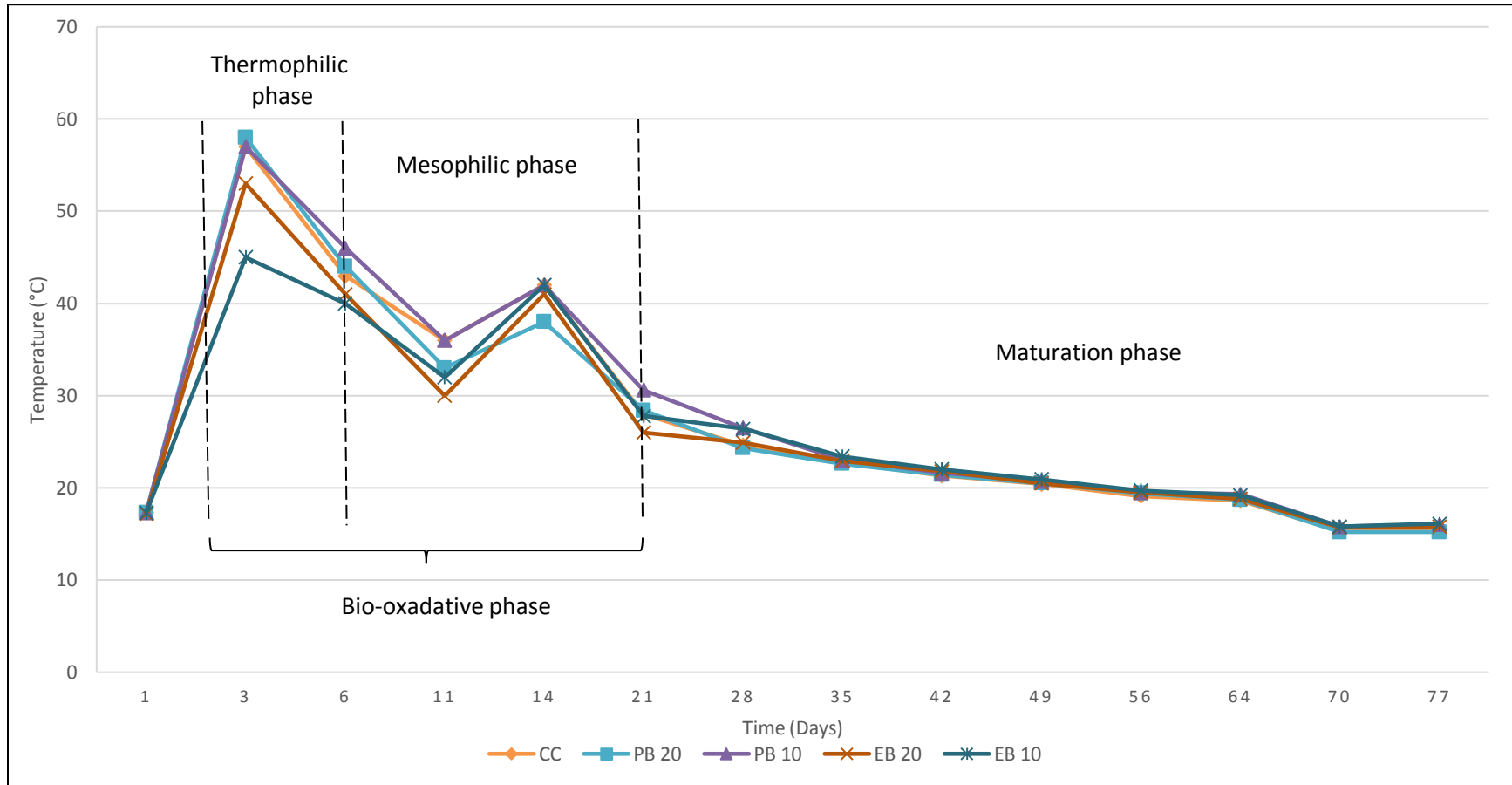
Figure 3.3 – Compost core temperature changes (°C) over time (days) during the composting period of the control (CC) and PB and EB biochar-containing compost.

The zig-zag pattern witnessed on the temperature graph is due to the turning of the compost piles. Results show that all piles reached the required thermophilic phase temperatures (45 - 64°C) at which the biodegradation rate is maximized and sanitation of the composting material takes place (Stentiford 1996). However, this temperature range was only maintained for three days before moving back into mesophilic stage, which was sustained for a total of 15 days before the start of the maturation phase. Temperature increases to thermophilic levels in the early composting process are due to the greater availability of easily degradable organic material. When the availability of easily degradable organic material decreases, microbes becomes less active and the composting process moves into the mesophilic phase (Brady and Weil 1999). These two phases combined are known as the bio-oxidative phase of composting where this active phase consists mostly of easily degradable material being transformed and mineralized into a stabile form. Following the active phase is the maturing/curing phase in which the stabilized organic material is converted into humic substances (Queda et al. 2002).

Table 3.4 - Average temperature of control and biochar-containing compost mixtures during the three different phases of the composting process. The phases correlates to the areas indicated in

Composts	Average temperature (°C)		
	Thermophilic phase	**Mesophilic phase**	**Maturation phase**
CC	50.0	35.3	19.0
PB 10	51.5	36.2	20.3
PB 20	51.0	33.1	19.7
EB 10	42.5	33.9	20.4
EB 20	47.0	32.3	20.0

Table 3.4 shows that PB10 exhibited the highest average temperature in both the thermophilic (51.5°C) and mesophilic stages (36.2°C), while EB10 averaged the highest temperature (20.4°C) by a small margin (0.1°C) over PB10 in the maturation phase. Both of the 10 % biochar treatments (PB10, EB10) had higher temperature averages than the 20 % biochar treatments (PB20, EB20) through the mesophilic and maturation phases, and PB10 and PB20 showed higher temperatures than EB10 and EB20 in the thermophilic phase. Figure 3.3 also illustrates that the biochar mixtures did not behave differently from the control (CC) even though the control had slightly higher average temperatures than PB20, EB10 and EB20 in the bio-oxidative phase, and the lowest temperature in the maturation phase. A general deduction can be made that the addition

of biochar as bulking agent did not alter the overall temperature dynamics, but could have affected the temperature range within each phase. The lower initial temperatures during composting of the eucalyptus biochar mixtures could be attributed to its higher fixed C content (Table 3.2). The lower amount of fixed carbon, high percentage of volatiles and higher reactivity of the pine biochar could all be aspects that contributed to the higher temperatures PB10 and PB20 over that of EB10 and EB20 in the thermophilic phase. The fact that the 10 % PB mixture had the highest average temperature in the bio-oxidative phase is similar to findings from Steiner et al. (2010), who found increased temperatures in compost amended with biochar. They theorized that biochar improves the oxygen availability thereby improving microbial activity and respiration in the bio-oxidative phase of the composting process. Furthermore, PB contained significantly higher amounts of macro nutrients such as P, Ca, and K which could enhance microbial activity (Table 3.3). This, however, seems only to be true to a certain extent as CC still outperformed EB10, EB20 and PB20 in the bio-oxidative phase of composting. When too large a percentage of easily degradable organic material is substituted with recalcitrant biochar, it could lower the available carbon which would result in lower composting temperatures. During the curing phase, the control had the lowest average temperature. This could indicate that the composting process had started to slightly oxidize the biochars surface or that the microorganisms could have started to use the biochar as a carbon source. Surface oxidation takes place when organic matter is adsorbed onto the biochars surface (Liang et al. 2006) or when biologically or abiotically mediated reactions with O_2 take place (Lehmann et al. 2011). If the surface of the biochar becomes oxidized, new hydrophilic functional groups are produced in the structure of the char which enhances microbial activity and promotes further degradation (Cheng et al. 2006).

3.3.2.2   C/N ratio

The organic matter (OM) content and carbon to nitrogen ratio values are widely recognized as measurements of mineralisation and stability. A C/N ratio of 10 or 12 is considered to be an indicator of a stable and decomposed organic product (Jimenez and Garcia 1992). The compost mixtures all started out with different initial C/N values ranging from 20.67 (PB10) to 26.63 (EB20) due to the heterogeneous nature of ingredients included in the mix (Table 3.2). Several authors

such as Baca et al. (1992) have however indicated that the initial C/N ratio is not a good indicator of the subsequent transformation of the organic material.
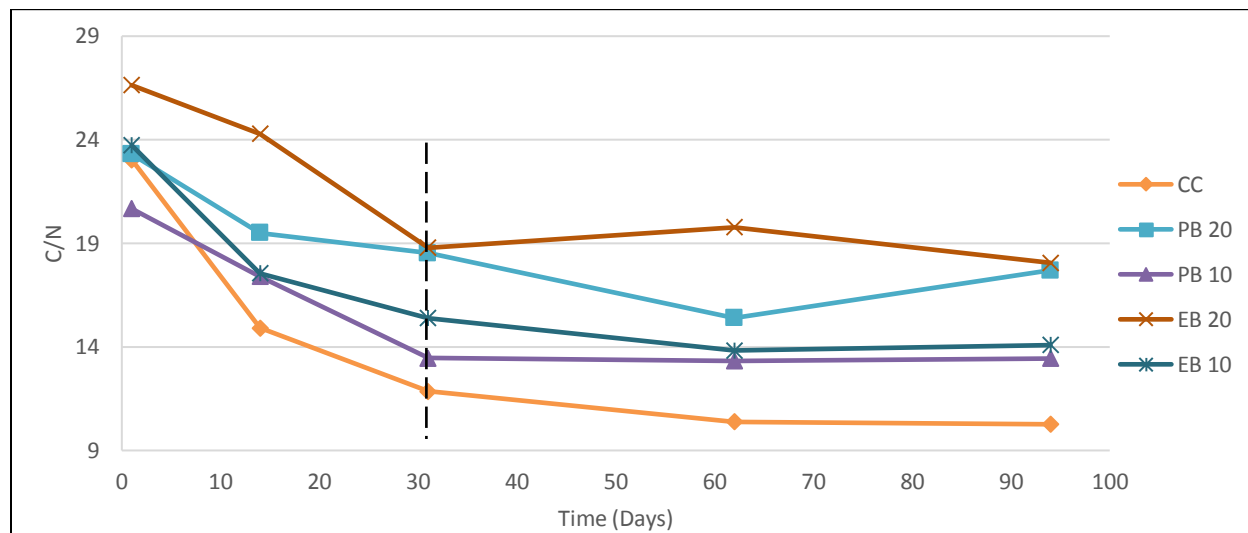
Figure 3.4 - Line graph illustrating the evolution of the C/N ratio for compost piles CC, PB10. PB20, EB10 and EB20 over composting time in days.

This is apparent in the evolution of the C/N ratios from the different compost piles. Figure 3.4 illustrates that all of the compost mixtures had a steady decrease of C/N ratio from day 1 to day 31, before stabilizing during the curing phase. Benito et al. (2003) found similar results when their C/N ratios also decreased mainly during the bio-oxidative phase due to the high decomposition rate of the OM. This correlates well with our results in which one can see that temperatures of the piles also started to merge after day 31 when the bio-oxidative phase was complete. After the 94-day composting period, CC exhibited the lowest C/N ratio (10.26) with EB20 having the highest (18.06). Piles PB20 (17.7) and EB20 (18.06) showed higher final C/N ratios than PB10 (13.44) and EB10 (14.09), whilst EB10 and EB20 also had higher values than the pine biochar piles containing the same amount of char (Figure 3.4).

This phenomenon is once again explained by the physical and chemical structure of the two types of char owed to their respective feedstocks and pyrolysis temperatures. The labile fraction of carbon in organic residues decompose at a faster rate and much more easily than the non-labile fractions (Bolan et al. 2012). According to further fractionation analysis by Bolan et al. (2012), the majority of C in biochar remains in a non-labile form contrary to that of the labile fraction in regular compost. The higher C/N ratios of the 20% biochar piles could then be a result of less labile carbon being available for degradation. This also explains the increased C/N ratios for EB10 and EB20 relative to PB10 and PB20, as proximate analyses of both biochars revealed that eucalyptus biochar has 75.6% fixed carbon whilst pine biochar only has 50.6% (Table 3.2). The biochars

quantities of fixed carbon and volatiles, resulted in the final compost products having clear differences in their fixed carbon contents (Table 3.5). EB20 had: 10 % more fixed carbon than PB20, 11 % more than EB10, and 20% more than PB10. The less condensed pine biochar produced at a lower temperature could therefore have more labile carbon which is more degradable and results in lower C/N ratios. The final C/N ratios for all the compost piles were below 20:1 but according to Jimenez and Garcia (1992) only CC reached a mature and fully stable state (10.26). However, Zucconi et al. (1981) stated that composts are matured when the C/N ratio is less than 15. If this parameter is used then CC, PB10 and EB10 are considered to be mature whilst PB20 and EB20 are not completely transformed and stable. However, it is important to recognize that the C/N maturity parameter is not necessarily applicable for biochar containing compost as it was developed for composts produced from non-pyrolysed organic material.

Table 3.5 - Proximate analysis results of the mature (94 days) control and biochar-containing composts.

Composts	Fixed C (%)	Volatiles (%)	Ash (%)
CC	3.09	28.93	67.98
PB 10	4.57	37.39	58.04
PB 20	12.84	31.93	55.23
EB 10	11.71	32.72	55.58
EB 20	22.55	33.37	43.27

3.3.2.3 pH and electrical conductivity

The pH and EC during the composting process are good indicators of reactions taking place and factors affecting the breakdown of the compost piles. In Figure 3.5 it can be seen that all the piles had different initial pH values ranging from 7.54 (CC) to 8.28 (PB10). After this they exhibited a steady increase towards the 14-day mark where all of the piles measured a similar pH value of approximately 8.8. This marked the final peak of the bio-oxidative phase where after pH values started to decrease towards their respective final resting points. The control compost showed the lowest initial and final pH which is explained by the inherent high pH of both biochars (Table 3.2) added to the other piles.

The pH increase took place during the bio-oxidative phase (Dias et al. 2010) as a consequence of the degradation and mineralisation of organic compounds (Benito et al. 2003). Specifically, processes such as ammonification and the dissolution of alkaline minerals are mainly responsible

(Dias et al. 2010; Lehmann et al. 2011). The main driving force behind the decrease in pH during the curing phase is the oxidation of C and the formation of acidic carboxyl groups (Lehmann et al. 2011). Electrical conductivity results show a trend of decrease for all piles from day 1 to 31, and a general increase thereafter (Figure 3.6). This is most easily explained by the addition of water throughout the bio-oxidative phase which resulted in the loss of soluble salts by leaching, despite mineralisation taking place (Benito et al. 2003). When temperatures stabilized, less water was added to the piles and salts could accumulate once again. The final pH values of all matured compost piles however, had a final pH ranging from 7.8 – 8.1 which falls within the expectable specifications (6.5 – 8.5) for both Belgium and Germany for compost maturation (Verdonck 1997). This means that all piles can be classified as matured and ready for application.
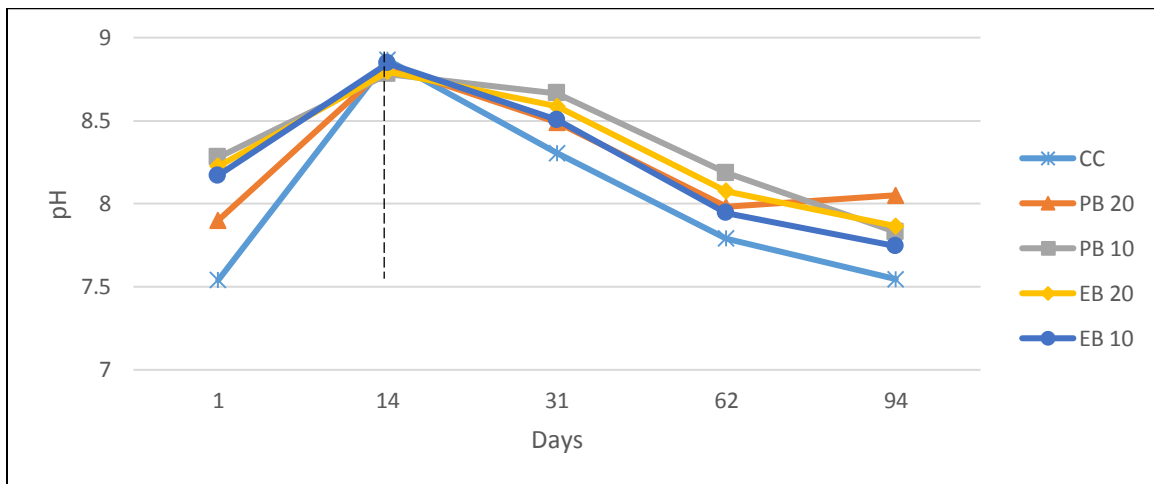
Figure 3.5 - The change in pH as measured in water (1:10) for all treatments during the composting process (94 days).
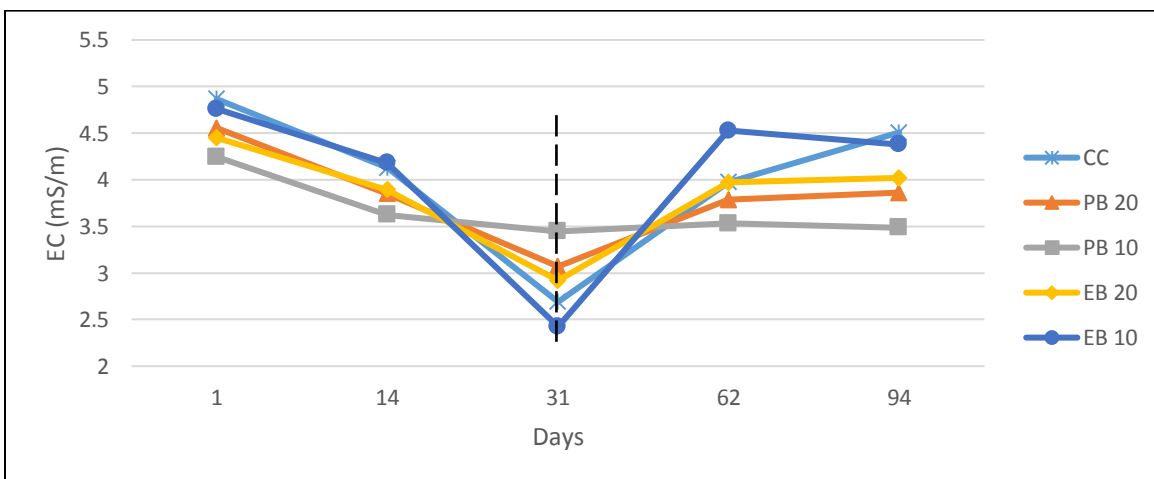
Figure 3.6 - The change in EC (mS/m) during the composting process of 94 days.

3.3.2.4 *Dehydrogenase activity*

Dehydrogenase activity (DA) is used to monitor oxidative microbial activity in soils and the composting process (Wong and Fang 2000). Triphenyltetrazolium chloride (TTC) is added to enhance the availability of certain dehydrogenases of which the chloride is then converged by a hydrolytic reaction to form formazan. The formazan product (TPF) is extracted with methanol and the concentration is determined spectrophotometrically (Tabatabai 1994). Figure 3.7 shows that all of the compost piles had similar trends of decreasing oxidative microbial activity from day 14 to day 94. Day 14 marked the final peak in the bio-oxidative phase after which all of the compost piles also showed reduced temperatures (Figure 3.3).
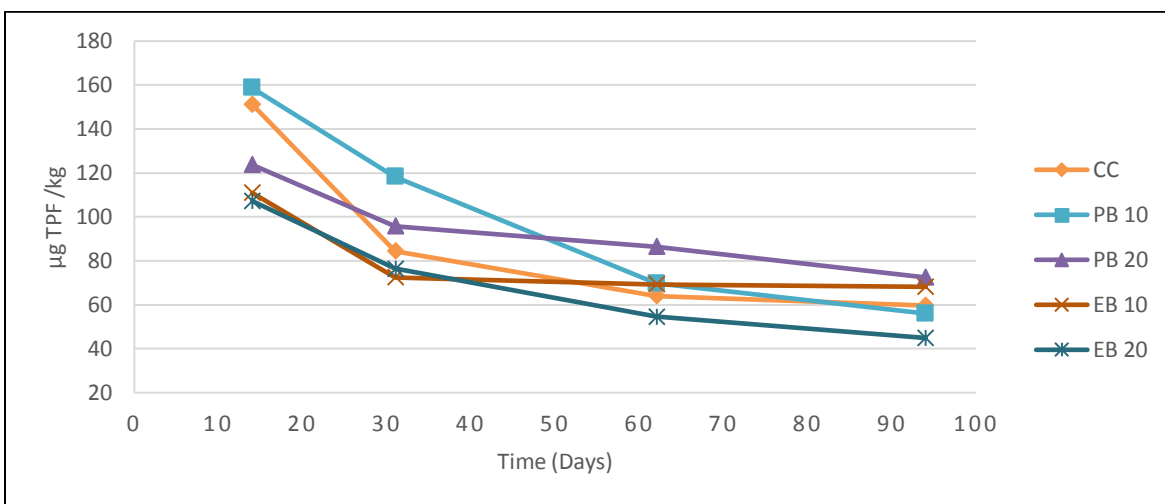
Figure 3.7 - The concentration of TPF produced (indicator of dehydrogenase enzyme activity) over time (days) during the compositing period of the control and biochar-containing compost mixtures.

This result is reinforced by various researchers who found correlations between DA and biochemical parameters such as temperature, nitrogen content and other enzymatic activities (Barrena et al. 2008). The rate at which the microbial activity decreased is however interesting. Power trend lines showed the strongest correlation to the rate of decreasing microbial activity (PB10 - 0.979, PB20 - 0.974, EB10 - 0.796, EB20 - 0.999, CC - 0.947) (Appendix, Figure A.1). The exponent, indicating the decaying rate of microbial activity before stabilizing, was the highest for PB10 (0.566) and the lowest for EB10 (0.248). The sudden decrease in microbial activity is due to the disappearance of easily degradable substrate (Benitez et al. 1999) as also seen in the progression of the C/N ratio over time (Figure 3.4).
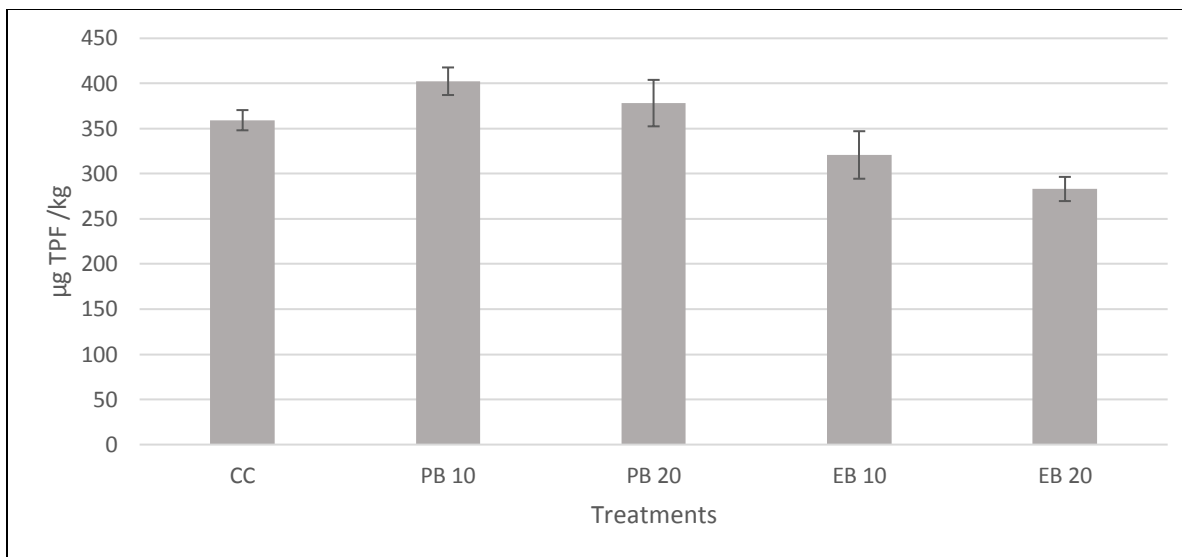
Figure 3.8 – Cumulative amount of TPF produced during the compositing period (0-94 days) of the control and biochar-containing compost mixtures with error bars

Cumulative TPF results in Figure 3.8 show that the pine biochar mixtures had the highest total dehydrogenase activity for all the compost piles. Although non-significantly higher than the other treatments except EB20, this trend is not unexpected as biochar has been found to increase microbial activity and diversity (Lehmann et al. 2011). The larger pore structure of the pine biochar seen in Figure 3.2 provides more macropores which are the perfect size for bacteria (Bhaduri et al. 2016). Oxygen availability is also critical during the composting process (De Bertoldi et al. 1983) and with biochar improving aeration along with dehydrogenase measurements being associated with oxidative microbial activity, it is to be expected that the PB10 and PB20 would have the highest dehydrogenase activity. On the other hand, smaller pores found in EB along with the high quantity of fixed carbon might not create the correct micro-climate for microbes, hence less microbial activity. The quantity of biochar added seemed to also have an effect, as the 10 % biochar piles had higher levels of TPF produced than their respective 20 % piles which also correlates to the temperature results from the mesophilic and maturation phases. According to the rate at which microbial activity decreased, the compost piles were all close to reaching their lower limits of microbial activity. It is difficult to assess whether the compost piles are fully matured, however (Tiquia et al. 2005) stated that the upper limit of TPF produced for a mature compost is 35 µg TPF /kg. This is however more of a guideline rather than an absolute, but according to this guideline none of the compost piles can be classified as mature. According to these guidelines PB20 is the closest to maturity.

3.4 Conclusions

The aim of this study was to investigate the effect of adding varying amounts of contrasting biochars to green and animal waste on the composting process and compost quality. Biochar characterization revealed that the high temperature, refined eucalyptus char had very different physical and chemical properties to the robust, low temperature pine biochar. Results from the composting trial showed that the addition of biochar did not affect the general temperature dynamics, as all piles went through the same typical thermophilic, mesophilic and maturation phases.

However, type of biochar did influence the results as eucalyptus biochar treated piles generally exhibited lower temperatures in the thermophilic (3.0 – 7.5°C) and mesophilic phase (1.4 – 3.0°C) relative to the control. This is related to pyrolysis temperature of the eucalyptus biochar, as higher temperatures reduce the ability of microbes to degrade the charred C, resulting in less microbial activity during composting, and lower compost temperatures. These lower composting temperatures could be cause for concern as high temperatures are required to ensure the sterilization of the organic material (Stentiford 1996). The C/N ratios of the compost piles showed good evolution as all the mixtures had a final ratio of <20 (Garcia-de-la-Fuente 2011). The difference in biochar type is however further illustrated by eucalyptus piles resulting in higher final C/N ratios, due to higher fixed C content, as well as lower pH values and decreased dehydrogenase activity relative to pine composts with the same quantity of biochar.

In general, quantity of biochar added to the piles also seemed to influence the final products as greater quantities of fixed C present in the piles containing 20 % biochar resulted in C/N ratios above the standard of 15:1 as prescribed by Zucconi et al. (1983). Dehydrogenase activity also reduced with an increase in biochar content, as seen through lower temperatures in the mesophilic and maturation phase, along with increased final pH values when more biochar is present.

Biochar type and content therefor did affect the composting dynamics however, not to such a degree that compost piles could not be classified as mature, stabile and usable. Although all piles reached the requirements to be classified as mature, further research and analyses are required to determine how these slight differences during composting could affect the composts potential for improving nutrient availability in an agricultural context whilst improving carbon sequestration from an environmental conscious aspect.

CHAPTER 4 – THE EFFECT OF COMPOSTED BIOCHAR ON MINERALISATION AND STABILITY

4.1 Introduction

Applying organic amendments in the hopes of improving the soils physical and chemical properties are well known in literature (Siqua et al. 2016), but research has shown a wide variety of impacts, depending on the type of amendment (Larney and Angers 2012). Biochar amendments especially have been claimed to achieve sustainability goals such as C sequestration, soil improvements, and energy production (Schulz et al. 2013).

The release of phosphorus from biochar has long been recognized (Tryon 1948). Combustion of organic materials greatly enhance P availability by disproportionately volatilizing C and cleaving organic P bonds which results in a residue of soluble P salts (DeLuca et al. 2006). The availability of phosphorus in the soil could also be greatly affected by other biochar related pH-dependent factors that influence the ratio of soluble-to-insoluble P pools such as; the interference of biochar with P sorption to Al and Fe oxides, biochar-induced changes to the ion exchange capacity; and the sorption of plant and microbial chelates onto biochar (DeLuca et al. 2006).

Nitrogen is mainly bound in proteins in fresh organic matter. During composting, these compounds are degraded or modified in several different ways to form inorganic (ammonium, nitrite, nitrate) and organic (microbial biomass, humic substances) N-containing components (Amlinger et al. 2003). This turnover is caused by different microbial processes such as: ammonification (proteins via amino acids to ammonium), nitrification (ammonium via nitrite to nitrate), denitrification (nitrate via nitrite, nitrous oxide via nitric oxide to elemental nitrogen), and immobilization (transformation of different nitrogen containing compounds to humic substances, lignin, amino acids and inorganic compounds into microbial proteins) (Korner and Stegman 2002). These N-transforming processes are all linked to specific composting factors are therefore dependent on compost parameters like the C/N ratio of the feedstock, composting conditions, decomposition or stabilization rate, the compost post-treatment, and the final compost quality with specific reference to the final C/N ratio and degradable amounts of C- and N-fractions (Amlinger et al. 2003).

The theory stands that the easily decomposable organic material in compost, combined with the contribution of C sequestration from the biochar, could induce long term carbon, nitrogen and phosphorus mineralisation (Siqua et al. 2016). Carbonized materials from incomplete organic combustion such as biochar provide high levels of stable organic matter that can release nutrients in the soil over a long period (Glaser et al. 2002). However, one of the core purposes of

composting is to degrade and transform organic material via microbial activity (Lasaridi and Stentiford 1998). The carbon compounds within the biochar could therefore be modified, and since N mineralisation/immobilization and phosphorus availability are predominantly linked to the degradability and balance of soil C pools, it is essential to understand and quantify these dynamics (Cambardella et al. 2013).

The aim of this study was therefore to quantify these dynamics by; (i) determining the effect of *composted biochar* on C, N and P mineralisation in comparison to the application of *biochar only* and a combination of *biochar and compost*; and (ii) investigating the longevity of composted biochar under field conditions and its effect on organic matter functional pools. These objectives were assessed by conducting a laboratory incubation study with 10 different treatments applied at 25 t/ha and a field trial with 6 treatments applied at 50 t/ha, to enhance possible differences between treatments when conditions are less than ideal.

4.2 Materials and Methods

4.2.1 Laboratory incubation

Three parameters were selected as representative indices to convey the effect of composted biochar on soils under controlled conditions; CO_2 respiration, N and P mineralisation. Two separate jar incubation experiments were constructed, one for measuring CO_2 respiration and the other for P- and N-mineralisation. For each jar incubation study, a soil of low fertility. The soil used for this trial was collected from a Kroonstad soil form in a fallow field in Brackenfell, Western Cape, (33°53'42.67" S, 18°43'26.982" E). The top 10 cm of the soil was removed and the soil was sampled to a depth of 1 m. The acidic pure sand of inherent low fertility (Table 4.1) was air dried and sieved (< 2mm) before use.

Table 4.1 - Physical and chemical properties of the control soil used for the incubation studies.

	Sand (%)	Silt (%)	Clay (%)	C (%)	N (%)	BrayII - P (mg/kg)	pH (water)	pH (KCl)
Control soil	97.6	1.9	0.5	0.16	0.03	11.07	4.45	4.28

Nine treatments were constructed by applying the five produced composts (CC, PB10, PB20, EB10, EB20), two biochars (PB, EB), and two CC mixed with both types of biochar (10 % dry weight) (CPB, CEB) at a rate 25 t/ha (Jones et al. 2010). A soil treatment with no amendments

was also included to serve as control (C) (Table 4.2). All of the incubations were performed in triplicate.

Table 4.2 - Description of different treatments added to sandy soil used in the Incubation study.

Treatment	Description of sandy soil treatments applied at a rate of 25 t/ha
CC	Control compost (no biochar)
PB10	Compost made with 10% (d/w) pine biochar (co-composted)
PB20	Compost made with 20% (d/w) pine biochar (co-composted)
EB10	Compost made with 10% (d/w) eucalyptus biochar (co-composted)
EB20	Compost made with 20% (d/w) eucalyptus biochar (co-composted)
CPB	Control compost mixed with 10% (d/w) pine biochar (not co-composted)
CEB	Control compost mixed with 10% (d/w) eucalyptus biochar (not co-composted)
PB	Pine biochar
EB	Eucalyptus biochar
C	Control soil only

4.2.1.1 *Water holding capacity*

Before incubations started, field water holding capacity (WHC) for each treatment was determined in duplicate by placing 20 g of an air dried sample in a funnel with filter paper mounted on a pre-weighed collection flask. Thereafter, precisely 15 g of distilled water was poured over the soil, then covered with aluminium foil, and left for 24 hours so that the excess water could drain and collect in the collection flask. Hereafter, each collection flask was weighed and the water content was calculated with the following formula:

$$\% \, WHC = (15 - W_p)/dwt \times 100$$

where W_p is the weight of the percolated water and *dwt* is the soils dry weight in grams (Cassel and Nielson 1986).

4.2.1.2 *CO_2 Respiration*

Carbon dioxide respiration was determined with a modified version of the CO_2 trap method described by Pell et al. (2006). Incubates consisted of 40 g of each soil treatment placed in a glass beaker which was then brought to field water-capacity. Each beaker, along with a 50 ml glass bottle containing 25 ml 0.05M NaOH, was placed inside a 1000 ml incubation jar before being sealed. At the start of the measurements, jars were opened and the glass bottles were replaced with new bottles containing 25 ml of 0.05 M NaOH. To determine the amount of CO_2 captured by the NaOH traps, 5 ml of 0.5 M $BaCl_2$ was immediately added after collection to precipitate as $BaCO_3$. The remaining OH- in solution was then determined by adding 4 drops of

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phenolphthalein and titrating with 0.05 M HCl to the endpoint of the indicator. The volume of 0.05 M HCl consumed was used to calculate the basal respiration rate (BAS) in units of µg CO_2 -C/g DW per hour with the following formula:

$$BAS = \frac{M_C \times (V_b - V_s) \times 0.5}{S_{dw} \times t \times 2} \times 10^3$$

where M_c is the molar weight of carbon; V_b and V_s the volume in ml of 0.05 M HCl consumed in the titration of the blanks and the sample; S_{dw} is the dry weight of the soil in grams; and t is the incubation time in hours. Two OH⁻ are consumed per CO_2 molecule precipitated and thus a factor of 2 is included in the formula. After the first 12 h measurement, this process was repeated at increasing time intervals over a time period of 2 months.

Figure 4.1 - Pictures of CO_2 respiration incubation jars containing the glass beaker with soil treatments and an open glass bottle with 0.05 M NaOH.

4.2.1.3 *N and P- availability*

The availability and mineralisation of nitrogen and phosphorus was determined by a modified version of the method described by Nelson et al. (2011). One hundred grams of each of the treatments was weighed and placed in a 500 ml flask. Distilled water was added to bring the soil to field water capacity and the flasks were sealed with ParafilmM laboratory film. The sealed flasks were then incubated at ±30°C for 60 days and soil samples were extracted for analysis at days 1, 3, 7, 14, 28, 42 and 60. Available mineral N was determined with the Mulvaney (1996) method by extracting 5 g of soil with 25 mL of 2M KCl. Available P was determined by extracting 2.5 g of soil with 25 mL Melich-3 solution (0.2 N acetic acid; 0.25 N NH4NO3; 0.015 NH4F; 0.013 N HNO3; 0.001 M EDTA) (Nelson et al. 2011). Soil moisture content was kept constant throughout the experiment by weighing each flask before and after sampling.

To measure the available N extracted from the soils, Merck's spectroquant® Nitrate (14773) and Ammonium (100683) test kits were used. Both nitrate and ammonium test kits come equipped with pre-mixed chemicals used to measure the quantity of either nitrate or ammonium in 2 M KCl

extracting solution. The standard procedure for measuring nitrate consists of placing 1 micro-scoop of test chemical NO_3-1 (as labelled by Merck) and 5 ml of NO_3-2 in a round cell and shaking it vigorously for 1 minute. Thereafter slowly pipetting 1.5 ml of the sample extract into the cell and allowing the colour to develop for 10 minutes before transferring the solution into a 10-mm cuvette and measuring the absorbance at 540 nm with a Jenway 7315 Spectrophotometer. Ammonium measurements followed a similar standard procedure, with 5 ml of test solution NH_4-1 being pipetted into the test tube along with 0.20 ml of the sample extract and 1 micro-spoon of test chemical NH_4-2. After shaking the solution vigorously until the solid substance was dissolved, 15 minutes of reaction time was allocated for colour development and the absorbance was measured in a 10 mm cuvette at 667 nm. An absorbance calibration for ammonium and nitrate was constructed by weighing off 0, 2, 5, 10, 20, 30 and 50 mg of NH_4^+-N and 0, 0.2, 1, 5, 10 and 20 mg of NO_3^- -N per litre of extracting solution and analyzing the standards with the same methods described previously.

For colourimetric P determination, 20 ml of the Mehlich-3 filtrate was pipetted into a 50 ml volumetric flask. Ten ml of Boric acid-molybdate solution was added followed by 10 ml of the reducing agent. The volume was made up to 50 ml using distilled water and the solution was allowed to stand for exactly 20 minutes. The visible absorbance was then measured at 660 nm using the spectrophotometer. An absorbance calibration was prepared by making a stock solution of 100 ppm and diluting it to an equivalent of 0, 2, 4, 6, 8 and 10 mg P L^{-1} where after 20 ml of each standard was analyzed for colour development.

Figure 4.2 - Digital image of a dilution series of NH_4^+ and the colour development of treatment soils after 7 days of incubation.

4.2.2 Field trial

The field trial was erected to determine the difference in stability and transformation of carbon between biochar- and non-biochar containing composts applied in soil over a 6-month period. The trial site was situated in Welgevallen farm, Stellenbosch (33°56'30.86" S; 18°51'53.47'' E), on a

piece of fallow land that has not been cultivated in five years and is classified according to the South African soil classification system as a Tukulu soil family with a sandy loam texture (Table 4.3). This site was selected due to little or no remnants of cultivation practices, while still agriculturally viable. Trial setup was done by clearing an area of roughly 2 × 1.5 m from all vegetation and the 5 cm of topsoil. Hereafter, 20 black nursery bags (with drainage holes) were filled with 10 kg of soil and taken to the lab for accurate dry weight (D/W) compost application.

Table 4.3 - Physical and chemical properties of the control soil used for the field trial.

	Sand (%)	Silt (%)	Clay (%)	C (%)	N (%)	Bray II - P (mg/kg)	pH (water)	pH (KCl)
Control soil	80	10	10	0.714	0.026	51.99	5.55	4.68

Samples from all bags were dried at 105°C for 24 hours to determine the moisture content. Each compost treatment was applied at 50 t/ha (Jones et al. 2011), which translated to 204 g of dried and sieved (<2 mm) compost being thoroughly mixed into 10 kg (dry weight equivalent) of soil and placed into the large nursery bags. All five composts (CC – PB10 – PB20 – EB10 – EB20) were applied in triplicate along with a no-treatment control (C) (total of 18 nursery bags). These bags were returned to the study site and buried in three rows that was 30 cm apart from one another and within each row the bags were 15 cm apart and buried at a depth of 25 cm so that the bags' soil surface is level with the surrounding soil (Figure 4.3). The trial was set up to start in the spring and continue until the end of summer (6 months) under natural climatic conditions in terms of moisture content and ambient temperatures. Representative core samples were taken from each bag at the start and end of the trial on which soil pH, EC, loss of ignition and density fractionation was performed. Cumulative soil respiration was measured with the use of the soda-lime method throughout the 6-month trial.
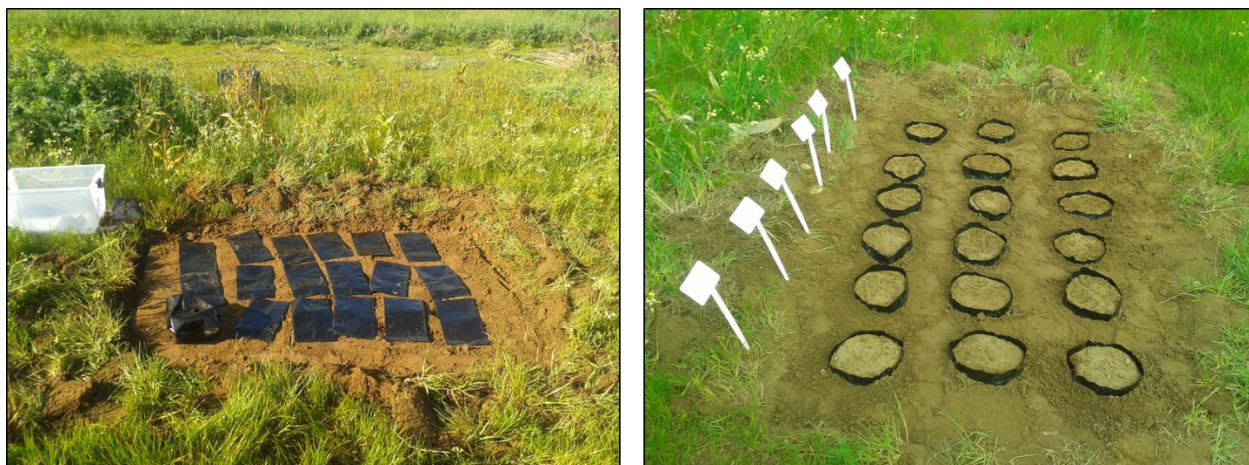
Figure 4.3 - Digital images illustrating the preparation of the field trial site and filling of the nursery bags as well as the final treated buried bags.

4.2.2.1 *Soil pH and EC*

Soil pH in water and in KCl was measured in a 1:2.5 supernatant solution (Sonmez et al. 2008). A 10 g soil sample was weighed of and placed into a 50 ml plastic bottle. For pH determination in water, 25 ml of distilled water was added whilst in the case of pH in KCl, 25 ml of 1 M KCl was added to the weighed soil fraction. The plastic bottles were closed and placed on a IKA KS260 shaking machine for 15 minutes where after the sample was given time to settle. pH readings were taken by placing the portable glass electrode of the pH meter into the soil solution. The pH value was only recorded after the reading stabilised.

4.2.2.2 *Soil respiration (CO2 efflux)*

The soda-lime method is a low-cost and reliable method used to quantify cumulative soil respiration and characterizes the loss of C between different treatments (Keith and Wong 2006). Soil respiration was estimated by weighing $\pm$ 10 g of soda-lime (granules consisting of NaOH and $Ca(OH)_2$) into a perforated tube then fixing it with glass wool and oven drying the tube at 100°C for 11 hours. The dry weight of the tube was then recorded and sealed in a Ziploc bag before being transported to the field site. At the field site, the tubes were removed from the Ziploc bag and positioned in a polyvinylchloride (PVC) chamber with a diameter of approximately 12 cm that was then placed in the soil to a depth of $\pm$ 4 cm. Connected to the sealed lid of the PVC chamber was an aeration tube filled with an unknown amount of soda-lime which was also fixed in place with glass wool. This outlet allows for the natural movement of O_2 in and out of the chamber whilst preventing CO_2 from entering the chamber from the atmosphere. The perforated tubes positioned in the PVC chambers were collected after 21-28 days, placed in Ziploc bags, transported to the laboratory, oven dried, and re-weighed. The chemical absorption of CO_2 is measured by the

weight gain due to the formation of Na_2CO_3 and $CaCO_3$ in the granules. Sample preparation, handling and chamber leakage could cause abnormal absorbance of atmospheric CO_2 and for this reason, two blank chambers with soda-lime tubes handled the same way as the treatments were also included in the study. The difference in weight before and after the field period along with the blank values were used to estimate the flux of CO_2. This method is not an exact yield measurement but does however allow one to compare soil respiration between treatments (Keith and Wong 2006).

Figure 4.4 - Example of soda lime traps installed on the field trial soils for CO_2 respiration measurement.

4.2.2.3 *Density fractionation of SOM functional pools*

Using density fraction technique, soil organic matter can be separated into three functional pools, namely, free particulate OM (least transformed, most labile C fraction), intra-aggregate particulate OM (physically occluded labile C fraction), and mineral bound (most stable and humified C fraction) (Sohi et al. 1994), The aim was to use this physical fractionation technique based on the procedures proposed by Sohi et al. (2001) and Cerli et al. (2012) to isolate (i) the free particulate organic matter (fPOM) (undecomposed material), (ii) the occluded particulate organic matter (oPOM) and (iii) the mineral-bound organic matter (MBOM) fraction.

The process starts by weighing 5 g of soil and adding 25 ml Sodium Iodide (NaI) (1:5 soil to solution ratio) with a density of 1.6 g cm^{-3}. The density of the NaI is specifically selected as OM usually has a density equal or less than 1.5 g cm^{-3} (Cerli et al. 2012). After adding NaI, the 50 ml

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centrifuge tube containing the aliquot was gently shaken to ensure wetting of the soil without disrupting the aggregates. Hereafter the suspension was allowed to stand for 1 hour before being centrifuged for 20 min at 5600g. A Millipore filtration funnel fitted with a 0.45 µm SUPELCO nylon membrane was then used to capture the floating fPOM under vacuum pressure. The NaI solution collected in the Buchner flask was recycled into the centrifuge tube and the centrifuge-filtering procedure was repeated four times to ensure that all of the fPOM had been quantitatively transferred. To extract the oPOM fraction from the soil, 25 ml of fresh NaI was added to the centrifuged sample and dispersed by ultrasound at an energy level of 200 j ml^{-1}. The suspensions were sonified with a sonicator (Qsonica Sonicators, Newton, USA) fitted with a probe tip (dimensions 13.8 cm x 1.3 cm) to a depth of 15 mm in suspension to attain the intra-aggregate organic matter by disrupting the aggregates (Smith 2014). This intra-aggregate fraction was retrieved using the same procedure as the fPOM fraction described above. Both fPOM and oPOM fractions were collected on the same membrane as organic matter levels were too low to extract and analyse separately. These collected fPOM and oPOM fractions were then rinsed with deionized water in a separate flask until the electrical conductivity of the freshly collected water was less than 50 µS cm^{-1}. These fractions were air dried at below 40°C, weighed and ball milled to ensure homogeneous samples for analysis. The soil samples remaining in the centrifuge tubes after the extraction of fPOM and oPOM now contained the mineral-bound OM fraction (MBOM). These remaining soils were dialyzed in a MEMBRA-CEL cellulose membrane dialysis tube and placed in a container filled with distilled water until the water tested free of salts in 0.1 M AgNO$_3^-$ whereafter it was oven dried for 72 hours at 40°C.The isolated fPOM, oPOM and MBOM pools were then weighed with a 5 decimal micro-scale and submitted for total C and N analysis by dry combustion using a Euro Vector elemental analyser (Nelson et al. 1996).

4.2.2.4 *Loss on ignition*

Loss on ignition is considered to be an excellent method to estimate soil organic matter in soils as it accounts for a greater fraction of OM than other methods such as wet combustion (Heiri et al. 2001, Koide et al. 2011). The method is based on the general assumption that non-thermally altered organic matter is oxidized at a temperature of 550°C in most soils. The loss on ignition was determined by the weighing of 2 g of oven dried soil from each field treatment into a platinum container and heating the samples in a muffled furnace at 550°C for 4 hours. Loss of ignition was performed in triplicate on soil samples from before and after the trial period to determine the change in carbon content over 6-months. The weight difference was used to calculate the percentage change of soil organic matter.

4.2.2.5 *Statistical analysis*

The analysis of variance (ANOVA) of each mineralisation data set was computed with the use of Statistika's (Statsoft STATISTIKA 13.0) repeated measures procedure to determine the interactions between, and effects of each treatment over time. The cumulative mineralisation results and independent nutrient information was submitted to a single factor One-Way ANOVA to analyze for significant differences between and within treatments with a Tukey HSD post hoc test.

4.3 Results and Discussion

4.3.1 Laboratory Incubation studies

4.3.1.1 *pH in water*

The pH of the laboratory incubated treatments was measured in water before and after the 2-month incubation to aid with interpreting relative mineralisation rates of C, N and P, as pH affects microbial activity and nutrient availability. The pH analyses revealed that all the treatments pH values increased over time (Table 4.4). This increase is likely due to the adsorption of H^+ onto the negatively charged organic material and biochar from solution, thereby decreasing H^+ in solution. The pine biochar treatments had a larger liming effect than the eucalyptus biochar treatments, which is most apparent in the pure biochar + sand treatments, PB (final pH of 8.87) and EB (final pH 4.78) (Table 4.4). It also interesting to note that the pH of pine biochar on its own measured in water (1:10) was slightly lower (pH 9.98) than the eucalyptus biochar on its own (pH 10.15) (Table 3.2). As previously discussed in Chapter 3, pine biochar contained less fixed C (aromatic C) than eucalyptus (Table 3.5), thus it likely contains more oxygen functional groups than eucalyptus and thus pH reading in water appeared lower due to this buffering effect. However, pine biochar contained more ash (Table 3.2) and basic cations than eucalyptus (Table 3.3), which would explain its greater liming effect when added to the poorly buffered sandy soil. Biochar has also been found to have amphoteric sites that can react both as an acid or a base depending on the number of oxide surfaces and the solution pH (Amonette and Joseph 2009). These amphoteric sites could be the reason why Chan et al. (2008) found that there is no direct relationship between the liming value and the pH of a biochar. What they did find however is that some biochars can have fairly high concentrations of carbonates (Chan et al. 2008). The amphoteric sites, could therefor explain the smaller changes in pH seen for the eucalyptus biochars, while the presents of carbonates could be a source of the liming potential exhibited in the pH measurements of pine treatments (Van Zwieten et al. 2007). In general, composting was found to improve the buffering capacity of the fresh biochar, by possibly increasing the surface

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reactivity (Borchard et al. 2012) and controlling and stabilizing the initial change is soil pH after application. This can be seen as the fresh biochars natural liming capabilities (PB – 7.63 and EB – 4.28) are still pronounced in the treatments containing fresh biochar and compost (CPB – 7.28 and CEB – 5.36), but is more buffered in the composted biochar treatments (PB10 – 6.54 and EB10 – 6.55).

Table 4.4 - Soil pH (1:2.5 water) measured at the start and end of the 60-day laboratory incubation of the compost and biochar amended sandy soil.

Treatment	Start	End	ΔpH
CC	6.42	7.31	0.89
PB 10	6.54	7.17	0.63
PB 20	6.65	7.48	0.83
EB 10	6.55	6.77	0.22
EB 20	6.51	7.01	0.50
CPB	7.28	7.72	0.44
CEB	5.36	6.86	1.50
PB	7.63	8.87	1.24
EB	4.28	4.78	0.50
C	4.11	4.45	0.34

4.3.1.2 *CO$_2$ respiration*

Figure 4.5 illustrates the release of CO_2 over 60 days for all treatments mixed with an acid, sandy soil in the laboratory incubation study. The CO_2 effluxes have been normalized to the total amount of C in each treatment so that the relative degradability of the materials can be assessed. There was an initial rush of C mineralisation followed by a steady decline to an average low point at day 14 (Fig. 4.5). The compost control (*CC* – 319.5 µg CO_2/g C) and treatments containing both pine biochar and compost (*CPB* – 362.47 µg CO_2/g C, *PB10* – 342.33 µg CO_2/g C, and *PB20* – 276.01 µg CO_2/g C) exhibited the highest initial release of CO_2, whilst eucalyptus biochar only (*EB* – 0.67 µg CO_2/g C) and the soil control (*C* – 0.056 µg CO_2/g C) showed very low amounts of respiration. After day 14, all treatments except CEB, PB, EB and C showed a slight increase in respiration towards day 21, whereafter they maintained a steady or declining respiration rate. The immediate rush of CO_2 release after application of the pine biochar containing composts (CPB, PB10, PB20) and the compost control (CC) can be explained by the high amounts of easily degradable C due to the lower quantity of fixed C and ash in these treatments (Table 3.5). Studies by Zimmerman
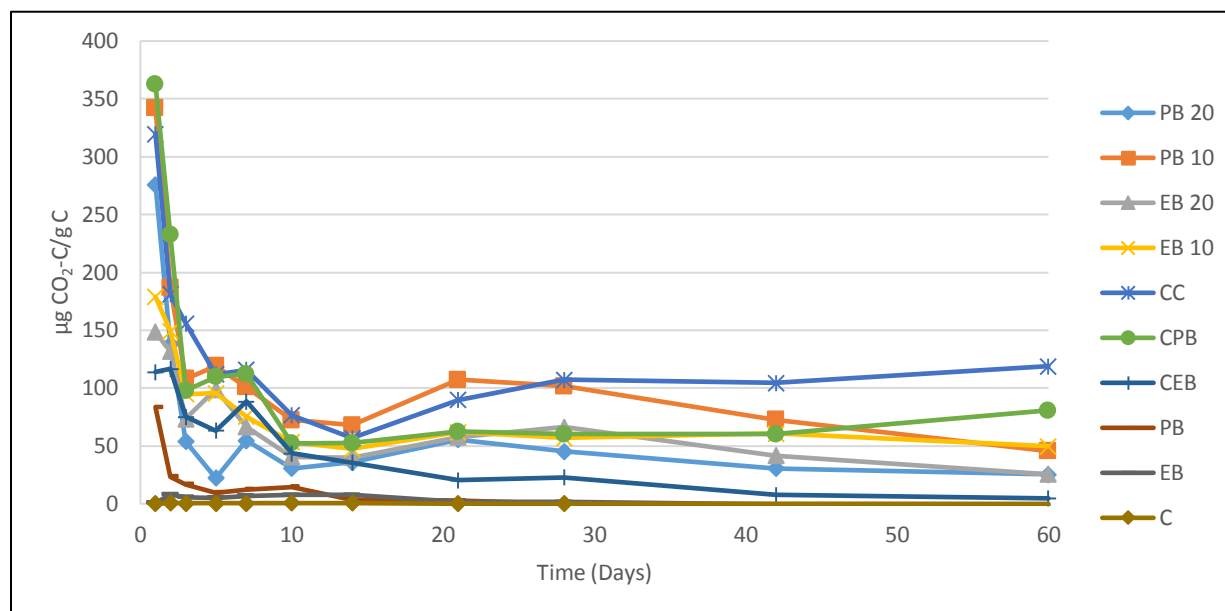
Figure 4.5 - Normalized CO$_2$ respiration in µg CO$_2$-C/g C released from the compost, biochar and control treatments during the 60-day laboratory incubation.

et al. (2011) and Liu et al. (2011) showed similar results of increased initial C mineralisation after the application of biochar in soils. High quantities of volatile matter, aliphatic C, and carboxyl and carbohydrates contained in the lower temperature biochar, may present a relatively labile fraction which could be responsible for the enhanced C mineralisation observed in the initial stages (Purakayashta et al. 2015).

Low initial CO$_2$ release by EB and C are explained by the high amounts of fixed C components (Table 3.2) (Lehmann et al. 2011) found in the eucalyptus char and the low inherent total C content of the sandy soil (Table 4.1). The evolution of CO$_2$ release, characterized by a rush of C mineralisation followed by a steady decrease towards day 14, and an increasing and stabilizing phase towards the latter stage of the incubation is similar to results obtain by Bhaduri et al. (2016). They found that microbial activity was highest when a large portion of labile C was available, whereafter exhausted sources of easily degradable C caused a decrease in C mineralisation, followed by a slight increase via microbial shifts to alternative, semi-labile C sources (Bhaduri et al. 2016*).*

Cumulative respiration results in Figure 4.6 shows that CC (1671.67 µg CO$_2$/g C) produced the most CO$_2$ over the 60-day incubation. This was significantly higher than all other treatments except for PB10. The soil control (C), EB and PB on the other hand showed significantly low amounts of CO$_2$ respiration, whilst PB20, EB10, EB20, CPB, and CEB resulted in similar total C mineralisation.
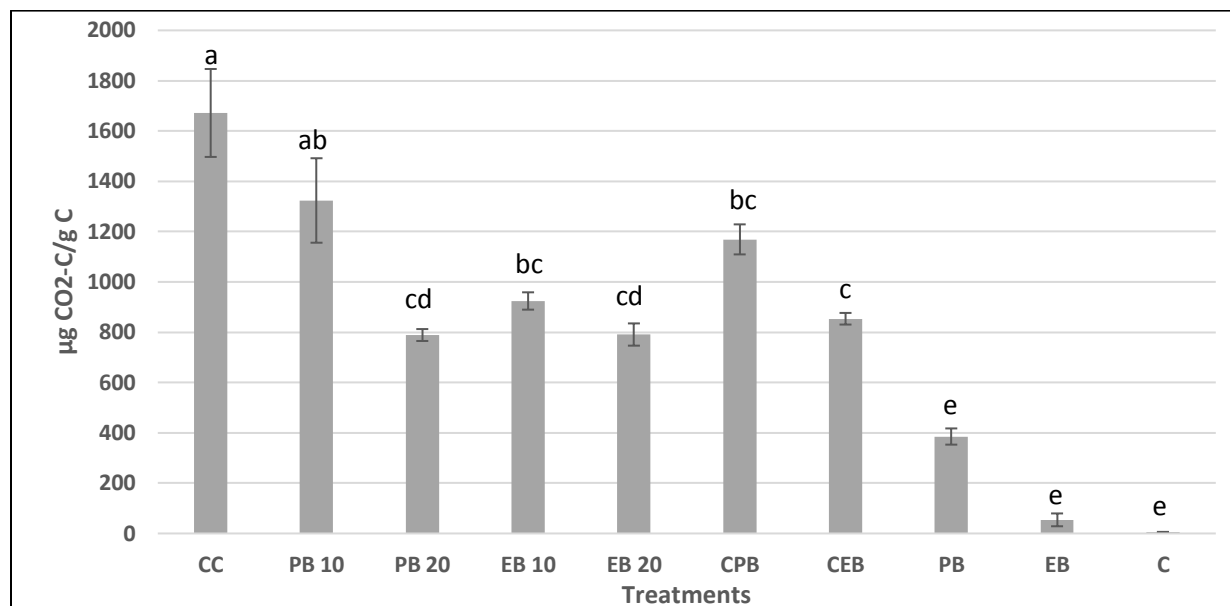
Figure 4.6 - Total cumulative CO$_2$ respiration (normalized to C content) from the compost, biochar and control treatments released over 60-day laboratory incubation. Standard error bars and letters of significance (p < 0.05) according to Tukey's HSD test are shown.

Although the pine biochar-containing compost mixtures (CPB, PB10 and PB20) exhibited a high initial release of CO$_2$ (Figure 4.5, Fig. A.4), the compost control (CC) still resulted in the highest total C mineralisation (Figure 4.6). This could arguably show that after the initial flush of C mineralisation in the biochar amendments, other factors such as C/N ratio, labile C content, pH and nutrient availability start to play a role (Purakayashta et al. 2015). Liu et al. (2016) found that biochar material with lower C/N ratios can greatly improve microbial activity which results in an enhanced C mineralisation rate due to increased C and N availability. Yang et al. (2014) reported that the increased stability of biochar after the initial loss of easily degradable C, is responsible for the reduced C mineralisation observed in the later stage of the incubation for the biochar treatments. This increased stability then translates to increased C contents in the soil and a larger C/N ratio (Purakayashta et al. 2015). The fact that CC produced the most CO$_2$ further substantiates this theory as it had the lowest recorded C/N ratio before application (Figure 3.4). A general trend, that pine biochar treatments resulted in slightly higher respiration than their respective eucalyptus biochar counterparts, can also be seen in the cumulative CO$_2$ graph. This trend (non-significant) testifies to the role that pyrolysis temperature plays in biochars chemical construction and nutrient availability. Lower temperature biochars, such as the pine biochar used in our study, has a higher volatile and labile C content (Table 3.2), which would naturally lead to increased microbial activity (Lehmann et al. 2011) and subsequently higher CO$_2$ respiration. The

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fact that the application of PB and EB only (relative to compost treatments), both produced very low amounts of respiration could also be related to the pH of these treatments in the soil. As seen in Table 4.4, PB resulted in a pH of 8.87 and EB in 4.78. Both of these pH values fall within the non-optimal pH ranges for microbial activity (Smith and Doran 1999) and could therefore have contributed along with the high amounts of fixed carbon (relative to compost treatments) to the reduced levels of respiration. However, the higher pH of PB still falls within the general functional range of most bacteria (pH 5 – 9) (Smith and Doran 1999) which could also have allowed, along with increased labile C, for more suitable conditions and therefor higher respiration in relation to EB.

When comparing the results of co-composted 10% biochar treatments (PB10 and EB10) versus adding 10% biochar post-composting (CPB and CEB), it was found that composting biochar did not significantly affect the total cumulative CO_2 respiration (Fig. 4.6). However, the same trend of higher cumulative respiration in the co-composted biochar treatments (PB10 and EB10) than post-composted addition (CPB and CEB) was observed (Fig. A.5) as PB10 had 11.7% more respiration than CPB and EB10 had 7.3% more than CEB. This trend illustrates that composting did have an effect on the degradability of the biochars. There are two mechanisms that explain the degradation of the biochar in compost, the first is abiotic oxidation and the second is microbial degradation. Abiotic oxidation involved the formation of new hydrophilic functional groups in the biochar structure (Cheng et al. 2006), which then allows for increased microbial populations, diversity and activity resulting in further biochar degradation. The composting process could also have enhanced degradation through fungi (Dias et al. 2010). Fungal mediated degradation was studied by Hofrichter et al. (1999), whom found that wood decaying fungi can partially decompose low-grade coals similar to biochar. However, this potential degradability is related to feedstock and charring temperature (Baldock and Smernik 2002), which is also why PB had a larger difference in degradation relative to the EB.

4.3.1.3 *Nitrogen mineralisation*

Figure 4.7 shows that all treatments resulted in similarly poor ammonium mineralisation over the course of the 60-day incubation. Descriptive statistics performed on the normalized data illustrated that the mean trend across all treatments consisted of a general increase in NH_4^+ release from day 1 to 7 followed by a steady decline in mineralisation towards day 42 and a subsequent increase at day 60. The most significant differences seen in the evolution of ammonium, is the high initial amount of NH_4^+ extracted from EB20 (0.267 mg/kg), which is nearly

double that of the second highest treatment (PB20: 0.138 mg/kg), and that CC produced the most NH_4^+ (0.205 mg/kg) at the average peak of the trial period (day 7).
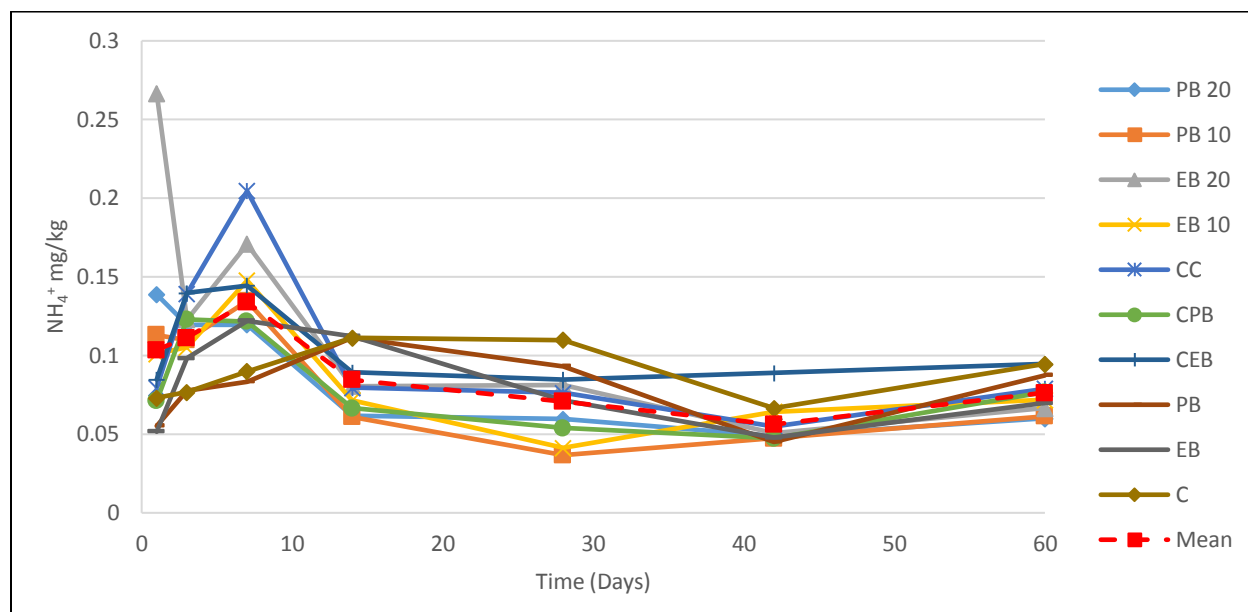
Figure 4.7 - Plant available ammonium (2 M KCl) extracted from the compost, biochar and control treatments during the 60-day laboratory incubation.

The general increase of ammonium release over the first 7 days can be explained by the process of ammonification, which is characterized by the breakdown of proteins into amino acids before being transformed into NH_4^+ (Korner and Stegman 1998). When the availability of easily degradable N became suppressed, ammonification rates would decrease. This decrease in ammonification along with the use of NH_4^+ for nitrification could account for the reduction in available NH_4^+ measured. The high initial amounts of NH_4^+ extracted from EB20 and PB20, is arguably due to the surface chemistry of the biochar itself, which released the exchangeable ammonium captured during the composting process. Cheng et al. (2006) researched on the oxidation of biochar in abiotic and biotic conditions under different temperature ranges and found that the biochar naturally oxidized when exposed to higher temperatures and longer incubation periods. This oxidation was largely initiated by the chemisorption of O_2 at unsaturated ring sites that led to the formation of carboxylic groups on the interior surface, which caused an increase in the biochars cation exchange capacity (Cheng et al. 2006). Pittman et al. (1999) reported similar findings of Ag^+ being absorbed (at pH 10) onto activated C adsorption sites mainly consisting of carboxyl and phenolic function groups, while Lehmann et al. (2003) found pronounced adsorption of ammonium on biochar particles after the exposure to manure extract. The combination of high

pH (10.15, Table 3.1) and surface area (623.85 m^2/g) of the eucalyptus biochar could therefore have resulted in the increased absorption of NH_4^+ mineralized during composting.
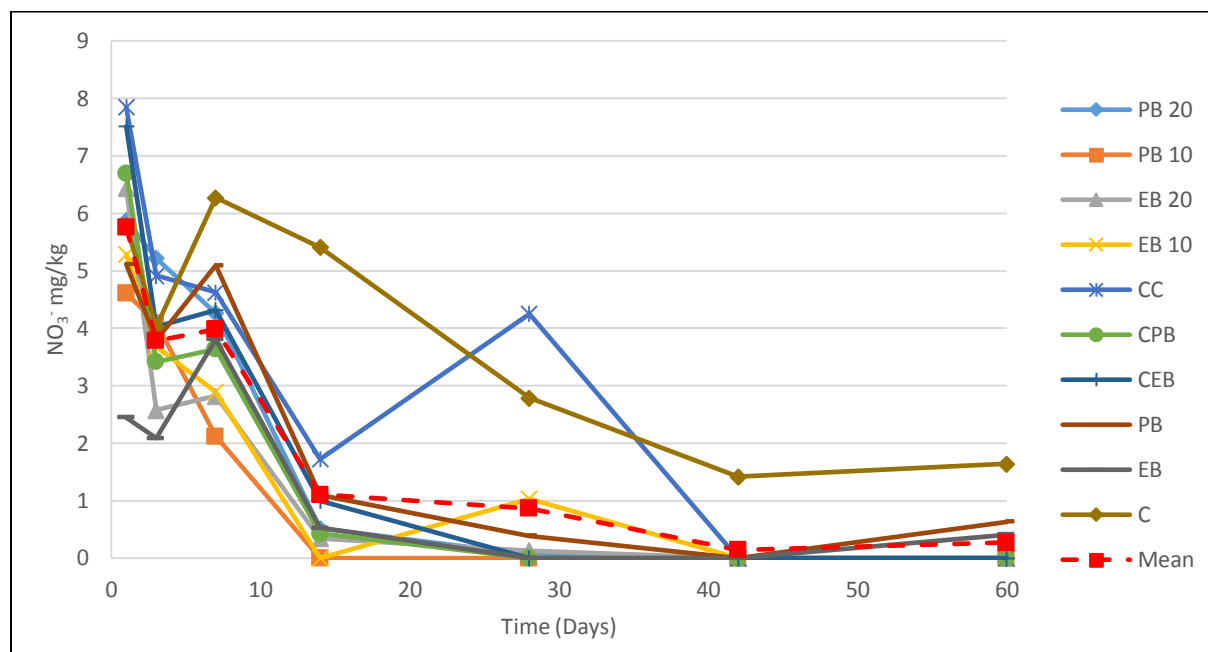
Figure 4.8 - Plant available nitrate (2M KCl) extracted from the compost, biochar and control treatments during the 60-day laboratory incubation.

Nitrate mineralisation in Figure 4.8 shows that net immobilization took place during the incubation study. The mean nitrification rate illustrates the average release of nitrate for all treatments, and shows that a slight increase was observed between day 3 and day 7 before further immobilization took place. This increase in NO_3^- content is best explained by the increased availability of ammonium after initial ammonification between day 1 and 7 (Figure 4.8), which could then be converted to nitrate via the process of nitrification (Korner and Stegman 1998).

The cumulative amount of 2M KCl extractable ammonium (Figure 4.9) and nitrate (Figure 4.10) further illustrates the total effect that different treatments had on the mineralisation in the incubation study. Only EB20 showed significantly higher amounts of total NH_4^+ released in comparison to the soil control (C) (Figure 4.7). All of the other treatments were significantly comparable to one another, which would mean that the incorporation of either compost or biochar did not significantly improve nitrification in the soil medium. The repeated factorial ANOVA (Tukey HSD) revealed that the cumulative significance of EB20 can be traced back to the high initial release of NH_4^+ (Day 1) followed by non-significant nitrification rates throughout the remainder of the trial in relation to other treatments (except for PB and C at day 7).
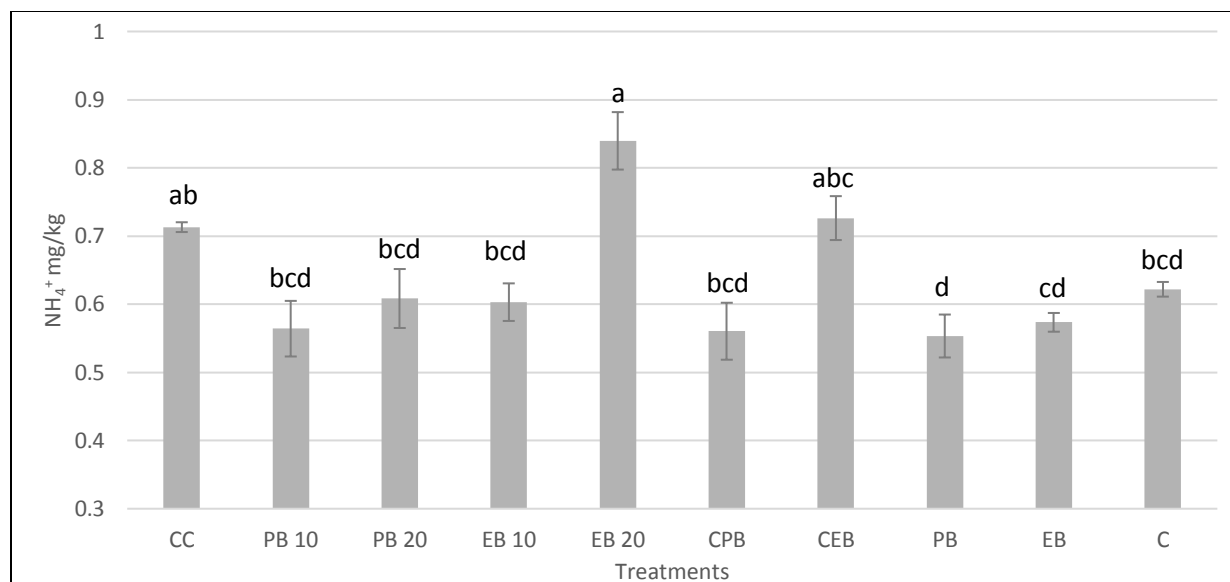
Figure 4.9 - Total cumulative available ammonium (2M KCl) extracted from the compost, biochar and control treatments over the 60-day laboratory incubation. Standard error bars and letters of significance (p < 0.05) according to Tukey's HSD test are shown.

Figure 4.10 shows the cumulative amount of nitrate that was extracted from the compost and biochar treatments during the 2-month incubation. In the graph it can be seen that the soil control (C) released the highest total amount of NO_3^- (27.346 mg/kg) which was significantly more than all treatments except for CC (23.357 mg/kg) and PB (16.126 mg/kg).
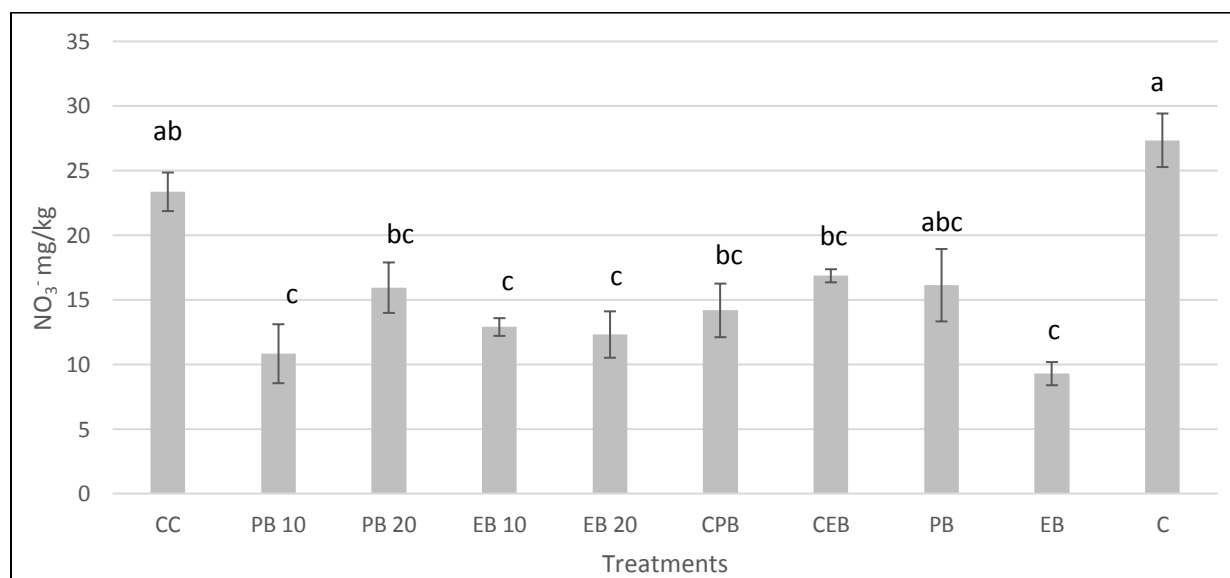
Figure 4.10 - Total cumulative available nitrate (2M KCl) extracted from the compost, biochar and control treatments over the 60-day laboratory incubation. Standard error bars and letters of significance (p < 0.05) according to Tukey's HSD test are shown.

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This also further substantiates the results seen in Figure 4.8, which illustrates that biochar caused net N immobilization and had a strong negative affect on nitrification. Nelson et al. (2011) found similar results in which N immobilization induced by biochar application, followed by persisting reduced NO_3^- concentrations over a 56-day incubation period took place.

After calculating the cumulative amount of NH_4^+ and NO_3^+ released, net change in total mineral N (ammonium and nitrate) from the start to the end of the 2-month incubation was calculated (Figure 4.11). Three deductions can be drawn from the net N mineralisation results; the first is that the application of compost (CC) did not increase soil mineralisation and resulted in net N immobilization, the second is that all biochar applications also caused reduced N availability, and the third is that 2-months under optimal temperature and moisture conditions might be too short to fully evaluate the effect of a long-term soil amendments such as biochar.
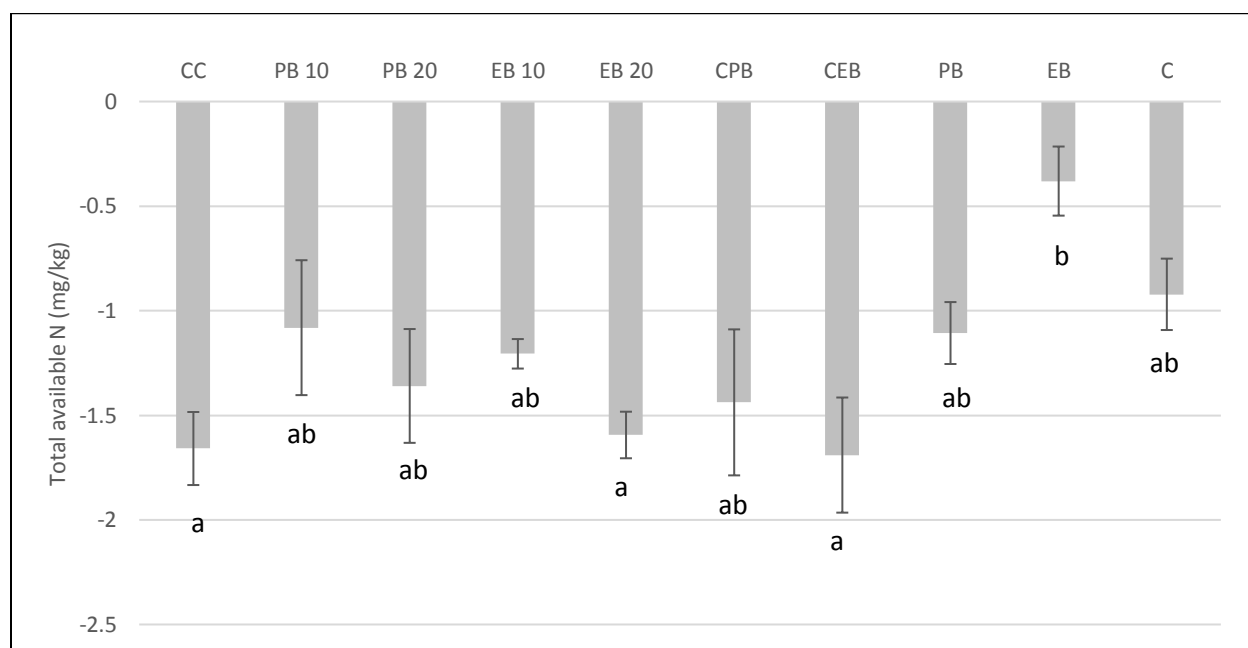
Figure 4.11 - Total net change in mineral nitrogen (2M KCl) extracted from the compost, biochar and control treatments over the 60-day laboratory incubation. Standard error bars and letters of significance (p < 0.05) according to Tukey's HSD test are shown.

The immobilization of N after the application of composts is a well-documented phenomenon in the literature. According to Amlinger et al. (2003), Berner et al. (1995) studied the N-mineralisation on 38 different composts over 12 weeks and found that, on average, only 2.1% of total compost N (between +8% and -11%) became mineralized. Hadas and Portnoy (1994) also studied mineralisation and showed that the rate of inorganic N release in compost is dependent on the availability of C and N and the ratio (C/N) at which they are present. It is therefore a general conception that compost maturity is a cause of N immobilization (Bernal et al. 1996), as less

mature composts are likely to have higher C/N ratios (Zucconi et al. 1981), resulting in less mineralisation (Hadas and Portnoy 1994). However, compost maturity (in terms of C/N ratio) and its effect on soil mineralisation was tested by Cambardella et al. (2003), who found

that composts produced under near-optimal conditions, with different levels of maturity, may not always result in net N mineralisation, as no correlation between C/N ratio and N mineralisation was found. The net immobilization seen for CC could thus only be explained through research done by Palm and Sanchez (1991) and Palm et al. (2001). They did research on the inorganic release of nitrogen from leguminous mulches and green manures and found that the biggest determining factor for nitrogen mineralisation, is the total N content, number of polyphenolic compounds, and quality of the organic material. If the N content was below 2.5%, and the ratio of polyphenolics-to-N was < 0.5 then N immobilization took place. They suggested that the polyphenolics bind to the proteins (source of N for ammonification) thereby forming complex compounds resistant to decomposition. Dry combustion results for the five composts mixtures (CC, PB10, PB20, EB10, EB20) (Appendix, Table A.1) seem to align with the findings from Palm et al. (2001) as none of them had a total N content of more than 2.5%.

The reduced availability of N with the application of biochar (composted or fresh) has been studied by many researchers and could be explained by a combination of inorganic nitrogen being converted into humic substances and microbial proteins (Korner and Stegman 1998), the probable loss of NH_4^+ through ammonia volatilization with an increase in pH (Lehmann and Joseph 2009), and the adsorption of NH_4^+ onto the biochars negatively charged interior surface (Lehmann et al. 2002). Yao et al. (2012) reported that negative affect in nitrification can be dependent on the soil pH, however, in this study, the influence of pH would be unlikely as the compost treatments had a pH value close to neutral (Schulz et al. 2013). Ammonia volatilization could therefor only marginally account for N loss in pine biochar and CC treatments as they have the more alkaline pH values.

The fact that there is no significant difference in N mineralisation between composted biochar, biochar with compost, and biochar only, shows that composting biochar does not improve the availability of N in soil under 2-months of ideal conditions. However, slight signs of later stage mineralisation seen in some biochar treatments in the CO_2 respiration, NH_4^+, and NO_3^- results (Figure 4.5, 4.7 and 4.8) could mean that the reduction of available N in the short term could evolve into net mineralisation over a longer time period.

4.3.1.4 *Phosphorus mineralisation*

The total available P recorded over time (Figure 4.12) shows that all treatments displays net P mineralisation across the 60-day incubation period. A general trend is observed of a slight decrease in available P from day 1 to 10 where after a net increase is seen towards the end at which point CC (117.73 mg/kg) had the highest and EB (59.33 mg/kg) the lowest amount of available P.
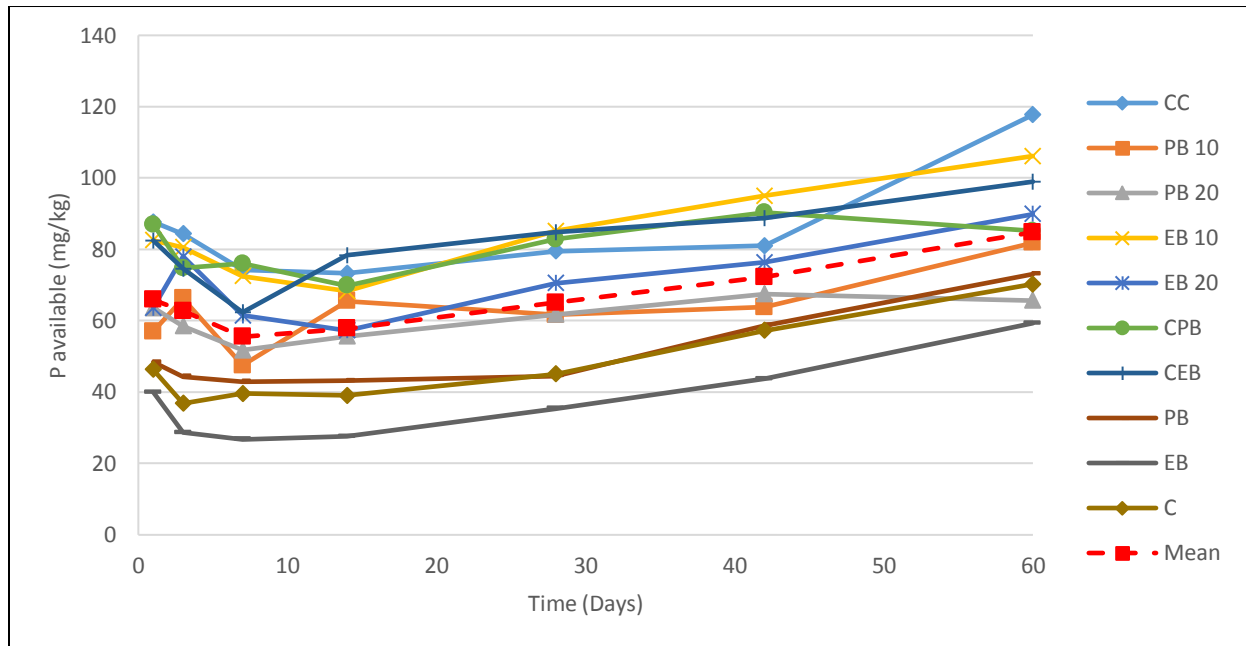
Figure 4.12 - Available P (Mehlich-3) extracted from the compost, biochar and control treatments during the 60-day laboratory incubation period.

The two plant-available forms of phosphorus ($H_2PO_4^-$ and HPO_4^{2-}) found in soils would be more easily extractable at higher pH values as anion exchange capacity decreases with an increase in pH. This means that the net increase in P availability (seen for all treatments) over time (Figure 4.12) could be a result of an increasing soil pH (Table 4.4). The results for total cumulative phosphorus availability for the various treatments (Figure 4.13) show that all compost treatments (CC, PB10, PB20, EB10, EB20, CPB, CEB) produced significantly more available P than PB, EB and C. The compost control (CC) had the highest total available P (597.80 mg/kg) with EB releasing the least (261.42 mg/kg). Compost and EB mixtures (CEB – 570.18 mg/kg) or composted EB (EB 10 – 590.26 mg/kg, EB20 – 497.07 mg/kg) proved to have higher amounts of P available than pine biochar based mixtures; CPB (565.80 mg/kg), PB10 (443.66 mg/kg) and PB20 (424.44 mg/kg). However, the application of PB and EB only, showed that PB (355.10 mg/kg) released more plant available P than EB.

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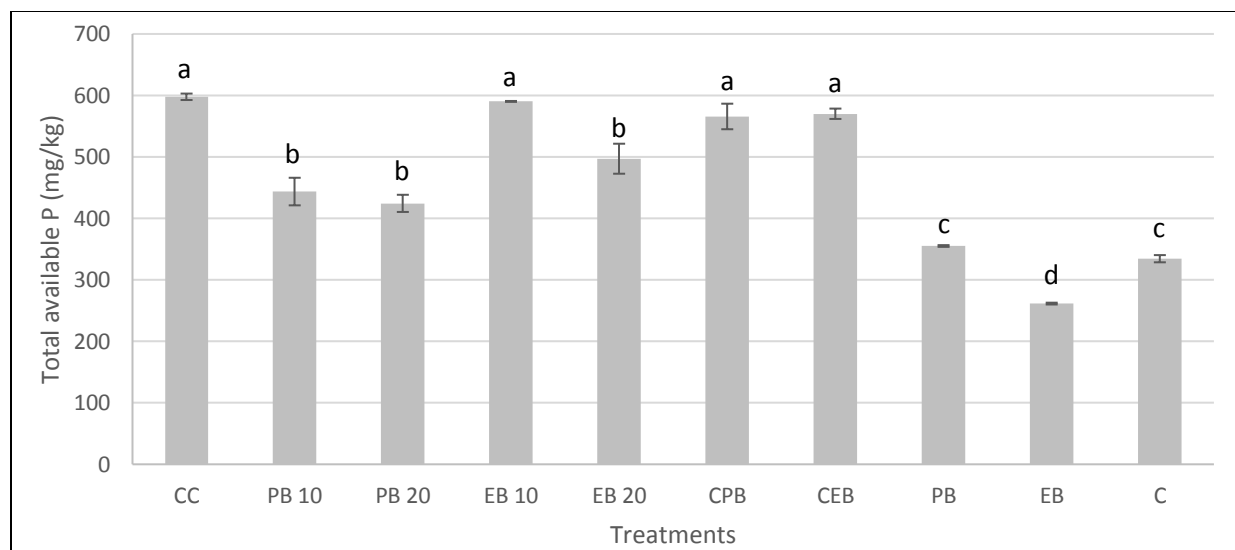
Figure 4.13 - Total cumulative available P (Mehlich-3) extracted from the compost, biochar and control treatments over the 60-day laboratory incubation. Standard error bars and letters of significance (p < 0.05) according to Tukey's HSD test are shown.

The high amounts of P extracted from all compost mixtures and especially CC could be due to organic forms of P captured in phospholipids, nucleic acids and phytyl which are broken down by microbes and transformed to phosphate ions, or the organic complexation of Al and Fe in the soil which protects phosphates from being fixed and becoming unavailable (McBride 1994). Higher concentrations of available P in the EB compost mixtures relative to PB and compost mixtures (difference of 24.8% between PB10 and EB10, 14.6% between PB20 and EB20) can be explained by findings by Nelson et al. (2011), which suggested that hydroxyapatite $(Ca_{10}(PO_4)_6(O)_2)$ is the most popular form of P found in wood based biochars (Amonette and Joseph 2009) and that, according to Lindsay (1979), the solubility of hydroxyapatites increases approximately 215 times with a decrease in each pH unit. The lower pH's seen for EB10, EB20, and CEB along with the lower quantities of Ca present in EB (Table 3.2) could have caused the dissolution of P minerals in these mixtures and thus increased concentrations of P. Yao et al. (2012) applied 13 different types of biochar produced at various temperatures and conducted absorbance and leaching test on these biochars. According to their results, the ability of a biochar to absorb phosphorus does not depend on feedstock or production temperatures as 5 of the 13 biochars showed phosphorus absorption while the rest resulted in P release (Yao et al. 2012). This research further illustrates that multiple mechanisms could be responsible for the retention of nutrients in biochar as Atkinson et al. (2010) also showed that microbes could influence the binding of nutrients in soil and biochar. The fact that PB released 26.4% more P than EB further substantiates the complexity of release mechanisms by biochar. One explanation for this could be explained by PB having more than

double the P content (1126.3 mg/kg) than EB (425.6 mg/kg) (Table 3.2), which along with the higher pH values (Table 4.4), could have resulted in the absorbance of phosphate by EB and the increased release thereof by PB. In general these results show that, even though biochar has the ability to retain nutrients (Beesley et al. 2011, Lehmann et al. 2011), it cannot be considered universal (Yao et al. 2012), and that the application of composted biochar or compost with biochar does increase the bioavailability of phosphorus (Qayyum et al. 2015) significantly more than applications of biochar only.

4.3.2 Field trial

4.3.2.1   pH and EC

Results from the pH measurements (Figure 4.14) of the amended soils showed that PB20 had the highest pH in both water and KCl at the start (water - 6.08; KCl – 6.07) and the end (water – 6.46; KCl – 5.66) of the trial. The general change in pH in both water and KCl can be seen in Figure 4.14, which illustrates that pH in water significantly increased over time (except for EB20 in which it was non-significant and C in which pH decreased), while the pH in KCl decreased significantly for all treatments. Table 1 also depicts that the PB treatments showed the largest difference in pH measured in water and KCl over the 6-month trial period. The increase of pH in water over time can be explained by the adsorption of H^+ onto the negatively charged sites located on the humic organic substrates (CC) as well as the biochar itself, while the decrease in pH measured in KCl over time is due the release of these absorbed H^+ through the substitution of K^+ on the organic, biochar and mineral surfaces. The difference in liming capabilities of the two chars are similar to what was seen in the incubation soils and can be explained by their different chemical construction (Chan et al. 2008).

Compost treatments all showed significantly higher electrical conductivity than the control soil at both the start and the end of the trial (Figure 4.15) which is to be expected as all composts contains higher amounts of ash and other ionic elements than the soil only (Schulz et al. 2013). After 6-months however, there were no significant differences in EC between various compost treatments. The large reduction in EC seen between the start and the end of the field trial is likely due to the leaching of salts from rainfall, or the adsorption thereof onto the organic matter and biochar that has an increased cation exchange capacity relative to the soil only. The EC therefor decreased as the pH increased over time for the compost treatments.
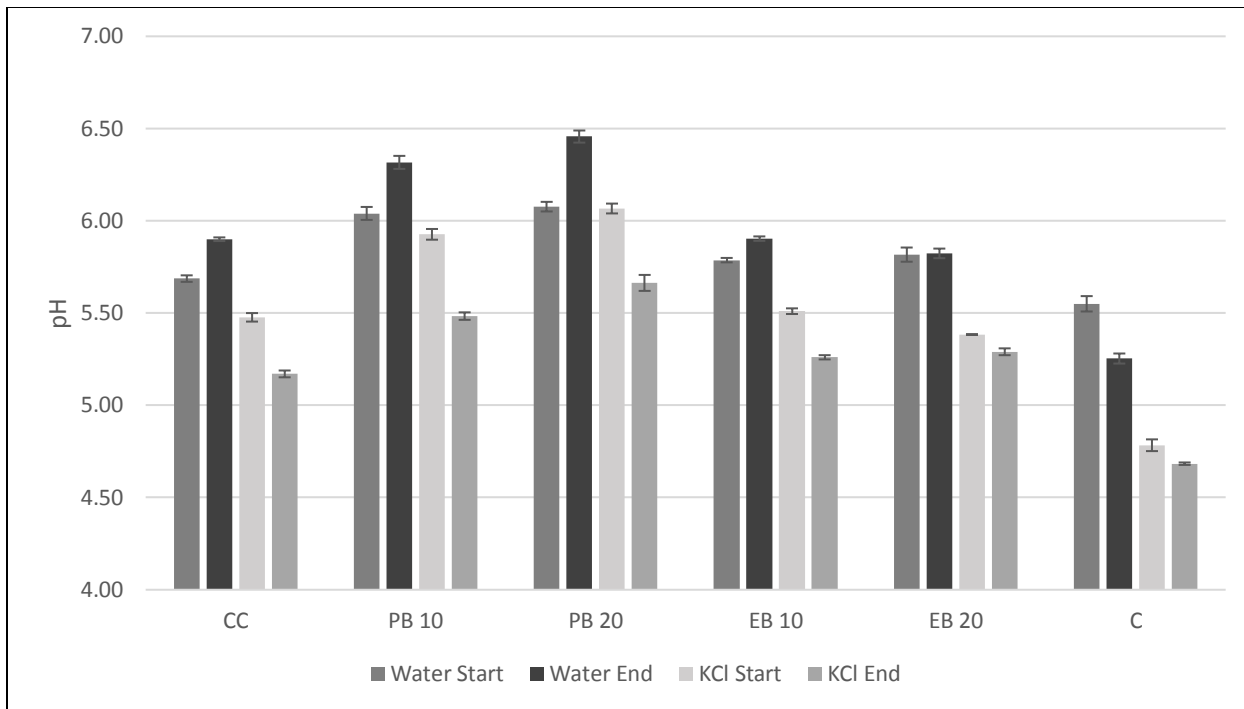
Figure 4.14 – Bar graph illustrating the differences in pH measured at the start and the end of the 6-month field trial in water and KCl
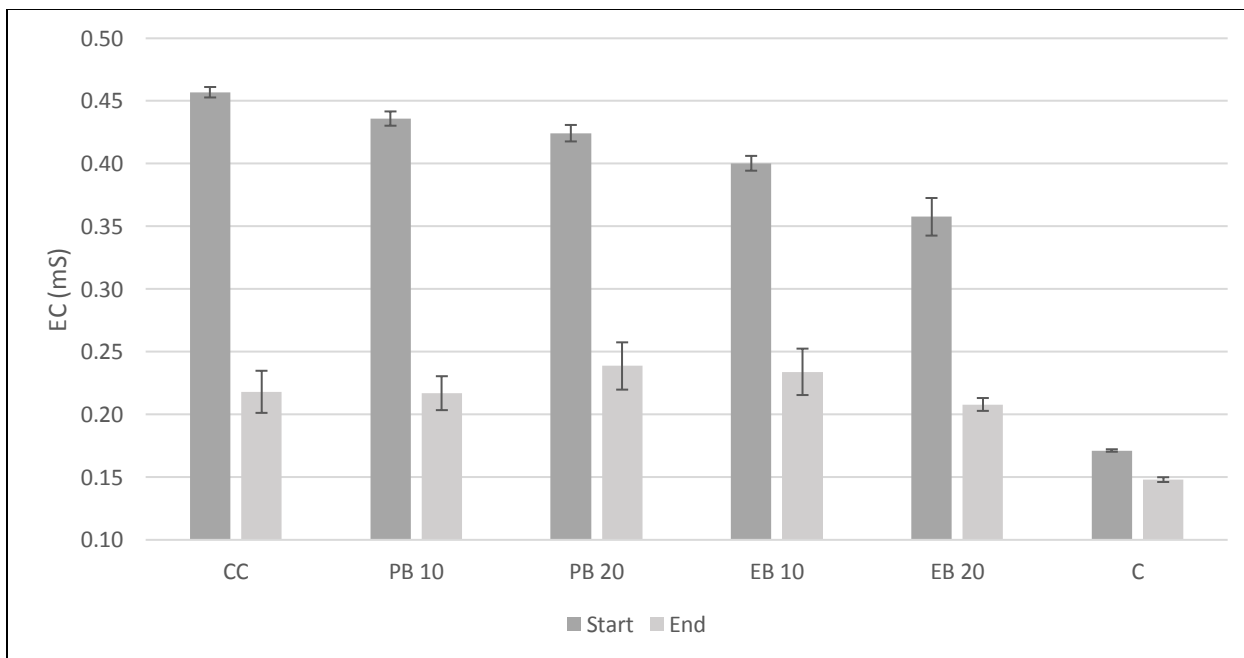
Figure 4.15 – Electrical conductivity (EC) measured in milli-Semens at the start and the end of the 6-month field trial.

4.3.2.2   Soil respiration

The CO_2 respiration normalized to total C in each treatment under 6-months of field conditions are illustrated in Figure 4.16. Repeated measurement ANOVA results showed that all compost treatments produced significantly higher amounts of CO_2 relative to the soil control from day 23 to 132. The only other significant difference over time, was found on day 112 at which point CC produced 0.0166 g CO_2/m^2, which was significantly higher than the other compost treatments and soil on its own. Similar trends in variation of CO_2 respiration can be seen for all treatments over the 6-month trial period. This is likely the result of microbial activity fluctuating due to environmental conditions such as temperature and rainfall. The effect of temperature on CO_2 respiration is illustrated by the peak of respiration found between mid-December and January which falls within the middle of the summer in the southern hemisphere.
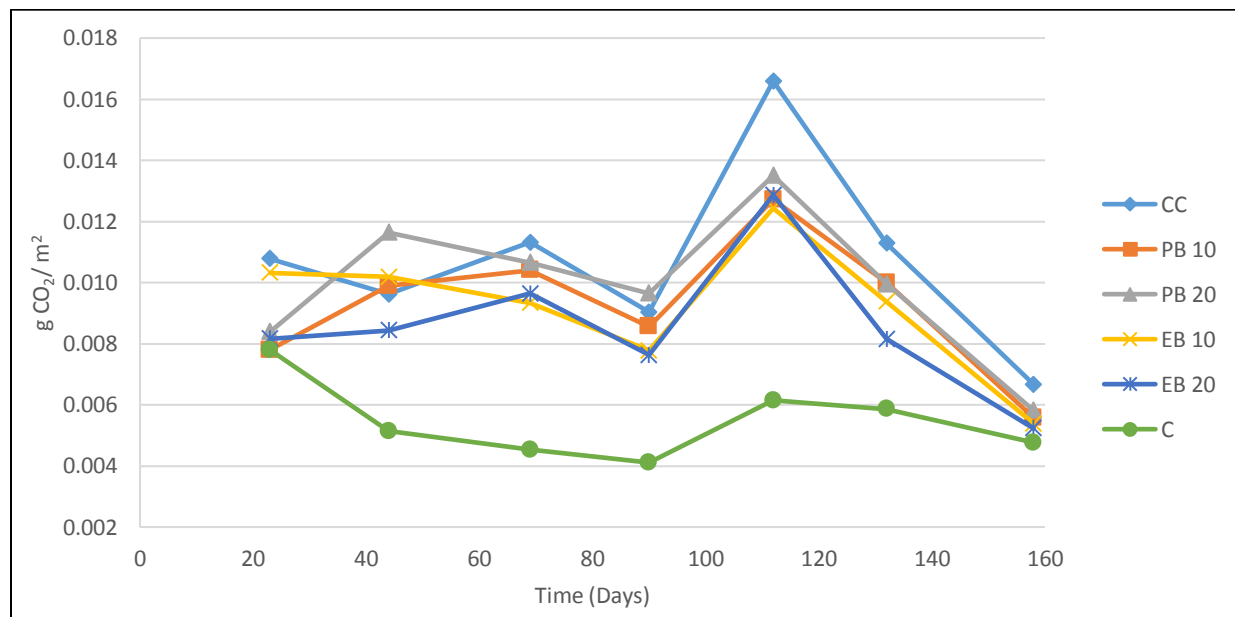
Figure 4.16 - Normalized CO_2 release (g CO_2/m^2) from compost and control treatments during the 160-day field study.

There were no statistically significant differences in total CO_2 respired between the compost treatments over the 6-month field study (Figure 4.17). The compost control (CC) released the greatest amount of carbon through respiration (0.075 g CO_2/m^2) while control soil (C) the least (0.048 g CO_2/m^2). The Tukey test between treatments revealed that the soil control (C) respired significantly less than all treatments except for EB20. However, EB20 did not produce significantly less CO_2 than other compost treatments. In general, it can be also seen that the composts that contain biochar (PB10, PB20, EB10, EB20) showed less CO_2 respiration than the compost only (CC) amendments.

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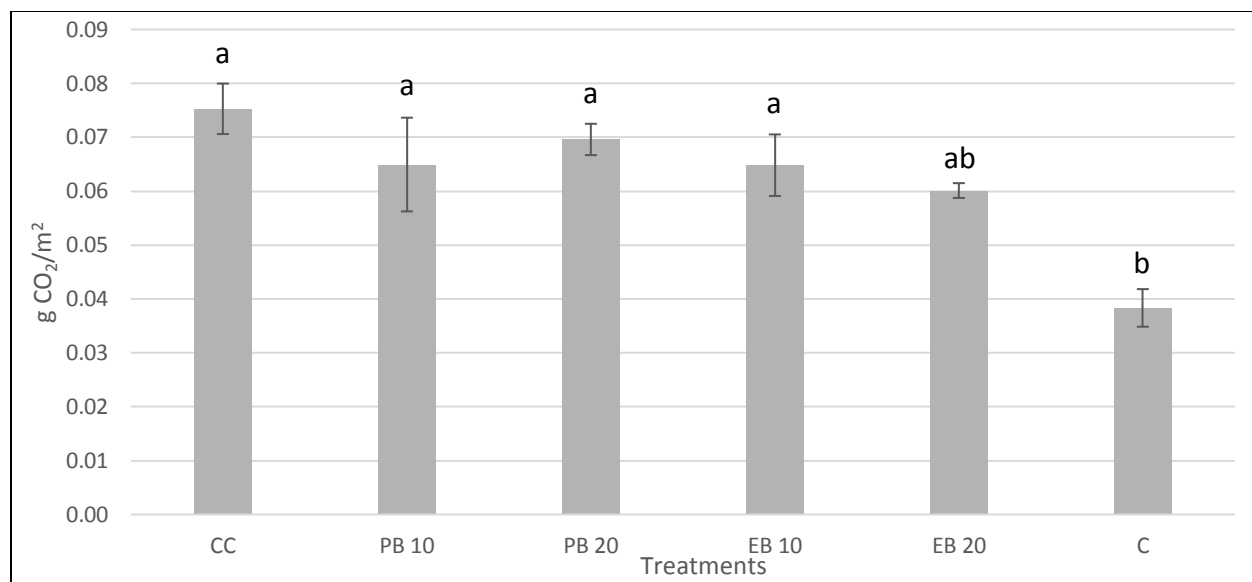
Figure 4.17 - Total CO_2 respired (g CO_2/m^2) from the compost and control treatments during the 160-day field trial. Standard error bars and letters of significance (p < 0.05) according to Tukey's HSD test are shown.

The importance of determining the amount of CO_2 released from soils and organic amendments lies within the stability of C and the impact thereof on climate change as well as its role in nutrient availability. Soil has been found to be a great source and sink for greenhouse gasses such as carbon dioxide, methane (CH_4) and nitrous oxide (N_2O) (Van Zwieten et al. 2009). The results from the soda lime traps could thus give us an indication as to the short-term (6 month) effect that composted biochar vs compost only has on C retention in soils.

From the results depicted above, differences in CO_2 can be discerned between applications containing biochar and also between the type of biochar applied. Once again the amount of labile or easily degradable carbon present in the various sources plays an important role in the breakdown and release of these C amendments. Since the majority of the biochar containing composts still consisted of labile components (Table 3.5), these mixtures were still able to release close to- or similar amounts of CO_2 than the control compost. The reduced CO_2 respiration measured for EB20 is arguably due to the lower quantity of volatile matter (33.37%, Table 3.5) and higher percentage of fixed carbon (23.35 %) for this mixture relative to the other composts. These properties are a result of the eucalyptus chars high pyrolysis temperature which makes it more resistant to decomposition (Novak et al. 2010; Siqua et al. 2016).

4.3.2.3 *Loss on ignition*

The total soil organic matter content results obtained from loss of ignition revealed that the compost treatments all contained very similar (non-significant) amounts of organic matter at the start (4.2% – 4.5%) as well as the end (3.8% – 4.02%) of the field trial (Table 4.5). The Tukey post hoc statistic showed that soil control (C) contained significantly lower quantities of organic matter than the other treatments, and that the difference in organic material from before and after the trial for all treatments were not significantly dissimilar. Despite the significance data, specific trends are visible. These trends are that compost mixtures with higher quantities of biochar (PB20, EB20) had higher total organic matter contents before and after the trial, and that pine biochar treatments showed the largest percentage loss of organic matter (PB10 – 13%, PB20 – 11%) versus the loss of EB10 (7.4 %) and EB20 (7.8%), and larger than the CC (10.6%)

Table 4.5 - Change in total soil organic matter contents (%) determined by LOI during 6-month field trial for the compost only (CC), pine biochar mixtures (PB10 and PB20), eucalyptus mixtures (EB10 and EB20) and the soil control (C) with letters of significance.

	Start	End	Diff	% of SOM lost
CC	4.244 [a]	3.793 [a]	0.451	10.6% [a]
PB 10	4.474 [a]	3.894 [a]	0.580	13.0% [a]
PB 20	4.514 [a]	4.019 [a]	0.495	11.0% [a]
EB 10	4.232 [a]	3.920 [a]	0.312	7.4% [a]
EB 20	4.318 [a]	3.980 [a]	0.339	7.8% [a]
C	3.353 [b]	3.119 [b]	0.234	7.0% [a]

The increased organic matter content seen in the 20% biochar relative to the 10% mixtures, can be correlated to the chemical properties of the biochar. Biochar is dense in carbon due to the fused aromatic C structures that form under increased temperatures (Brewer 2012). This means that an increase in biochar content will result in an increase of total carbon content. However, the same logic would mean that higher temperature biochars should have even more fused aromatic C rings (Lehmann et al. 2009), which would result in even higher organic matter contents in the soil. However non-significant, this was seen to not be the case as the pine biochar mixtures showed slightly higher initial organic matter contents than the eucalyptus composts (Table 4.5).

One of the limitations associated with the loss on ignition method, according to Koide et al. (2011), is the over estimation of soil organic matter in soil when a substantial amount of carbonate is present. This non-significant difference in initial organic matter content between the pine and eucalyptus could therefore be explained by a higher number of carbonates in PB10 and PB20 which cause an overestimation of total SOM. The higher percentage of SOM loss in pine treatments relative to CC, which showed more CO_2 respiration, along with the higher pH measurements of the pine could all be on account of these carbonates that perhaps may have leached (Schulz et al. 2013; Iqbal et al. 2015). The final observation is that the higher temperature eucalyptus biochar once again resulted in the least amount of carbon lost or transformed in relation to the PB10, PB20 and CC. This is a testament to the stability and recalcitrant nature of the higher temperature EB that has increased quantities of fixed C (Table 3.2) and is slow to decompose (Novak et al. 2010), which resulted in a very similar amount of total SOM loss than the control soil, even though the control soil has significantly less SOM. These results show that composts that contain EB amendments would be best for the purpose of building up C stocks in soil through C sequestration.

4.3.2.4 *Density fractionation*

The amounts of labile (particulate) and mineral-bound (stabilized) SOM fractions extracted using density fractionation from the soil field trial after 6 months can be used to assess the relative extent of humification that occurred in the different soil compost treatments.

The relative percentages of fPOM + oPOM and mineral bound in relation to total SOM measured in the soil are illustrated in Figure 4.18. With these results it can be seen that the majority of C (74.89% - 89.47%) is fixed in the mineral fraction of the soil, whereas only 10.54% (CC) to 25.11% (PB10) are found in free and occluded forms. Although Tukey HSD analysis showed that there are no significant differences in OM content between treatments of the particulate or stabilized form, it can be seen that the biochar containing compost treatments has between 14.57% (PB10) and 12.903% (PB20) more free and occluded OM than the control soil, and 7.15% - 5.48% more than CC. The exact opposite is found for the mineral fraction in which the largest % of mineral-bound SOM is seen in the soil control with the biochar treatments displaying the least
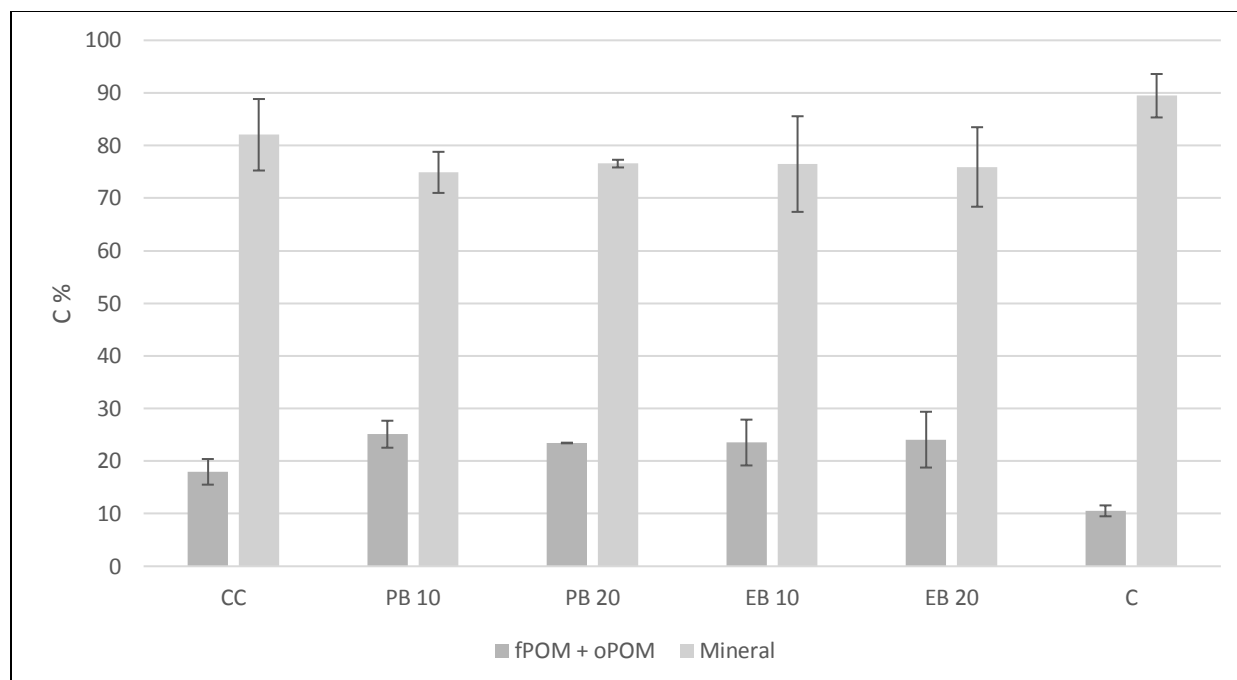
Figure 4.18 - Relative soil particulate (fPOM + oPOM) and stabilized (Mineral) C content (expressed as percentage of total soil C) in the compost and control treatments after 6-months in the field. Standard error bars are shown indicating no significant difference between treatments.

The results from the density fractionation further illustrates the relative degradability and stability of the two types of biochars by showing comparable results to that of CO_2 respiration. In the particulate fraction (fPOM and oPOM), the composted biochar treatments (PB10, PB20, EB10, EB20) showed the least amount of C degradation by having the highest non-humified C content at the end of the 6-month trial. This is similar to decreased C mineralisation witnessed in the field respiration study in which the soda lime traps captured the least amount of CO_2 (Figure 4.16) during this time period.

The general difference in mineral bound C between the biochar composts and the compost and soil control (CC and C) is once again related to the relative degradability of the C fractions. With the addition of biochar in the soil, less labile C is available for microbial activity (Lehmann and Rondon 2006) thereby resulting in less C mineralisation/transformation taking place. This means that the composting process did not significantly affect the biochars resistance to decomposition under field conditions and that composted biochar will still have good capabilities of suppressing C mineralisation and improving soil C stocks, especially with higher application rates. Field conditions did not show specific trends in terms of SOM fractions related to biochar type or quantity. This is arguably related to the environmental aspects such as temperature and water

content that did not create ideal circumstances for microbial activity, as well as the low amounts of biochar applied to the soil thereby reducing the opportunity for apparent differences to arise.

4.4 Conclusion

The aim of this chapter was to determine the effect of composted biochars on soil nutrient mineralisation in comparison to the application of compost or biochar only and a combination of biochar and compost. Results from the laboratory incubation study revealed that composted 10% biochar released 11.7% to 7.6% more CO_2 than compost mixed with 10% biochar. Although non-statistically significant, this indicates that composting of biochar increases the degradability of the biochar through surface oxidation and microbial breakdown. It was also observed that the lower pyrolysis temperature pine biochar exhibited higher rates of respiration (77.6% between PB and EB, 30.2% between PB10 and EB20, 26.9% between CPB and CEB) relative to that of eucalyptus. This is attributable to the lower level of fixed C found in the pine biochar. Nitrogen mineralisation results proved to be in accordance with what previous researchers found with the application of biochar that reduced N availability through N immobilization and apparent inhibition of nitrification, with no clear difference between type and quantity of biochar applied. The net immobilization witnessed in CC was construed to be a result of the low total N content and possible high content of polyphenolics (Palm and Sanchez 1991; Palm et al. 2001). The further adsorption and entrapment of NH_4^+ on biochar surfaces could have been one of the leading causes for net loss of available N over the short incubation period in all biochar containing treatments. Composting of biochar did not prove to affect the availability of nitrogen in relation to non-composted biochar, however inclusion of biochar during the composting process allowed for the absorption of ammonium onto the biochar surfaces, which was then releases immediately upon application, thereby resulting in an initial rush of available NH_4^+. A slight increase of available NH_4^+ and NO_3^+ towards the end of the two-month incubation could prove however that biochar might only start to mineralize N at a later stage and improve N availability on a longer time-scale. The phosphorus mineralisation results showed that both co-composted biochar and compost with biochar treatments increased P mineralisation significantly more than the application of biochar only. The availability of P was also found to be correlated to the pH of the soil and thus the liming capabilities of the two different types of biochar. In general however, composting showed to improve the availability of P in eucalyptus treated composts relative to pine with 14.6% and 24.8%, whereas biochar only applications had pine resulting in 26.3% more available P than eucalyptus. The composting process therefor allowed for more of the dominant form of P found in wood biochars, hydroxyapatite, to become soluble and plant available in eucalyptus treatments.

The incubation study as well as the 6-month field trial both illustrated that pine biochar has a much larger liming capability than the eucalyptus char. This is explained by certain types of biochar (EB) having amphoteric sites that can react both as an acid or a base depending on the solution pH (Amonette and Joseph 2009), as well as the possible high concentration of carbonates found in the pine char, thereby significantly increasing the soils pH. Carbon dioxide respiration, LOI, and density fractionation analysis all showed very similar results with regards to the degradability of the two types of composted biochar in relation to CC and the soil control (C). Respiration data proved that all composts that contain biochar in general, showed between 7.6% and 20.1% less CO_2 released into the atmosphere than CC. It also illustrated that the eucalyptus char treatments released the least amount of carbon dioxide. This result was further substantiated when LOI proved that EB10 and EB20 also showed the smallest % of total SOM loss (7.4% and 7.8%) relative to the other compost treatments. Density fractionation showed that all biochar containing compost treatments had 14.57% to 12.9% more free and occluded SOM than the control soil and 7.15% - 5.48% more than the control compost. This indicates that composting did not significantly inhibit biochars capabilities for C sequestration under field conditions by drastically reducing its degradability.

The laboratory incubation therefor displayed that composting of biochar alters its reactivity in the soil relative to fresh biochar and compost with biochar by, buffering the fresh biochars natural liming capabilities, increasing CO_2 respiration, improving initial ammonium release through ammonium uptake during composting, and improving P availability of eucalyptus. The field trial, showed that eucalyptus compost is the compost treatment that releases the least amount of CO_2 into the atmosphere, and is the least biodegradable in the soil.

The results from this chapter means that the application of composted biochar by a small-scale farmer could improve soil by reducing the risk for over-liming, increasing the C stocks, and improving phosphorus availability, with the possible loss of nitrogen mineralisation regardless of type of biochar applied. The price difference between the high-quality eucalyptus and robust pine biochars, along with the poor nitrogen mineralisation by both chars, slight differences in phosphorus availability, and non-significant differences in C sequestration, would mean that from a socio-economic aspect, composted pine biochar would be the best for a poor, small-scale sustainable farmer.

CHAPTER 5 – NEAR INFRARED REFLECTANCE SPECTROSCOPIC QUANTIFICATION OF BIOCHARS IN COMPOST AND SOIL

5.1 Introduction

Biochar has been found to be very stable and inert to degradation (Kuzayakov et al. 2014). This stability determines its longevity in the soil, capabilities to remain sequestered in contribution to mitigating climate change, and long term agricultural benefits (Lehmann et al. 2009). Properties such as heterogeneity, chemical complexity, and the inherent recalcitrant nature of the C compounds have posed several challenges to quantifying biochar (Manning and Lopez-Capel 2009). Quantification is however important for several reasons such as; carbon accounting (Mathews 2008), verification and distinguishing it from other forms of black carbon, and quantifying its longevity and impact on soil systems for different research fields (Manning and Lopez-Capel 2009).

Near infrared reflectance spectroscopy (NIRS) is a rapid and cost affective technique used for the quantification of many soil properties (Chang et al. 2001, Knadel et al. 2013). Some of the soil properties found to be spectrally relevant in NIR are soil moisture, particle size variation, minerals (mostly clay, iron oxide, primary minerals, salt, carbonates and phosphates) and organic material (Ben-Dor et al. 2008; Vohland and Emmerling 2011). In recent years, appreciable efforts have been given to the construction of spectral libraries (Brown et al. 2006; Sankey et al. 2008) and the development of portable NIR-based technology which can be used for in situ characterization of soil properties (Bricklemeyer and Brown 2010; Knadel et al. 2013). The strength of NIR-spectroscopy lies within the possibility of measuring several constituencies concurrently. Depending on the constituencies present in the soil, bending or stretching of individual bonds caused by the radiation will adsorb light at different degrees (Stenberg et al. 2010). Each vibration can then be corresponded to a specific series of overtone combinations in the NIR spectra. Several choices of absorptions of different intensities containing the same chemical information can therefore be provided, thus serving as a built-in dilution series (Shetty and Gislum 2010).

Various calibration methods based on NIR are used to predict soil properties such as organic material. The partial least-squares regression (PLSR) method is the standard method used due to its simplicity and robustness (Vasques et al. 2008, Nawar et al. 2016). PLS maximizes the correlation between measured spectral data and specific parameters to produce a probable collinear relationship that can be used for quantifying various soil properties simultaneously or individually (Rossel and Behrens et al. 2010, Nawar et al. 2016, Alamprese et al. 2016). The

robust and all-encompassing nature of NIR combined with PLS calibrations could therefore allow for a sufficiently accurate and cost-effective method to measure the quantity of biochar and its stability of degradation over time.

The goal of this study was to, (1) develop a NIR-based method for the quantification of biochar in soil and compost mixtures, and to (2) apply the soil methods to evaluate whether the prediction models can be used to estimate biochar content in soils. In order to achieve this goal, both pine and eucalyptus biochar was mixed with soil and compost in various amounts and subsequently analyzed by NIR spectroscopy. Hereafter, several methods of spectral pre-processing were tested before applying the method that would result in the best PLS regression for quantifying each biochar in both mediums. These methods were used to measure biochar content in the mature composts produced in Chapter 3 and soil biochar content at the start and end of the 6-month field trial conducted in Chapter 4.

5.2 Material and Methods

5.2.1 Calibration preparation

The study was performed on the same pine (PB) and eucalyptus biochars (EB) along with the mature control compost (CC) reported in chapter 3. The soil used for the NIR calibration was the the control soil (C) also used in the field trial, which was dried and sieved (<2 mm) before preparing the calibration concentrations. For each biochar, ten soil mixtures (PB S, EB S) were prepared (100 g each) by adding different percentages of biochar: 0.1 – 0.3 – 0.5 – 0.8 – 1 – 1.5 – 2 – 2.5 – 3 – 4 – 5 % (D/W). Ten compost mixtures of each biochar (PB C, EB C) was also produced but in a smaller quantity (10 g each) and different percentages: 1 – 3 – 5 – 10 – 15 – 20 – 25 – 30 – 35 – 40 – 50 % (D/W). A control containing 0 % biochar was included in each calibration range. After all mixtures were constructed, 5 g samples were weighed off and analysed with NIR spectroscopy.

5.2.2 Spectra acquisition

Calibration and analysis sample spectra were both collected using a Fourier transform NIR spectrophotometer (MPA, Bruker Optics, Germany) fitted with a rotating integrating sphere and measured at a wavelength range from 12,500 to 3,800 cm^{-1}. Soil based samples were scanned with a resolution of 8 cm^{-1} and accumulating 128 scans for the sample and the background (Alamprese et al. 2016), while compost mixtures were scanned at the same resolution with only 64 scans (Galvez-Sola et al. 2015). All of the soil and compost calibration samples were scanned in 5 replicates, whilst field trial soils used for analysis was only scanned in duplicate.
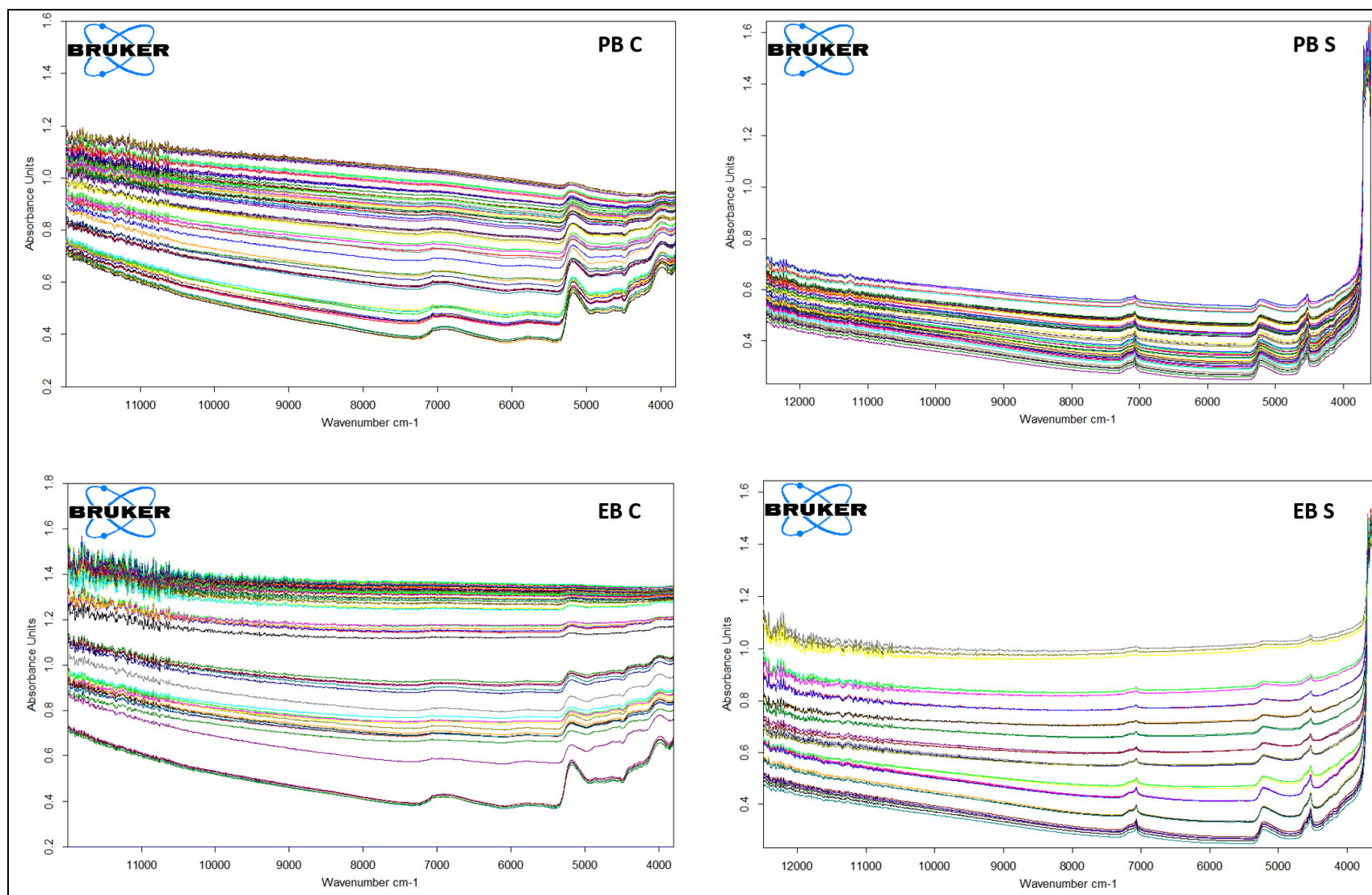
Figure 5.1 - Near infrared spectrum obtained for the different calibration sets of pine biochar (PB) and eucalyptus biochar (EB) in soil (S) and compost (C). Spectrum is displayed in wavelength (cm^{-1}) over absorbance.

Spectra processing and data processing was performed on OPUS software (v. 7.2, Bruker Optics, Germany).

5.2.3 Data processing

A key step for the successful analysis of NIR spectra is preprocessing. Preprocessing can be defined as the mathematical manipulation of NIR spectral data to enhance and/or remove spectral features prior to the development of a calibration model (Shetty and Gislum 2010). Spectral data from all biochar mixtures was separately subjected to pre-processing methods, including Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), first and second derivative spectra, constant offset elimination, and Min-Max normalization. The spectral pre-processing method which provided the lowest root mean square error of calibration (RMSEC) was selected and used for developing the PLS calibration model (Shetty et al. 2010, Alamprese et al. 2016; Magwaza et al. 2016). Outliers were identified and removed from the calibration ranges with the use of a residual variance exercise (Magwaza et al. 2016). Hereafter cross-validation (Westad et al. 2007) was used as a spectroscopic technique to provide a more accurate estimate of model performance for new samples (Wight et al 2015; Shetty and Gislum 2010). The leave-one-out cross validation is a simple method which excludes one sample from the calibration range and uses it to measure and rate the models performance. PLS regression is a multivariate regression method used to correlate spectroscopic data (X-variables) with chemical or physical data (y-variables). The model is based on PLSR components of which the decomposition of X during regression is guided by the variation in y, at which point the co-variation between X and y is maximized (Shetty and Gislum 2010). The PLS regression results are evaluated and described by values such as; RMSEC, root mean square error of cross-validation (RMSECV), regression coefficient for predicted versus actual biochar content (R^2), and the residual predictive deviation (RPD) (Wight et al. 2016; Magwaze et al. 2016). The models reported for each calibration set were selected for their higher R^2 and RPD, and lower RMSEC and RMSECV values. These models were then used to analyze biochar content in soil samples collected before and after the field trial.

5.3 Results and Discussion

5.3.1 Spectral interpretation

Figure 5.1 shows the absorbance spectrums measured for the four different calibration sets of which the absorbance varied from 400 to 2500 nm (12,500 to 3,800 cm^{-1}). The NIR region is generally constructed from highly overlapped combination bands of fundamental vibrations which makes it difficult to distinguish between them visually (ElMasry et al. 2013). This is especially apparent in biological material, such as the compost, which are characterized by complex hydrogen bonds and overtones for cellulose, lignin and humic acid (Ben-Dor et al. 1997). The spectral lines do however illustrate important absorption peaks related to the composition of the compost and the soil mixtures. Some of the pronounced peaks in the compost samples are found at 4000, 5200 and 7000 cm^{-1}, whilst less distinct, but still noteworthy peaks are seen between 4300 and 4400 cm^{-1} (Figure 5.1). These pronounced peaks have been characterized by previous researchers as OH stretching (7000 cm^{-1}) (Oinuma and Hayashi 1965), OH in water, cellulose, lignin, glucan and humic acid (5200 cm^{-1}) (Ben-Dor et al. 1997), and carbonates (4000 cm^{-1}) (Clark et al. 1990; Hunt 1977). The less distinct peaks in the composts (4300 – 44000 cm^{-1}) are all related to similar organic compounds such as carbohydrates (White 1971), aliphatic C-H stretching, methyls and aromatic ring stretch (Ben-Dor et al. 1997). The soils absorbance spectral clearly illustrates three distinct peaks at 4600, 5200 and 7100 cm^{-1}, which is a response to soil moisture (Chang and Laird 2002), kaolin O-H stretching (Oinuma and Hayashi 1965) and organic material (Ben-Dor et al. 1997). The effect of adding both pine and eucalyptus biochar to compost is seen by a general increase of absorbance, without specific peaks becoming more apparent.

5.3.2 Calibration and validation of PLS models

Table 5.1 shows a summary of the results from the PLS multivariate data analysis performed on the spectra shown in Figure 5.1. A total of six models were created from the four spectral ranges. Calibration results for both soil and compost mediums had high coefficient of determinations (R^2) ranging between 95.99 (PB + EB C) and 99.92 (EB C) as well as very strong predictive performance with RPD's from 20 to 21.4 in soil and 14.8 and 4.99 in composts. After the validation of each model, R^2 values along with RPD's showed a slight decrease whilst error values (RMSECV) increased, however all models still had high correlation coefficients between 95.07 and 99.59. The accuracy and precision of the PLRS models are measured by their respective RMSECV and RPD values. Results show that the prediction models for biochar in soil had lower RMSECV values than those in compost, with EB S (0.094) having the lowest error value and (PB + EB) C having the highest error (3.360). This means that biochar content is more accurately

measured in soil than in compost. The precision of the models is measured by the RPD values, which can be classified into three classes: category A includes models that accurately predict a given property (RPD > 2), category B has limited prediction capabilities (1.4 < RPD > 2) and category C has no predictive ability (RPD < 1.4) (Nawar et al. 2016). The models displayed values of 4.51 and higher, which according to Davey et al. (2009), would be considered as excellent models based on biological material. The RPD values of both EB S (15.7) and PB S (11) also show that biochar can be quantified with higher precision in soil, especially when separate models are used for each type of biochar. The stronger prediction capabilities of biochar in soil is best explained by the chemical complexity of organic matter. Organic matter makes it difficult for the compost containing model to relate specific absorption bands to particular biochar wavelengths (Ben-Dor and Banin 1995). Spectral predictive mechanisms could therefore be influenced by various heterogeneous aspects of compost such as the decomposition stage, nature of the organic compounds and the texture (Vohland and Emmerling 2011). These factors could distort the models capabilities of recognizing biochar components in the compost mixtures, thereby affecting the accuracy and precision of the models. However, results still indicate that all of the models developed has excellent predictive capabilities for pine and eucalyptus biochar.

Table 5.1 - The results from PLSR calibrations and validations for the different models created for estimating biochar content in soil (S) and compost (C).

Treatment	Calibration			Validation					
	R^2	RMSEC	RPD	R^2	RMSECV	Offset	Slope	Corr. C	RPD
PB S	99.78	0.069	21.4	99.18	0.124	0.029	0.982	0.9959	11.00
EB S	99.77	0.074	20.9	99.59	0.094	0.008	1.004	0.998	15.70
PB + EB S	99.75	0.076	20	98.30	0.187	0.033	0.966	0.9917	7.70
PB C	99.53	1.200	14.6	99.00	1.610	0.229	0.989	0.995	10.00
EB C	99.92	0.401	14.8	98.69	1.450	0.084	0.969	0.994	8.99
PB + EB C	95.99	3.090	4.99	95.07	3.360	0.891	0.951	0.9751	4.51

R^2 – regression coefficient
RMSEC – root mean square error of calibration
RMSECV – root mean square error of calibration validation
Corr. C – correlation coefficient
RPD – residual predictive deviation

5.3.3 Quantitative analysis of mature compost and field trial soil samples

The results from applying the three compost-based biochar quantification models are displayed in Table 5.2. From the results obtained, it appears that the PB + EB C model yielded better results than the EB C or PB C models, as biochar contents were much lower than the target 10 or 20 % added by mass to the composts prior to composting. It is more likely that the composts would become enriched in biochars due to the preferential decomposition of the green materials during composting period. The EB biochar appears to be more poorly detected than the PB biochars (Table 5.2). The improved prediction capability of the combined model could be a result of the heterogeneity of the compost mixtures influencing the spectral absorbance of the compost mixtures, which is compensated for by the combination of both biochars calibration specral data (Figure 5.1).

The results from applying the three soil-based biochar quantification models are displayed in Table 5.3. From the results obtained, it can be seen that the model created by combining the calibration ranges of both pine and eucalyptus biochars (PB + EB S) is not an adequate method for quantifying the change of biochar over time. This can be seen by the negative values measured for eucalyptus applications (EB10, EB20) and the large inconsistent differences between the start and the end of the field trial for both treatments. The fact that this model was not accurate at quantifying both biochars simultaneously can be attributed to the physiochemical differences of PB and EB which altered the level of absorbance measured in the calibration sets (Figure 5.1). Prediction models developed for each biochar separately (PBS and EBS) do however showcase values that are in accordance with the expected results (Table 5.3). The model EB S predicted a total of 0.1571% biochar in EB20 and 0.0889% in EB10 at the start and 0.1436% and 0.0631% at the end of the field trial. These results are very similar to predictions by PB S which estimated a total of 0.1043% (PB20) and 0.0909% (PB10) at the beginning and 0.081% and 0.0681% at the end of the trial. The promising thing about these results are the relationship seen between theoretical 20% and 10% biochar mixtures which aligns with the loss on ignition results (Table 4.5). In both instances, higher percentages of organic matter or biochar were found in the mixtures that originally contained more biochar.

Table 5.2 - Pine and eucalyptus biochar content estimated in mature composts with the (PB C, EB C, PB + EB C) PLSR prediction models.

	PB C		EB C		PB + EB C			
	PB 10	PB 20	EB10	EB20	PB 10	PB 20	EB10	EB20
Mature compost	3.854	9.018	2.109	3.631	5.486	14.765	9.485	10.917

Table 5.3 - Pine and eucalyptus biochar content estimated in field trial soils with three different (PB S, EB S, PB + EB S) PLSR prediction models, along with the calculated % biochar lost over the 6 months.

	PB S		EB S		PB + EB S			
	PB 10	PB 20	EB10	EB20	PB 10	PB 20	EB10	EB20
Start	0.0909	0.1043	0.0889	0.1571	0.1112	0.0942	-0.2609	-0.2776
End	0.0681	0.0810	0.0631	0.1436	0.0088	0.0689	-0.3967	-0.3207
Difference	0.0228	0.0233	0.0259	0.0135	0.1024	0.0253	0.1358	0.0431
% Biochar lost	25.0	22.3	29.1	8.6	92.1	26.9	-52.0	-15.5

The differences in biochar content measured for each treatment at the start and end of the field study provide some insight into the relative degradability and stability of composted pine and eucalyptus biochar in the soil (Table 5.3). Results show that PB10, PB20 and EB10 had very similar amounts (0.0228 % – 0.0259 %) of loss and that EB 20 (0.0135 %) showed the least amount of biochar loss. This translates to roughly a 8.6-29.1 % of biochar being degraded or leached over a period of 6 months. Many studies have looked at the decay of biochar over time but the determination and quantification thereof has been especially problematic due to the heterogeneity and complexity of the biochar itself (Lehmann and Joseph 2009). One such example is an incubation conducted by Brodowski (2005) who found that 48 percent of maize and rye biochar applied decomposed in the first six months, while only 3 percent were lost in the following 18 months. The results obtained through the NIR prediction model could therefore very well fall within the realistic range for biochar decay over a six-month trial. Bulk density of the soils were however not determined which means that the effect of soil consolidation on the change in concentration of biochar could not be quantified. The small biochar differences between PB10, PB20 and EB10 are also in line with the CO_2 respiration results reported in chapter 4. There it

can be seen that these treatments were not significantly different from one another and that the error bars were rather large. The low amounts of biochar loss reported for EB20 could be explained by the decreased respiration and decomposition of the higher temperature char (Bolan et al. 2012; Harvey et al. 2012) (also seen in Figure 4.16), but also by the detection limits of the model due to the calibration range selected. In the results from both models it can be seen that PB10, PB20, and EB10 reported values slightly higher or mostly lower than the lowest amount of biochar (0.1 %) used in the calibration construction. The biochar content within the soil could therefore fall on the bottom range of the calibration spectrum which could possibly cause a certain degree of detection error or variation as a result of the prediction lines offset (Table 5.1). If this is the case, then the difference estimated for EB20 could possibly be the most accurate measurement and also the reason as to why it is different from the other treatments. Biochars also undergo chemical composition changes in soil over time (Lehman and Joseph 2009), which could perhaps invalidate some of the assumption used in the correlations. These changes could also cause slight variations for measurements and predictions of biochar contents in soils.

To validate the prediction capabilities of the NIR method, calculations were performed to estimate the biochar content in the mature composts and at the start of the field trial using the proximate analysis results of the biochars (Table 3.1) and mature composts (Table 3.4). It was decided to use the fixed C content from the proximate analyses of the biochars as this is less likely to change during composting and serves as relatively stable marker of biochar. As the compost control also contained some (2.98%) fixed C (Table 3.4) an equation had to be set up to derive the fraction of fixed C coming from the biochar and from the compost control (Equation 5.1). Proximate analysis results presented us with the total amount of fixed C found in each compost treatments as well as the percentage of fixed C in PB and EB (Table 3.1 and 3.4). With this information, an unknown variable equation was constructed to determine exactly how much of the fixed C seen in each mature compost treatment comes from the char itself, in relation to that found in the control compost:

$$Compost\ treatment\ FC\ (\%) = x(CC\ FC\%) + y(BC\ FC\%) \qquad \text{(Equation 5.1)}$$

Where x is the unknown fraction of compost control (CC) and y is the unknown fraction of biochar (BC), and FC% being the percentage of fixed C determined through proximate analysis. An example of this calculation for determining biochar content of PB20 from information in Table 3.1 and 3.4 is:

$$12.416 = x(2.98) + y(50.606)$$
$$and: \quad (x + y) = 1$$
$$\therefore \ 12.416 = (1 - y)(2.98) + y(50.606)$$
$$\therefore \ y = 0.1981$$
$$\therefore BC \ \% \ in \ PB20 = 0.1981 \times 100$$
$$\therefore \ BC \ \% \ in \ PB20 = 19.81$$

The calculated biochar content (%) in the mature composts (Table 5.4), was then used to calculate the biochar content (%) in the field soil at the start of the trial after applying 204 g of compost to 10 kg of soil (Table 5.4). The proximate-calculated biochar contents in the mature composts was generally similar to the intended 10 and 20% biochar contents by mass; for example: PB20 proximate-calculated biochar contents was 19.8%, while EB10 was 11.2% and EB20 was 26.9% (Table 5.3). The PB10 had the lowest calculated biochar content of 3%, which could be due to the heterogeneous nature of the composts which resulted in variable proximate analyses or an error during the initial mixing of the ingredients of the composts. It was expected that especially the higher temperature eucalyptus biochar containing composts (EB10 and EB20) should become enriched in biochar as the green materials would be more likely to decompose, and this appears to be demonstrated by the proximate-calculated results (Table 5.4).

The NIR predicted biochar contents of the mature composts (based on PB + EB C prediction models) were generally much lower (9.5-60.4%) than the proximate-calculated results, except for PB10 which higher (81%) was (Table 5.4). This would indicate the NIR models were able to predict biochar contents in the composts within the same order of magnitude values, but tended to underestimate the biochar content. The organic matrix of the composts most likely contains very similar organic functional groups as the biochar and this makes it difficult to accurately distinguish the biochar from humified organic matter in the composts. It is also possible that biochar changed (oxidised) during composting process thus making it more difficult to detect.

Table 5.4 - Calculated biochar content in mature composts and starting field trial soils according to fixed C content (proximate analysis) compared with NIR predicted biochar contents (using PB + EB C, PB S and EB S PLSR models).

	PB 10	PB 20	EB 10	EB 20
Proximate-calculated biochar content (%) in mature compost	3.019	19.805	11.248	26.914
NIR predicted (PB + EB C model) biochar content (%) in mature composts	5.486	14.765	9.485	10.917
% of under- or overestimation relative to proximate-calculated contents	+81.7%	-25.4%	-9.5%	-60.4%
Proximate-calculated biochar content (%) in amended soil at start of field trial	0.062	0.404	0.229	0.549
NIR predicted biochar content in amended soil (%) at start of field trial (PB S and EB S models, resp.)	0.091	0.104	0.089	0.157
% of under- or overestimation relative to proximate-calculated contents	+47.8%	-75.3%	-61.1%	-71.4%

The NIR-predicted soil biochar contents were generally much lower (61-75%) than the proximate-calculated values, except for PB10 which was around 48% higher (Table 5.4). The proximate analysis calculated results are also not be interpreted as 100% accurate since compost mixtures are extremely heterogeneous which means that sampling differences and biological factors could play a role in the accuracy of both prediction methods, however ultimately, what it does show is that the NIR prediction falls within a range within the same order of magnitude that can be used as an indication of biochar content in composts and soils. This result is in accordance with the core purpose of NIR spectroscopy which is an analytical method used for rapid and cost affective

quantification of soil properties with reasonable reliability (Knadel et al. 2013). Biochar quantification with NIR could therefore be a good method to easily estimate biochar contents in soil especially for soil carbon stock assessments.

5.4 Conclusions

The aim of this study was to develop a rapid and cost affective technique for the quantification and determination of biochar stability in compost and soils. The NIR based prediction models were successfully constructed and showed that strong correlation (98.69 – 99.57) and prediction factors (8.99 – 15.7) can be obtained when separate calibration data sets are used for pine and eucalyptus biochar quantification. NIR predictive models are therefore pyrolysis temperature and/or feedstock specific which means that different models need to be constructed for different types of biochar. Validation of the PLSR models also revealed that biochar is more accurately determined in soil mixtures as opposed to compost mixtures, which is due to the heterogeneous nature and similar colour of the organic material distorting the model's capabilities of recognizing biochar. According to RPD values however, all methods constructed were found to be excellent for the prediction of biochar content. The models produced for biochar quantification in compost and soil were then applied to mature composts and field trial soils with unknown quantities of biochar.

Use of the predictive models illustrated that the combined compost model (PB +EB C) is the most accurate for quantifying pine and eucalyptus char in compost, whilst separate models for each biochar was more accurate in soils. The difference in the calibrations spectral ranges for each biochar showed to improve prediction capabilities in a heterogeneous medium such as compost, while the same difference was possibly the cause for the poor prediction of combined models in the homogenous soils. By comparing the prediction results with other organic matter and C analyses previously performed on the same soils, it was deduced that NIRS can be used to predict biochar content in compost and soil, and estimate changes of biochar content in soil over time. These estimations are slightly robust by possibly having some degree of error if actual biochar content are outside of the calibration range, or the biochar underwent some form of physiochemical change in the soil over time. However, results are still relatively accurate by being in the same order of magnitude as shown by the calculated estimation using the proximate analysis results and the compost application rate.

Further research is required to investigate the effect of chemical changes of biochar on NIR accuracy as well as comparative analytical studies focused on quantifying biochar in laboratory and field conditions. NIR spectroscopy prediction models could also be improved by combining

other variables to the PLSR method such as total carbon content or C/N ratios. This could expand the models capabilities of distinguishing between biochar and other organic compounds in different substrates. Lastly, models could be constructed using biochar that has been exposed to field conditions or chemical changes to reduce factors that could interfere with the recognition of biochar in compost or soil. In order for analytical methods to gain wide acceptance, they need to achieve some form of compromise between price, reliability and accuracy (Manning and Lopez-Capel 2009). With further research, biochar quantification by means of NIR and PLSR models, could be used as a cost and time effective method.

GENERAL CONCLUSION AND FUTURE RESEARCH

The Centre for Excellence in Food Security is striving to find means to improve food systems that can overcome ecological, social, economic, and physical challenges in poor, vulnerable and marginal populations. This led to investigating the use of biochar for its adsorption, C sequestration and agricultural qualities, as poor small-scale farmers could possibly use biochar to construct biochar based filters to treat agricultural effluent, where after the spent biochar could be sterilized through composting before being applied as soil conditioner. This however posed challenges as to the effect that biochar might have on the composting process, and how the process would alter nutrient availability and C sequestrating capabilities of the biochar itself. Previous research on biochar have shown that the pyrolysis temperature and feedstock properties can affect the chemical and physical character of the final product, which in turn could determine its react ability in soils and composts. There was found that higher temperature biochar tends to have increases in condensed aromatic C content, which results in an increased ash-to-carbon ratio, greater surface area, decreased CEC and loss of volatile matter. These physiochemical properties can play a cardinal role in effecting the biochars capacity and affinity to sorb organic pollutants, microbial activity in composts and soils, and nutrient availability. The general goal of this project was therefor to evaluate how the addition of two contrasting biochars could affect the (i) composting process and the final compost products in terms of (ii) nutrient mineralisation and long-term C sequestration capabilities due to possible alterations of the biochars chemical degradability and chemical structure, and (iii) quantifying biochar content in compost in soil with NIR-based method.

The first objective was to investigate how the addition of two contrasting biochars (10% and 20% d/w) to fresh composting materials would affect the composting process and properties. Biochar characterization proved that the robust, low temperature pine char (50.61%) only had 2/3 the fixed C of the eucalyptus (75.66%), but double the nutrient content, and less than a tenth of the surface area (59.9 m^2. g^{-1} vs 623.9 m^2. g^{-1}). The composting process for all compost treatments was deemed to be a success as all piles could be classified as mature, stabile and usable at the end of the 94-day composting period. The differences of the biochars resulted in eucalyptus based compost piles having lower temperatures in the thermophilic (3.0 – 7.5°C lower) and mesophilic stages (1.4 – 3.0°C lower) than the control, higher relative C/N ratio's than the control and equivalent pine mixtures, lower pH values, and decreased microbial activity. The temperatures were of highest concern as temperature plays a cardinal role in compost sterilization. Quantity of biochar also seemed to have affected composting dynamics as increased quantities of biochar

resulted in greater quantities of fixed C and higher C/N ratio's. Dehydrogenase activity also decreased with an increase of biochar content on account of less labile C available. Twenty percent biochar compost mixtures also had lower temperatures in the mesophilic and maturation phases along with higher final pH values.

The second objective was to determine the effect of the *composted biochars* on soil nutrient mineralisation in relation to *compost and biochar* and *biochar only* applications. The incubation study to assess mineralisation under optimal conditions revealed that composted biochar increased respiration with 7.6 – 11.7% in relation to compost with biochar. Indicating that the composting process increased the degradability of the biochar through surface oxidation and microbial activity. Composted pine biochar treatments were also found to respire more than composted eucalyptus biochar as PB10 and PB20 produced 20.8 - 52.8% less CO_2 than CC, whilst both EB10 and EB20 respired significantly less (44.7 - 52.7%) than CC. Composting improved the biochars' respective soil reactions in terms of pH, by allowing chemical surface changes during composting to act as a buffer upon application in the soil. Type and quantity of biochar along with composting proved not to have an influence on the net N mineralisation, as all biochar containing treatments showed N immobilization and apparent inhibition of nitrification throughout the 60-day incubation. The compost control also exhibited N immobilization as a result of the low total N content and possible high content of polyphenolics. Phosphorus availability did prove to be related to biochar type and composting, as composted eucalyptus treatments released 14.6 – 24.8% more P than composted pine treatments, whilst fresh pine biochar had 26.3% more available P than fresh eucalyptus. The composting process therefore allowed for more of the dominant form of P found in wood biochars, hydroxyapaptite, to become soluble and plant available in eucalyptus treatments.

Carbon dioxide mineralisation was further assessed in a field trial of 6-months and similar to what was found in the laboratory incubation study, pine biochar compost had a larger liming capability than eucalyptus biochar compost. Furthermore, under field conditions, the eucalyptus biochar compost also released the least amount of CO_2 into the atmosphere, although not statistically significant. This was further substantiated through loss on ignition (LOI) results whereby eucalyptus biochar composts lost the least amount of SOM (7.4 and 7.8%) in relation to the pine biochar composts (11.0 and 13.0 %) or the compost control (10.6%). Density fractionation results further showed that biochar containing composts had the largest fPOM and oPOM fractions (14.6 to 12.9%) and smallest mineral bound fractions (7.2% to 5.5%) in the soil. The LOI and density fractionation results were not statistically significant. Results did however indicate that all biochar

containing treatments improved C sequestration in relation to the control compost, and that eucalyptus biochar served as a better medium to improve C stocks in the soil.

The final objective was to develop a NIR method to cheaply and rapidly quantify biochar content in composts and soils amended with biochar. PLSR models using NIR were successfully constructed to show strong correlations (R^2 = 98.69 to 99.57) and prediction factors (RPD = 8.99 to 15.7) for quantifying biochar in soils and composts. These models were then used to predict the quantity of biochar in mature compost and field trial soils that contain unknown quantities of biochar. To determine biochar content in composts, it was found that a combined model created from both pine and eucalyptus biochars calibration spectra was the most suitable and could correctly quantify biochar within the same order of magnitude. Biochar quantification in soil was also found to be successful and relatively accurate when separate models, constructed from pine and eucalyptus calibration individually, was used. These results also showed to have some degree of error within the same order of magnitude, however, in accordance with the purpose of NIR, this analytical method is suitable quantify biochar in both composts in soil. The revolutionary result could provide environmental companies with a great platform to conduct soil carbon stock assessments in a cost-effective and reliable fashion.

In general, it can be concluded that the objectives of this research project was successfully evaluated. The results show that biochar can be incorporated into compost and that a lower temperature, robust char, at a general lower (10% d/w) application rate is more suitable for the composting process by providing microbes with more easily degradable C and nutrients. This translates to higher composting temperatures, lower C/N ratios and increased microbial activity. The incorporation of the produced composts along with other biochar treatments proved that composting increased the two types of biochars degradability, and also their respective soil reactivity by buffering the biochars natural liming capabilities. In terms of mineralisation, it was found that eucalyptus char with a low labile C content respires the least when applied to soils and that both types of biochar, regardless of quantity or composting, results in reduced N availability through immobilization and that composting did however improve eucalyptus biochars P release more than that of pine biochar. Finally, the field trial proved that the higher quality expensive biochar is the best candidate for C sequestration as the eucalyptus composts produced the least CO_2 and exhibited the smallest % of SOM between all the compost treatments.

Future research can include an up scaled composting experiment with larger compost pile volumes and the use of spent biochar filters to assess whether pathogens are eliminated during composting. It would also be advisable to undergo mineralisation studies over a longer time period

in both laboratory and field conditions die see whether composted biochar might promote N and P mineralisation in the long run. This could also include a pot or crop trial to evaluate nutrient availability in relation effects on yield. Finally, further research needs to be conducted on the use of NIR to improve the accuracy and predictability of the method with more calibration sets in relation to other biochar linked indices such as black C content.

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APPENDIX

Table A.1 – C/N ratio evolution for compost piles over time (days). Final nitrogen % of compost piles also displayed.

	1	14	31	62	94	Final N (%)
CC	23.0412	14.9198	11.8458	10.3891	10.2629	1.8880
PB 10	20.6710	17.4007	13.4588	13.3215	13.4448	1.9835
PB 20	23.3016	19.4938	18.5294	15.3949	17.6998	1.7348
EB 10	23.7349	17.5551	15.3921	13.8268	14.0907	1.8617
EB 20	26.6332	24.2935	18.7890	19.7858	18.0582	1.6528

Table A.2 – Measured pH values in water for composts at different days during the composting process.

	1	14	31	62	94
CC	7.54	8.87	8.31	7.79	7.55
PB 10	8.28	8.78	8.67	8.19	7.83
PB 20	7.90	8.83	8.49	7.98	8.05
EB 10	8.17	8.85	8.51	7.95	7.75
EB 20	8.23	8.80	8.59	8.08	7.87

Table A.3 – Electrical conductivity (mS/cm) at different days during the composting process for each treatment.

	1	14	31	62	94
CC	4.865	4.130	2.690	3.975	4.505
PB 10	4.245	3.625	3.445	3.530	3.485
PB 20	4.555	3.850	3.070	3.785	3.860
EB 10	4.760	4.180	2.425	4.525	4.380
EB 20	4.450	3.890	2.920	3.970	4.020

Table A.4 – Dehydrogenase activity (µg TPF/kg) measured at different days during the composting process. Total amount of dehydrogenase produced is also included.

	14	31	62	94	Total
CC	151.1940	84.4563	64.0299	59.6375	359.3177
PB 10	158.6567	118.1450	69.6588	56.0128	402.4733
PB 20	123.6887	95.7143	86.4179	72.4307	378.2516
EB 10	110.9382	72.4733	69.2324	68.1663	320.8102
EB 20	107.2281	76.3966	54.6055	44.8827	283.1130

$y = 245.91x^{-0.264}$ $y = 363.56x^{-0.459}$ $y = 743.81x^{-0.566}$ $y = 514.41x^{-0.493}$ $y = 195.88x^{-0.248}$
$R^2 = 0.9741$ $R^2 = 0.9991$ $R^2 = 0.9786$ $R^2 = 0.9469$ $R^2 = 0.7955$
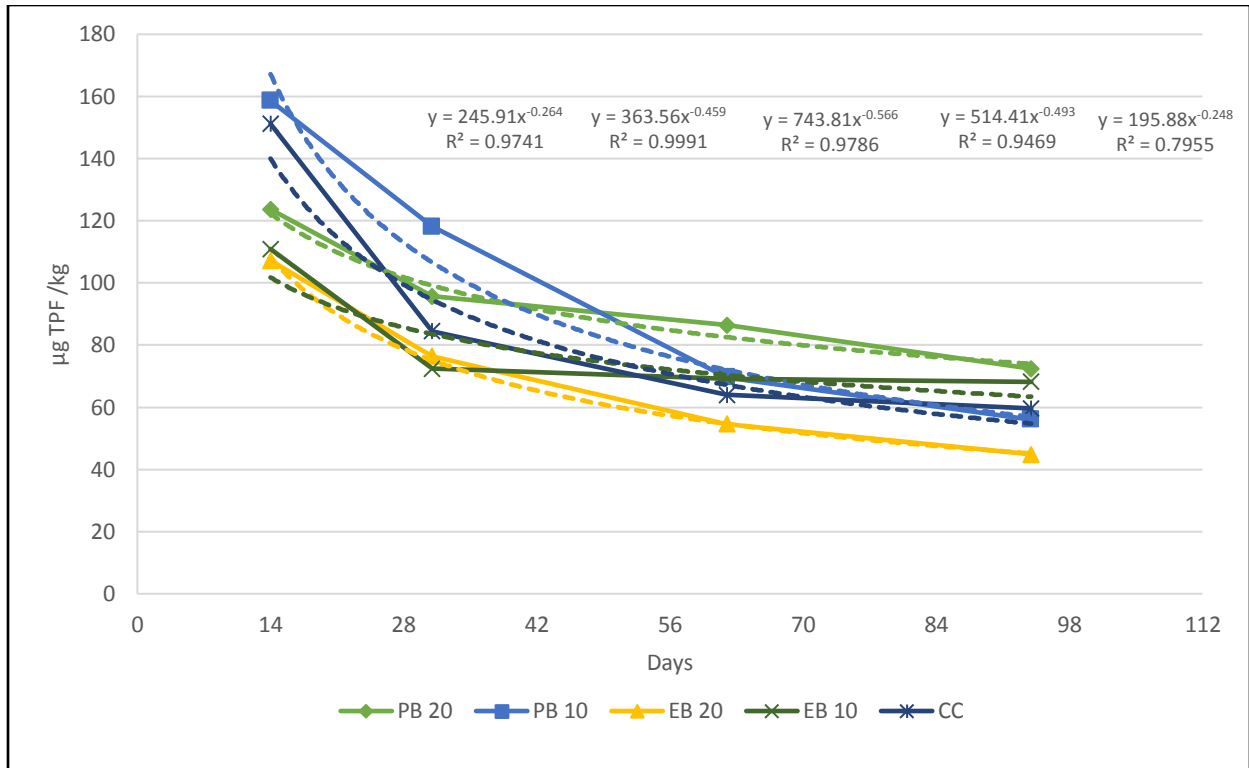
Figure A. 1 – Dehydrogenase activity (µg TPF/kg) over time in days with power trend lines and their R^2 values and equations.
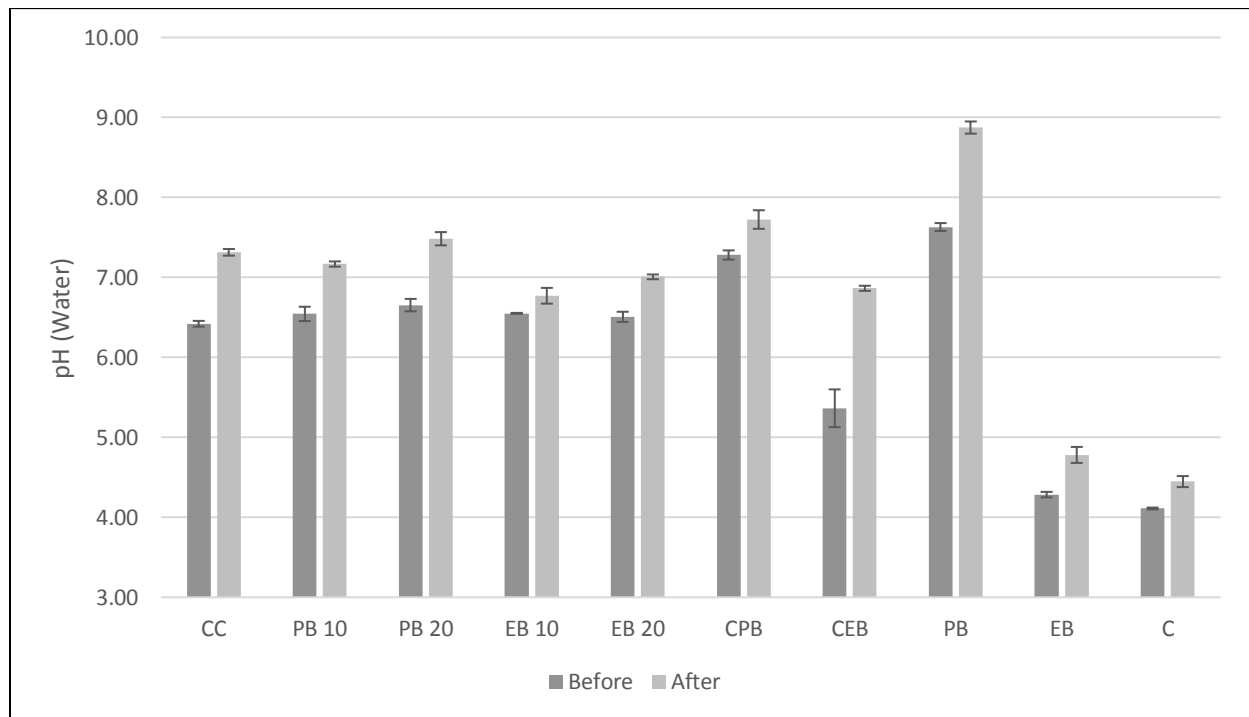
Figure A. 2 – Graphic illustration of the change in pH (water) for the different treatments from the start to the end of the 2-month incubation with error bars included.

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Table A.5 – CO₂ respiration measured (µg CO2-C/g carbon per hour) over time in days along with the total amount of CO₂ produced for each treatment in the incubation study.

|	1	2	3	5	7	10	14	21	28	42	60	Total
CC	477.1	232.0	147.9	169.2	113.8	72.0	70.2	94.2	96.6	97.3	101.3	1671.7
PB 10	342.3	186.8	108.0	119.0	100.5	72.3	67.9	107.1	101.6	72.8	45.5	1323.8
PB 20	280.1	138.9	54.7	26.8	65.4	30.0	36.2	55.5	45.3	30.6	25.2	788.8
EB 10	178.9	149.3	94.8	95.6	75.5	53.3	47.6	61.9	56.8	60.7	49.8	924.2
EB 20	148.3	131.8	73.5	98.8	66.7	40.6	39.9	57.8	66.4	41.6	25.5	790.8
CPB	263.6	149.4	128.6	92.4	95.6	63.0	46.9	74.1	88.5	86.2	80.8	1169.1
CEB	236.8	152.0	63.9	71.7	73.4	34.1	34.3	40.8	39.3	39.4	67.9	853.6
PB	46.6	47.7	30.8	25.9	36.2	17.8	14.5	8.3	9.3	3.2	1.6	241.8
EB	50.8	8.7	10.0	2.0	5.0	6.1	1.7	0.4	0.4	0.0	0.0	54.0
C	0.5	0.7	0.5	0.4	0.6	0.6	0.6	0.2	0.1	0.0	0.0	4.2
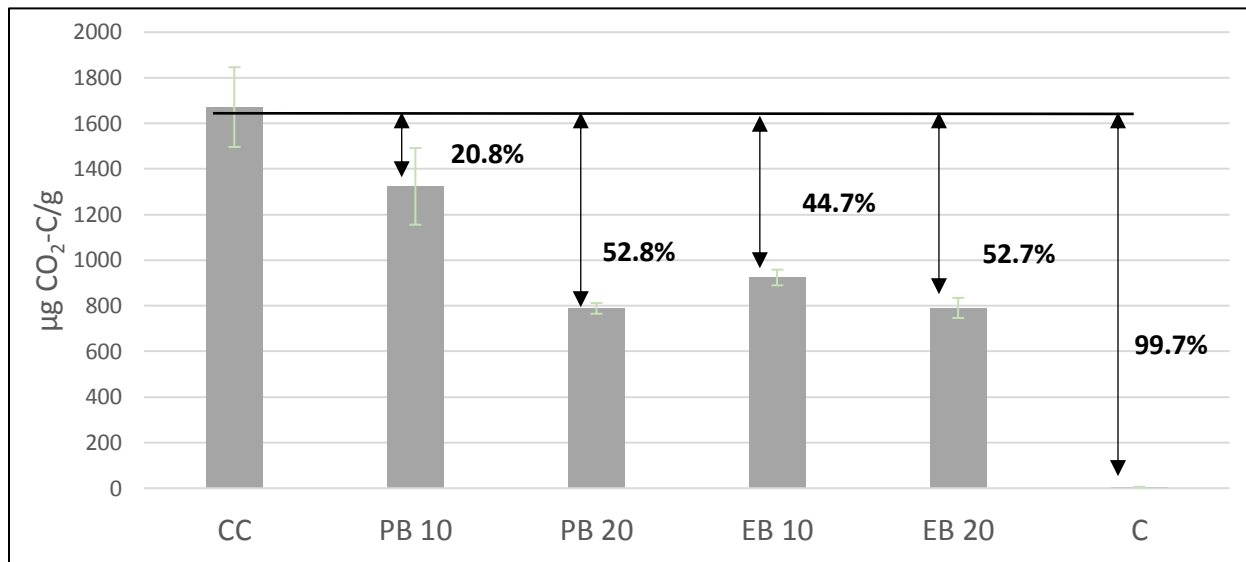
Figure A.4 - Percentage differences of CO_2 respiration for biochar and control treatments relative to the compost control.
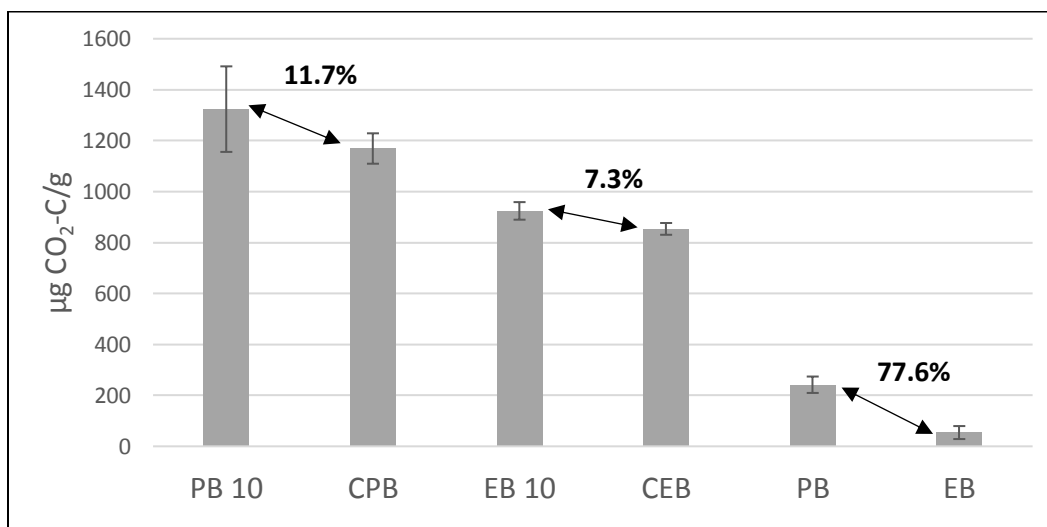
Figure A.5- Percentage differences of CO_2 respiration between composted biochar and biochar with compost

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Table A.7 – Ammonium extracted (mg/kg) from treatments soils at regular intervals over the 2-month incubation study, along with the total amount available.

	1	3	7	14	28	42	60	Total
CC	0.0800	0.1393	0.2045	0.0796	0.0763	0.0549	0.1057	0.7403
PB 10	0.1131	0.1099	0.1346	0.0609	0.0367	0.1001	0.0633	0.6186
PB 20	0.1388	0.1197	0.1197	0.0619	0.0595	0.0488	0.0600	0.6083
EB 10	0.1010	0.1052	0.1476	0.0717	0.0414	0.0642	0.0875	0.6186
EB 20	0.2665	0.1229	0.1710	0.0805	0.0814	0.0507	0.0665	0.8396
CPB	0.0712	0.1229	0.1215	0.0665	0.0539	0.1062	0.0749	0.6172
CEB	0.0847	0.1397	0.1444	0.0894	0.0847	0.0889	0.0945	0.7263
PB	0.0553	0.0777	0.0833	0.1113	0.0931	0.0451	0.0875	0.5533
EB	0.0516	0.0982	0.1220	0.1122	0.0717	0.0479	0.0698	0.5734
C	0.0731	0.0768	0.0898	0.1113	0.1099	0.0665	0.0945	0.6219

Table A.8 - Nitrate extracted (mg/kg) from treatment soils at regular intervals over the 2-month incubation study, along with the total amount available.

	1	3	7	14	28	42	60	Total
CC	7.7813	4.9158	4.6234	1.7228	4.2491	0.0000	0.3532	23.6456
PB 10	4.6117	5.8047	2.1205	0.0000	0.0000	0.0000	0.0000	12.5368
PB 20	5.8749	5.2199	4.2608	0.7392	0.3064	0.0129	0.1298	16.5439
EB 10	5.2901	3.6760	2.8924	0.1649	1.0327	0.0000	0.0000	13.0561
EB 20	6.4363	2.5766	2.8222	0.4819	0.2713	0.0000	0.0713	12.6596
CPB	6.6936	3.4187	3.6409	0.6573	0.0000	0.0000	0.3170	14.7275
CEB	7.5123	4.0269	4.3193	1.2421	0.0480	0.0000	0.0000	17.1485
PB	5.1146	3.7930	5.0912	1.1029	0.3895	0.0000	3.0444	18.5357
EB	2.4480	2.0854	3.8047	0.5520	0.0713	0.0000	0.7029	9.6643
C	5.7930	4.0269	6.2725	5.4070	2.7871	1.4187	1.6409	27.3462

Table A.9 – Available phosphorus as extracted (mg/kg) from treatment soils at regular intervals over the 2-month incubation study, along with the total amount available.

|	1	3	7	14	28	42	60	Total
CC	87.6764	84.3917	74.1727	73.3212	79.5255	80.9854	117.7251	597.7981
PB 10	57.0195	66.2652	47.5304	65.5353	61.6423	63.8321	81.8370	443.6618
PB 20	63.5888	58.4793	51.7883	55.6813	61.7640	67.4818	65.6569	424.4404
EB 10	82.5669	80.6204	72.4696	68.3333	85.1217	94.9757	106.1679	590.2555
EB 20	63.5888	77.9440	61.5207	57.2628	70.5231	76.3625	89.8662	497.0681
CPB	86.9465	74.7810	75.9976	69.7932	82.8102	90.3528	85.1217	565.8029
EPB	82.4453	74.5377	62.3723	78.3090	84.7567	88.7713	98.9903	570.1825
PB	48.3820	44.3674	42.9075	43.1509	44.4891	58.7226	73.0779	355.0973
EB	39.9878	28.6740	26.7275	27.5791	35.3650	43.7591	59.3309	261.4234
C	46.3139	36.8248	39.6229	39.0146	45.0973	57.2628	70.2798	334.4161

Table A.10 – Measured pH and EC for the field trial treatments at the start and the end of the 6-month trial period.

	pH (Water)			pH (KCl)			EC (mS/cm-1)		
	Start	End	ΔpH	Start	End	ΔpH	Start	End	ΔEC
CC	5.69	5.90	0.21	5.17	5.48	0.31	0.457	0.218	0.239
PB 10	6.04	6.32	0.28	5.48	5.93	0.44	0.436	0.217	0.219
PB 20	6.08	6.46	0.38	5.66	6.07	0.40	0.424	0.239	0.186
EB 10	5.79	5.90	0.12	5.26	5.51	0.25	0.400	0.234	0.166
EB 20	5.82	5.82	0.01	5.29	5.38	0.09	0.358	0.208	0.150
C	5.55	5.25	-0.30	4.68	4.78	0.10	0.171	0.148	0.023

Table A.6 – CO_2 captured (g CO_2 m^{-2}. day^{-1}) by the Soda lime traps in the field trial for each treatment.

	23	44	69	90	112	132	158	Total
CC	0.0108	0.0096	0.0113	0.0090	0.0166	0.0113	0.0067	0.0753
PB 10	0.0078	0.0099	0.0104	0.0086	0.0127	0.0100	0.0056	0.0649
PB 20	0.0084	0.0116	0.0107	0.0097	0.0135	0.0100	0.0058	0.0696
EB 10	0.0103	0.0102	0.0093	0.0078	0.0124	0.0094	0.0054	0.0648
EB20	0.0082	0.0084	0.0096	0.0076	0.0129	0.0082	0.0052	0.0601
C	0.0078	0.0051	0.0045	0.0041	0.0061	0.0059	0.0048	0.0383
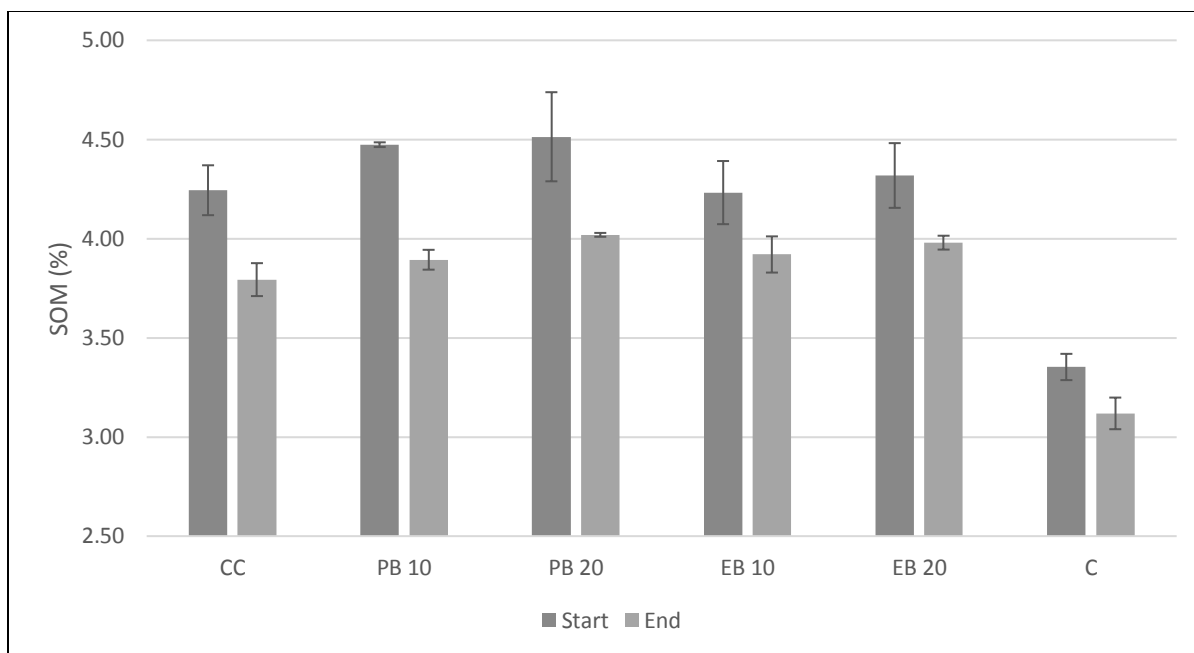
Figure A.6 – Graphic illustration of the total (%) soil organic matter (SOM) for each treatment at the start and the end of the 6-month field trial with error bars included.

Table A.7 – Density fractionation results at the end of the field trial to illustrate the free and occluded (particulate) organic material (POM) and mineral bound organic material in g/kg as well as % of total SOM

	POM (g/kg)	MBOM (g/kg)	Total SOM	POM (%)	MBOM (%)
CC	2.4095	11.0094	13.4188	18.5368	81.4632
PB 10	3.7727	11.2534	15.0261	25.2779	74.7221
PB 20	2.6302	8.5917	11.2219	23.4312	76.5688
EB 10	3.6906	11.9968	15.6874	24.8756	75.1244
EB 20	3.1433	9.9120	13.0553	24.2347	75.7653
C	1.5517	13.1769	14.7286	10.6350	89.3650