Evaluating effects of pyrogenic organic matter and biochar amendments on soil structure, soil chemical properties and soil fauna dynamics in tropical agroecosystems

Mbau Solomon Kamau

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University of Nairobi

College of Agriculture and Veterinary Sciences

Faculty of agriculture

Department of Land Resource Management and Agricultural Technology

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DECLARATION

This thesis is submitted with the approval of my supervisors.

Signature…… Date……..

Mbau Solomon Kamau

(Candidate)

Supervisors

Signature…… Date……..

Prof. Nancy K. Karanja.

(University of Nairobi)

Signature…… Date……..

Dr. Fredrick O. Ayuke

(University of Nairobi)

Signature…… Date……..

Dr. Edmundo Barrios

(World Agroforestry Centre – ICRAF)

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GENERAL ABSTRACT

During vegetation clearance to cultivated lands, charcoal making eliminates canopy effects on soil associated with trees while at the same time creating new conditions in and around such charcoal-making spots due to increased concentration of pyrogenic organic matter (PyOM). It is unclear how and/or whether such (un)intentional management practices affect the abundance, diversity and distribution patterns of soil macrofauna. However, biochar has been proposed as an amendment for improving soil fertility as well as a means of sequestering C into the soil. Nonetheless, biochar effects on soil macrofauna has received little attention despite the profound role they play in soil ecosystems. Therefore, the objectives of this work were to: i) assess effects of *Croton megalocarpus* and *Zanthoxylum gilletii* trees on the abundance, spatial distribution of soil macrofauna, and soil aggregation, ii) assess influence of converting these trees to PyOM on spatial distribution of soil macrofauna, iii) evaluate influence of PyOM from these trees on earthworm cast production in a mesocosm study, and iv) evaluate potential of biochar and fertiliser + biochar blends in restoring fertility of a nutrient deficient soil and the effects on soil fauna abundance and diversity. Biochar used in this study was prepared from *Prosopis juliflora*, a common invasive shrub found in the semi-arid parts of Kenya. In the field study, soil macrofauna samples were collected at increasing distances from the tree stems and the centre of charcoal-making spots. In the greenhouse study, earthworm casts were collected after every two days and at the end of experiment (at 30 days) as a measure of earthworm activity. In the on-station trial, soil and soil fauna samples were collected six and eight weeks after crop emergence.

Highest soil macrofauna abundance was found below the tree canopies than away from the tree, though the trends differed with specific macrofauna group. Earthworms were prominent under the canopy of *Z. gilletii*, whereas beetles occurred in higher numbers under *C. megalocarpus*. Soil aggregate analysis showed higher small macro-aggregates and micro-aggregate fractions under the canopy of *Z. gilletii*. However, C content in these two aggregate fractions decreased by more than 50% in soils with longer duration of cultivation, with the greatest magnitude of differences under

the canopy of *Z. gilletii*. In contrast to trees, soil macrofauna (with the exception of centipedes) declined with distance from charcoal-making spots, with most notable trends in spots where *Z. gilletii* was used in charcoal making. The number of centipedes decreased with increasing distance from the centre of spots rich in *Z. gilletii* PyOM. Beetles, termites and crickets were significantly higher in spots rich in *C. megalocarpus* PyOM than *Z. gilletii* PyOM, though sampling distance had no significant influence. The mesocosm study showed that the weight of earthworm casts declined by as much as 30% on mesocosms with PyOM compared to the control. In the on-station field study, application of biochar and fertiliser + biochar blends led to more than 70% increase in C, N, available P and exchangeable K compared to the control, though these effects lasted only for a season. There were no significant difference between soil treated with biochar or fertiliser + biochar. The population of earthworms observed in plots treated with *P. juliflora* biochar increased with increasing amounts of biochar, whereas a decline in nematodes population, particularly bacterivores occurred in plots which received biochar, regardless of the amounts.

The study shows detrimental effects of converting trees to PyOM, especially the endogeic earthworms which are known to rely heavily on soil organic matter for nourishment. This could perhaps be caused by high recalcitrance of PyOM compared to the litter from trees and root biomass, which are more palatable. Other soil macrofauna may not directly be influenced by PyOM since PyOM was not the main subtrate food substrate. Application of biochar resulted in an increased content of major soil nutrients, which shows that *P. juliflora* biochar can potentially be a valuable soil amendment for improving nutrients in soil that are severely deficient in N and P and low in organic matter. Blending fertilisers with biochar however, seems not to have had much effects in terms of soil nutrient retention as it was hypothesised. Further research with long-term application of biochar could be of great benefit in expounding their impacts on soil fertility and soil fauna, since seasonal variations could have affected the observed results at short-term scales.

CHAPTER ONE

General introduction

1.1 Background information

In order to sustain high levels of crop productivity, soils need to be replenished often with organic inputs such as animal and green manures, crop residues or integrating trees into the annual cropping system (Mbau et al., 2015). Thus it is a common practice that smallholder farmers intercrop trees with annual crops for various reasons such as provision of food, forage, wood or charcoal, among other products (Akinnifesi et al., 2010; Nyaga et al., 2015). Incorporation of trees into agroecosystems could have significant effects on belowground biodiversity through litter inputs and/or root turnover (Gill and Jackson, 2000; Iversen and O'Brien, 2010). Apart from organic carbon and nutrient inputs, habitat provision is a major service provided by trees to belowground biodiversity largely through microclimate regulation (Lavelle et al., 2003; Lin, 2010; Barrios et al., 2012a). This supports the concept that trees constitute 'hotspots' of biological activity in agricultural landscapes and thus play a protective role to these organisms during periods of climatic stress (Barrios et al., 2012a).

However, in many agroecosystems, it is also a common practice that trees are felled and charcoal made on site. Conversion of trees to charcoal eliminates canopy effects associated with living trees while at the same time creating new conditions in and around such spots due to increased concentration of pyrolysed materials, often referred to as pyrogenic organic matter (PyOM). It is unclear, whether and/or how such unintentional PyOM additions play a role in the abundance and distribution patterns of soil macrofauna. However, early studies with biochar (another form of pyrolysed material) proposed that application of more recalcitrant forms of C can be one way of decreasing the rate of soil organic matter (SOM) loss. This is especially key in tropical agroecosystems which deplete SOM rapidly due to high temperatures.

Therefore, biochar has in recent times received global interest as a possible means of sequestering C into the soil as it is considered to be relatively stable to microbial attack (Lehmann et al., 2006; Lehmann et al., 2007; Chan et al., 2008). As such, biochar application could have its own set of unique effects on soil macrofauna abundance, diversity and distribution patterns through a cascade of effects within the soil food web (Domene et al., 2014). These effects can not only be affected by physical and chemical properties of biochar, but also may vary with the recipient soil and the prevailing environmental conditions (Verheijen et al., 2010). Though many studies have been done in relation to biochar effects on soil nutrients and microbial dynamics, its effects on soil macrofauna has received little attention (Lehmann et al., 2011; Ameloot et al., 2013). Such information is of importance, especially where large amounts of biochar are to be applied given that once applied, its removal from the soil, if needed, would be practically impossible (Verheijen et al., 2010).

Soil macrofauna are vital part of a soil ecosystem due to their profound direct and indirect influence on soil processes and functions (Lavelle et al., 1997; Barrios, 2007; Brussaard et al., 2007; Ayuke et al., 2009; Mbau et al., 2015). For instance, earthworms and termites, have been shown to ingest considerable amounts of soil mineral and organic matter which are further redistributed across the soil profile through excretions. Such activities could play an important role in aggregation process, thus affecting soil structure and its associated functions such as water and gas transport, and nutrient retention and availability (Ayuke, 2010; Fonte et al., 2010). In addition, earthworm casts and termite mounds are important micro-habitats for soil microbes to colonise. On the other hand, soil macrofauna's feeding and burrowing habits could be affected by soil management practices that alter the quality and quantity of organic inputs (Lavelle et al., 2001). Thus, what affects soil macrofauna either positively or negatively, may indirectly affect soil functions and consequently plant growth and productivity.

1.2 Problem statement and justification of the study

Sustainable management of soil fertility in agroecosystems has been a major concern since time immemorial (Venkateswarlu et al., 2013). Intensification in crop production, for instance, has led to continuous nutrients loss and increase in disease incidences and thus human beings have always been trying to find the best options to address the challenges. The situation is increasingly worsening in smallholder farms where continuous cultivation with little or no inputs has led to nutrient mining, thus a reduced capacity of the soils to produce sustainably (Kathuku et al., 2007; Ayuke, 2010; Kimaro et al., 2015). Apart from nutrient mining, loss of SOM has also been reported to have devastating effects on multiple functions of the soil. For instance, its loss has been linked to unresponsiveness to fertiliser, loss of soil biodiversity, poor soil structure and increased susceptibility of the soil to erosion among other challenges (Mbau et al., 2015). Thus, many options have been proposed to address this challenge. One of the options that has been suggested is use of biochar (Lehmann et al., 2011). Its use as a soil amendment is vested on its high recalcitrance, i.e. most of its C is unavailable to majority of soil microbes (Lehmann et al., 2006; Woolf et al., 2010; Wang et al., 2011; Felber et al., 2012). Though use of biochar has gained global interest in recent times, anthropogenic improvement of soil fertility dates back over thousands of years ago, with an example of the *Terra Preta* soils of the Amazon (Liang et al., 2006; Glaser, 2007; Grossman et al., 2010; Downie et al., 2011). The discovery of these ancient, carbon-rich soils has ignited interest and a series of global research into the potential impacts of amending soil with biochar have been undertaken. The *Terra Preta* soils have been shown to have elevated and long-term-sustained level of fertility demonstrating the benefits of amending soil with pyrolysed organic biomass (Glaser, 2007; Downie et al., 2011). The enhanced fertility has been linked to improvement in soil physical and chemical characteristics, such as enhanced water holding capacity, cation

adsorption, amelioration of soil pH and high soil organic matter stocks (Liang et al., 2006; Grossman et al., 2010; Downie et al., 2011).

In this regard, numerous studies have been conducted to compare processes and functions of the *Terra Preta* soils with either unamended adjacent soils or with soils whose biochar has been incorporated more recently. Some of these studies have shown significant shifts in soil microbial biomass and abundance (Warnock et al., 2007; O'Neill et al., 2009), community structure (O'Neill et al., 2009; Lehmann et al., 2011) and functional ecology of these microbes (DeLuca et al., 2009; Lehmann et al., 2011). These observations could be linked to alteration in soil nutrient release and carbon availability dynamics, changes in habitat properties such as soil pH and bioavailability of toxic elements, or protection of microbes by biochar (Lehmann et al., 2011). This could in turn have potential positive or negative impacts on soil organisms. However, despite the considerable potential impacts of biochar on soil macrofauna, only a few studies have addressed this area (Lehmann et al., 2011). Given the great role of soil macrofauna in moderating soil processes and functions and thus soil productivity, in-depth research addressing effects of biochar on the spatial distribution and activities of soil macrofauna could be a starting point towards achieving sustainable farming systems. This is especially important since such decisions could impact on socio-economic welfare of millions of people in Africa who rely on subsistence farming as a source of their livelihood.

1.3 Scope of the study

This thesis attempts to establish how PyOM and biochar affect soil macrofauna abundance, diversity and activity, soil aggregate stability and soil chemical properties. Activities conducted in this study included: (i) evaluation on how conversion of selected trees into PyOM affect soil macrofauna abundance, diversity and spatial distribution patterns. Results generated an understanding on the positive or negative effects of converting organic biomass to biochar or

biochar-like material (PyOM), (ii) assessing the causes of the observed responses by soil macrofauna to addition of PyOM in soil. This was achieved through a mesocosm study, and (iii) evaluating effects of biochar and fertiliser + biochar blends on soil chemical properties and soil macrofauna.

1.4 Study objectives

1.4.1 General objective

Enhance sustainable agroecosystems through use of biochar

1.4.1 Specific objectives

1. To evaluate soil macrofauna abundance under dominant tree species increases along a soil degradation gradient.
2. To determine soil aggregation and carbon allocation along a soil degradation gradient as affected by dominant tree species.
3. To evaluate spatial variation of soil macrofauna and nutrients in tropical agricultural systems influenced by historical charcoal production
4. To evaluate the effects of PyOM on casting activity by earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) under controlled conditions.
5. To evaluate the effects of biochar and fertiliser + biochar blends on soil chemical properties and soil fauna abundance and diversity in humid tropical highlands Nitisols.

1.5 Study hypotheses

1. Soil macrofauna abundance will decrease with increasing duration of cultivation, but differences will be moderated by tree species.
2. Soil aggregate and aggregate-associated soil C will decrease with increasing soil degradation and distance from the tree trunk.
3. Soil macrofauna abundance will increase with increasing distance from the charcoal-making spots.
4. Earthworm cast production will decrease with increasing amount of pyrogenic organic matter (PyOM).
5. Soil fauna abundance will decrease with increased amounts of biochar, but the magnitude of these effects would be modulated by type of inorganic fertiliser.

1.6 Outline of thesis

This thesis is composed of eight chapters which are structured as follows; Chapters one and two are on general introduction and literature review. Chapter three examines how three most dominant tree species (*Croton megalocarpus*, *Eucalyptus grandis* and *Zanthoxylum gilletii*) affects abundance and spatial distribution of seven key macrofauna groups along a soil degradation gradient. This was achieved by sampling from four different zones of tree influence, below and away from the canopy of the trees. Chapter four looks at the detailed effects of the same tree species on aggregate stability and C storage, as influenced by ecosystem engineers (earthworms and termites). Chapter five gives the detailed account of the effects of charcoal-making spots, where two of the three dominant tree species (*Croton megalocarpus* and *Zanthoxylum gilletii*) were used in charcoal making. Sampling was done following the same protocol as the tree species. This was in attempt to examine the effects of converting trees to PyOM on soil macrofauna abundance and spatial distribution patterns. This could in essence

provide a broader picture of the implications of management practices, such as biochar application, could have on soil biota. To further this topic, in chapter six, PyOM from the two trees studied in chapter five was prepared in the same way as the farmers prepare charcoal in their farms. The effects of PyOM on earthworm (*Pontoscolex corethrurus*) cast production was assessed as a measure of earthworm activity. Here, the PyOM was treated with either 2M HCl or acetone with the aim of removing substances that could be potentially toxic to earthworms, thus affecting their response to PyOM/biochar application. Chapter seven examines the effects of biochar on soil macro/mesofauna abundance and diversity. This was an on-station trial which was meant to assess the potential of biochar from *P. juliflora*, a common shrub in the semi-arid parts of Kenya which is termed a noxious weed. Chapter eight provides an integrated summary of the results described in previous chapters, conclusions and recommendations.

CHAPTER TWO

Literature review

2.1 Definition of biochar/PyOM and their preparation

Biochar is a solid, carbon-rich material produced through partial combustion or heating of organic biomass in an oxygen-limited kiln (pyrolysis) and targeted to be used as a soil amendment (Lehmann and Joseph, 2009; Liesch et al., 2010; Sohi et al., 2010; Felber et al., 2012). Thus, biochar differs from other forms of pyrogenic organic matter (PyOM) in that, the former is specifically intended for soil carbon management or agricultural use. Traditional methods of pyrolysis simply involved burning the organic materials in pit or earth-mound kilns (FAO, 1987). Modern pyrolysis technology is done in a controlled environment to minimise emissions and generally allows larger quantities of biomass to be processed with ease and less labour (Brown, 2009; Downie and Van Zwieten, 2013; Laird et al., 2009). The temperature at which materials are heated and the time they are exposed to heating generates biochar with substantially varying chemical composition since elements volatilise at different temperatures (DeLuca et al., 2009; Downie et al., 2009). Further, elements in a compound structure may be converted from one form to another, thereby affecting their concentrations in the soil. For instance, DeLuca et al. (2009) noted that ammonium ions (NH_4^+) could be oxidised to nitrates (NO_3^-) at higher temperatures.

Physical properties of biochar such as surface area and porosity are also affected by pyrolysis temperature. The organic compound structures such as hemicellulose, cellulose and lignin, decompose at different temperatures and therefore their proportions in the biomass could have significant effects on physical properties of the biochar produced (Downie et al., 2009). Inorganic compounds such as ash could also undergo certain reactions with the organic compounds which could ultimately affect the physical conditions of biochar. The relative

proportions of crystalline aromatic and aromatic-aliphatic organic compounds are important indicators of biochar structure and are often formed at different pyrolysis temperature (Downie et al., 2009). When complemented with inorganic compounds and voids within the biochar matrix, these organic compounds influence molecular structure of biochar (Downie et al., 2009). Therefore, chemical composition of feedstock and pyrolysis conditions significantly influence both yield and quality of biochar (Warnock et al., 2007; DeLuca et al., 2009; Downie et al., 2009; Laird et al., 2009; Sohi et al., 2010).

2.2 Changes in soil ecosystem processes and functions in response to biochar application

Intensive cultivation contributes to the decline of organic matter contents in tropical soils, a situation further aggravated by the high temperature that favours soil organic matter (SOM) decomposition (Six et al., 2002). Low nutrient and water retention capacity and weak structure in many agricultural soils has partly been attributed to low SOM content (Glaser et al., 2002; Hamza and Anderson, 2005). Therefore, deliberate efforts have been made across the tropical agro-ecosystems to improve such soils through management practices that encourage return of organic residues such as farm and agro-industrial organic wastes. However, the high amounts of organic residues required to improve soil properties and retain significant SOM contents, are discouraging adoption of these strategies. In this regard, biochar has been proposed as a possible means of improving soil fertility, increasing nutrient retention capacity and carbon sequestration (Liang et al., 2006; Thies and Rillig, 2009; Lehmann et al., 2011) among other potential benefits. Changes in soil characteristics can play a great role in modification of soil life (Verheijen et al., 2010). This could in turn have either direct or indirect effects on soil functions and ecosystem services such as soil structure stability, cycling of soil organic carbon and other nutrients, soil aeration, water use efficiency and disease resistance and therefore affect plant growth and productivity (Rillig and Mummey 2006; Barrios, 2007; Warnock et al.,

2007; Wardle et al., 2008; Liang et al., 2010). However, these changes can also vary depending on the inherent properties of biochar and the soil type. For instance, application of biochar has been shown to increase the abundance of cellulose-hydrolyzing bacteria (Kumar et al., 1987) and positively affect mycorrhizal fungi (Warnock et al., 2007; Solaiman et al., 2010). On the contrary, a decrease in plant-microbe symbiosis has also been reported, which could result from increased nutrient supply and therefore reducing the need for such interaction. Elmer and Pignatello (2011) observed a marked reduction in root lesions in asparagus caused by the *Fusarium spp.* on addition of biochar to soil. They attributed this to the increased Arbuscular Mycorrhiza (AM) colonization which suppresses infection of asparagus by *Fusarium.* On the other hand, an increase in AM colonization was attributed to the binding of allelochemicals. The biochar pore spaces have also been suggested as possible refuge or microhabitats for microorganisms to shelter from predators (Thies and Rillig, 2009).

2.3 Impacts of biochar on soil macrofauna

Several studies have shown significant direct impact of biochar application on soil macrofauna communities and their activities. However, the mechanisms and consequences behind these effects is poorly understood as only a few studies have been dedicated towards this topic (Lehmann et al., 2011). The studies have also shown that, the impact of biochar application on soil macrofauna depend greatly on the quality of biochar added. It is suggested that changes in soil macrofauna composition can result from short-term release of organic molecules from freshly added biochar (Lehmann et al., 2009; Liang et al., 2010; Lehmann et al., 2011). This could affect the macrofauna that ingest the soil and other organic materials applied in soil. Application of biochar could also alter physical properties of soil such as porosity, aeration and water transport which in turn affects soil fauna. Changes in soil community structure may in turn influence soil functions such as soil structure stability, soil nutrients transformations, soil

aeration and water use efficiency (Rillig and Mummey 2006; Warnock et al., 2007; Wardle et al., 2008; Thies and Rillig 2009; Liang et al., 2010).

Among the key soil macrofauna groups studied, only earthworms' interaction with biochar has received wide attention from researchers, perhaps due to their significant contribution towards soil processes and functions. Some studies have shown significant changes in earthworm growth and behaviour on soils amended with biochar in both field and laboratory conditions. These changes have been linked to the physical and chemical characteristics of biochar which in turn are affected by the type of feedstock and pyrolysis conditions. For instance, when Liesch et al. (2010) conducted a 28-day toxicity study of two biochar types (poultry litter and pine chip biochar) on earthworms (*Eisenia foetida*), they observed a higher mortality and weight loss on soil treated with the two highest biochar application rates (67.5 and 90 Mg ha^{-1}) which they attributed to drastic increase in pH to intolerable levels over the course of incubation. In addition, the authors observed a rapid mortality of the worms on soil amended with poultry-litter biochar which they suggested could have been caused by release of ammonia from the soil + biochar mixture. In their studies, Gomez-Eyles et al. (2011) reported that biochar could have played a role in decreasing the concentration of contaminants in the earthworms' body. Nonetheless, Gomez-Eyles et al. (2011) noted that earthworms in biochar treated soil also lost significantly higher weight compared unamended control soil. The authors speculated that the loss in weight could have been caused by a decrease in consumption of biochar-amended soil as the worms tried to avoid ingesting biochar particles. It has been suggested that some biochar/PyOM types may be too reculcitrant to support soil microbiota, which may ultimately affect larger organisms such as earthworms that feed on the microbiota or their metabolites. Thus, it was not clear if the reduced consumption of contaminated soil could have led to the lower concentration of polycyclic aromatic hydrocarbons (PAHs).

Besides the quality characteristics of biochar, the quantities applied could also influence the behaviour earthworm exhibit in the soil. Li et al. (2011) observed that earthworms (*E. foetida*) avoided soil containing 100 and 200 g/kg of biochar made from apple wood chips but not on soil receiving 10 g/kg of the same treatment. The authors investigated whether the avoidance was being caused by nutrition deficiency in soil, desiccation from the dry biochar or presence of toxic PAHs in biochar. After the tests, they ruled out the possibility that the observed avoidance was being caused by traces of toxic PAHs in biochar or nutrients deficiency in soil. After pre-wetting biochar before application, the authors observed that the avoidance test was no longer statistically significant compared to unamended control.

It has been documented that changes in soil properties following application of biochar may differ from one soil type to another and this affect the response of soil macrofauna. For instance, Van Zwieten et al. (2010) reported slightly higher preference of earthworms (*E. foetida*) to biochar-amended Ferrosol over unamended soil. However, no significant differences were recorded on biochar-amended and unamended Calcarosol. In another related study, Topoliantz and Ponge (2005) reported higher cast production of earthworms (*Pontoscolex corethrurus*) on soil + charcoal mixture than on pure soil or pure charcoal. The authors suggested that soil + charcoal mixture could have been preferentially used as a living substrate, perhaps due to positive influence of biochar in improving chemical and physical properties of the soil. In yet another study, Topoliantz et al. (2005) observed higher cocoon and juvenile numbers on an acidic Oxisol treated with char and manioc peel compared to other organic amendments. The authors attributed this to an increase in soil pH from the char added, and therefore favoured survival of juveniles which are more sensitive to soil acidity than the adults. These studies demonstrate that, the behaviour of earthworms and perhaps of other soil macrofauna could vary depending on the type of soil receiving the biochar.

2.4 Biochar effects on soil N and C transformations

Soil organic carbon (SOC) is the largest carbon sink in terrestrial biosphere (Akala and Lal, 2001; Wu et al., 2003). However, due to its high vulnerability, small changes in SOC pools can significantly influence the global C balance and to a greater extent affect global climate (Akala and Lal, 2001). Use of biochar has been suggested as a possible means of capturing atmospheric carbon dioxide (CO_2) and sequestering it into soils in the form of SOC through conversion of crop residues and agroforestry products into recalcitrant carbon (Lehmann et al., 2006; Woolf et al., 2010; Wang et al., 2011; Felber et al., 2012). Woolf et al. (2010) estimated that adoption of biochar technology could reduce the current global annual net anthropogenic emission of greenhouse gases (methane, carbon dioxide and nitrous oxide) by up to 12% (1.8 Pg CO_2-C equivalent). This could be attributed to the higher carbon-residence time of biochar in soil, which varies from hundreds to thousands of years (Czimczik and Masiello, 2007; Lehmann et al., 2009; Jeffery et al., 2011). In addition, the relatively high resistance of biochar from microbial attack avoids conversion of terrestrial organic carbon to atmospheric carbon (CO_2) and this has been viewed as the strength in using it as a climate mitigation strategy (Sohi et al., 2010; Zhang et al., 2010; Lehmann et al., 2011). Therefore, processing organic biomass C to biochar C could sequester more carbon than direct application of residues to soil (Lehmann et al., 2006). In this regard, Lehmann et al. (2006) proposed that slash-and-char strategy could effectively be used to replace the traditional slash-and-burn practice which contributes immensely to emission of greenhouse gasses. Slash-and-char can therefore be an important strategy of converting organic residues derived from the farm to a valuable soil amendment, replacing the tradition practice of burning these organic residues during land preparation.

CHAPTER THREE

Soil macrofauna abundance under dominant tree species increases along a soil degradation gradient

Authors: Solomon Kamau [a, b*], Edmundo Barrios [b], Nancy K. Karanja [a], Fredrick O. Ayuke [a], Johannes Lehmann [c, d]

[a] Department of Land Resource Management and Agricultural Technology, College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053, Nairobi, Kenya.

[b] World Agroforestry Centre, United Nations Avenue, P.O. Box 30677, Nairobi, Kenya.

[c] Soil and Crop Science, Cornell University, Ithaca, NY 14853, USA.

[d] Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14853, USA

[*]Corresponding author:

E-mail address: solkam08@gmail.com (S. Kamau)

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Abstract

Soil macrofauna contribute to key soil functions underpinning soil-mediated ecosystem services. There is limited understanding about the role of trees as 'resource islands' for soil macrofauna in agricultural landscapes and how this interaction is affected by soil degradation status. The study assessed the spatial influence of three dominant trees namely, *Croton megalocarpus, Eucalyptus grandis* and *Zanthoxylum gilletii*, on soil macrofauna abundance, along a soil degradation gradient resulting from continuous cultivation for 10, 16 and 62 years. It was hypothesised that spatial variation in soil macrofauna abundance is affected by duration of cultivation, tree species and distance from the tree trunk. Soils cultivated for 10 years showed highest soil nutrient levels. Notably, soil C and N were higher below the canopy of *C. megalocarpus* (64.6 g kg^{-1} C; 6.7 g kg^{-1} N), than *E. grandis* (58.7 g kg^{-1} C; 5.9 g kg^{-1} N) and *Z. gilletii* (54.5 g kg^{-1} C; 5.6 g kg^{-1} N) after 10 years of cultivation. Similar trends were also found after 16 and 62 years of cultivation, although the mean values for the two elements were below 40.0 g kg^{-1} and 4.0 g kg^{-1}, respectively. Higher soil macrofauna abundance was found after 16 and 62 years of cultivation, though this was dependent on tree species and soil macrofauna group. Earthworm abundance was highest below the canopy of *Z. gilletii* averaging 389 individuals and 160 individuals m^{-2}, respectively, compared to 14 individuals m^{-2} after 10 years of cultivation. Conversely, beetles showed higher numbers under *E. grandis* and *C. megalocarpus* than under *Z. gilletii*. Highest numbers of termites and centipedes were found under *E. grandis* after 16 years of cultivation. These findings support the importance of a diverse tree cover in agricultural landscapes to conserve soil macrofauna communities and the contribution of their activity to soil ecological functions.

Keywords: *Croton megalocarpus*; *Eucalyptus grandis*; organic resource quality; soil biodiversity; spatial variation; *Zanthoxylum gilletii*

3.1 Introduction

Soil biota is a central constituent of any ecosystem, whether natural or managed, due to their role in regulating key soil functions such as organic matter decomposition, nutrient cycling and soil structure maintenance (Brussaard et al., 1997; Barrios, 2007). Soil macrofauna constitute an important component of soil biota given the significant impact of their activities on soil properties (Lavelle, 1997; Ayuke et al., 2009). Earthworms and termites, for example, have earned recognition as 'ecosystem engineers' due to their significant effects on soil structure and functions through their soil-feeding, nesting and burrowing habits (Jones et al., 1994). However, their activities could be affected by management practices largely through changes in organic inputs to soil which affect food availability, and through soil disturbance (e.g. tillage) which often kill the larger species (e.g. earthworms) or the structures they inhabit and interfere with their activities (Lavelle et al., 2003; Ayuke et al., 2011a; Mbau et al., 2015). Furthermore, these management practices can also contribute to the spatial heterogeneity in soil properties which underlies the distribution of soil macrofauna. Consequently, soil macrofauna are usually not uniformly distributed within the soil in any given space and time, but rather, aggregated in 'hotspots' of carbon-rich areas such as the rhizosphere, soil aggregates and organic detritus (Beare et al., 1995; Lavelle, 1997; Barrios et al., 2012a; Kuzyakov and Blagodatskaya, 2015). Therefore, farmer practices involving tillage, application of agricultural inputs and/or the types of plants grown on their farms may have significant positive or negative effects on soil macrofauna abundance and distribution in any given location.

Smallholder farmers often intercrop trees with annual crops for various reasons such as provision of food, forage, wood and/or charcoal, among other products (Akinnifesi et al., 2010; Nyaga et al., 2015). In some occasions, farmers deliberately retain indigenous trees during conversion of forest to cultivated lands for similar reasons (Fonte et al., 2010; Pauli et al., 2012). Trees are known to modify conditions beneath the canopy through shading, root

turnover and litter inputs which significantly influence soil moisture, temperature, carbon substrate availability and nutrient regimes (Lavelle et al., 2003; Lin, 2010; Barrios et al., 2012a). Earlier research has shown predictable patterns in the variation of soil properties resulting from individual trees where litter deposition around the trees produces characteristic concentric rings of influence that are proportional to the size of the crown (Rhoades, 1997). Other studies have shown gradual decline in the content of organic carbon, nitrogen, phosphorus and exchangeable bases with increasing distance from the tree stem due to differences in litter deposition (Kater et al., 1992; Tomlinson et al., 1998; Jonsson et al., 1999). Root turnover is also a critical component of soil carbon and nutrients and therefore an important driver of belowground processes and ecological functions (Gill and Jackson, 2000; Iversen and O'Brien, 2010). Due to the feeding preference of some soil macrofauna groups for specific organic substrate types, the quality of litter and its deposition patterns as well as root turnover may therefore affect their distribution (Lavelle et al., 2003, Pauli et al., 2010). For instance, Warren and Zou (2002), Caner et al. (2004) and Frouz et al. (2013) have reported differential effects of litter quality on soil macrofauna in different systems. Further, in their recent review, Korboulewsky et al. (2016) highlighted that the litter quality from a given tree species can significantly contribute to the changes observed in soil fauna communities.

Besides tree leaf litter and root turnover, stemflow could also contribute nutrients to the soil at the base of trees through the washing of dust, insect remains or bird droppings from the leaves and bark (Rhoades, 1997). Changes in soil chemistry beneath the tree could potentially affect the occurrence of soil macrofauna since soil chemical properties have been used to partially explain the variations in distribution of soil macrofauna (Ayuke et al., 2009; Pauli et al., 2011; Mbau et al., 2015). The spatial patterns of soil macrofauna abundance are thus expected to be structured in a manner that corresponds to the heterogeneity of soil resources around the tree (Korboulewsky et al., 2016). The soil beneath tree canopy can therefore be hypothesised as a

distinct area of favourable or unfavourable conditions to the abundance of some soil macrofauna group(s), thus becoming an important determinant of their spatial distribution patterns. As such, in-depth research that addresses spatial-temporal patterns of soil macrofauna abundance as affected by tree attributes under contrasting soil degradation levels could significantly contribute towards the design of sustainable farming systems (Barrios *et al*., 2012a). Though the spatial arrangement of single trees has been shown to affect soil properties (Belsky et al., 1989; Kater et al., 1992; Rhoades, 1997; Tomlinson et al., 1998; Jonsson et al., 1999; Amiotti et al., 2000), little is known about the magnitude and pattern of their influence on soil macrofauna abundance in agricultural landscapes particularly in tropical Africa.

In this study, I assessed effects of three dominant tree species; *Croton megalocarpus* Hutch., *Eucalyptus grandis* W.Hill and *Zanthoxylum gilletii* (De Wild.) P.G.Waterman, on soil macrofauna abundance and biomass across three catchments that represent a soil degradation gradient resulting from different times since conversion from primary forest to agriculture (Kimetu et al., 2008). This provided a chronosequence experimental set-up where short/medium term effects of tree species and long-term effects of land-use change could be systematically studied. It was hypothesised that i) soil nutrient stocks and availability would decrease with increasing duration of cultivation and distance from the tree trunk, and ii) soil macrofauna abundance and biomass would decrease with distance from the tree trunk and duration of cultivation but the magnitude of these effects would be modulated by tree species.

3.2 Materials and methods

3.2.1 The study sites

The study site is located in Kapchorwa region, Nandi County in several farms along the Kakamega-Nandi forest complex which lies between Latitude 0° 9' 00" and 0° 10' 00" N and Longitude: 35° 0' 00" and 35° 1' 00" E. Altitude ranges from 1600 to 1900 m above sea level. The area receives an annual precipitation of approximately 2000 mm; the rainfall is bimodal, with 'long rains' occurring between April and June (approximately 1200 mm), and 'short rains' between August and October (approximately 800 mm) (Güereña et al., 2015a). Being near the equator, temperatures are relatively constant throughout the year with an average maximum daily temperature of 26°C, an average minimum of 11°C and a mean annual temperature of 19°C. Soils are classified as kaolinitic Acrisols (FAO/UNESCO classification) or Ultisols (USDA classification) showing deep reddish-brown coloration and thick humic topsoil with 45-49% clay, 15-25% silt and 26-40% sand on predominantly heavier-textured Ultisols and 11-14% clay, 21-27% silt and 59-68% sand, on lighter-textured Ultisols (Kimetu et al., 2008). The indigenous vegetation is primarily highland rainforest, an extension of Guinean-Congolian belt, and dominated by *Funtumia africana* (Benth.) Stapf, *Prunus africana* (Hook.f.) Kalkman, *Ficus* spp., *Croton* spp. and *Celtis* spp. (Glenday, 2006). The farms are dominated by cereal cultivation and rarely use any form of inorganic inputs. If applied, the amounts used are barely enough to meet crop needs. Farmlands are therefore characterised by low soil fertility and low crop productivity. Study farms were selected from three catchments which were under continuous cultivation for 10 years, 16 years and 62 years, since conversion from primary forest to agricultural lands. The three microcatchments are located within an area of 6 km^2, with their sizes ranging from 9-14 ha. Detailed description of these catchments can be found in Recha et al. (2013) and Güereña et al. (2015a).

3.2.2 Identification and selection of tree species

Selection of tree species of interest was conducted using participatory action research tools in the context of focus group discussions involving randomly-selected farmers from all the three catchments (Barrios et al., 2012b). A ranked list of the most common trees within the area of study was identified and the top three most abundant trees were selected, namely, *Croton megalocarpus, Eucalyptus grandis* and *Zanthoxylum gilletii*. Selection of trees to be sampled within the three catchments was based on the following criteria: (i) dominance: for each species selected, at least three single trees could be located within each catchment. Each tree species represented a treatment while each single tree acted as a replicate; (ii) distribution: the selected trees occurred singly within the farms and were located at least 4 times their crown diameter from other trees, thus free from tree interferences; (iii) attributes: the height, shape and age of the single trees were comparable; (iv) farm management practices: study trees were all found under small-holder maize-based cropping system, involving minimal superficial disturbance at planting (e.g. hand hoe) and manual weeding, across all sampling distances.

3.2.3 Soil macrofauna sampling protocol

In order to study the effects of tree species and canopy on soil macrofauna, soil monoliths (0.25 by 0.25 by 0.30 m) were excavated following the standard Tropical Soil Biology and Fertility Programme (TSBF) sampling protocol (Anderson and Ingram, 1993) at predetermined points around the tree (Figure 3.1). The area around the selected trees was subdivided into four concentric zones, A, B, C and D based on modifications to the method used by Bayala et al. (2004). Modifications included: i) Zone A was located 0.25 m from the tree stem on all the occasions, whereas in the former it could vary from 0 to 2 m and ii) Zone D was located away from edge of the tree crown at an equivalent distance to that between A and C, whereas in the former it was located 2 m from the edge of the crown. Zone B and C were not modified and

remained at the middle of the tree crown and at the tree crown edge respectively. Soil monoliths were excavated from each concentric zone following four transects at right angles from each other, for a total of 16 monoliths per tree. Sampling was conducted towards the end of the short rain season in the month of November 2014. The excavated soil was placed in plastic trays and large clods gently broken to enable hand picking of soil macrofauna. All soil macrofauna were first placed in 75% ethanol. At the end of the sampling exercise, the macrofauna (except earthworms) were transferred into fresh 75% ethanol and sealed in vials. Earthworms were transferred into 4% formaldehyde for preservation. The preservative solution was replaced when coloration change was observed. Soil macrofauna were separated into seven broad taxonomic units (orders or families), i.e. earthworms (Oligochaeta), ants (Hymenoptera), termites (Isoptera), centipedes (Chilopoda), millipedes (Diplopoda), beetles (Coleoptera) and spiders (Araneae). Other soil invertebrates included crickets (Orthoptera), cockroaches (Blattodea) and earwigs (Dermaptera); due to their low numbers, they were pooled together as 'other soil macrofauna'. Soil macrofauna abundance was calculated as number of individuals per square meter (individuals m^{-2}) and their biomass in grams per square meter (g m^{-2}).

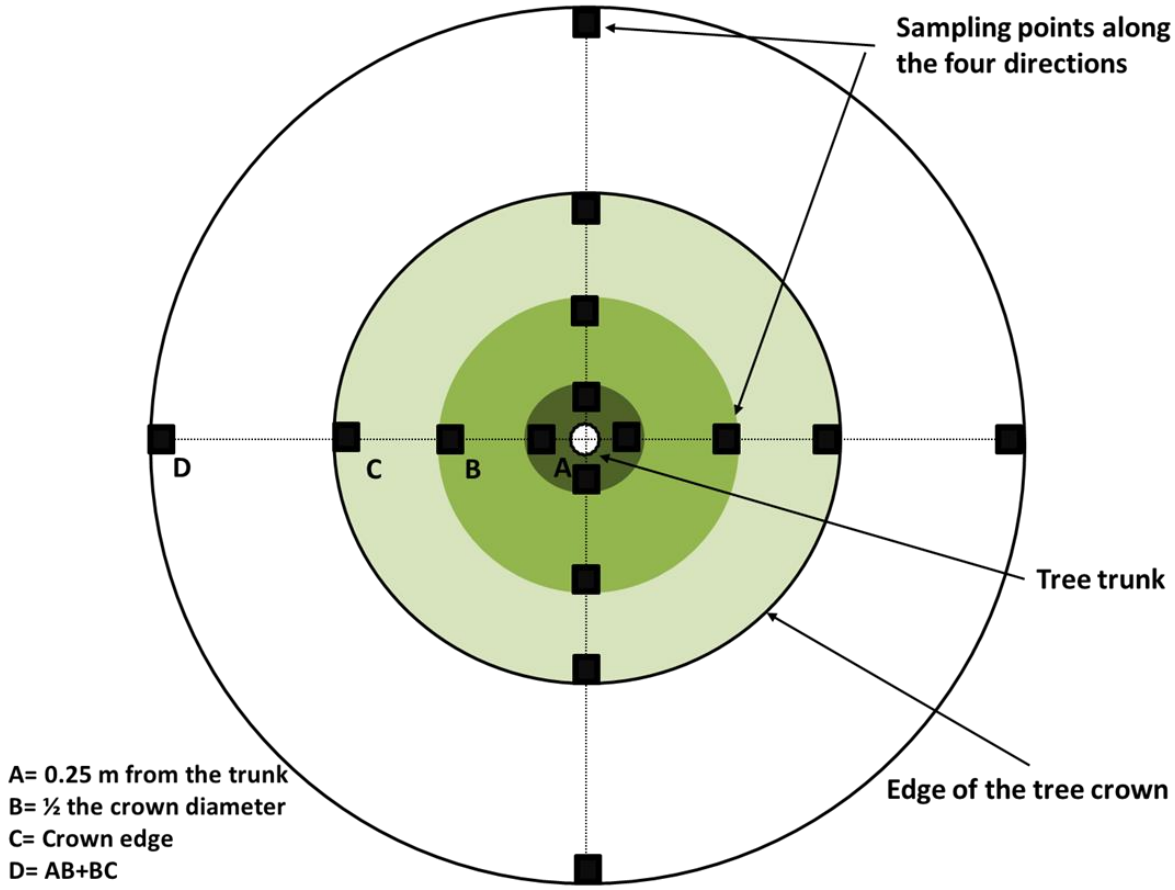

Figure 3.1: **Schematic representation of the sampling protocol from beneath the trees.**

3.2.4 Collection and chemical characterisation of tree litter and roots

Leaf litter was collected in January 2015, at the beginning of the dry season when leaf fall takes place, using litter traps placed below the selected trees for a period of two weeks. Fine roots (< 5 mm diameter) were dug out from several locations below the canopy concurrent with soil macrofauna sampling. After collection, the materials were air dried in the field, bulked and taken to the laboratory, where they were further dried in the oven at 60°C to a constant weight. The dried samples were then ground and passed through a 2 mm sieve. Total macro-elements (total nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg)), total carbon (C), lignin and polyphenols were analysed from the samples. Total C and N were determined using CN-analyser while P, K, Ca and Mg were extracted through closed-vessel microwave-assisted digestion system (Miller, 1998) and determined using inductively coupled

plasma atomic emission spectroscopy (Isaac and Johnson, 1998). Lignin were analysed using the acid detergent fibre method, while polyphenols were analysed using the Folin-Denis method (Anderson and Ingram, 1993).

3.2.5 Soil sampling and nutrient analysis

Immediately after handpicking the soil macrofauna, soil from each tree zone for the four directions (Figure 3.1), was mixed thoroughly to make a composite sample of about 500 g for analysis. All soil samples were initially scanned using near-infrared (NIR) spectroscopy for the selection of 10% of total samples as reference samples to undergo conventional soil chemical analysis (Shepherd and Walsh, 2007). Soil parameters measured included: total N and C, available P, soil pH and exchangeable bases (Ca, Mg and K). Total C and N were determined using a CN-analyser, while P and the bases were extracted by the Mehlich-3 procedure (Mehlich, 1984) and measured through inductively coupled plasma atomic emission spectroscopy (Isaac and Johnson, 1998). Soil pH was determined using a pH meter with soil-water ratio of 1:2.5 (Anderson and Ingram, 1993). Soil chemical data from these reference soil samples was used to generate a calibration curve using partial least-squares regression analysis through mid-infrared spectroscopy (MIR) which was then used to determine soil parameters of the remaining 90% of the samples.

3.2.6 Statistical analysis

Given that the soil macrofauna data showed deviation from normality, based on Shapiro-Wilk test, and lack of homogeneity of variance (Levene's test), coupled with the complex sampling design, generalised linear mixed models (GLMM) were used to test the effects of duration of cultivation (i.e. catchment conversion age), tree species and zone of sampling using the package lme4 (Bates et al., 2015) in R (R Core Team, 2015). Further, given that the data had a

considerable proportion of zero values, negative binomial regression was chosen as an extension of the Poisson distribution, using (1|Tree replicates: Tree species : Duration of cultivation) as a random term. However, it should be noted that 'duration of cultivation' is not a randomly allocated treatment and the differences between catchments could be due to other factors in addition to the duration of cultivation. The best fitting models were chosen based on the lowest Akaike Information Criterion (AIC). The high frequency of zeros values in the biomass data, however, meant that the usual statistical models were not appropriate since the models available either describe discrete distributions in which the variable can take only a few specific values or continuous distributions in which the variable can take any value in a range. In my case, there was a mixture of a continuous distribution of non-zero values and a clump of zero values. Hence I did the analysis in two stages. First, I performed a logistic regression analysis to determine whether the response outcome was positive (e.g. presence/absence). Conditional on the outcome being positive, the second stage was to determine how these positive outcomes depended on the explanatory variables using the log-normal distribution. Multivariate principal component analysis (PCA) and Monte Carlo test were performed to assess the influence of tree species and duration of cultivation. I also conducted a redundancy analysis (RDA) to determine factors explaining soil macrofauna abundance. Soil macrofauna were entered as dependent variables whereas soil chemical properties as explanatory variables. The analysis was conducted using the Vegan package of R (Oksanen et al., 2015).

3.3 Results

3.3.1 Quality parameters of litter derived from tree species

Tree species had a considerably greater influence on litter quality parameters than the duration of cultivation (Table 3.1). All the chemical elements were significantly different between the different tree species. Duration of cultivation only influenced the lignin content and the L/N ratio of leaf litter, decreasing in *Z. gilletii* with time. Total C was higher in *E. grandis* (514.0 g kg^{-1}) and low in *C. megalocarpus* tree litter (472.7 g kg^{-1}), whereas total N content was low in *E. grandis* (9.1 g kg^{-1}) compared to *Z. gilletii* (13.5 g kg^{-1}) and *C. megalocarpus* (18.1 g kg^{-1}). Thus C/N values were low in the litter of native trees, *C. megalocarpus* and *Z. gilletii*, with values of 27:1 and 36:1, respectively, compared to 58:1 in litter derived from the exotic tree *E. grandis*. Phosphorous was more than 3 times higher in *C. megalocarpus* and *Z. gilletii* litter than that of *E. grandis*. Like the C/N, the C/P ratios were higher in *E. grandis* than the native trees. Therefore, the quality of *E. grandis* litter was very low as measured by C/N and C/P ratios, compared to that of the two indigenous trees, *C. megalocarpus* and *Z. gilletii*. Exchangeable bases (K, Ca and Mg) were remarkably higher in *C. megalocarpus* litter than that of the other two trees species. *C. megalocarpus* contained the lowest concentration of polyphenols (6.5%) whereas *E. grandis* had the highest (13.5%). Due to the low concentration of N in *E. grandis*, the ratios L/N, PP/N and (L+PP)/N, were also higher in *E. grandis* litter.

Table 3.1: Tree litter quality parameters (mean ± SE) as influenced by duration of cultivation and tree species.

Parameter	Tree species/Duration of cultivation												*p*-value	
	Croton megalocarpus				***Eucalyptus grandis***				***Zanthoxylum gilletii***					
	10 years	**16 years**	**62 years**	**Mean†**	**10 years**	**16 years**	**62 years**	**Mean†**	**10 years**	**16 years**	**62 years**	**Mean†**	**Duration of cultivation**	**Species**
C (g kg^{-1})	466.3 (3.0)	473.7 (3.0)	478.0 (5.0)	**472.7 (2.7)**	511.0 (3.0)	514.7 (1.0)	516.3 (1.0)	**514.0 (3.0)**	492.7 (1.0)	492.3 (4.0)	477.0 (5.0)	**487.3 (3.6)**	0.255	**<0.001*****
N (g kg^{-1})	16.2 (0.8)	21.2 (0.3)	17.0 (0.3)	**18.1 (0.4)**	9.1 (1.4)	8.4 (0.3)	9.7 (1.0)	**9.1 (0.7)**	14.7 (1.7)	13.1 (1.0)	12.8 (0.4)	**13.5 (0.9)**	0.545	**<0.001*****
P (g kg^{-1})	0.8 (0.1)	1.2 (0.1)	1.2 (0.1)	**1.1 (0.1)**	0.2 (0.1)	0.2 (0.0)	0.4 (0.1)	**0.3 (0.1)**	0.8 (0.1)	0.6 (0.0)	0.6 (0.1)	**0.7 (0.1)**	0.115	**<0.001*****
K (g kg^{-1})	17.2 (2.0)	16.2 (2.0)	15.8 (0.2)	**16.4 (1.9)**	6.5 (1.0)	4.1 (1.0)	5.9 (0.2)	**5.5 (0.8)**	5.8 (2.0)	6.9 (1.0)	8.4 (4.0)	**7.1 (3.6)**	0.756	**<0.001*****
Ca (g kg^{-1})	33.8 (2.0)	23.7 (3.0)	28.8 (2.0)	**28.8 (2.3)**	15.7 (2.0)	13.1 (0.2)	10.5 (0.3)	**13.1 (1.1)**	18.4 (5.0)	16.9 (1.0)	22.3 (6.0)	**19.2 (4.2)**	0.239	**<0.001*****
Mg (g kg^{-1})	4.9 (0.1)	5.5 (1.0)	4.3 (1.0)	**4.9 (0.9)**	1.4 (0.2)	1.3 (0.1)	1.4 (0.2)	**1.4 (0.2)**	2.8 (0.3)	2.8 (1.0)	3.0 (1.0)	**2.9 (0.9)**	0.859	**<0.001*****
C/N	28.9 (1.5)	22.6 (0.6)	27.9 (0.7)	**26.5 (0.8)**	59.6 (11.1)	61.4 (2.0)	54.1 (4.3)	**58.4 (5.2)**	34.2 (3.1)	37.7 (2.0)	37.4 (0.9)	**36.4 (1.8)**	0.975	**<0.001*****
C/P	606.7 (56.7)	418.4 (43.5)	406.4 (48.1)	**477.2 (42.3)**	2320.1 (192.7)	2209.5 (65.8)	1595.6 (379.6)	**2041.7 (219.7)**	673.5 (70.7)	804.4 (50.8)	782.2 (53.6)	**753.4 (53.9)**	0.075	**<0.001*****
L (%)	33.5 (0.6)	35.5 (0.9)	36.1 (0.4)	**35.1 (0.6)**	30.9 (0.5)	30.7 (0.8)	31.1 (0.9)	**30.9 (0.6)**	37.2 (2.8)	23.4 (1.4)	18.6 (0.5)	**26.4 (1.8)**	**<0.001*****	**<0.001*****
PP (%)	6.0 (0.7)	6.6 (0.9)	6.8 (0.5)	**6.5 (0.6)**	12.8 (0.6)	13.9 (0.2)	13.9 (0.1)	**13.5 (0.5)**	9.7 (0.8)	8.9 (0.5)	8.3 (0.6)	**9.0 (0.6)**	0.843	**<0.001*****
L/N	20.8 (1.2)	16.8 (0.4)	21.3 (0.3)	**19.6 (0.8)**	35.7 (5.9)	36.6 (1.0)	32.5 (1.9)	**34.9 (2.7)**	25.5 (0.9)	17.8 (0.7)	14.6 (0.6)	**19.3 (0.8)**	**0.014***	**<0.001*****
PP/N	3.7 (0.5)	3.1 (0.4)	4.0 (0.3)	**3.6 (0.5)**	15.0 (3.1)	16.5 (0.4)	14.5 (1.1)	**15.3 (1.5)**	6.7 (0.4)	6.9 (0.5)	6.5 (0.6)	**6.7 (0.5)**	0.958	**<0.001*****
(L+PP)/N	24.5 (1.4)	19.9 (0.8)	25.3 (0.5)	**23.2 (0.9)**	50.7 (9.1)	53.2 (1.3)	47.0 (3.1)	**50.3 (4.3)**	32.2 (1.3)	24.7 (1.1)	21.1 (1.2)	**26.0 (1.2)**	0.118	**<0.001*****

† This mean gives the aggregate tree effect. Abbreviations: C=carbon, N=nitrogen, P=phosphorous, K=potassium, Ca=calcium, Mg=magnesium, L=lignin, PP=polyphenols. Means in bold are significant different: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3.2 Quality parameters of fine roots derived from tree species

Similar to the litter, duration of cultivation had little influence on root quality parameters (Table 3.2). Only root C content was significantly affected by duration of cultivation, particularly in *Z. gilletii*. All the chemical elements (except K and Mg) differed significantly among the tree species. Total N was very low in *E. grandis* (5.5 g kg^{-1}) compared *C. megalocarpus* (14.0 g kg^{-1}) and *Z. gilletii* (17.1 g kg^{-1}). Phosphorous was at least 60% higher in *C. megalocarpus* and *Z. gilletii* roots than those of *E. grandis*. Due to the low N and P content, C/N and C/P ratios were large in *E. grandis* with values exceeding 75:1 and 700:1, respectively compared to lowest values <40:1 and <400:1, respectively recorded in *C. megalocarpus* fine roots. Contrary to the other elements, Ca was significantly higher (16.9 g kg^{-1}) in *E. grandis* roots, while averages of 13.3 g kg^{-1} and 10.9 g kg^{-1} were found in *C. megalocarpus* and *Z. gilletii* roots, respectively. Lignin was about 25% and polyphenols about 8% in *E. grandis* roots compared to *C. megalocarpus* (14.1%; 0.9%) and *Z. gilletii* (13.3%; 2.6%).

Table 3.2: Tree root quality parameters (mean ± SE) as influenced by the duration of cultivation and tree species.

Parameter	Tree species/Duration of cultivation												*p*-value	
	Croton megalocarpus				*Eucalyptus grandis*				*Zanthoxylum gilletii*					
	10 years	**16 years**	**62 years**	**Mean†**	**10 years**	**16 years**	**62 years**	**Mean†**	**10 years**	**16 years**	**62 years**	**Mean†**	**Duration of cultivation**	**Species**
C (g kg^{-1})	437.5 (2.8)	433.1 (6.8)	431.5 (1.5)	434.2 (4.7)	434.3 (1.1)	432.4 (0.9)	430.8 (1.7)	432.5 (1.2)	442.1 (12.1)	465.7 (8.4)	415.7 (17.7)	441.2 (12.7)	**0.047***	0.448
N (g kg^{-1})	15.3 (1.3)	13.5 (1.8)	13.1 (2.6)	**14.0 (1.3)**	5.7 (0.6)	5.5 (0.9)	5.2 (0.2)	**5.5 (0.7)**	15.6 (2.0)	23.1 (3.4)	12.6 (2.6)	**17.1 (2.6)**	0.119	**<0.001*****
P (g kg^{-1})	1.6 (0.04)	1.3 (0.3)	1.3 (0.4)	**1.4 (0.2)**	0.6 (0.03)	0.5 (0.1)	0.6 (0.03)	**0.6 (0.3)**	0.7 (0.02)	1.5 (0.5)	0.7 (0.1)	**0.9 (0.3)**	0.420	**<0.001*****
K (g kg^{-1})	7.5 (0.8)	6.9 (0.6)	7.6 (1.4)	7.3 (0.5)	8.3 (0.7)	8.7 (0.6)	8.2 (0.3)	8.4 (0.5)	5.5 (0.3)	13.0 (4.2)	6.7 (1.2)	8.4 (2.3)	0.232	0.657
Ca (g kg^{-1})	13.1 (0.7)	13.3 (0.1)	13.4 (0.4)	**13.3 (0.5)**	16.3 (1.0)	16.7 (1.1)	17.6 (1.0)	**16.9 (1.0)**	9.9 (1.4)	10.5 (0.7)	12.3 (1.2)	**10.9 (1.3)**	0.221	**<0.001*****
Mg (g kg^{-1})	2.2 (0.3)	1.9 (0.3)	2.1 (0.4)	2.1 (0.3)	1.7 (0.3)	1.7 (0.2)	1.8 (0.1)	1.8 (0.1)	1.7 (0.2)	2.0 (0.2)	1.8 (0.2)	1.8 (0.1)	1.000	0.276
C/N	29.0 (2.6)	33.1 (4.0)	36.0 (8.0)	**32.7 (3.2)**	77.2 (6.8)	82.6 (11.2)	83.5 (3.1)	**81.1 (7.3)**	29.3 (3.5)	21.2 (3.2)	35.2 (5.5)	**28.6 (4.1)**	0.272	**<0.001*****
C/P	267.3 (6.4)	398.2 (12.8)	400.6 (13.8)	**355 (9.0)**	755.5 (40.6)	830.1 (82.3)	703.1 (35.2)	**769.2 (42.7)**	614.2 (7.1)	391.0 (15.3)	654.1 (60.5)	**553.1 (27.6)**	0.705	**<0.001*****
L (%)	12.1 (3.0)	13.5 (2.3)	16.8 (3.4)	**14.1 (1.8)**	28.2 (2.1)	22.0 (0.3)	25.1 (0.8)	**25.1 (0.8)**	18.4 (6.2)	9.5 (0.7)	11.9 (1.9)	**13.3 (2.9)**	0.233	**<0.001*****
PP (%)	1.0 (0.2)	1.1 (0.2)	0.8 (0.1)	**0.9 (0.1)**	7.9 (0.9)	8.1 (0.2)	8.6 (0.6)	**8.2 (0.6)**	2.4 (0.8)	3.3 (0.4)	2.0 (0.9)	**2.6 (0.7)**	0.381	**<0.001*****
L/N	8.0 (1.8)	10.8 (3.1)	13.8 (3.7)	**10.9 (2.9)**	49.6 (1.8)	41.8 (5.3)	48.6 (2.0)	**46.7 (3.0)**	11.9 (3.4)	4.5 (1.1)	9.9 (1.9)	**8.8 (2.4)**	0.102	**<0.001*****
PP/N	0.7 (0.1)	0.9 (0.1)	0.7 (0.1)	**0.8 (0.1)**	14.1 (2.1)	15.6 (2.3)	16.5 (1.0)	**15.4 (1.9)**	1.4 (0.4)	1.5 (0.3)	1.8 (1.0)	**1.6 (0.5)**	0.686	**<0.001*****
(L+PP)/N	8.7 (1.7)	11.7 (3.1)	14.4 (3.7)	**11.6 (3.2)**	63.7 (2.6)	57.4 (7.6)	65.1 (2.7)	**62.1 (4.3)**	13.4 (3.3)	5.9 (1.3)	11.7 (1.8)	**10.3 (2.7)**	0.136	**<0.001*****

† This mean gives the aggregate tree effect. Abbreviations: C=carbon, N=nitrogen, P=phosphorous, K=potassium, Ca=calcium, Mg=magnesium, L=lignin, PP=polyphenols. Means in bold are significant different: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3.3 Effects of duration of cultivation and tree species on soil chemical properties

Duration of cultivation and tree species had significant effects on soil chemical properties (Table 3.3). Soil below *E. grandis* was slightly lower in pH with values of 5.9 compared to the other two tree species *C. megalocarpus* and *Z. gilletii*, both with values of 6.2. There was significantly higher C and N in soil after 10 years of cultivation compared 16 and 62 years (Table 3.4). Notably, these elements were higher below the canopy of *C. megalocarpus*, (64.5 g kg^{-1} C; 6.7 g kg^{-1} N), compared to *E. grandis* (58.7 g kg^{-1} C; 5.9 g kg^{-1} N) and *Z. gilletii* (54.5 g kg^{-1} C; 5.6 g kg^{-1} N). The C and N content under the trees in soil after 16 and 62 years of cultivation was generally below 40.0 g kg^{-1} and 4.0 g kg^{-1}, respectively, except under the canopy of *C. megalocarpus* in soil after 16 years of cultivation. Thus due to the lower N content, the soil C/N ratios were relatively higher in the farms with longer duration of cultivation compared to farms with shorter conversion age. Exchangeable Ca and Mg showed a similar trend with the highest values recorded below the canopy of *C. megalocarpus* (4.8 g Ca; 376.9 mg Mg kg^{-1}) and lowest on *Z. gilletii* (3.9 g Ca; 352.3 mg Mg kg^{-1}) in soil after 10 years of cultivation. Available P was significantly different as a function of duration of cultivation but not between tree species. In particular, P was higher in soil after 16 and 62 years (15.8 mg and 15.5 mg kg^{-1} respectively), than 10 years (11.3 mg kg^{-1}) of cultivation. This was contrary to all the other nutrient elements which, on average, were higher shortly after forest conversion and decreased with duration of cultivation. Generally, soil under *E. grandis* had a lower concentration of chemical elements compared to the two indigenous tree species. Projection of differences based on PCA showed significant ($p < 0.01$) separation of *E. grandis* from *C. megalocarpus* and *Z. gilletii* along the second axis of Figure 3.2a. Separation between soils after 10 years of cultivation, which had considerably higher stocks of soil nutrients than after 16 and 62 years of cultivation, was also evident. The first principal component axis thus expressed a significant ($p < 0.001$) gradient in soil degradation (Figure 3.2b).

Table 3.3: *p*-values associated with the soil chemical properties as influenced by duration of cultivation, tree species and tree zone.

Soil chemical parameter	*p*-value					
	Duration of cultivation	**Tree species**	**Tree zone**	**Duration × Species**	**Species × Zone**	**Duration × Species × Zone**
pH (water)	**<0.001*****	**0.050***	0.091	**0.033***	0.091	0.315
Total C	**<0.001*****	0.263	**0.009****	0.292	**<0.001*****	0.136
Total N	**<0.001*****	0.184	**0.009****	0.213	**0.001****	0. 061
C/N ratio	**<0.001*****	**0.027***	0.6107	**<0.001*****	0.8130	0.7854
Available P	**0.011***	0.220	0.452	0.122	0.833	0.404
Exchangeable K	**<0.001*****	**0.042***	0.740	**0.050***	0.910	0.394
Exchangeable Ca	**<0.001*****	**0.033***	0.063	0.102	0.060	0.508
Exchangeable Mg	**<0.001*****	0.374	**0.030***	**0.042***	0.395	0.556

Abbreviations: C=Carbon, N=Nitrogen, P=Phosphorous, K=Potassium, Ca=Calcium, Mg=Magnesium. Values marked in bold are significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3.4: Soil chemical properties (mean and *SE*) as influenced by the duration of cultivation, tree species and tree zone.

Soil parameter	Tree species											
	Croton megalocarpus				***Eucalyptus grandis***				***Zanthoxylum gilletii***			
	Tree zone											
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
10 years of cultivation												
pH (water)	6.6 (0.1)	6.6 (0.0)	6.6 (0.0)	6.1 (0.0)	6.6 (0.1)	6.6 (0.1)	6.4 (0.1)	6.5 (0.0)	6.3 (0.1)	6.3 (0.1)	6.5 (0.1)	5.9 (0.0)
Total C (g kg^{-1})	66.1 (3.1)	65.6 (3.0)	62.2 (4.8)	47.0 (3.7)	55.1 (3.1)	60.6 (5.3)	60.5 (4.4)	61.1 (4.5)	54.5 (2.9)	54.5 (2.5)	54.6 (2.7)	45.4 (3.0)
Total N (g kg^{-1})	7.0 (0.5)	6.8 (0.5)	6.4 (0.5)	5.8 (0.3)	5.5 (0.4)	6.0 (0.5)	6.2 (0.5)	6.3 (0.5)	5.5 (0.3)	5.7 (0.3)	5.6 (0.3)	5.1 (0.3)
C/N ratio	9.5 (0.4)	9.7 (0.4)	9.7 (0.2)	8.1 (0.3)	10.2 (0.4)	10.2 (0.3)	9.8 (0.2)	9.7 (0.2)	9.8 (0.2)	9.6 (0.2)	9.8 (0.1)	8.9 (0.2)
Av. P (mg kg^{-1})	11.9 (1.4)	10.9 (0.4)	10.8 (0.5)	11.0 (0.7)	13.9 (2.1)	13.2 (2.6)	12.4 (0.8)	10.7 (0.9)	10.5 (0.5)	10.2 (0.2)	10.8 (0.3)	9.2 (0.9)
Exc. K (mg kg^{-1})	455.4 (9.1)	446.4 (9.8)	437.8 (8.0)	455.7 (14.9)	472.1 (23.4)	446.8 (55.9)	455.9 (11.5)	451.4 (15.8)	433.4 (51.2)	438.6 (39.8)	443.0 (41.5)	417.9 (26.0)
Exc. Ca (g kg^{-1})	4.9 (0.2)	4.9 (0.2)	4.6 (0.3)	4.3 (0.3)	4.0 (0.3)	4.4 (0.4)	4.4 (0.3)	4.4 (0.2)	3.8 (0.4)	3.9 (0.3)	3.9 (0.3)	3.2 (0.2)
Exc. Mg (mg kg^{-1})	387.2 (0.0)	371.0 (7.2)	372.4 (7.2)	356.7 (5.6)	351.0 (7.2)	346.1 (2.8)	367.0 (9.6)	359.8 (7.4)	356.4 (6.8)	353.3 (8.3)	347.3 (2.4)	345.8 (5.4)
16 years of cultivation												
pH (water)	6.2 (0.3)	6.1 (0.2)	6.1 (0.1)	5.2 (0.0)	5.4 (0.0)	5.4 (0.0)	5.4 (0.0)	5.5 (0.1)	6.0 (0.4)	6.0 (0.4)	5.8 (0.3)	5.8 (0.3)
Total C (g kg^{-1})	48.9 (5.7)	48.4 (0.9)	39.9 (2.2)	36.1 (4.2)	38.8 (2.5)	38.3 (2.8)	39.6 (3.5)	38.1 (2.9)	33.0 (3.9)	32.0 (4.5)	32.2 (4.9)	30.7 (2.5)
Total N (g kg^{-1})	5.0 (0.6)	4.9 (0.2)	3.9 (0.3)	3.6 (0.4)	3.5 (0.1)	3.5 (0.2)	3.7 (0.4)	3.5 (0.3)	2.9 (0.4)	2.7 (0.5)	2.9 (0.5)	2.1 (0.2)
C/N ratio	9.8 (0.5)	9.8 (0.5)	10.2 (0.9)	10.0 (0.3)	11.0 (0.7)	10.9 (0.5)	10.8 (0.3)	11.0 (0.2)	11.3 (0.3)	11.9 (1.0)	11.3 (1.0)	14.6 (0.7)
Av. P (mg kg^{-1})	20.8 (1.4)	18.3 (1.2)	16.7 (1.3)	14.8 (0.9)	15.3 (4.0)	16.0 (3.7)	15.6 (4.1)	17.2 (3.3)	14.5 (2.5)	15.4 (3.8)	11.4 (1.9)	13.3 (1.1)
Exc. K (mg kg^{-1})	385.6 (9.4)	331.4 (30.7)	265.4 (60.4)	305.1 (41.4)	198.7 (41.7)	214.9 (35.9)	205.7 (20.6)	240.9 (28.4)	386.1 (39.3)	391.2 (82.1)	337.9 (20.9)	338.5 (26.9)
Exc. Ca (g kg^{-1})	3.5 (0.4)	3.4 (0.2)	2.5 (0.4)	2.7 (0.2)	1.6 (0.1)	1.6 (0.0)	1.8 (0.1)	1.8 (0.1)	1.9 (0.4)	1.8 (0.5)	1.8 (0.5)	1.7 (0.1)
Exc. Mg (mg kg^{-1})	325.7 (6.5)	327.5 (5.4)	289.1 (8.0)	297.2 (24.7)	203.8 (23.3)	213.2 (15.8)	212.3 (19.1)	222.7 (31.5)	276.5 (39.3)	285.5 (59.2)	246.0 (24.1)	225.4 (22.0)
62 years of cultivation												
pH (water)	5.9 (0.2)	5.9 (0.2)	5.8 (0.2)	5.8 (0.2)	5.8 (0.2)	5.9 (0.1)	5.8 (0.2)	5.8 (0.2)	6.3 (0.1)	6.3 (0.0)	6.3 (0.1)	5.6 (0.1)
Total C (g kg^{-1})	31.4 (7.0)	31.6 (6.8)	31.9 (6.7)	26.3 (5.4)	40.1 (1.7)	37.2 (0.1)	38.6 (1.2)	36.4 (1.0)	36.1 (5.4)	35.8 (4.7)	36.7 (5.0)	35.2 (3.3)
Total N (g kg^{-1})	2.8 (0.8)	2.8 (0.7)	2.8 (0.7)	2.7 (0.6)	3.6 (0.2)	3.2 (0.2)	3.3 (0.2)	3.1 (0.1)	3.3 (0.6)	3.2 (0.6)	3.4 (0.7)	2.1 (0.4)
C/N ratio	11.5 (0.9)	11.5 (0.8)	11.5 (0.9)	9.7 (0.9)	11.1 (0.6)	11.7 (0.5)	11.6 (0.4)	11.6 (0.2)	11.0 (0.6)	11.3 (1.3)	11.2 (1.3)	16.8 (0.4)
Avail. P (mg kg^{-1})	18.1 (3.8)	19.9 (2.3)	19.7 (2.2)	13.2 (2.6)	12.7 (0.7)	10.7 (1.2)	12.3 (1.2)	12.3 (1.2)	16.7 (2.6)	18.5 (1.0)	19.2 (3.8)	12.4 (2.2)
Exc. K (mg kg^{-1})	317.9 (9.3)	305.7 (8.1)	306.5 (6.4)	318.5 (74.4)	361.9 (33.0)	376.8 (5.0)	348.1 (21.2)	348.1 (21.2)	455.6 (4.3)	443.5 (4.7)	435.5 (37.2)	423.8 (18.5)
Exc. Ca (g kg^{-1})	2.1 (0.6)	2.1 (0.7)	2.0 (0.6)	1.7 (0.4)	1.9 (0.1)	1.8 (0.1)	1.6 (0.7)	1.6 (0.7)	2.2 (0.3)	2.4 (0.2)	2.5 (0.5)	2.1 (0.2)
Exc. Mg (mg kg^{-1})	255.1 (6.7)	238.1 (9.2)	236.8 (9.7)	235.2 (36.6)	299.5 (17.6)	287.0 (20.7)	267.9 (15.3)	267.9 (15.3)	297.4 (27.6)	300.3 (24.9)	308.4 (21.4)	247.0 (24.5)

Abbreviations: C=Carbon, N=Nitrogen, P=Phosphorous, K=Potassium, Ca=Calcium, Mg=Magnesium. Avail. = available; Exc. = exchangeable

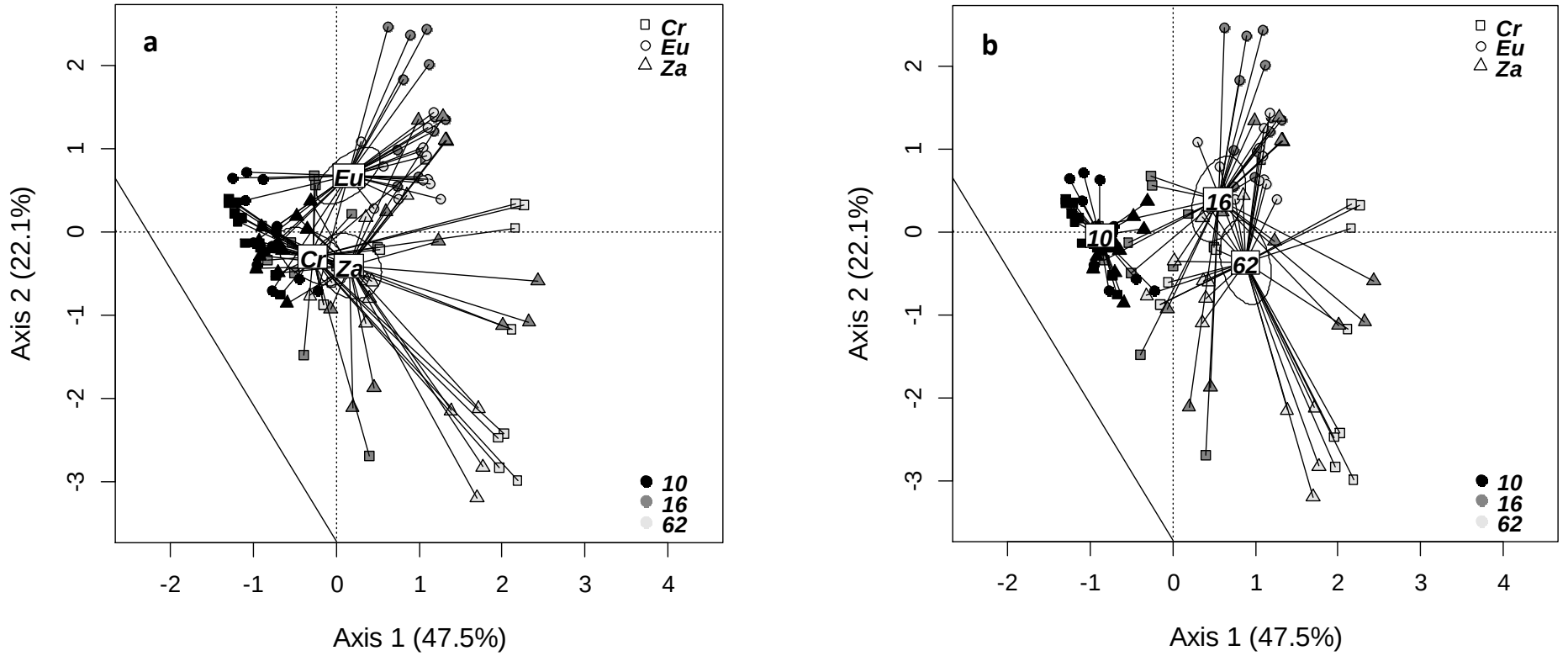

Figure 3.2: Projection of soil chemical parameters sampling points along the two principal component (PC) axes using the *ordiellipse* and *ordispider* functions in package Vegan. The ellipses are standard errors, while the letters indicate the location of centroids for each (a) tree species and (b) duration of cultivation. Abbreviations; Cr = *Croton megalocarpus*, Eu = *Eucalyptus grandis*, Za = *Zanthoxylum gilletii*. The numbers 10, 16 and 62 represent the years of cultivation. $p < 0.001$ for both tree species and duration of cultivation; Monte Carlo permutation test is based on 999 random permutations.

3.3.4 Effects of duration of cultivation and tree species on soil macrofauna abundance

Ten soil macrofauna groups were identified across the study area, but four of these; earthworms, beetles, ants and termites, were the dominant groups. Generally, the abundance of soil macrofauna was influenced differently by tree species (Table 3.5). Though there was evidence of tree species effects on earthworms abundance, this was dependent on duration of cultivation as shown by the interactions of the two factors. For instance, a significantly high number of earthworms was found below the canopy of *Z. gilletii* in the farms after 16 and 62 years of cultivation with an average of 389 individuals m^{-2} and 160 individuals m^{-2} respectively, compared to only 14 individuals m^{-2} in the farms after 10 years of cultivation under the same tree species (Table 3.6). These values represented 40%, 16% and 1% of the total earthworm counts beneath tree canopies, respectively. The number of earthworms associated with *E. grandis* and *C. megalocarpus* followed a similar trend to that of *Z. gilletii*, but with lower abundances. Beetles showed a contrasting trend to that of earthworms with higher numbers associated with *E. grandis* and *C. megalocarpus* and lower below the canopy of *Z. gilletii*. However, unlike earthworms, duration of cultivation had no significant influence on beetles. An exceptionally high number of termites was found to be associated with *E. grandis* after 16 years of cultivation with an average of 82 individuals m^{-2}, representing about 38% of the total termite counts. Centipedes were significantly higher below the canopy of *E. grandis* with an average of 11 individuals m^{-2} constituting 56% of total centipede counts, compared to an average of 4 individuals m^{-2} recorded below the canopy of the other two tree species, *C. megalocarpus* and *Z. gilletii*. Based on the duration of cultivation, the abundance of centipedes was higher in soils after 16 years of cultivation than after 10 or 62 years of cultivation. Ants showed a similar trend to that of termites and centipedes, except that soils after 10 years of cultivation also showed relatively high numbers associated with *E. grandis*. Higher spider numbers were found to be associated with *C. megalocarpus* in soils after 16 years of cultivation

with an average of 8 individuals m^{-2}, constituting 28% of the total spider counts beneath the trees. Generally, soil macrofauna under *Z. gilletii* showed significant separation ($p < 0.01$) from *E. grandis* and *C. megalocarpus* as shown by PCA along the first axis (Figure 3.3a). A clear separation was also observed along the first principal component axis ($p < 0.001$) between soils after 10 years of cultivation and those with greater duration of cultivation (Figure 3.3b).

Table 3.5: Summary of *p*-values associated with the soil macrofauna abundance as influenced by the duration of cultivation, tree species and tree zone.

Soil macrofauna group	***p*-value**					
	Duration of cultivation	**Tree species**	**Tree zone**	**Duration × Species**	**Species × Zone**	**Duration × Species × Zone**
Ants	0.122	**0.008****	0.555	0.474	0.088	1.000
Beetles	0.176	**0.017***	**0.031***	0.779	0.298	0.307
Centipedes	**0.013***	**0.006****	0.062	0.078	0.965	0.365
Earthworms	**<0.001*****	0.149	0.083	**<0.001*****	0.299	0.130
Millipedes	0.072	0.110	0.805	0.226	0.482	1.000
Spiders	0.792	0.309	0.911	**0.019***	**<0.001*****	1.000
Termites	0.836	0.804	0.196	**0.003****	0.859	0.339
Other soil macrofauna	0.564	0.805	0.469	0.931	0.205	0.319

Table 3.6: Soil macrofauna abundance (mean individuals m^{-2} ± SE) as influenced by the duration of cultivation, tree species and tree zone.

Macrofauna group	Tree species											
	Croton megalocarpus				*Eucalyptus grandis*				*Zanthoxylum gilletii*			
	Tree zone											
	A	B	C	D	A	B	C	D	A	B	C	D
10 years of cultivation												
Ants	8.0 (4.6)	33.3 (21.8)	13.3 (6.2)	17.3 (14.5)	16.0 (7.9)	9.3 (3.1)	94.7 (73.5)	33.3 (17.1)	10.7 (5.3)	10.7 (10.7)	9.3 (8.0)	4.0 (2.1)
Beetles	26.7 (5.7)	34.7 (9.6)	34.7 (11.8)	22.7 (5.7)	33.3 (13.4)	28.0 (6.6)	41.3 (10.1)	38.7 (17.2)	20.0 (4.9)	20.0 (5.6)	26.7 (7.7)	12.0 (5.3)
Centipedes	0.0	1.3 (1.3)	5.3 (2.3)	2.7 (1.8)	4.0 (2.1)	10.7 (6.9)	6.7 (2.4)	4.0 (2.9)	1.0 (1.0)	1.3 (1.3)	0.0	0.1 (0.1)
Earthworms	8.0 (4.2)	30.7 (10.9)	29.3 (14.5)	10.7 (4.1)	26.7 (8.7)	36.0 (18.6)	26.7 (8.2)	24.0 (6.4)	8.0 (3.7)	20.0 (7.1)	13.3 (4.8)	4.0 (2.9)
Millipedes	0.0	0.0	14.7(13.3)	0.0	2.7 (2.7)	1.3 (1.3)	0.0	2.7 (2.7)	0.0	1.3(1.3)	0.0	0.0
Spiders	0 .0	0.0	5.3(2.3)	0.0	1.3 (1.3)	1.3 (1.3)	2.7 (2.7)	1.3 (1.0)	0.0	4.0 (2.9)	4.0 (2.9)	1.3 (1.0)
Termites	58.7 (54.4)	26.7 (26.7)	2.7 (1.8)	24.0 (13.1)	22.7 (19.8)	8.0 (5.4)	1.3 (1.3)	2.7 (1.8)	6.7 (6.7)	44.0 (37.1)	0.0	12.0 (8.2)
Other soil macrofauna	4.0 (3.1)	0.1 (0.1)	6.7 (3.1)	2.7 (1.8)	0.0	4.0 (2.1)	5.3 (4.1)	1.3 (0.5)	4.0 (2.9)	8.0 (4.2)	9.3 (4.2)	4.7 (2.0)
16 years of cultivation												
Ants	4.0 (2.9)	46.7 (30.1)	36.0 (11.3)	36.0 (21.9)	96.0 (49.6)	2.7 (2.7)	32.0 (22.1)	48.0 (35.2)	10.7 (6.6)	13.3 (8.1)	17.3 (7.7)	6.7 (5.4)
Beetles	42.7 (7.2)	48.0 (11.3)	72.0 (18.9)	26.7 (6.0)	73.3 (14.0)	45.3 (10.6)	49.3 (16.5)	37.3 (8.4)	20.0 (5.3)	52.0 (20.5)	24.0 (7.0)	20.0 (4.0)
Centipedes	4.0 (4.0)	9.3 (4.6)	4.0 (2.9)	2.7 (1.8)	24.0 (9.3)	10.7 (3.6)	22.7 (9.5)	14.7 (4.6)	10.7 (3.6)	10.7 (6.9)	8.0 (3.1)	6.7 (3.1)
Earthworms	62.7 (26.1)	42.7 (12.0)	26.7 (7.5)	42.7 (12.0)	85.3 (29.0)	92.0 (20.4)	89.3 (15.3)	68.0 (20.9)	381.3 (59.6)	370.7 (91.8)	414.7 (72.8)	186.7 (41.5)
Millipedes	2.7 (1.8)	0.0	4.0 (2.1)	4.0 (2.9)	1.3 (1.3)	0.0	1.3 (1.3)	5.3 (4.1)	5.3 (3.0)	4.0 (2.9)	2.7 (2.7)	1.3 (1.3)
Spiders	2.7 (1.8)	9.3 (5.4)	12.0 (5.3)	1.3 (1.3)	0.0	0 .0	4.0 (2.3)	2.7 (1.8)	1.3 (1.3)	0.0	2.7 (1.8)	0.0
Termites	0.0	2.7 (1.8)	2.7 (2.7)	0.0	197.3 (117.2)	20.0 (11.2)	29.3 (20.7)	65.3 (32.1)	18.7 (6.8)	58.9 (24.8)	34.7 (24.8)	0.1 (0.1)
Other soil macrofauna	5.3 (3.0)	13.3 (5.9)	16.0 (7.1)	2.7 (1.8)	5.3 (2.3)	1.3 (1.3)	5.3 (2.3)	1.3 (1.0)	6.7 (2.4)	5.3 (2.3)	2.0 (1.0)	1.3 (1.0)
62 years of cultivation												
Ants	2.7 (1.8)	2.7 (1.8)	33.3 (20.5)	21.3 (9.3)	8.0 (4.6)	0.0	30.7 (17.4)	6.7 (5.4)	25.3 (21.3)	0.0	6.7 (6.7)	6.7 (3.1)
Beetles	57.3 (17.1)	34.7 (6.5)	49.3 (10.9)	32.0 (8.4)	41.3 (13.7)	24.0 (4.6)	33.3 (13.7)	38.7 (10.9)	24.0 (5.4)	20.0 (5.9)	36.0 (9.5)	33.3 (7.2)
Centipedes	2.7 (2.7)	4.0 (2.1)	5.3 (2.3)	4.0 (3.7)	5.3 (3.0)	6.7 (3.1)	4.0 (2.1)	2.7 (2.7)	5.3 (2.3)	1.3 (1.3)	1.3 (1.3)	0.0
Earthworms	222.7 (99.0)	93.3 (24.7)	98.7 (32.7)	161.3 (32.9)	66.7 (18.4)	92.0 (25.2)	122.7 (19.4)	94.7 (32.4)	150.7 (57.5)	161.3 (24.0)	166.7 (43.2)	164.0 (46.2)
Millipedes	6.7 (3.1)	5.3 (3.6)	9.3 (4.6)	9.3 (3.7)	4.0 (2.9)	4.0 (2.9)	4.0 (2.1)	1.3 (1.3)	2.7 (1.8)	4.0 (2.9)	2.7 (2.7)	1.3 (1.3)
Spiders	2.7 (1.8)	1.3 (1.3)	6.7 (3.1)	1.3 (1.3)	1.3 (1.3)	0.0	2.7 (1.8)	0.0	1.3 (1.3)	2.7 (1.8)	0.0	4.0 (2.1)
Termites	8.0 (4.6)	10.7 (10.7)	68.0 (36.0)	25.3 (17.3)	20.0 (20.0)	1.3 (1.3)	12.0 (5.6)	12.0 (10.6)	1.3 (1.3)	0.0	9.3 (9.3)	5.3 (4.1)
Other soil macrofauna	4.0 (2.1)	5.3 (2.3)	13.3 (5.5)	2.0 (0.1)	1.3 (1.3)	5.3 (3.0)	5.3 (3.0)	1.3 (1.3)	2.7 (1.8)	10.7 (4.1)	2.7 (1.8)	2.3 (1.3)

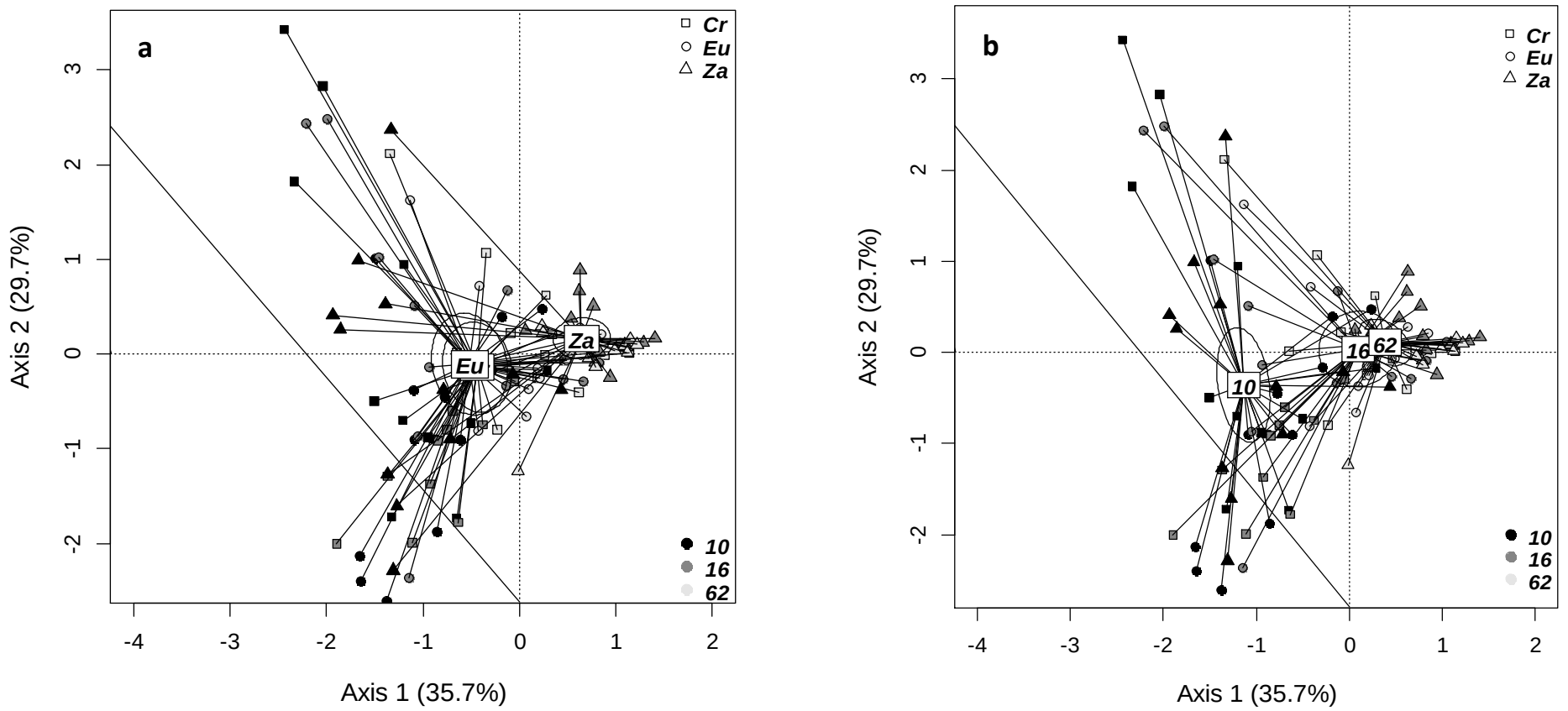

Figure 3.3: Projection of soil macrofauna sampling points along the two principal component (PC) axes using the *ordiellipse* and *ordispider* functions in package Vegan. The ellipses are standard errors, while the letters indicate the location of centroids for each (a) tree species and (b) duration of cultivation. Abbreviations; Cr = *Croton megalocarpus*, Eu = *Eucalyptus grandis*, Za = *Zanthoxylum gilletii*. The numbers 10, 16 and 62 represent the years of cultivation. $p < 0.01$ (tree species) and $p < 0.001$ (duration of cultivation); Monte Carlo permutation test is based on 999 random permutations).

3.3.5 Effects of duration of cultivation and tree species on soil macrofauna biomass

Except for a few soil macrofauna groups, the biomass was not significantly affected by tree species, duration of cultivation or the zone of sampling (Table 3.7). Earthworm biomass was greatest in soils after 16 years of cultivation (20.4 g m^{-2}), compared to 10 years (17.1 g m^{-2}) and 16 years (16.4 g m^{-2}) (Table 3.8). Tree species played a significant role in determining the biomass of termites only, but this occurred at specific time under cultivation. For instance, an average of 4.1 g m^{-2} or 36% of the total termite biomass, was associated with *E. grandis* in soils after 16 years of cultivation. Of all the soil macrofauna groups, only biomass of spiders showed significant differences between tree zones. Higher biomass values were found below *E. grandis* and *C. megalocarpus* in soils after 62 years of cultivation, while *E. grandis* and *Z. gilletii* in soils after 10 years of cultivation showed greater biomass away from the trees.

Table 3.7: Summary of *p*-values from the two-part analysis associated with soil macrofauna biomass from the best-fitting models.

Soil macrofauna group	***p*-value**					
	Duration of cultivation	**Tree Species**	**Tree zone**	**Duration × Tree**	**Tree × Zone**	**Duration × Tree × Zone**
Presence/absence analysis						
Ants	**0.006****	**0.012***	0.951	0.879	**0.009****	1.000
Beetles	0.108	0.167	**0.036***	0.928	0. 7845	**0.048***
Centipedes	**0.021***	**0.039***	0.101	0.067	0.680	0.348
Earthworms	**<0.001*****	0.349	0.437	**<0.001*****	0. 222	0. 506
Millipedes	**0.002****	0.203	0.930	0.410	0. 498	0.421
Spiders	0.961	0.330	0.803	0.071	**<0.001*****	1.000
Termites	0.189	0.240	0.417	**0.023***	0. 425	**0.034***
Other soil macrofauna	0.645	0.808	0.425	0.944	**0.043***	0.973
Non-zero values						
Ants	0.669	0.122	0.337	0.402	0.263	0.230
Beetles	0.973	0.736	0.994	0.260	0.528	0.135
Centipedes	0.465	0.951	0.054	0.236	0.562	0.699
Earthworms	**<0.001*****	0.720	0.103	0.222	0.558	0.185
Millipedes	0.220	0.641	0.699	0.906	0.321	0.223
Spiders	0.291	0.700	**0.044***	0.644	0.177	1.000
Termites	0.156	0.220	0.403	**0.033***	0.542	1.000
Other soil macrofauna	0.067	0.962	**0.018***	0.144	0.478	1.000

Table 3.8: Soil macrofauna biomass (g m^{-2}) identified across the three catchments (mean ±SE) as influenced by the tree species and tree zones.

Soil macrofauna group	Tree species											
	Croton megalocarpus				*Eucalyptus grandis*				*Zanthoxylum gilletii*			
	Tree zone											
	A	B	C	D	A	B	C	D	A	B	C	D
10 years of cultivation												
Ants	0.9 (0.5)	1.0 (0.5)	1.4 (0.7)	0.6 (0.4)	0.3 (0.2)	2.2 (0.8)	4.5 (1.6)	4.8 (3.0)	0.3 (0.2)	0.2 (0.2)	1.7 (0.8)	1.5 (0.8)
Beetles	43.8 (18.4)	37.1 (17.7)	17.7 (11.7)	9.5 (3.2)	38.1 (11.2)	9.5 (2.4)	22.1 (8.5)	18.9 (7.5)	46.6 (20.4)	35.5 (15.7)	39.1 (17.4)	47.5 (17.8)
Centipedes	0.0	0.1 (0.1)	5.5 (5.3)	0.1 (0.1)	0.1 (0.1)	0.3 (0.2)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.0	0.1 (0.1)
Earthworms	5.6 (4.2)	6.2 (2.7)	5.8 (3.8)	2.9 (1.3)	12.3 (6.7)	13.8 (6.2)	24.3 (16.8)	10.8 (4.2)	44.3 (14.1)	14.1 (6.3)	26.6 (9.4)	38.2 (11.8)
Millipedes	0.0	0.0	2.4 (1.6)	0.0	8.2 (8.2)	10.5 (5.8)	0.0	0.5 (0.4)	0.0	13.0 (11.3)	0.0	0.0
Spiders	0.0	0.0	0.5 (0.2)	0.0	0.2 (0.1)	0.9 (0.5)	1.9 (1.6)	0.8 (0.8)	0.0	3.0 (0.3)	1.8 (1.2)	0.2 (0.2)
Termites	4.3 (4.0)	1.8 (1.8)	0.2 (0.2)	1.7 (1.1)	0.1 (0.1)	0.8 (0.7)	0.1 (0.1)	0.1 (0.1)	0.4 (0.3)	1.2 (1.2)	0.0	2.5 (2.0)
Other soil macrofauna	0.1 (0.1)	0.2 (0.2)	0.5 (0.2)	16.3 (16.2)	0.0	2.9 (1.9)	2.1 (1.6)	0.9 (0.8)	0.2 (0.1)	0.9 (0.5)	3.6 (1.8)	2.2 (1.8)
16 years of cultivation												
Ants	6.2 (3.2)	0.1 (0.1)	5.3 (4.4)	2.4 (1.2)	1.1 (0.4)	0.7 (0.3)	5.6 (2.9)	3.2 (1.6)	0.5 (0.2)	0.1 (0.1)	1.3 (0.5)	0.9 (0.8)
Beetles	34.0 (20.0)	23.7 (10.4)	22.8 (11.8)	72.3 (40.3)	43.1 (12.2)	59.9 (25.6)	50.4 (21.0)	26.6 (11.1)	54.0 (22.9)	8.3 (3.9)	40.2 (17.7)	28.1 (20.1)
Centipedes	0.2 (0.1)	0.3 (0.2)	0.5 (0.2)	0.2 (0.2)	0.6 (0.3)	0.3 (0.1)	0.9 (0.3)	0.4 (0.2)	0.3 (0.2)	1.1 (0.7)	0.2 (0.1)	0.1 (0.1)
Earthworms	5.0 (2.2)	1.3 (0.6)	13.0 (8.9)	23.1 (11.1)	41.5 (12.4)	20.8 (5.5)	17.1 (5.7)	29.8 (11.5)	16.4 (6.3)	19.9 (5.4)	22.6 (9.6)	34.4 (23.1)
Millipedes	3.0 (3.0)	0.0	1.8 (1.8)	2.9 (2.9)	0.1 (0.1)	0.0	0.9 (0.9)	1.6 (1.5)	1.6 (1.4)	2.1 (1.9)	9.1 (9.0)	0.3 (0.3)
Spiders	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.9 (0.6)	0.0	0.0	0.2 (0.1)	0.3 (0.2)	1.6 (1.6)	0.0	0.1 (0.1)	0.0
Termites	0.0	0.8 (0.5)	0.1 (0.1)	0.0	9.9 (6.2)	1.0 (0.5)	1.3 (1.0)	3.5 (1.6)	0.4 (0.4)	0.1 (0.1)	0.4 (0.3)	0.1 (0.1)
Other soil macrofauna	0.1 (0.1)	1.6 (1.4)	1.9 (1.9)	0.9 (0.6)	0.7 (0.4)	1.1 (1.1)	1.2 (1.0)	1.6 (1.1)	0.2 (0.2)	5.3 (3.2)	0.3 (0.2)	0.2 (0.2)
62 years of cultivation												
Ants	0.6 (0.3)	0.2 (0.2)	1.3 (1.1)	0.6 (0.3)	0.6 (0.4)	0.2 (0.1)	1.7(0.8)	1.1 (0.6)	0.7 (0.5)	0.0	0.1 (0.1)	0.5 (0.3)
Beetles	14.0 (4.3)	22.7 (10.4)	23.0 (11.5)	32.8 (20.0)	20.5 (11.8)	12.5 (3.5)	20.1 (10.6)	11.2 (7.0)	24.1 (11.5)	18.5 (8.6)	24.4 (11.5)	24.1 (11.3)
Centipedes	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	1.6 (0.9)	0.3 (0.2)	0.5 (0.2)	0.2(0.1)	0.4 (0.2)	0.1 (0.1)	0.1 (0.1)	0.0
Earthworms	1.0 (0.5)	4.0 (2.5)	8.2 (5.0)	1.0 (0.7)	29.7(8.1)	22.0 (5.7)	31.4 (8.7)	18.4 (4.0)	11.2 (3.5)	20.1 (7.3)	19.9 (5.2)	29.9 (8.7)
Millipedes	0.1 (0.1)	3.5 (3.5)	0.4 (0.1)	0.2 (0.1)	9.2 (7.9)	2.6 (2.5)	1.9 (0.2)	0.1 (0.1)	3.0 (2.7)	1.9 (1.8)	3.2 (3.2)	0.3 (0.3)
Spiders	0.1 (0.1)	1.1 (1.0)	0.1 (0.1)	1.9 (0.9)	0.1 (0.1)	0.0	0.1 (0.1)	0.0	0.7 (0.6)	0.8 (0.7)	0.0	0.5 (0.3)
Termites	0.3 (0.3)	3.1 (2.5)	0.2 (0.1)	0.9 (0.7)	0.4 (0.1)	1.7 (0.9)	1.5 (1.2)	0.1 (0.1)	0.2 (0.2)	0.0	0.2 (0.2)	0.1 (0.1)
Other soil macrofauna	2.7 (2.6)	1.2 (1.0)	1.1 (0.5)	5.0 (1.9)	0.2 (0.1)	4.6 (3.4)	1.0 (0.6)	0.3 (0.2)	0.7 (0.6)	2.2 (1.1)	0.4 (0.3)	2.0 (1.5)

3.3.6 Correlation of tree litter/root quality parameters and soil macrofauna abundance

Earthworms, centipedes and termites showed significant correlation with litter quality parameters (Table 3.9). Earthworm abundance correlated negatively with litter K, lignin and C/P, while correlation with P was significantly positive. Centipedes on the other hand were significantly correlated with all the chemical parameters measured (except K and lignin). They were positively correlated with C and plant tissue quality indicators (e.g. C/N, C/P, L/N, PP/N, L+PP/N), but negatively correlated with N, P, Ca and Mg of litter. Like centipedes, termites were also positively and significantly correlated with Mg and all the ratios, but negatively and significantly correlated with N and P. Beetles, millipedes, ants and spiders showed no significant correlation with any of the tree litter quality parameters. Only earthworms and centipedes showed significant response towards root quality parameters (Table 3.9). Earthworm abundance was positively correlated with root C, N, P and K but negatively correlated with lignin. Centipedes were positively correlated with K, Ca, polyphenols and PP/N ratio of roots.

Table 3.9: Pearson correlation matrix between soil macrofauna and selected tree litter and root quality parameters.

Soil macrofauna group	Tree litter quality parameters												
	C	N	P	K	Ca	Mg	C/N	C/P	L	PP	L/N	PP/N	(L+PP)/N
Ants	0.22	0.07	0.15	0.23	0.06	0.13	0.04	0.10	-0.10	-0.10	-0.07	-0.10	-0.10
Beetles	-0.06	-0.02	0.14	0.16	-0.1	-0.15	0.11	0.17	-0.03	-0.10	0.01	-0.04	-0.01
Centipedes	**0.53****	**-0.42***	**-0.43***	-0.32	**-0.48***	**-0.44***	**0.48***	**0.55****	-0.12	**0.48***	**0.42***	**0.51****	**0.46***
Earthworms	-0.26	-0.33	**0.49***	**-0.65****	-0.15	-0.05	-0.07	**-0.48****	**-0.60****	0.03	-0.20	-0.10	0.03
Millipedes	-0.32	0.10	0.17	-0.04	-0.31	0.02	-0.18	-0.24	0.02	-0.13	-0.08	-0.13	-0.13
Spiders	-0.09	0.32	0.28	0.23	-0.29	-0.07	-0.21	-0.05	-0.03	-0.09	-0.09	-0.14	-0.14
Termites	0.01	**-0.68****	**-0.53****	-0.29	0.01	**0.43***	**0.74****	**0.50****	**0.54****	0.18	**0.77****	**0.44***	**0.85****
	Tree roots quality parameters												
Ants	-0.01	-0.29	-0.19	-0.06	0.18	-0.16	0.38	0.27	0.27	0.33	0.36	0.32	0.38
Beetles	0.09	-0.08	0.14	-0.03	0.20	0.14	0.02	-0.17	-0.04	-0.03	-0.01	-0.04	-0.02
Centipedes	0.16	-0.07	-0.01	**0.43***	**0.44***	0.03	0.32	0.17	0.08	**0.42***	0.28	**0.40***	0.32
Earthworms	**0.38***	**0.54****	**0.40***	**0.66****	-0.23	0.15	-0.29	-0.26	**-0.44***	-0.09	-0.32	-0.19	-0.29
Millipedes	0.01	0.19	0.35	0.21	0.11	0.04	-0.13	-0.33	-0.32	-0.23	-0.19	-0.18	-0.19
Spiders	-0.17	0.04	-0.05	-0.26	-0.15	-0.12	-0.20	-0.03	-0.14	-0.34	-0.20	-0.30	-0.23
Termites	0.10	-0.04	0.02	0.22	0.03	0.06	0.19	0.10	-0.04	0.16	0.11	0.22	0.14

Abbreviations: C=carbon, N=nitrogen, P=phosphorous, K=potassium, Ca=calcium, Mg=magnesium, L=lignin, PP=polyphenols. Correlation between variables with values marked in bold are significant. * $p < 0.05$; ** $p < 0.01$.

3.3.7 Correlation between soil macrofauna and selected soil chemical properties

Soil degradation status varied considerably as shown by the redundancy analysis (RDA). The sampling points were aligned along axis 1 which corresponded to the different duration of cultivation, and the separation was significant ($p < 0.001$; Figure 3.4). The axis (45.9% of explained variance) clearly revealed that there was a difference in soil chemical properties amongst soils with different time under cultivation. All the elements entered into the RDA (except available P), were projected on one side along the first axis, therefore revealing a degradation gradient between soils after relatively short-term cultivation and long-term cultivation. Soil macrofauna abundance tended to increase with duration of cultivation and therefore negatively correlated with most soil chemical properties along the first axis. Notably, however, earthworms and millipedes were strongly correlated with available P. On the other hand, correlations between either centipedes or termites with available P were generally weak. The second axis (9.3%) reflected the variability within catchments and/or tree species.

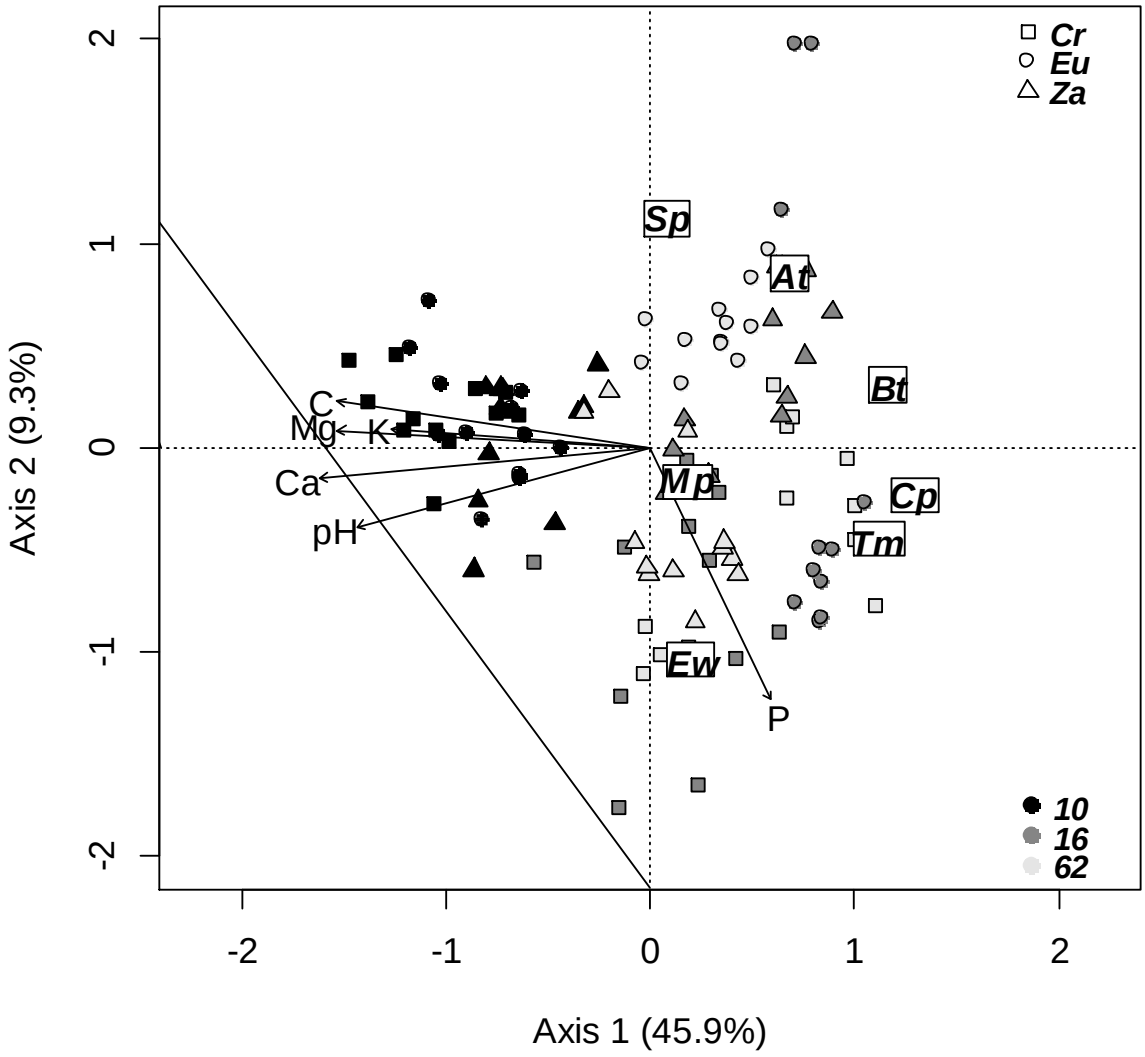

Figure 3.4: A Redundancy Analysis (RDA) biplot showing correlation between soil macrofauna groups and soil chemical properties. Abbreviations; At = Ants, Bt = Beetles, Cp = Centipedes, Ew = Earthworms, Mp = millipedes, Sp = Spiders and Tm = Termites. The numbers 10, 16 and 62 represent the years of cultivation.

3.4 Discussion

3.4.1 Effects of duration of cultivation and tree species on soil chemical properties

There are at least two major mechanisms which could explain the observed higher soil nutrients under the canopy of the trees: i) trees often exploit nutrients from deep layers in the soil profile, or laterally, and redistribute them under the canopy in the form of organic inputs aboveground (litter) and belowground (root turnover) or ii) leguminous trees fix N which goes back to the soil through the first mechanism (Rhoades, 1997; Schroth et al., 2003). In this study, only the first mechanism can explain the nutrient increases below the tree canopy since none of the three species are N-fixing trees. Therefore, the differences in nutrient elements below the canopy of the three tree species could be a reflection of their organic input quality and/or deposition patterns. The higher nutrient contents below the canopy of *C. megalocarpus* and *Z. gilletii* could therefore be attributed to either a higher nutrient content in their litter (as observed in Table 3.1), and/or a higher rate of litter deposition (which was not measured in this study). On the other hand, trees with higher nutrient use efficiency have been reported to produce litter with lower nutrient contents (Aerts and Chapin, 1999). In an early study done by Poggiani (1985), it was reported that *Eucalyptus saligna* Sm. produced litter with lower N and K concentration compared to that of *Pinus caribaea var. hondurensis* (Sénécl) Barr. et Golf. In addition, the author reported that the amount of litter deposited by *E. saligna* was nearly half of that deposited by *P. caribaea*. In this case, the tree had the capacity to reduce nutrient loss through two ways; reabsorption of the nutrients before leaf senescence and reduced shedding of leaves. In another study, Kater et al. (1992) noted that the lower available Ca and K in the upper soil layers under the canopy of *Parkia biglobosa* G.Don compared to *Vitellaria paradoxa* C.F.Gaertn. may be an indication of the high capacity of *P. biglobosa* to absorb and retain scarce soil nutrients. This could suggest that the exotic *E. grandis* has greater capacity to hold nutrients in its biomass than *C. megalocarpus* and Z. *gilletii*. Such outcome emphasises

the importance of native trees as 'resource islands' supporting nutrient cycling in farms where no inputs or only minimal external nutrients are available to farmers.

On the other hand, intensive cultivation with minimal or no external inputs leads to degradation of soil, manifested in form of nutrient depletion, poor soil structure, and low soil biodiversity (Lal, 2009). In this study, soils from farms with greater duration of cultivation were particularly lower in soil nutrients compared to the younger farms. This trend is expected since some of the farms had been cultivated with low, if any nutrient inputs, for over 60 years since conversion from the native forest compared to the younger farms which were barely 10 years under cultivation. These trends are in agreement with those reported by Recha et al. (2013) who had previously worked in the same area. The authors attributed the lower nutrient contents in older farms to losses through crop off-take, as well as microbial mineralisation and leaching losses. It should be noted, however, that since duration of cultivation was not randomly allocated to catchments and there was only one replicate of each, other differences between the catchments instead of, or in addition to, time of cultivation will be implicated in observed differences. Nevertheless, the mechanism described above along with the fact that there are no other striking differences in soils or topography of the catchments provide good evidence that duration of cultivation has a dominant effect. Contrary to other nutrients, available P was higher in the farms with longer duration of cultivation. In their study, Recha et al. (2013) reported that farmers did not apply organic or inorganic fertilisers in the young farms while estimated applications of P fertiliser in soils after 16 and 62 years of cultivation was 2.8 and 4.1 kg ha^{-1} respectively. Despite the small amounts of fertiliser-P applied, the low mobility of P in the soil matrix could have resulted in the accumulation with increasing duration of cultivation. Therefore, in addition to the contribution of trees to the redistribution of soil P, the observed higher soil available P in the older farms could have been influenced by the small, but repeated external P inputs.

3.4.2 Tree effects on soil macrofauna abundance

Trees differ in the quantity and quality of their aboveground and belowground organic inputs, which potentially determines the patterns of influence on soil macrofauna (Korboulewsky et al., 2016). Vohland and Schroth (1999), for instance, reported that the overall faunal abundance were significantly higher under *Bactris gasipaes* Kunth and *Bixa orellana* L. compared to that obtained under *Bertholletia excelsa* Bonpl. and *Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum as a result of differences in plant tissue quality. In this study, it was noted that there was a significant difference between the litter and root tissue quality of native trees *C. megalocarpus* and Z. *gilletii*, and exotic tree *E. grandis*. These two organic inputs, through litter decomposition and root turnover, could have played a key role in shaping the observed differences in the soil macrofauna population abundance below the trees. For instance, it was observed that earthworms were strongly and positively correlated with P of the litter, and N and P of the roots, which were both higher in *C. megalocarpus* and Z. *gilletii* roots compared to that of *E. grandis*. Further, Z. *gilletii* particularly recorded exceptionally higher number of earthworms in soils after 16 years of cultivation which corresponded to the higher N and P content observed in the roots of this tree in that specific catchment. In agreement with these findings, Barrios et al. (2005) reported highest earthworm counts under slash and mulch of *Tithonia diversifolia* (Hemsl.) Gray known to accumulate soil P in plant tissues given its profuse root development and association with native arbuscular mycorrhizal fungi (Sharrock et al., 2004). Furthermore, Mbau et al. (2015) reported that P could have been the main driver of the high number of earthworms recorded in plots treated with filtermud compost. The higher soil macrofauna generally recorded below the canopies of *C. megalocarpus* and Z. *gilletii* could therefore, be associated with the higher quality of litter and root turnover than that of *E. grandis*. Differences found in C/N and (L+PP)/N ratios, frequently used as measures of organic resource quality (Tian et al. 1997; Vanlauwe et al. 2005; Cobo et al. 2002), support the

argument that plant tissue quality can significantly contribute to differences in abundance of soil macrofauna. In this study, the C/N ratio of the litter and fine roots was relatively lower in the litter and roots derived from the two native trees, *C. megalocarpus* and Z. *gilletii*, than that from *E. grandis*. In addition, lignin and polyphenols contents were also lower in the litter and fine roots of the native trees. This suggests that organic inputs derived from native trees would likely be more palatable to some soil macrofauna than those from *E. grandis*. In contrast to other soil macrofauna, termites showed higher abundance under the canopy of *E. grandis*. Termites are known to produce a large variety of enzymes from the associated gut microflora which enables them to digest low quality organic matter (Lavelle, 1997). Due to this diverse preference in food substrates, the quality of organic inputs below tree canopies could therefore be an important determinant of soil macrofauna abundance. Like termites, centipedes were also higher under the canopy of *E. grandis*. Since centipedes are known to be predators, the high numbers under this tree may not be linked directly to the litter or root biomass as substrates, but rather to the presence of prey.

Apart from soil organic matter influence, the differences observed in soil macrofauna in the current study may partly be attributed to tree influence on microclimatic conditions of the soil under its canopy. It has been documented in several studies that trees intercept significant amount of incident solar radiation depending on the size and species (Belsky *et al*., 1989; Lott et al., 2009). In an early study in Tsavo, Kenya, Belsky et al. (1989) reported a reduction in solar irradiance of between 45–65% under *Adansonia digitata* L. and *Acacia tortilis* (Forssk.) Hayne, which led to a 5–12°C lower temperature below the two trees than in the open grassland. Vandenbeldt and Williams (1992) reported that a nearly leafless *Faidherbia albida* (Delile) A.Chev. intercepted almost half of the incoming radiation resulting in a soil temperature decrease of up to 10°C at 0.02 m depth. More recently, Ong et al. (2000) and Lott *et al*. (2009) reported amelioration of soil temperature as a result of shading from *Grevillea*

robusta A.Cunn. while Lin (2010) and De Souza et al. (2012) observed that incorporating trees in coffee farms helped in protecting extreme fluctuations in soil temperature. Such moderation in temperature also reduces the rate of evapotranspiration hence soil moisture content is likely to be higher than in the adjacent open sites. Apart from shading, some trees/shrubs such as *Piliostigma reticulatum* (DC.) Hochst. and *Guiera senegalensis* J. F. Gmel. have also been shown to directly increase moisture of the surface soil by drawing out water from the subsoil through hydraulic redistribution processes (Diedhiou-Sall et al., 2013; Kizito et al., 2012). Though soil moisture and temperature below the tree canopies was not measured, I believe that differences in these two parameters may have also contributed to the observed patterns in soil macrofauna, given the sensitivity of numerous soil organisms to soil moisture and temperature regimes (Pflug and Wolters, 2001; Lindberg et al., 2002; Tsiafouli et al., 2005).

3.4.3 Effects of duration of cultivation on soil macrofauna abundance

Land-use change from natural forest to plantations, pasture or cultivated lands often results in intense and rapid changes in soil that are likely to affect soil macrofauna abundance and distribution patterns (Beare et al., 1997; Giller et al., 1997; Decaëns et al., 2004). The effects are linked to direct induced mortality through physical destruction or loss of food resources (Fragoso et al., 1993; Palm et al., 1996; Blanchart and Julka, 1997) or indirectly through changes in microhabitats resulting from damage of nests and burrows (Ayuke et al., 2011a; Orgiazzi et al., 2016). This is especially notable in cropped lands, perhaps due to the higher levels of intensification and disturbance (Decaëns et al., 2004; Eggleton et al., 2005; Rossi and Blanchart, 2005). Nonetheless, even in intensively cultivated lands, there is usually a re-establishment of soil macrofauna after such disturbances. The re-establishment is, however, largely dependent on the soil macrofauna group in consideration and the soil management practices applied. Agricultural practices which increase soil organic matter inputs therefore,

may be vital in accelerating the rate of soil macrofauna survival and re-establishment. The observed greater soil macrofauna abundance with increasing time under cultivation supports the increasing importance of trees as 'resource islands' (Liu et al., 2011; Dossa et al., 2013). Furthermore, Pauli et al. (2010) and Diedhiou-Sall et al. (2013) also found greater soil biological activity beneath trees with increasing soil resource and environmental limitations in Central America and the Sahel respectively, thus supporting the role of trees in contributing to greater functional resilience in agro-ecosystems (Barrios et al., 2015). In the study area, it was evident that farms on soils after 62 years of cultivation had more trees incorporated as live fences or hedgerows to delineate farm boundaries, intercropped with annual crops or as small pockets of woodlots. This is a common practice in smallholder farms in Kenya as the farm fragmentation increases as highlighted by Nyaga et al. (2015). On the farms after 10 years of cultivation, however, the plant cover was predominantly annual crops (maize and beans) including only a few sparse trees within the farms. The increased importance of trees could therefore be one of the contributors to the observed impacts of duration of cultivation on soil macrofauna abundance. Numerous studies have also reported similar outcomes. For instance, Mathieu et al. (2005) reported that the species richness of soil macrofauna fell from an initial 76 species in the primary forest to 30 in deforested plots under rice crop. However, the authors noted that soil macrofauna re-established based on the land-use type, with the higher recovery being observed on old fallow plots. The higher population in the fallow plots, they noted, could have been as a result of the higher litter retention and creation of microclimatic conditions that resemble more closely those in the forests. This shows that following soil disturbance in the form of cultivation, soil macrofauna may generally re-establish where the management options provide them with better living conditions. Furthermore, agroforestry practices used to restore degraded and eroded soils safeguard the already accumulated soil organic matter, and enhance the availability of vital food resource to a large number of soil-dwelling organisms (Barrios et

al., 2005). This could therefore have indirectly favoured soil macrofauna proliferation on the older farms compared to the younger ones. However, some soil macrofauna may be more sensitive to disturbance and changes in soil, and still are negatively affected by agriculture. This could partly explain why, in this study, some soil macrofauna such as millipedes, spiders, crickets, cockroaches and earwigs occurred in low numbers. Most of these occurred either below the tree canopy or in the farms with greater duration of cultivation, perhaps attracted by better microclimatic conditions under the trees or improved soil conditions, respectively. Therefore, introduction of trees is likely to play a major role in shaping spatial patterns of soil macrofauna distribution and abundance.

3.5 Conclusions

Land-use change from natural forest to cultivated lands often results in soil nutrient losses that are likely to negatively affect soil macrofauna. As hypothesised, soil nutrient content decreased with increasing distance from the tree stem. In addition, the nutrient stocks decreased with increasing duration of cultivation, but the magnitude of differences were moderated by the specific tree species. However, soil macrofauna responded differently to soil degradation and tree species. Generally, higher soil macrofauna were found in farms with longer duration of cultivation under specific trees, thus highlighting significance of these trees as 'resource islands' in such degraded soils. The quality of tree organic inputs also showed a dominant effect on soil macrofauna abundance and spatial distribution. Thus, increasing diversity of tree species in agroecosystems can play a major role in enhancing soil biodiversity.

CHAPTER FOUR

Soil aggregation and carbon dynamics along a soil degradation gradient as affected by dominant tree species

Authors: Solomon Kamau[1, 2*], Edmundo Barrios[2], Nancy K. Karanja[1], Fredrick O. Ayuke[1], Johannes Lehmann[3, 4]

[1] Department of Land Resource Management and Agricultural Technology, College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053-00625, Nairobi.

[2] World Agroforestry Centre, United Nations Avenue, P.O. Box 30677-00100, Nairobi.

[3] Soil and Crop Science, Cornell University, Ithaca, NY 14853, USA.

[4] Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14853, USA.

[*]Corresponding author:

E-mail address: solkam08@gmail.com (S. Kamau)

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Abstract

Soil organic matter (SOM) and soil structure have been proposed as key indicators of soil quality and thus critical components in defining sustainable land uses. In particular, SOM is considered an important determinant of soil fertility in tropical agroecosystems, and its loss has been shown to have significant negative effects on soil productivity. Though numerous studies have shown the value of agroforestry in improving crop yield and soil nutrients, very few have addressed spatial influence of trees on soil aggregation and C storage along a soil degradation gradient, especially in sub-Saharan Africa. A study was conducted in South Nandi (Kenya) to assess spatial influence of three dominant trees; *Croton megalocarpus*, *Eucalyptus grandis* and *Zanthoxylum gilletii*, on soil aggregation and C storage. The study was conducted in a chronosequence experimental set-up where farms were continuously cultivated for 10, 16 and 62 years since conversion from primary forest. It was hypothesised that soil aggregate stability and C storage, would be affected by duration of cultivation, with the magnitude of influence being moderated by the tree attributes and abundance of earthworms and termites below the tree canopies. Higher small macro-aggregates and micro-aggregate were recorded under the canopy of *Z. gilletii* with an average weight of 61.9 g and 9.4 g 100 g^{-1} soil compared to 53.1 g and 3.1 g 100 g^{-1} soil under *C. megalocarpus* and 49.0 g and 3.8 g 100 g^{-1} soil under *E. grandis*, respectively. Notably, the weight of these aggregate fractions generally increased with duration of cultivation. The other fractions were significantly higher under *C. megalocarpus* and *E. grandis* than *Z. gilletii*. The C content decreased by more than 50% along degradation gradient in all aggregate fractions. The highest magnitude of differences were observed under the canopy of *Z. gilletii*. This study shows the importance of specific trees in shaping soil aggregate stability and C dynamics which could have far reaching implications on the long-term C storage and SOM stabilisation and thus on nutrient cycling and soil fertility.

Keywords: Carbon storage; *Nematogenia lacuum*; Soil organic matter; Soil aggregates

4.1 Introduction

Soil organic matter (SOM) content and soil structural stability have been proposed as key indicators of soil quality and thus critical elements in defining sustainable land uses (Lal, 2004; Lehmann and Kleber, 2015). SOM is also considered an important determinant of soil fertility in tropical agro-ecosystems, and its loss has been shown to have significant negative effects on soil productivity in such ecosystems (Barrios et al., 2005; Solomon et al., 2007). Low SOM content has been linked to decreasing crop productivity and increased soil degradation in tropical agroecosystems (Fonte et al. 2010; Six et al. 2002). Furthermore, the little or no-external inputs and high temperatures especially in the small-scale farming systems in sub-Saharan Africa, contributes to further losses of SOM (Sanchez, 1976; Mbau et al., 2015). Retention or addition of organic materials to soil to foster SOM accrual can be considered the most direct intervention by farm managers in the regulation of soil structure and fertility. For instance, agroforestry has been promoted as an effective way of sustaining high levels of SOM with the aim of restoring the degraded farm lands (Barrios et al., 2012a).

Integration of trees into the annual cropping systems has thus become a common practice in many small-scale farms. Through shading, root turnover and litter fall, trees create microclimatic conditions beneath the tree canopy that can significantly influence soil properties (Lin, 2010). The significance of single trees in creating predictable patterns of soil influence that are proportional to the canopy size has been well established (Zinke 1962; Rhoades, 1997). For instance, organic C, N, P and exchangeable bases have been shown to decrease with increasing distance from the tree stem (Kater et al., 1992; Tomlinson et al., 1998; Jonsson et al., 1999). These patterns have been linked to the deposition of litter under and near the trees which increases SOM levels that upon decomposition release nutrients to the soil. Soil OM content plays a key role in stabilisation of soil aggregates as they constitute binding agents such as polysaccharides and aromatic components (Tisdall and Oades, 1982; Schmidt et al., 2011).

Furthermore, the dynamics of SOM cannot be dissociated from soil biological activity, since SOM is the primary source of C and nutrients for soil biota and, in turn, soil biota modify soil structure through their activities (Coleman et al., 2004). For instance, soil macrofauna induced decomposition and redistribution of SOM through excretions are known to affect the aggregate stabilisation and C protection (Barrios, 2007). Notably, earthworms and termites, which are recognised as ecosystem engineers, incorporate organic matter into their excretions and thus protecting it from microbial breakdown (Ayuke et al., 2011a). Spatial arrangement of the trees within the farms have been shown to play a key role in determining the patterns of soil macrofauna distribution as described in chapter 3, casting activity (Pauli et al., 2010), and significantly influencing soil aggregation and aggregate stability (Fonte et al. 2010). Although numerous studies have shown the value of agroforestry in improving crop yield and soil nutrients, very few have addressed the systematic impacts of duration in such systems, especially in sub-Saharan Africa. Most of the mechanistic knowledge on the effects of soil macrofauna on soil aggregation are extrapolated from microcosm studies, and thus fails to recognize ecosystem interactions involved and their potential impact at larger scales.

The objectives of this study were to determine the effects of duration of cultivation, tree species and tree zones of influence on (i) soil aggregate size distribution (ii) aggregate-associated soil C and (iii) relationships between aggregate-associated soil-C and the abundance of earthworms or termites. It was hypothesised that i) soil aggregate distribution and aggregate-associated soil C would decrease with increasing soil degradation and distance from the tree trunk, ii) the presence of earthworms and/or termites would increase soil aggregation and aggregate-associated soil C, iii) trees that increase the abundance and activity of soil earthworms and/or termites would contribute to greater soil C storage.

4.2 Materials and methods

4.2.1 Site description

The study was conducted in Kapchorwa region, Nandi County, Kenya. The area is located between Latitude 0° 09' 00" and 0° 10' 00" N and Longitude 35° 0' 00" and 35° 1' 00" E. At an average altitude of about 1800 m above sea level, mean daily temperature ranges between 11 and 26°C whereas mean annual temperature of 19°C. Annual rainfall occurs in a bimodal pattern, with an annual total of about 2,000 mm; 1200 mm falls between April and June and 800 mm between August and October (Güereña et al., 2015a). Soils are predominantly kaolinitic Acrisols (FAO/UNESCO classification) or Ultisols (USDA classification) (Kimetu et al., 2008; Recha et al., 2013). Farms used in the study are found near the Kakamega-Nandi forest complex, a remnant of the greater Guinean-Congolian rainforest. Selected farms differed based on the time of cultivation since conversion from indigenous forest, the longest duration of conversion being 62 years, medium term 16 years and the youngest conversion 10 years. Except for the time of conversion, the farms were similar in many aspects, including soil types, land use history and hydrology (Recha et al., 2013; Güereña et al., 2015a). Maize and beans are the major crops, with the average farm size being less than 0.5 ha per household. Detailed description of the study site can be found in Recha et al. (2013), Güereña et al. (2015a).

4.2.2 Soil sampling below the tree canopies

Trees used in the study were selected using participatory action research tools (Barrios et al., 2012b). Through focus group discussions, in the context of a knowledge sharing workshop, randomly-selected farmers from the three catchments were involved in identification and ranking of the most common tree species in the area. From the list, the top three most abundant trees were selected for this study, namely: *Croton megalocarpus* Hutch., *Eucalyptus grandis* W.Hill and *Zanthoxylum gilletii* (De Wild.) P.G.Waterman. Detailed description of the process

and criteria used for the selection of study trees can be found in chapter 3. Soil monoliths (0.08 by 0.08 by 0.30 m) were excavated adjacent to the points where soil macrofauna monoliths were sampled at the predetermined points in the concentric zones of tree influence, A, B, C and D as described in chapter 3. The excavated soil was placed in plastic trays and large clods gently broken along the lines of weakness and air dried prior to transporting to the laboratory. Each sample were placed in a zip-lock bag and packed in a special container with cotton wool round it to maintain integrity of the samples during transportation.

4.2.3 Extraction of water stable aggregates through wet sieving

The air-dried soil was gently passed through a 10 mm sieve. Water-stable aggregates were determined using wet sieving method (Elliott, 1986). Using this method, the soil was separated into four water-stable aggregate size classes: large macro-aggregates (LM; >2000 μm), small macro-aggregates (SM; 2000-250μm), micro-aggregates (m; 250–53μm) and silt and clay sized fraction (s+c; <53 μm). Briefly, thirty-two grams (32 g) of each air-dried soil sample were transferred into eight 2 mm sieve units held by a mechanical shaker, each sieve carrying 4 g of the soil. These sieve units were then immersed into stainless steel pans with sufficient deionised water to fully cover the sample, and left to slake for 5 minutes. The sieve was then moved up and down 100 times for 3 minutes. This process was repeated using the fractions that went through the 2000 μm sieve, using another set of eight 250 μm sieves and finally with a set of eight 53 μm sieves. The fractions retained on each screen size were backwashed into pre-weighed beakers. All the fractions were oven-dried at 60 °C overnight and weighed. All fractions were then expressed in g of fraction per 100 gram of soil sample.

4.2.4 Fractionation of macro-aggregates

The small amount of the large macro-aggregates did not allow separate fractionation of this fraction. Therefore, after oven-drying, the large and small macro-aggregates fractions were combined into one sample, named thereafter as total macro-aggregates (TM) and further fractionated as described by Six et al. (2002). Briefly, a five grams sub-sample of TM was placed into a transparent fiberglass tube with a 250 μm sieve at the bottom. The fiberglass tube contained enough deionised water to saturate the sample and was attached to a mechanical shaker. Thirty (30) glass beads (each, 4 mm in diameter) were also placed into the tube to enhance the process of sample breakup into different fractions during shaking. The sample was shaken for 3 minutes, after which it was flushed with deionised water and the soil slurry poured into a 53 μm sieve inside a larger container such that all aggregates < 53 μm in diameter were collected in the container, whereas those that were > 53 μm were retained on the sieve. Additional deionised water was passed through the 250 μm sieve to ensure that all the fractions were flushed out into their respective sieves. This process yielded three aggregate size fractions: coarse particulate organic matter (cPOM; >250 μm), micro-aggregates within macro-aggregates (mM; 250-53μm) and silt and clay sized fraction within macro-aggregates (s+cM; <53 μm). The fractions retained in the 250 μm and 53 μm sieves were backwashed into pre-weighed 250 ml beakers. All the fractions were oven-dried as described above.

4.2.5 Soil C analysis

About 20 mg of the whole soil samples before fractionation and from the aggregate fractions collected at the two steps of fractionation steps were weighed into aluminium foil capsules and scanned using Mid-infrared (MIR) spectroscopy for the selection of 10% of total samples as reference samples for C analysis. Total C were analysed using FLASH 2000 NC Analyser (ThermoFisher Scientific, Cambridge, UK). The data generated by reference C analysis was

used to generate a calibration curve to predict the results in all the other soil samples using partial least-squares (PLS) regression analysis through mid-infrared (MIR) spectroscopy.

4.2.6 Statistical analysis

Linear mixed-effects models were used to test the effects of duration of cultivation, tree species and zone of sampling on soil aggregate fractions and C in the aggregates using the package lme4 (Bates *et al*., 2015) in R (R Core Team, 2015). Using tree replicates as a random variable, several models were built, from which the best fitting models were chosen. The choice of the models was based on the Akaike Information Criterion (AIC), with those with the lowest AIC values being chosen. When analysis of variance (ANOVA) showed significant main or interactive effects, Tukey's post-hoc comparisons were performed at $\alpha = 0.05$. Further, correlation analysis was conducted to determine the relationship between soil aggregate fractions and C and soil macrofauna regarded as ecosystem engineers (earthworm and termites). Soil aggregate fraction weight and their C content were entered as dependent variables whereas earthworms and termites abundance as explanatory variables. Earthworms and termites data used in the correlation analysis was obtained from chapter 3 of this thesis.

4.3 Results

4.3.1 Effects of trees on water-stable soil aggregates

Tree species had the greatest influence on soil aggregate fractions (Table 4.1). The average weight of large macroaggregates (LM) was significantly higher below the canopy of *C. megalocarpus* and *E. grandis* trees (43.2 g and 46.8 g 100 g^{-1} soil, respectively) compared to *Z. gilletii* tree (28.5 g 100 g^{-1} soil) (Table 4.2). In contrast, the average weight of small macroaggregates (SM) was significantly higher (61.9 g 100 g^{-1} soil) under the canopy of *Z. gilletii* than *C. megalocarpus* and *E. grandis* trees (53.1 g and 49.0 g 100 g^{-1} soil, respectively).

Significantly higher microaggregates weight was also recorded under the canopy of *Z. gilletii* with an average weight of 9.4 g 100 g^{-1} soil compared to 3.8 g 100 g^{-1} soil under *E. grandis* and 3.1 g 100 g^{-1} soil under *C. megalocarpus*. Notably, however, the weight of microaggregates increased with the duration of cultivation below the canopy of the two native trees, *C. megalocarpus* and *Z. gilletii*, from 1.9 g and 8.5 g 100 g^{-1} soil after 10 years of cultivation to 4.4 g and 10.7 g 100 g^{-1} soil after 62 years of cultivation, respectively. On the other hand, the weight of microaggregates decreased with duration of cultivation under the canopy of the exotic tree *E. grandis*. Based on the tree zone, only the native trees (*C. megalocarpus* and *Z. gilletii*) showed distinct trends (Table 4.2). The fraction LM was lower under the canopy of the two trees than away, with an average of 42.6 g 100 g^{-1} under *C. megalocarpus* compared to 53.2 g 100 g^{-1} away from this tree after 10 years of cultivation and 37.0 g 100 g^{-1} under the tree compared to 44.4 g 100 g^{-1} away from the tree after 62 years of cultivation. Under *Z. gilletii* tree, the fraction LM was 23.2 g 100 g^{-1} compared to 33.6 g 100 g^{-1} away from the tree after 10 years of cultivation and 29.8 g 100 g^{-1} under the tree compared to 36.6 g 100 g^{-1} away from the tree after 62 years of cultivation. Conversely, SM and m were significantly higher under the trees' canopy than away. Under *C. megalocarpus* tree, the fraction SM was 54.4 g 100 g^{-1} compared to 44.7 g 100 g^{-1} away from the tree after 10 years of cultivation and 57.8 g 100 g^{-1} under the tree compared to 50.5 g 100 g^{-1} away from the tree after 62 years of cultivation. Below *Z. gilletii*, the fraction SM was 67.7 g 100 g^{-1} compared to 59.1 g 100 g^{-1} away from the tree after 10 years of cultivation and 59.2 g 100 g^{-1} under the tree compared to 52.4 g 100 g^{-1} away from the tree after 62 years of cultivation. The fraction m only showed trends in 10 years after cultivation only. Under *C. megalocarpus* tree the fraction m was 2.1 g 100 g^{-1} compared to 1.3 g 100 g^{-1} away from the tree whereas under *Z. gilletii* tree the values were 9.0 g 100 g^{-1} compared to 7.1 g 100 g^{-1} away from the tree. The silt and clay fraction (s+c) was not significantly affected by duration of cultivation, tree species or tree zone.

Following fractionation of total soil macroaggregates only mM and cPOM showed significant trends (Table 4.1). The two indigenous trees *C. megalocarpus* and *Z. gilletii* showed a decrease in mM fraction with increasing duration of cultivation from the highest values of 77.4 g and 67.1 g 100 g^{-1} soil after 10 years of cultivation to the lowest of 65.3 g and 66.3 g 100 g^{-1} soil after 62 years, respectively. Conversely, under *E. grandis*, the fraction mM was lower after 10 years (72.3 g 100 g^{-1} soil) and higher after 62 years (76.9 g 100 g^{-1} soil). Based on the tree zone, only the native trees showed distinct trends. Higher mM weight (71.6 g 100 g^{-1}) was recorded under *C. megalocarpus* tree than away (66.2 g 100 g^{-1}) after 16 years of cultivation. Similarly, higher values were recorded under the same tree (66.3 g 100 g^{-1}) than away (62.0 g 100 g^{-1}) after 62 years of cultivation. Under *Z. gilletii* tree, the fraction showed significant trend only after 16 years of cultivation, with 71.1 g 100 g^{-1} under the tree compared to 66.9 g 100 g^{-1} away from the tree. The fraction cPOM showed opposite trend to that of mM. The fraction s+cM was not significantly affected by duration of cultivation, tree species or tree zone.

Table 4.1: *p*-values associated with the soil aggregate fractions as influenced by duration of cultivation, tree species and sampling zone.

Soil fraction	*p*-value					
	Duration of cultivation	**Tree species**	**Sampling zone**	**Duration × Species**	**Species × Zone**	**Duration × Species × Zone**
LM	0.736	**<0.001*****	0.989	0.247	**0.050***	0.677
SM	0.481	**0.002****	0.416	0.161	**0.050***	0.833
m	0.413	**<0.001*****	0.497	**0.010***	0.716	**0.050***
s+c	0.259	0.415	0.907	0.253	0.139	0.112
cPOM	0.651	0.765	0.907	**0.016***	**0.003****	**0.037***
mM	0.749	0.322	0.318	**0.003****	**0.003****	**0.050***
s+cM	0.227	0.070	0.892	0.090	0.243	0.865

Abbreviations; LM=large macro-aggregates (> 2000 μm), SM=small macro-aggregates (250-2000 μm), m=micro-aggregates (53–250 μm), s+c=silt and clay (<53 μm), cPOM=coarse particulate organic matter (>250 μm), mM=micro-aggregates within macro-aggregates (53–250 μm), s+cM=silt and clay within macro-aggregates (<53 μm). Values marked in bold are significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4.2: Soil aggregate fractions weight distribution (means ± (SE)) in the soil as influenced by the duration of cultivation, tree species and tree zone.

Soil fraction (g 100 g^{-1} soil)	Tree species														
	Croton megalocarpus					*Eucalyptus grandis*					*Zanthoxylum gilletii*				
	Sampling zone														
	A	B	C	D	Mean†	A	B	C	D	Mean†	A	B	C	D	Mean†
10 years of cultivation															
LM	**42.5 (2.6)^{b}**	**40.0 (2.6)^{b}**	**45.3 (2.5)^{b}**	**53.2 (2.9)^{a}**	**45.3 (2.6)^{A}**	48.4 (4.0)^{a}	46.0 (5.4)^{a}	43.3 (5.0)^{a}	45.5 (5.1)^{a}	**45.8 (2.4)^{A}**	**21.8 (1.3)^{b}**	**26.2 (3.3)^{ab}**	**21.6 (1.3)^{b}**	**33.6 (4.9)^{a}**	**25.8 (1.7)^{B}**
SM	**54.4 (2.2)^{ab}**	**57.2 (2.4)^{a}**	**51.6 (2.3)^{b}**	**44.7 (3.8)^{c}**	**52.0 (2.5)^{B}**	45.2 (3.6)^{a}	48.2 (5.0)^{a}	51.9 (4.5)^{a}	49.5 (4.5)^{a}	**48.7 (2.2)^{B}**	**69.5 (1.3)^{a}**	**64.3 (2.8)^{ab}**	**69.3 (1.4)^{a}**	**59.1 (4.2)^{b}**	**65.6 (1.4)^{A}**
m	**2.2 (0.4)^{a}**	**2.0 (0.4)^{a}**	**2.2 (0.4)^{a}**	**1.3 (0.2)^{b}**	**1.9 (0.2)^{C}**	6.3 (1.6)^{a}	5.6 (0.6)^{a}	4.6 (0.6)^{a}	4.9 (0.6)^{a}	**5.3 (0.5)^{B}**	**8.9 (0.8)^{a}**	**9.1 (0.8)^{a}**	**8.9 (0.7)^{a}**	**7.1 (0.6)^{b}**	**8.5 (0.4)^{A}**
s+c	0.9 (0.4)^{a}	0.7 (0.3)^{a}	0.9 (0.4)^{a}	0.5 (0.2)^{a}	0.8 (0.2)^{A}	0.1 (0.0)^{a}	0.1 (0.0)^{a}	0.2 (0.1)^{a}	0.1 (0.0)^{a}	0.3 (0.1)^{A}	0.2 (0.1)^{a}	0.4 (0.3)^{a}	0.2 (0.0)^{a}	0.2 (0.0)^{a}	0.2 (0.1)^{A}
cPOM	8.7 (1.1)^{a}	8.9 (1.1)^{a}	9.3 (1.2)^{a}	9.4 (1.4)^{a}	**9.1 (0.6)^{B}**	18.6 (4.0)^{a}	17.0 (2.9)^{a}	9.9 (1.0)^{a}	11.1 (1.5)^{a}	**14.1 (1.4)^{A}**	7.4 (1.3)^{a}	6.6 (0.8)^{a}	12.6 (1.7)^{a}	10.1 (2.0)^{a}	**9.2 (0.8)^{B}**
mM	77.2 (1.5)^{a}	79.0 (1.3)^{a}	76.6 (1.4)^{a}	76.8 (1.5)^{a}	**77.4 (0.7)^{A}**	62.5 (4.3)^{a}	64.0 (3.6)^{a}	69.1 (2.3) ^{a}	72.7 (1.8)^{a}	**72.3 (1.6)^{B}**	73.4 (1.0)^{a}	73.5 (1.2)^{a}	69.7 (2.2)^{a}	72.7 (2.2)^{a}	**67.1 (0.9)^{C}**
s+cM	10.9 (0.7)^{a}	9.3 (0.7)^{a}	10.9 (0.6)^{a}	12.1 (1.0)^{a}	10.8 (0.4)^{A}	12.5 (1.7)^{a}	13.2 (1.9)^{a}	16.2 (2.5)^{a}	11.3 (1.1)^{a}	13.3 (0.9)^{A}	10.4 (1.5)^{a}	10.3 (1.4)^{a}	8.7 (1.6)^{a}	9.9 (1.6)^{a}	9.8 (0.8)^{A}
16 years of cultivation															
LM	46.5 (4.5)^{a}	44.5 (3.8)^{a}	43.1 (2.5)^{a}	47.6 (3.4)^{a}	**45.4 (1.6)^{B}**	52.2 (6.5)^{a}	55.3 (5.7)^{a}	54.0 (5.8)^{a}	52.7 (5.5)^{a}	**53.5 (2.9)^{A}**	29.9 (3.8)^{a}	32.4 (3.5)^{a}	27.3 (3.4)^{a}	23.3 (4.5)^{a}	**28.2 (2.1)^{C}**
SM	50.5 (4.3)^{a}	52.4 (3.7)^{a}	53.3 (2.3)^{a}	49.1 (3.2)^{a}	**51.3 (1.7)^{B}**	44.0 (5.9)^{a}	41.0 (5.2)^{a}	42.6 (5.4)^{a}	44.5 (5.3)^{a}	**43.0 (2.6)^{C}**	61.6 (3.3)^{a}	59.4 (4.8)^{a}	63.3 (2.7)^{a}	65.5 (2.6)^{a}	**62.5 (1.7)^{A}**
m	2.6 (0.4)^{a}	2.7 (0.3)^{a}	3.3 (1.0)^{a}	3.0 (0.4)^{a}	**2.9 (0.3)^{B}**	3.4 (0.6)^{a}	3.4 (0.4)^{a}	3.3 (0.6)^{a}	2.6 (0.6)^{a}	**3.2 (0.3)^{B}**	8.3 (0.8)^{a}	7.9 (1.0)	9.0 (0.9)^{a}	10.8 (1.3)^{a}	**9.0 (0.5)^{A}**
s+c	0.5 (0.2)^{a}	0.4 (0.2)^{a}	0.4 (0.2)^{a}	0.3 (0.2)^{a}	0.4 (0.1)^{A}	0.5 (0.3)^{a}	0.4 (0.2)^{a}	0.1 (0.0)^{a}	0.2 (0.1)^{a}	0.3 (0.1)^{A}	0.3 (0.1)^{a}	0.4 (0.1)^{a}	0.3 (0.1)^{a}	0.4 (0.1)^{a}	0.3 (0.1)^{A}
cPOM	**13.4 (1.3)^{b}**	**14.1 (1.9)^{b}**	**14.6 (1.3)^{b}**	**17.8 (1.8)^{a}**	**15.0 (1.0)^{A}**	18.9 (2.6)^{a}	18.3 (2.7)^{a}	19.0 (2.7)^{a}	18.2 (2.8)^{a}	**18.6 (1.3)^{A}**	**10.2 (0.8)^{a}**	**9.8 (0.5)^{a}**	**10.6 (0.3)^{a}**	**8.4 (0.5)^{b}**	**9.8 (0.4)^{C}**
mM	**71.8 (1.5)^{a}**	**72.2 (2.1)^{a}**	**70.8 (1.2)^{a}**	**66.2 (1.8)^{b}**	70.3 (1.1)^{A}	67.3 (2.8)^{a}	68.5 (3.0)^{a}	67.6 (3.1)^{a}	70.5 (2.8)^{a}	68.5 (1.4)^{A}	**71.5 (1.2)^{a}**	**71.4 (1.0)^{a}**	**70.4 (1.3)^{a}**	**66.9 (1.2)^{b}**	70.1 (0.6)^{A}
s+cM	11.8 (0.6)^{a}	10.6 (1.1)^{a}	11.0 (0.6)^{a}	11.6 (0.8)^{a}	11.2 (0.4)^{A}	10.0 (1.0)^{a}	9.5 (0.6)^{a}	9.9 (0.6)^{a}	8.5 (0.6)^{a}	9.5 (0.4)^{A}	9.7 (0.5)^{a}	10.5 (0.4)^{a}	9.7 (0.6)^{a}	9.5 (0.5)^{a}	9.8 (0.3)^{A}
62 years of cultivation															
LM	**38.6 (3.0)^{b}**	**35.3 (2.4)^{b}**	**37.0 (3.2)^{b}**	**44.4 (2.6)^{a}**	**38.8 (1.9)^{A}**	47.4 (5.0)^{a}	41.3 (4.5)^{a}	35.6 (3.9)^{a}	39.9 (5.9)^{a}	**41.0 (2.4)^{A}**	**30.8 (2.2)^{b}**	**28.6 (2.3)^{b}**	**30.1 (3.2)^{b}**	**36.6 (2.2)^{a}**	**31.5 (2.1)^{B}**
SM	**56.8 (2.7)^{a}**	**58.3 (2.3)^{a}**	**58.3 (2.9)^{a}**	**50.5 (2.3)^{b}**	56.0 (1.8)^{A}	49.5 (4.6)^{a}	54.8 (4.2)^{a}	60.9 (3.7)^{a}	55.8 (5.6)^{a}	55.3 (2.3)^{A}	**58.5 (2.5)^{a}**	**60.3 (2.4)^{a}**	**58.9 (2.4)^{a}**	**52.4 (2.2)^{b}**	57.5 (1.7)^{A}
m	4.3 (0.4)^{a}	4.1 (0.5)^{a}	4.5 (0.3)^{a}	4.8 (0.7)^{a}	**4.4 (0.2)^{B}**	2.6 (0.5)^{a}	3.2 (0.5)^{a}	3.3 (0.4)^{a}	3.0 (0.4)^{a}	**3.0 (0.2)^{C}**	10.5 (1.1)^{a}	10.9 (1.0)^{a}	10.9 (0.9)^{a}	10.6 (1.2)^{a}	**10.7 (0.5)^{A}**
s+c	0.4 (0.1)^{a}	0.3 (0.1)^{a}	0.2 (0.1)^{a}	0.3 (0.1)^{a}	0.3 (0.1)^{A}	0.5 (0.3)^{a}	0.6 (0.4)^{a}	0.3 (0.1)^{a}	1.3 (0.9)^{a}	0.7 (0.3)^{A}	0.2 (0.04)^{a}	0.2 (0.1)^{a}	0.2 (0.01)^{a}	0.5 (0.3)^{a}	0.3 (0.1)^{A}
cPOM	**15.7 (1.1)^{b}**	**15.1 (0.9)^{b}**	**15.6 (0.8)^{b}**	**17.3 (0.7)^{a}**	**15.9 (0.6)^{A}**	8.5 (0.8)^{a}	7.0 (0.7)^{a}	7.8 (0.5)^{a}	7.3 (0.5)^{a}	**7.7 (0.3)^{C}**	13.0 (2.0)^{a}	14.0 (1.2)^{a}	14.3 (1.7)^{a}	13.0 (1.5)^{a}	**13.6 (0.8)^{B}**
mM	**67.2 (1.0)^{a}**	**66.3 (0.9)^{a}**	**65.5 (1.0)^{a}**	**62.0 (1.5)^{b}**	**65.3 (0.6)^{B}**	76.1 (1.3)^{a}	78.4 (1.3)^{a}	77.2 (0.8)^{a}	76.1 (1.2)^{a}	**76.9 (0.6)^{A}**	66.3 (1.8)^{a}	66.2 (1.4)^{a}	65.8 (1.8)^{a}	66.8 (1.3)^{a}	**66.3 (0.8)^{B}**
s+cM	12.5 (0.7)^{a}	14.2 (0.7)^{a}	14.2 (0.9)^{a}	13.5 (0.4)^{a}	13.6 (0.4)^{A}	12.4 (1.1)^{a}	10.7 (1.0)^{a}	11.4 (0.9)^{a}	12.3 (0.9)^{a}	11.7 (0.5)^{A}	9.9 (0.8)^{a}	8.6 (0.4)^{a}	8.8 (0.5)^{a}	9.2 (0.9)^{a}	9.1 (0.3)^{A}

† This mean gives aggregate tree effect. Within rows, means in bold and followed by different letters in superscript are significantly different at $p < 0.05$. Uppercase superscript letters indicate the differences based on tree species while lowercase superscript letters indicate the differences within sampling zones. Abbreviations; LM=large macro-aggregates (> 2000 µm), SM=small macro-aggregates (250-2000 µm), m=micro-aggregates (53–250 µm), s+c=silt and clay (<53 µm), cPOM=coarse particulate organic matter (>250 µm), mM=micro-aggregates within macro-aggregates (53–250 µm), s+cM=silt and clay within macro-aggregates (<53 µm).

4.3.2 Effects of trees on aggregate-associated soil carbon

The duration of cultivation had significant influence on the C content of most of the aggregate fractions (Table 4.3). The C content in TM significantly decreased with increasing duration of cultivation with higher values of 63.3 mg, 62.9 and 53.8 mg g^{-1} below the canopy of *C. megalocarpus*, *E. grandis* and *Z. gilletii*, respectively, after 10 years of cultivation compared to 37.2 mg and 30.5 mg g^{-1} under *E. grandis* and *Z. gilletii*, respectively, after 16 years and 34.2 mg g^{-1} under *C. megalocarpus* after 62 years (Table 4.4). Similarly, C content in microaggregates decreased with increasing duration of cultivation, but these trends were more pronounced under the canopy of *Z. gilletii*. Under this tree, the microaggregates C was 5.0 mg g^{-1} in soil after 10 years of cultivation, compared to 3.0 mg g^{-1} in soils after 16 years and 4.4 mg g^{-1} after 62 years of cultivation, respectively. Based on tree zone, only the indigenous trees showed identifiable trends, though this was catchment specific. Higher C in TM (64.6 mg g^{-1}) was recorded under the canopy of *C. megalocarpus*, than away from the tree (59.5 mg g^{-1}) after 10 years of cultivation and 35.1 mg g^{-1} below the same tree compared to 31.5 mg g^{-1} away from the tree after 62 years of cultivation. Under *Z. gilletii*, the fraction showed significant trend only after 16 years of cultivation, with 31.1 mg g^{-1} of C in TM recorded below the tree compared to 28.5 mg g^{-1} away fro the tree. Under the canopy of *C. megalocarpus*, C in fraction m showed significant trends after 10 years of cultivation only, with a higher value recorded below the tree (1.4 mg g^{-1}) than away (0.7 mg g^{-1}) from the tree. On the other hand, under the canopy of *Z. gilletii*, C in fraction m showed significant trends after 16 years only with a lower value (2.9 mg g^{-1}) under the canopy than away (3.5 mg g^{-1}).

Duration of cultivation also significantly influenced C content of mM and cPOM within total macroaggregates. Significantly higher C content in mM was observed under the canopies of all tree species studies in soil with shorter duration than longer duration of cultivation. For

instance, below the canopy of *C. megalocarpus*, *E. grandis* and *Z. gilletii*, the C content in mM was 53.3 mg, 47.9 mg and 45.0 mg g^{-1}, respectively, after 10 years of cultivation, compared to 30.8 mg g^{-1} under *E. grandis* and 25.6 mg g^{-1} under *Z. gilletii* after 16 years and 26.2 mg g^{-1} under *C. megalocarpus* after 62 years of cultivation. Based on tree zone, differences in C content of mM fraction were more pronounced under the two native trees, *C. megalocarpus* and *Z. gilletii*, after 16 years of cultivation. The C in mM was higher below the canopy (37.4 mg g^{-1}) of *C. megalocarpus* than away (29.5 mg g^{-1}) from the tree after 16 years of cultivation and 27.2 mg g^{-1} below the canopy of the same tree compared to23.2 mg g^{-1} away from the tree, after 62 years of cultivation. The C in mM under *Z. gilletii* showed significant trends after 16 years of cultivation only, with higher values recorded below the tree (26.1 mg g^{-1}) than away (23.9 mg g^{-1}) from the tree. The C content in cPOM showed the same trend to that of mM.

Table 4.3: *p*-values associated with the soil C in the aggregate fractions as influenced by duration of cultivation, tree species and sampling zone.

Soil aggregate fraction	*p*-value					
	Duration of cultivation	**Tree species**	**Sampling zone**	**Duration × Species**	**Species × Zone**	**Duration × Species × Zone**
TM	**0.008****	0.584	**0.008****	0.581	**0.050***	0.236
m	0.766	**<0.001*****	0.567	0.242	**0.044***	0.299
s+c	0.250	0.163	0.625	0.182	0.971	0.621
cPOM	**0.017***	0.513	0.569	0.507	**0.050***	0.277
mM	**<0.001*****	0.618	0.280	0.446	**0.015***	0.085
s+cM	0.230	**0.025***	0.318	0.388	0.612	0.129

TM = total macro-aggregates (> 250 µm), m = micro-aggregates (53–250 µm), s+c = silt and clay (<53 µm), cPOM = coarse particulate organic matter (>250 µm), mM = micro-aggregates within macro-aggregates (53–250 µm), s+cM = silt and clay within macro-aggregates (<53 µm). Values marked in bold are significant.

Table 4.4: Distribution of soil C in the aggregate fractions (means ± (SE)) as influenced by duration of cultivation, tree species and sampling zone.

C content in the fraction (mg g^{-1} soil)	Tree species														
	Croton megalocarpus					*Eucalyptus grandis*					*Zanthoxylum gilletii*				
	Sampling zone														
	A	B	C	D	Mean†	A	B	C	D	Mean†	A	B	C	D	Mean†
10 years of cultivation															
TM	**66.2 (0.4)^{a}**	**64.8 (2.5)^{a}**	**62.9 (2.1)^{ab}**	**59.5 (1.6)^{b}**	**63.3 (1.5)^{A}**	62.2 (6.1)^{a}	65.7 (6.8)^{a}	60.7 (3.9)^{a}	63.0 (0.9)^{a}	**62.9 (2.2)^{A}**	54.8 (1.4)^{a}	53.3 (2.4)^{a}	53.9 (3.2)^{a}	53.3 (6.2)^{a}	**53.8 (2.0)^{B}**
m	**1.4 (0.5)^{a}**	**1.3 (0.2)^{a}**	**1.4 (0.3)^{a}**	**0.7 (0.1)^{b}**	**1.2 (0.3)^{C}**	3.9 (0.6)^{a}	3.6 (0.3)^{a}	2.8 (0.4)^{a}	3.1 (0.1)^{a}	**3.4 (0.4)^{B}**	4.6 (0.9)^{a}	5.6 (0.8)^{a}	5.7 (1.0)^{a}	4.2 (1.3)^{a}	**5.0 (0.9)^{A}**
s+c	0.5 (0.4)^{a}	0.5 (0.4)^{a}	0.5 (0.3)^{a}	0.2 (0.1)^{a}	0.4 (0.3)^{A}	0.1 (0.0)^{a}	0.1 (0.0)^{a}	0.1 (0.1)^{a}	0.1 (0.0)^{a}	0.1 (0.0)^{A}	0.2 (0.1)^{a}	0.4 (0.3)^{a}	0.1 (0.0)^{a}	0.2 (0.1)^{a}	0.2 (0.1)^{A}
cPOM	3.0 (1.2)^{a}	3.9 (1.2)^{a}	2.7 (0.3)^{a}	2.8 (0.5)^{a}	**3.1 (0.8)^{AB}**	6.9 (2.6)^{a}	7.0 (3.0)^{a}	2.5 (0.4)^{a}	3.3 (1.0)^{a}	**4.9 (2.1)^{A}**	1.6 (0.9)^{a}	1.4 (0.8)^{a}	3.7 (0.5)^{a}	2.4 (1.0)^{a}	**2.3 (0.9)^{B}**
mM	55.6 (1.4)^{a}	55.5 (3.8)^{a}	52.7 (2.3)^{a}	49.3 (3.8)^{a}	**53.3 (2.8)^{A}**	45.8 (7.9)^{a}	47.4 (8.9)^{a}	46.7 (4.9)^{a}	51.8 (0.4)^{a}	**47.9 (5.6)^{AB}**	46.0 (0.9)^{a}	45.2 (3.2)^{a}	44.3 (3.2)^{a}	44.5 (5.3)^{a}	**45.0 (3.0)^{B}**
s+cM	7.6 (0.6)^{a}	5.4 (1.1)^{a}	7.5 (0.7)^{a}	7.4 (1.1)^{a}	**7.0 (1.0)^{B}**	9.5 (0.9)^{a}	11.3 (1.3)^{a}	11.4 (1.9)^{a}	7.9 (0.3)^{a}	**10.0 (1.2)^{A}**	7.1 (1.7)^{a}	6.6 (0.8)^{a}	5.9 (1.5)^{a}	6.4 (1.7)^{a}	**6.5 (1.1)^{B}**
16 years of cultivation															
TM	47.2 (9.4)^{a}	49.4 (4.0)^{a}	38.6 (3.9)^{a}	36.1 (5.0)^{a}	**42.8 (6.0)^{A}**	36.6 (5.0)^{a}	39.8 (2.4)^{a}	37.4 (4.3)^{a}	35.0 (5.0)^{a}	**37.2 (3.8)^{AB}**	**32.3 (2.0)^{a}**	**30.7 (1.5)^{ab}**	**30.1 (1.7)^{ab}**	**28.5 (1.1)^{b}**	**30.5 (3.8)^{B}**
m	1.4 (0.3)^{a}	1.4 (0.2)^{a}	1.2 (0.3)^{a}	1.2 (0.3)^{a}	**1.3 (0.3)^{B}**	1.5 (0.4)^{a}	1.5 (0.3)^{a}	1.4 (0.2)^{a}	1.2 (0.2)^{a}	**1.4 (0.3)^{B}**	**2.8 (0.2)^{b}**	**2.8 (0.3)^{b}**	**3.0 (0.4)^{ab}**	**3.5 (0.3)^{a}**	**3.0 (0.6)^{A}**
s+c	0.2 (0.1)^{a}	0.2 (0.1)^{a}	0.2 (0.1)^{a}	0.2 (0.1)^{a}	0.2 (0.1)^{A}	0.4 (0.3)^{a}	0.2 (0.1)^{a}	0.1 (0.0)^{a}	0.1 (0.0)^{a}	0.2 (0.1)^{A}	0.2 (0.0)^{a}	0.2 (0.0)^{a}	0.2 (0.0)^{a}	0.2 (0.0)^{a}	0.2 (0.0)^{A}
cPOM	**1.5 (0.4)^{a}**	**1.0 (0.3)^{ab}**	**0.9 (0.2)^{b}**	**0.7 (0.2)^{b}**	1.1 (0.5)^{A}	1.0 (0.3)^{a}	0.9 (0.1)^{a}	1.1 (0.3)^{a}	0.8 (0.1)^{a}	1.0 (0.2)^{A}	**0.9 (0.2)^{a}**	**0.5 (0.2)^{ab}**	**0.6 (0.1)^{ab}**	**0.4 (0.2)^{b}**	0.6 (0.2)^{A}
mM	**38.7 (3.7)^{a}**	**41.6 (3.2)^{a}**	**31.9 (1.4)^{b}**	**29.5 (3.2)^{b}**	**35.4 (3.2)^{A}**	29.9 (4.4)^{a}	32.8 (2.6)^{a}	30.7 (4.1)^{a}	29.9 (4.6)^{a}	**30.8 (3.5)^{AB}**	**27.2 (2.7)^{a}**	**25.7 (2.1)^{ab}**	**25.4 (2.2)^{ab}**	**23.9 (0.6)^{b}**	**25.6 (2.2)^{B}**
s+cM	7.0 (1.0)^{a}	6.8 (1.6)^{a}	5.9 (0.9)^{a}	5.7 (0.6)^{a}	**6.3 (1.0)^{A}**	5.7 (0.8)^{a}	6.1 (0.3)^{a}	5.6 (0.7)^{a}	4.4 (0.6)^{a}	**5.4 (0.8)^{AB}**	4.2 (0.8)^{a}	4.5 (0.5)^{a}	4.3 (0.6)^{a}	4.1 (0.5)^{a}	**4.3 (0.4)^{B}**
62 years of cultivation															
TM	**35.9 (2.6)^{a}**	**33.4 (1.9)^{ab}**	**35.9 (2.2)^{a}**	**31.5 (1.9)^{b}**	34.2 (2.9)^{A}	43.3 (1.5)^{a}	41.0 (2.6)^{a}	41.2 (1.0)^{a}	40.8 (2.0)^{a}	41.6 (1.7)^{A}	33.4 (4.3)^{a}	32.3 (5.2)^{a}	36.6 (9.3)^{a}	33.5 (76.6)^{a}	34.0 (5.7)^{A}
m	1.9 (0.5)^{a}	1.6 (0.5)^{a}	1.6 (0.5)^{a}	1.6 (0.5)^{a}	**1.7 (0.3)^{B}**	1.2 (0.2)^{a}	1.5 (0.3)^{a}	1.4 (0.3)^{a}	1.4 (0.3)^{a}	**1.4 (0.4)^{B}**	4.4 (0.7)^{a}	4.4 (1.0)^{a}	4.5 (1.1)^{a}	4.3 (0.9)^{a}	**4.4 (0.8)^{A}**
s+c	0.2 (0.0)^{a}	0.2 (0.0)^{a}	0.1 (0.0)^{a}	0.2 (0.0)^{a}	0.2 (0.0)^{A}	0.3 (0.2)^{a}	0.4 (0.3)^{a}	0.2 (0.0)^{a}	0.8 (0.6)^{a}	0.4 (0.3)^{A}	0.1 (0.0)^{a}	0.2 (0.0)^{a}	0.1 (0.0)^{a}	0.2 (0.2)^{a}	0.2 (0.1)^{A}
cPOM	0.8 (0.2)^{a}	0.5 (0.1)^{a}	0.7 (0.1)^{a}	1.2 (0.7)^{a}	0.8 (0.3)^{A}	0.8 (0.2)^{a}	0.4 (0.0)^{a}	0.5 (0.0)^{a}	0.6 (0.1)^{a}	0.6 (0.3)^{A}	1.3 (0.8)^{a}	0.9 (0.3)^{a}	1.3 (0.7)^{a}	1.1 (0.4)^{a}	1.1 (0.5)^{A}
mM	**28.3 (1.4)^{a}**	**25.6 (1.1)^{ab}**	**27.8 (0.7)^{a}**	**23.2 (1.4)^{b}**	**26.2 (2.1)^{B}**	36.0 (1.2)^{a}	35.2 (2.6)^{a}	34.8 (1.3)^{a}	33.8 (1.2)^{a}	**35.0 (1.5)^{A}**	27.8 (3.2)^{a}	27.2 (4.6)^{a}	30.8 (8.3)^{a}	27.9 (5.6)^{a}	**28.4 (5.0)^{AB}**
s+cM	6.8 (1.7)^{a}	7.3 (1.8)^{a}	7.4 (0.6)^{a}	7.1 (1.0)^{a}	**7.2 (1.3)^{A}**	6.4 (0.2)^{a}	5.4 (0.6)^{a}	5.8 (0.6)^{a}	6.4 (1.0)^{a}	**6.0 (0.4)^{A}**	4.4 (0.3)^{a}	4.3 (0.4)^{a}	4.6 (0.5)^{a}	4.6 (0.8)^{a}	**4.5 (0.4)^{B}**

† The mean gives aggregate tree effect. Within rows, means in bold and followed by different letters in superscript are significantly different at $p < 0.05$. Uppercase superscript letters indicate differences based on tree species while lowercase superscript letters indicate the differences within sampling zones. TM = total macro-aggregates (>250 μm), m = micro-aggregates (53–250 μm), s+c = silt and clay (<53 μm), cPOM = coarse particulate organic matter (>250 μm), mM = micro-aggregates within macro-aggregates (53–250 μm), s+cM = silt and clay within macro-aggregates (<53 μm).

4.3.3 Correlation of soil macrofauna with soil aggregate fractions and C

Of the two soil macrofauna commonly classified as ecosystem engineers, only earthworms showed significant correlation with aggregate fractions and the C in these fractions (Table 4.5). However, this effect strongly depended on the tree species and the soil aggregate fraction considered. Earthworms were positively and significantly correlated with the fractions m, cPOM and s+cM, but negatively correlated with mM, under the canopy of *C. megalocarpus* tree. Under *Z. gilletii* tree, earthworms were significantly and positively correlated with SM and m, but negatively correlated with LM. There were no significant correlation between earthworms and the soil fractions under the canopy of *E. grandis*. The C content in the fractions showed a similar trend under all the three tree species (Table 4.5). In this, earthworms showed significantly and strong negative correlation with C content in all the fractions, except s+c, m and s+cM under the canopy of *C. megalocarpus* tree and s+c under the canopy of *Z. gilletii* trees. None of the aggregate fractions or C in the aggregates showed significant correlation with termite abundance.

Table 4.5: Correlation coefficients of the relationship between the soil aggregate fractions, C content and earthworms and termites abundance.

Variable	Tree species					
	Croton megalocarpus		***Eucalyptus grandis***		***Zanthoxylum gilletii***	
	Earthworms	**Termites**	**Earthworms**	**Termites**	**Earthworms**	**Termites**
Soil aggregate fractions						
LM	-0.18	0.05	-0.03	0.28	**-0.36***	0.07
SM	0.15	-0.03	0.02	-0.26	**0.40***	-0.06
m	**0.48****	-0.08	0.05	-0.21	**0.34***	-0.09
s+c	-0.27	0.06	0.14	-0.15	-0.17	0.09
cPOM	**0.45****	0.08	0.02	0.28	0.04	-0.23
mM	**-0.45****	-0.10	0.04	-0.27	-0.04	0.22
s+cM	**0.34***	0.07	-0.17	-0.20	0.08	-0.02
C content in the fractions						
TM	**-0.57*****	-0.01	**-0.64*****	-0.21	**-0.66*****	0.08
m	0.10	-0.17	**-0.43****	-0.18	**-0.37***	0.01
s+c	-0.22	0.05	**0.47***	-0.11	-0.03	-0.02
cPOM	**-0.34***	0.24	**-0.59*****	-0.01	**-0.59*****	-0.17
mM	**-0.61*****	-0.04	**-0.56*****	-0.25	**-0.65*****	0.10
s+cM	-0.07	0.03	**-0.47****	-0.09	**-0.48****	0.06

Abbreviations; LM=large macro-aggregates (> 2000 µm), SM=small macro-aggregates (250-2000 µm), m=micro-aggregates (53–250 µm), s+c=silt and clay (<53 µm), cPOM=coarse particulate organic matter (>250 µm), mM=micro-aggregates within macro-aggregates (53–250 µm), s+cM=silt and clay within macro-aggregates (<53 µm). Values marked in bold are significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4.4 Discussion

4.4.1 Effects of duration of cultivation, trees and soil macrofauna on soil aggregation

Variation in quality and quantity of litter and root turnover as moderated by tree-specific attributes has great effects on abundance, diversity and spatial distribution patterns of soil macrofauna as described in chapter 3. Such differences could therefore have great influence on the spatial and temporal differences in the activities of soil macrofauna below the trees (Pauli et al., 2010). In the current study, there are indications that earthworms played a great role in the observed differences in soil aggregate fractions. Apart from microbially-mediated process, earthworms play a critical role in initiating the process of soil aggregates formation through the following mechanisms i) secretion of calcium humate in the earthworms' gut which acts as a cementing compound, ii) polysaccharides either in the earthworms' mucus or by that produced by microbes in the earthworms' gut which are reported to strengthen bonds between organic and mineral components, and iii) mechanical binding by vascular bundles from ingested plant materials, or by enmeshment from fungal hyphae that could grow after cast secretion (Shipitalo and Le Bayon, 2004; Six et al., 2004). However, the magnitude of earthworms' effects on aggregation is dependent on their ecological (e.g. epigeic, endogeic or anecic) (Shipitalo and Le Bayon, 2004; Six et al., 2004; Pulleman et al., 2005) and/or functional attributes (e.g. compacting or decompacting) (Rossi 2003; Guéi et al., 2012). While endogeic and anecic species may play a major role in soil aggregation, the epigeic species are usually weakly correlated with soil structure (Rossi, 2003; Shipitalo and Le Bayon, 2004; Six et al., 2004). Since there were no anecic group in this study, the observed trends could thus be attributed to the dominance of endogeic earthworm species, *Nematogenia lacuum* (Table S1). Given that this is a small species and produces small excrements compared to other species, as noted by Ayuke et al. (2011a), the species may fragment large macro-aggregates into small macro-aggregates and micro-aggregates fractions. This could, to some extent, explain the

relatively lower large macro-aggregates but higher small macro-aggregates and micro-aggregates under the canopy of *Z. gilletii*, where very high number of this earthworm species was found. The observed significant negative correlation between large macro-aggregates and earthworms and positive correlation with small macro-aggregates and micro-aggregates and earthworm abundance could support this suggestion. Decreasing amounts of the fraction micro-aggregates within macro-aggregates (mM) with increasing duration of cultivation could also have been caused by fragmentation of large macro-aggregates and thus the observed negative correlation between this fraction and earthworm abundance.

Termites are also critical in soil aggregation process by moving large amounts of soil which may result in the breakdown of large macro-aggregates into smaller macro-aggregates or micro-aggregates. Their role is especially notable in low-C soils where the activity of other soil macrofauna such as earthworms is relatively low (Ayuke et al., 2011a). However, in this study, there were no specific trends that could be associated with the termites. For instance, despite the high number of termites under the canopy of *E. grandis*, as described in chapter 3, there were no unique patterns in soil aggregate fractions under that specific tree. This shows that the role of this group of macrofauna in explaining the trends observed could be limited. The only termite species collected in the study site, *Microtermes spp.* (Table S1), are known to have sophisticated methods of growing fungus in their nests (Nobre et al., 2010) and therefore could have contributed less to soil aggregation process. In addition, termites are highly mobile compared to earthworms, and thus their role in aggregation could be restricted mainly to areas near their nests or the galleries and sheetings they make while gathering food. This could explain the weak correlation between termites and soil aggregates. Coarse particulate organic matter (cPOM) increased with duration of cultivation under the native trees (*C. megalocarpus* and *Z. gilletii*) but decreased under the exotic (*E. grandis*). This could perhaps be attributed to

a lower litter deposition and/or root turnover rate by *E. grandis*, a strategy this tree could be using to conserve the little nutrients available in the more degraded soils.

4.4.2 Effects of duration of cultivation, trees and soil macrofauna on C dynamics

Results of this study shows that the observed trends in soil-aggregate-C are better explained by effects of duration of cultivation and presence of earthworms. Based on the concept of soil aggregate hierarchy, the organic matter content increases with the aggregate size class, since the larger aggregates are composed of smaller aggregates and organic materials that bind them together (Tisdall and Oades 1982; Jastrow et al., 1996; Fonte et al., 2010). The decrease in aggregates-C with duration of cultivation shows that organic matter that was once protected in macro-aggregates could have been lost to decay. This trend is expected since the farms, especially in the older catchments, have been under continuous cultivation for over 60 years with minimal, if any, organic or inorganic inputs (Recha et al., 2013). These results are in agreement with similar studies which have reported loss of soil C following conversion of primary forest to agriculture (Solomon et al., 2007, Kimetu et al., 2008, Fonte et al., 2010). Nonetheless, agroforestry has been proposed as a better farm management practice in restoring and reclaiming degraded soils (Barrios et al., 1997, 2005; Lal, 2004; Lamb et al., 2005). Though trees can play an important role as 'resource islands', especially in highly degraded soils, results here indicate that the amount of litter deposited below the canopy and/or root turnover was not enough to compensate for the C lost over the years in the older farms.

Presence of large number of earthworms under the trees could also have played a significant role in the trends observed in soil aggregate-C. Studies have shown increased stabilisation and protection of C in cast derived micro-aggregates (biogenic), thus a higher C content relative to physicogenic micro-aggregates (Six et al., 2004; Pulleman et al., 2005). Therefore many of these studies have shown positive correlation between cast-derived micro-aggregates and

micro-aggregate C. However, the results obtained from soils under *Z. gilletii* in the current study was contrary to this suggestion in that, the presence of earthworms (*N. lacuum*) could be linked to the observed decrease in C content in almost all the aggregate fractions. As noted previously in section 4.4.1, *N. lacuum* could have been involved in fragmenting large macro-aggregates to small macro-aggregates and micro-aggregates. Disrupting large macro-aggregates may expose the physically protected C to degradation by soil microbes (Six et al., 2004), which could explain the relatively lower C content of the aggregate fractions below the canopy of *Z. gilletii*. In addition after passage through the earthworm's gut, stability of the newly formed micro-aggregates, and thus the protected C, may vary depending on physicochemical attraction between organic and mineral components and the lability, size and location of the organic matter (Tisdall and Oades 1982; Jastrow et al., 1996). These components may, on the other hand, be influenced by earthworm species, soil type and quantity and quality of organic materials being used as food substrate by the earthworms (Pulleman et al., 2005). In chapter 3, spatial variation of soil macrofauna reported higher number of earthworms under *Z. gilletii*, which could be linked to higher quality litter and root biomass derived from this tree. Higher quality organic matter could attract a higher population of microbes, and thus the abundance of soil fauna which benefit from feeding on such microbes. Thus, C losses through CO_2 evolution from micro-foodwebs is likely to increase in such soils and this may partly explain the observed lower C content in aggregates under the canopy of *Z. gilletii*. Physical and biochemical processes after excretion of casts could also affect stability of the new micro-aggregates (Shipitalo and Le Bayon, 2004), thus further determining the fate of aggregate-C. Since there were no other remarkable difference between the tree species, this gives a good evidence that *N. lacuum* played a significant role in reduction of micro-aggregates C. Given that the litter and root turnover from the trees is a major source of C in low input agroforestry systems, interaction between organic inputs and earthworms' species as shaped by specific

trees could be instrumental in soil aggregation process, and thus have far reaching implications on long-term C storage and SOM stabilisation.

4.5 Conclusion

In this study, the results show that small macro-aggregate and microaggegates were higher under the *Z. gilletii* tree, and decreased with distance from the tree stem. Further, these two fractions increased with increasing duration of cultivation, and this was particularly conspicuous in micro-aggregate fraction under the canopy of *Z. gilletii*. As hypothesised, aggregate associated C decreased with increasing duration of cultivation, but the magnitude of differences varied with the tree species. Most notable trend was observed under the canopy of *Z. gilletii* where micro-aggregate C increased with distance from the stem, a trend which was attributed to the presence of earthworm species, *N. lacuum*. While this earthworm species may be important in decompacting soil dominated by the compacting earthworm species, fragmenting macro-aggregates could expose the previously protected C to degradation. Thus, promoting tree diversity is critical in maintaining biodiversity, which is vital in a healthy soil ecosystem, while at the same time reducing negative effects such as accelerated soil C loss that can be induced by dominance of a given species.

CHAPTER FIVE

Spatial variation of soil macrofauna and nutrients in tropical agricultural systems influenced by historical charcoal production in South Nandi, Kenya

Authors: Solomon Kamau [a, b*], Edmundo Barrios [b], Nancy K. Karanja [a], Fredrick O. Ayuke [a], Johannes Lehmann [c, d]

[a] Department of Land Resource Management and Agricultural Technology, College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053, Nairobi, Kenya.

[b] World Agroforestry Centre, United Nations Avenue, P.O. Box 30677, Nairobi, Kenya.

[c] Soil and Crop Science, Cornell University, Ithaca, NY 14853, USA.

[d] Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14853, USA

[*]Corresponding author:

E-mail address: solkam08@gmail.com (S. Kamau)

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Abstract

The charcoal sector constitutes an important source of employment and revenue for many tropical agroecosystems. Better understanding of the effects of charcoal-making is thus warranted to guide actions aimed at minimising environmental externalities. Conversion of trees to charcoal eliminates canopy effects associated with the living trees while at the same time creates new conditions in and around spots where the charcoal is produced due to increased concentration of pyrogenic organic matter (PyOM). It is unclear, whether such unintentional PyOM additions play a role in the abundance and distribution patterns of soil macrofauna. A study was conducted in South Nandi (Kenya) to assess effects of PyOM on soil macrofauna, taking advantage of abandoned traditional charcoal kilns, where *Croton megalocarpus* and *Zanthoxylum gilletii* trees were used in charcoal making. Soil and soil macrofauna samples were collected at increasing distances from the centre of the spots. Total C, non-pyrogenic C (non-PyC) and total N progressively increased with increasing distance from the centre of the spots, whereas soil pH, pyrogenic C (PyC), available P and exchangeable K decreased. The number of earthworms and centipedes in *Z. gilletii* spots (119 and 14 individuals m^{-2}, respectively) was twice that in *C. megalocarpus* (47 and 7 individuals m^{-2}, respectively). However, the two showed contrasting trends in that while earthworms increased, centipedes decreased with increasing distance from the centre of the spots. Conversely, beetles, termites and crickets were significantly higher in *C. megalocarpus* than *Z. gilletii* spots, though sampling distance had no significant influence. As hypothesised, source of PyOM played a major role in determining soil properties and macrofauna distribution patterns thus showing the value of abandoned charcoal-making spots in contributing to a mosaic of soil conditions that could ultimately affect soil productivity in tropical agricultural systems.

Keywords: Charcoal-making spots; Pyrogenic carbon (PyC); Pyrogenic organic matter (PyOM); Soil macrofauna

5.1 Introduction

Similar to many tropical agroecosystems world-wide, the charcoal sector significantly contributes to Kenya's economy with 1.6 billion US dollars per year, employing close to 900 000 people in production and trade (SEI/UNDP 2016). In these agroecosystems, it is a common practice that trees are felled and charcoal made on site (FAO, 1987). Smallholder farmers deliberately retain indigenous trees during conversion of forest to cultivated land or intercrop trees with annual crops for fuel, fodder, timber and fruits among other products (Nyaga et al., 2015). Some trees are harvested to make charcoal for household consumption or for sale to supplement household income. Charcoal making is usually done by traditional earth-mound kilns, where pieces of felled trees and branches are carbonised at 360 °C to 470 °C for several days (Coomes and Miltner, 2016). Once charcoal making is complete, these kilns are usually abandoned. This practice possibly creates a mosaic of soil conditions in such areas because during the process of charcoal production a substantial amount of soil organic matter (SOM) is lost in and around the charcoal-making spots (kilns) (Ketterings and Bigham 2000; Knicker 2007). Furthermore, large amounts of pyrolysed materials, often referred to as pyrogenic organic matter (PyOM), also remain *in situ* after charcoal production (Güereña et al., 2015a) which may bring about changes in the structure and composition of soil biota. On the other hand, soil biota could modify the properties of PyOM/biochar through, for example, fragmentation into smaller pieces after ingestion by large organisms such as earthworms which increases their surface area, thus enhances or limits further effects of PyOM on other soil biota (Gomez-Eyles et al., 2013). Apart from effects of PyOM, operations during kiln construction or intense heat during charcoal production could also cause soil biota to suffer dramatic short or long-term alteration in such areas. Soil biota are essential components of the soil ecosystem as they drive vital soil functions such as nutrient cycling, soil structure modification, biological control of soil borne pests and diseases among others (Barrios, 2007; Brussaard et al., 2007).

Thus, changes in soil biota could have profound effects on productivity of low-input farming systems which are characteristic to agriculture in tropical Africa.

Soil macrofauna constitute an important component of soil biota given the significant impact of their activity on soil properties (Lavelle, 1997) and their role as bioindicators of potential unintended impacts of biochar applications to soil (Castracani et al., 2015). Given their larger body size, soil macrofauna are more susceptible to physical damage or destruction, loss of their habitat, and even removal of food substrates (Ayuke et al. 2009; Mbau et al., 2015). For instance, the loss of existing SOM during charcoal making, and its replacement with PyOM, could alter the soil microbial communities and dynamics, and change the carbon substrates and nutrients available for soil macrofauna through a cascade of effects within the soil food web. As noted by Lehmann et al. (2011), if a large proportion of C in pyrolysed materials is chemically stable, the microbes may not be able to readily utilise the C as an energy source. Chemical composition of feedstock also greatly affects the quality of pyrolysed materials (Warnock et al., 2007; Downie et al., 2009; Laird et al., 2009) and thus persistence of C which influence the growth of soil microorganisms. Such changes may in turn affect the abundance and diversity of the soil macrofauna which benefits from feeding on microorganisms found on the PyOM (Domene et al., 2015a). High concentration of PyOM in charcoal-making spots may also cause changes in soil physico-chemical properties (Glaser et al., 2002; Oguntunde et al., 2004), which could further affect soil macrofauna. For instance, addition of pyrolysed materials has been shown to alter tensile strength and bulk density of the soil, which can affect the soil water dynamics and gas transport (Lehmann et al., 2011; Masiello et al., 2015). In addition, application of these materials has also been shown to affect soil pH and therefore the amounts of available nutrients such (Warnock et al., 2007; Ippolito et al., 2015). Therefore, tree-felling and concomitant charcoal production may trigger significant changes in soil chemical and physical properties as well as shifts in soil macrofauna abundance and diversity on these soils

for extended periods of time. Such changes, with potential negative effect on soil productivity thus impacting socio-economic welfare of millions of people in Africa, are rarely addressed. In addition, soil fauna are among the least well-studied components of soil biota as affected by PyOM and biochar (Lehmann et al., 2011; Ameloot et al., 2013; Castracani et al., 2015).

Therefore, this study aimed at investigating spatial effects of PyOM on the abundance and distribution patterns of seven key soil macrofauna groups: earthworms, beetles, centipedes, millipedes, spiders, termites and ants. I took advantage of existing charcoal-making spots derived from traditional earth-mound kilns where *Croton megalocarpus* Hutch. and *Zanthoxylum gilletii* (De Wild.) P.G.Waterman had been used for charcoal making *in situ*. It was hypothesised that PyOM additions modify soil chemical properties and consequently soil macrofauna abundance and spatial distribution. Given the significant differences in plant tissue quality reported in chapter 3 of this thesis for the same tree species, I expected that this would likely be reflected in charcoal-making spots and hence influence the abundance and spatial distribution of soil macrofauna.

5.2 Materials and methods

5.2.1 Description of the study site

The study was conducted in the Kapchorwa region of Nandi County (Kenya) on farmers' fields, approximately 20 km Southwest of Kapsabet town. The region lies along the Kakamega-Nandi forest complex, an extension of the Guinean-Congolian forest (Latitude 0° 10' 00" N and Longitude 35° 0' 00" E), at an average altitude 1800 m above sea level (Güereña et al., 2015a). Rainfall occurs in a bimodal pattern, with an annual total of about 2000 mm, distributed between April and June (1200 mm) and August and October (800 mm). Temperatures are fairly constant throughout the year with mean minimum and maximum annual temperatures of about 18 and 27 °C, respectively. Soils are classified as kaolinitic Acrisols based on the

FAO/UNESCO classification (Recha et al., 2013). The indigenous vegetation is dominated by *Funtumia africana* (Benth.) Stapf, *Prunus africana* (Hook.f.) Kalkman, *Ficus* spp., *Croton* spp., and *Celtis* spp. (Glenday, 2006). The area was originally occupied by a sparse population of former forest dwelling human communities who practiced shifting cultivation, hunting and gathering (Mbau et al., 2015). However, high population growth rate and immigration into the area has reduced average land holding to less than 0.5 ha per household. The farms are dominated by cereal cultivation, with maize and beans being the predominant crops often intercropped with indigenous and exotic trees as described in chapter 3.

5.2.2 Selection of charcoal-making spots used in the study

Identification of charcoal-making spots to be used in the study was guided by participatory action research tools involving randomly-selected farmers within the area of study (Barrios et al., 2012a). A total of 52 spots were identified in this process, with an average diameter of about 15 m, which were spread at an area of 28.9 ha. The criteria used in selection of charcoal-making spots to be used in this study were: (i) history of the spots: the type of tree used and the time since charcoal making were known. Each tree species used in charcoal making represented a treatment; (ii) distribution: the charcoal-making spots selected occurred isolated within the farms and thus free from interferences by trees. Only spots where *C. megalocarpus* and *Z. gilletii* were used in charcoal making, fulfilled the selection criteria in the study area. Five spots of each tree type were selected for the study. All charcoal-making spots had been abandoned 2 years before sampling. At the time of sampling, all the spots were under maize-beans intercrop.

5.2.3 Soil macrofauna sampling

The area around selected charcoal-making spots was subdivided into four concentric zones, W, X, Y and Z based on an adaptation of the sampling described in chapter 3. Zone W was located

0.25 m from the centre of the spot, X at the middle of the spot, and Y located at the edge. Zone Z was located away from edge of the charcoal-making spots at an equivalent distance to that between W and Y. Soil monoliths (0.25 × 0.25 × 0.30 m) were collected using the standard Tropical Soil Biology and Fertility Institute (TSBF) method as described by Anderson and Ingram (1993), in each concentric zone following four transects at right angles from each other, for a total of 16 monoliths per spot. Soil monoliths were hand-sorted in trays and all soil macrofauna seen with the naked eye were collected, counted, weighed and preserved in 75% alcohol, except for the earthworms which were first placed in 75% ethanol and then fixed in 4% formaldehyde and stored in sealed and labelled vials. The preservative solution was replaced when a change in colour was observed. Soil fauna were identified at least to genera or species, except a few (centipedes, earwigs and two of the beetles' families) where the identification keys only allowed identification to family level. Earthworms were further separated into ecological groups: epigeic and endogeic. The abundance of the soil fauna is reported as mean individuals per square metre (individuals m^{-2}).

5.2.4 Soil and PyOM chemical analyses

Fragments of PyOM were collected from charcoal-making spots at the points where soil monoliths, described in section 5.2.3, were excavated. The PyOM collected was air dried in the field before being transferred into paper bags for laboratory analysis. Once in the laboratory, the samples were further dried in the oven at 60°C to a constant weight, ground and passed through a 2 mm sieve and stored in bags. In addition, after removal of soil macrofauna, soil from each of the 4 monoliths by sampling zone was thoroughly mixed, and a sample of about 500 g collected for analysis. The PyOM samples were analysed for C, N, P, K, Ca and Mg (expressed as mg per g of PyOM dry weight) as well as lignin and polyphenol (expressed as percentage values). Total C and N were determined by FLASH 2000 NC Analyser

(ThermoFisher Scientific, Cambridge, UK) while P, K, Ca and Mg were extracted through a closed-vessel microwave-assisted digestion system (Miller, 1998) and determined using inductively coupled plasma atomic emission spectroscopy (Isaac and Johnson, 1998). Lignin content was analysed using the acid detergent fibre method while total polyphenols were measured by the Folin-Denis method (Anderson and Ingram, 1993). Soil parameters measured included: total N and C, plant-available P and bases (Ca, Mg and K) (expressed as mg per g of soil dry weight except P, expressed as mg per kg of soil dry weight) and soil pH. Total C and N were determined using NC Analyser, while P and the bases were extracted following Mehlich-3 procedure (Mehlich, 1984) and determined using inductively coupled plasma atomic emission spectroscopy. Soil pH was determined using a pH meter with a soil-water ratio of 1:2.5 (Anderson and Ingram, 1993). Soil PyC was determined using partial least-squares (PLS) regression analysis of mid-infrared (MIR) spectroscopy data using spectral calibration from previous work done in the same study area by Güereña et al. (2015a).

5.2.5 Statistical analyses

All statistical analyses were carried out using R software version 3.2.2 (R Core Team 2015). Soil macrofauna abundance data was modelled using generalised linear mixed models (GLMM) as a function of source of PyOM and zone of sampling, including the replicates as a random factor using R package lme4 (Bates et al., 2015). Several models were built based on the formula (Variable ~ Species + Zone + Species : Zone + (1|Replicate : Species), such that terms could be added or removed from the model. The term 'Species' referred to the tree species used in charcoal making, whereas 'Zone' was the sampling zone as related to the distance from the centre of charcoal-making spots. Negative binomial regression analysis was chosen as an extension of the Poisson distribution to allow for the count data with a significant proportion of zero values. When analysis of variance (ANOVA) showed significant main or

interactive effects, Tukey's HSD post-hoc comparisons were performed at $\alpha = 0.05$. Further, relative differences in soil chemical parameters between zones in charcoal-making spots (W, X and Y) and away from the spot (Z) were assessed.

5.3 Results

5.3.1 Quality parameters of PyOM fragments

The elements P, Mg and K were significantly higher in PyOM derived from *Z. gilletii* (0.7, 2.0 and 2.8 mg g^{-1}, respectively) compared to *C. megalocarpus* (0.4, 1.3 and 1.9 mg g^{-1}, respectively) (Table 5.1). On the contrary, Ca was higher in *C. megalocarpus* PyOM (20.1 mg g^{-1}) than that of *Z. gilletii* PyOM (11.2 mg g^{-1}). Thus, due to its lower P content, the C/P ratio of *C. megalocarpus* PyOM was more than double the value recorded in *Z. gilletii* PyOM. The ratio PP/N was significantly higher in *Z. gilletii* PyOM than in *C. megalocarpus* PyOM.

Table 5.1: Quality parameters (mean ± SE) of PyOM collected in charcoal-making spots.

Parameter	***Croton megalocarpus***	***Zanthoxylum gilletii***	***p*-value**
C (mg g^{-1})	587.0 (4.0)^{a}	572.0 (3.0)^{a}	0.257
N (mg g^{-1})	7.9 (0.7)^{a}	6.9 (1.1)^{a}	0.443
P (mg g^{-1})	**0.4 (0.1)^{b}**	**0.7 (0.1)^{a}**	**0.012**
K (mg g^{-1})	**1.9 (0.2)^{b}**	**2.8 (0.1)^{a}**	**0.050**
Ca (mg g^{-1})	**20.1 (4.9)^{a}**	**11.2 (0.3)^{b}**	**0.010**
Mg (mg g^{-1})	**1.3 (0.1)^{b}**	**2.0 (0.4)^{a}**	**0.035**
C/N	58.0 (5.9)^{a}	60.3 (10.4)^{a}	0.852
C/P	**1296.9 (81.4)^{a}**	**582.3 (76.1)^{b}**	**0.015**
L (%)	37.6 (3.5)^{a}	39.2 (2.7)^{a}	0.819
PP (%)	0.1 (0.1)^{a}	0.2 (0.1)^{a}	0.064
L/N	48.2 (4.6)^{a}	58.0 (5.0)^{a}	0.305
PP/N	**0.1 (0.03)^{b}**	**0.3 (0.2)^{a}**	**0.044**
(L+PP)/N	48.2 (4.6)^{a}	58.2 (4.8)^{a}	0.077

Within rows, means followed by different lower case letters in superscript are significantly different at $p < 0.05$. Means were separated based on Tukey's honest significant difference (HSD) test.

5.3.2 Effect of charcoal making on soil chemical properties

Seven of the nine soil chemical parameters measured were significantly affected by charcoal making, and the magnitude of the differences depended on the type of tree used in charcoal making and the sampling zone (Table 5.2). Total C, non-PyC and total N were higher in spots where *C. megalocarpus* was used in charcoal making (37.0 mg, 34.0 mg and 3.5 mg g^{-1}, respectively) than in *Z. gilletii* spots (29.9 mg, 25.6 mg and 2.6 mg g^{-1}, respectively) (Table 5.3). On the other hand, PyC, P and K were higher in spots rich in *Z. gilletii* PyOM (4.4 mg, 27.2 mg and 0.5 mg g^{-1}, respectively) than those rich in *C. megalocarpus* PyOM (3.6 mg, 18.0 mg and 0.4 mg g^{-1}, respectively). Sampling zone significantly affected soil pH, PyC, available P and exchangeable K, with magnitude of the differences decreasing with increasing distance from the centre of the spot (Figure 5.1). Higher differences in soil pH were recorded in spots rich in *Z. gilletii* PyOM and progressively declined from 6.7 in zone W at the centre of the spot to the lowest 6.2 in zone Z away from the spot. PyC concentration was greatest, 6.8 and 4.9 mg g^{-1}, at the centre of *Z. gilletii* and *C. megalocarpus* charcoal-making spots respectively, compared to 1.8 mg g^{-1} away from the spots. In this case, the proportion of PyC in total C was highest, 23% and 14%, at the centre of the spots compared to 6% and 5% away from the spots, respectively. Soil available P in the spots was greatly affected by tree species and sampling zone. This element was highest at the centre (zone W) of *Z. gilletii* charcoal-making spots (44.4 mg kg^{-1}) and progressively declined to 18.6 mg kg^{-1} in zone Z outside the charcoal-making spots. The concentration of available P in soil at the centre of *Z. gilletii* spots was therefore more than twice as high as in the soil outside the spot. A similar, but less contrasting soil available P pattern was observed across sampling distances in *C. megalocarpus* spots. Soil exchangeable K also decreased from 0.5 mg g^{-1} in zone W to 0.4 mg g^{-1} in zone Z in *C. megalocarpus* spots and from 0.6 mg g^{-1} in zone W to 0.4 mg g^{-1} in Z in *Z. gilletii* spots.

Table 5.2: Summary of *p*-values generated from fitting soil chemical properties as a function of source of PyOM and zone of sampling using generalised linear models (GLM) (n=5).

Soil chemical parameter	*p*-value		
	Species	**Zone**	**Species*Zone**
pH (water)	0.389	0.803	**0.050**
Total C	**0.017**	0.963	0.999
PyC	**0.041**	**0.027**	0.563
PyC (as % of Total C)	**0.040**	**0.003**	0.220
Non-PyC	**0.013**	0.851	0.997
Total N	**0.012**	0.937	0.999
Extractable P	**0.003**	**0.015**	**0.048**
Extractable K	**0.050**	**0.021**	0.714
Extractable Ca	0.274	0.894	0.592
Extractable Mg	0.641	0.242	0.580

Table 5.3: Soil chemical properties (mean ± SE) as influenced by the charcoal-making spots (n=5).

Soil chemical parameter	*Croton megalocarpus*					*Zanthoxylum gilletii*				
	Sampling zone									
	W	**X**	**Y**	**Z**	**Mean†**	**W**	**X**	**Y**	**Z**	**Mean†**
pH (water)	6.34 (0.23)^{a}	6.30 (0.22)^{a}	6.14 (0.23)^{a}	6.04 (0.24)^{a}	6.21 (0.11)^{A}	**6.67 (0.09)^{a}**	**6.63 (0.06)^{a}**	**6.33 (0.01)^{b}**	**6.23 (0.02)^{b}**	6.21 (0.11)^{A}
Total C (mg g^{-1})	36.39 (5.39)^{a}	35.58 (4.68)^{a}	36.98 (5.36)^{a}	39.10 (5.76)^{a}	**37.01 (2.5)^{A}**	29.25 (6.14)^{a}	29.34 (3.45)^{a}	30.13 (3.78)^{a}	31.00 (4.93)^{a}	**37.01 (2.5)^{A}**
PyC (mg g^{-1})	**4.93 (0.30)^{a}**	**3.95 (0.87)^{a}**	**3.71 (1.01)^{a}**	**1.78 (0.79)^{b}**	**3.60 (0.3)^{B}**	**6.81 (0.27)^{a}**	**5.33 (1.07)^{a}**	**3.67 (0.72)^{b}**	**1.77 (0.80)^{c}**	**3.60 (0.3)^{B}**
PyC (as % of Total C)	**13.51 (3.19)^{a}**	**11.10 (3.01)^{a}**	**10.03 (3.63)^{ab}**	**4.50 (0.60)^{b}**	**9.79 (1.2)^{B}**	**23.28 (5.80)^{a}**	**18.17 (6.08)^{ab}**	**11.74 (4.02)^{bc}**	**5.71 (0.81)^{c}**	**9.79 (1.2)^{B}**
Non-PyC (mg g^{-1})	32.00 (5.13)^{a}	32.74 (4.41)^{a}	33.56 (3.91)^{a}	37.67 (5.18)^{a}	**33.99 (1.9)^{A}**	23.43 (9.80)^{a}	24.21 (10.44)^{a}	26.41 (7.01)^{a}	28.36 (8.14)^{a}	**33.99 (1.9)^{A}**
Total N (mg g^{-1})	3.30 (0.60)^{a}	3.30 (0.50)^{a}	3.50 (0.60)^{a}	3.70 (0.60)^{a}	**3.45 (0.27)^{A}**	2.50 (0.50)^{a}	2.50 (0.40)^{a}	2.60 (0.40)^{a}	2.60 (0.60)^{a}	**3.45 (0.27)^{A}**
Extractable P (mg kg^{-1})	**20.35 (3.54)^{a}**	**21.14 (4.00)^{a}**	**16.69 (1.54)^{ab}**	**13.70 (1.21)^{b}**	**17.97 (1.46)^{B}**	**44.40 (8.52)^{a}**	**25.57 (4.58)^{ab}**	**20.34 (1.93)^{b}**	**18.57 (0.25)^{b}**	**17.97 (1.46)^{B}**
Extractable K (mg g^{-1})	**0.47 (0.05)^{a}**	**0.44 (0.05)^{a}**	**0.41 (0.05)^{a}**	**0.35 (0.05)^{b}**	**0.42 (0.02)^{B}**	**0.57 (0.02)^{a}**	**0.55 (0.03)^{a}**	**0.47 (0.04)^{ab}**	**0.44 (0.03)^{b}**	**0.42 (0.02)^{B}**
Extractable Ca (mg g^{-1})	2.51 (0.49)^{a}	2.34 (0.44)^{a}	2.48 (0.53)^{a}	2.62 (0.59)^{a}	2.49 (0.24)^{A}	2.09 (0.04)^{a}	2.17 (0.07)^{a}	2.12 (0.01)^{a}	2.12 (0.29)^{a}	2.49 (0.24)^{A}
Extractable Mg (mg g^{-1})	0.29 (0.02)^{a}	0.29 (0.03)^{a}	0.28 (0.02)^{a}	0.28 (0.03)^{a}	0.29 (0.01)^{A}	0.30 (0.02)^{a}	0.30 (0.03)^{a}	0.27 (0.02)^{a}	0.27 (0.02)^{a}	0.29 (0.01)^{A}

† This mean gives aggregate effect of tree species used in charcoal-making. Within rows, means in bold and followed by different letters in superscript are significantly different at $p < 0.05$ (n=5). Uppercase letters indicate the differences based on tree species used in charcoal making while lowercase letters indicate the differences within sampling zones. Means were separated based on Tukey's honest significant difference (HSD) test.

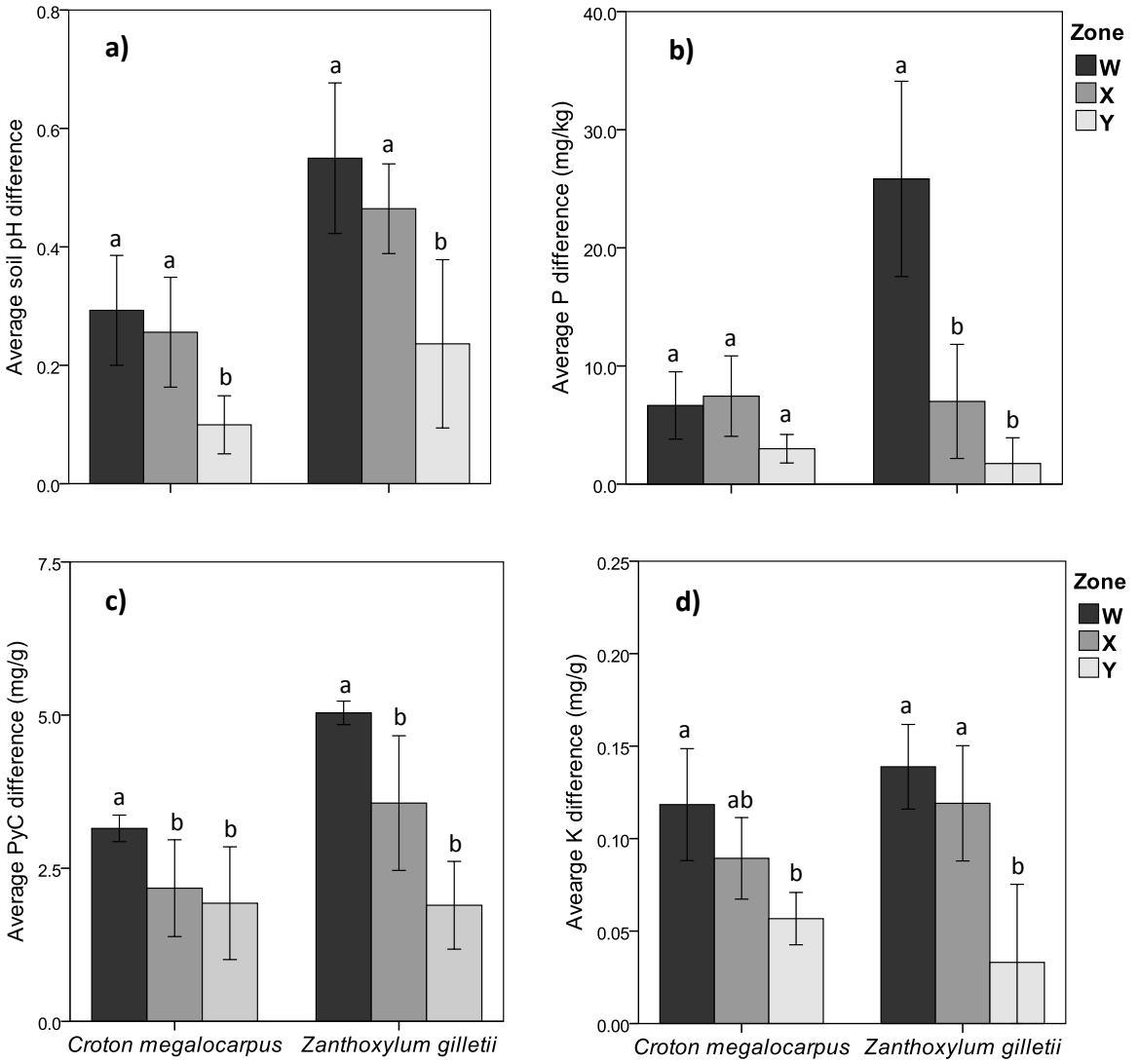

Figure 5.1: Absolute differences in pH, available P, Pyrogenic C and exchangeable K in zones W, X and Y in charcoal-making spots compared to Z away from the spots (means and standard errors). Different letters indicate significant differences between the zones within a given tree species at $p<0.05$ (n=5). Means were separated based on Tukey's honest significant difference (HSD) test.

5.3.3 Effect of charcoal making on soil macrofauna abundance

The abundance and spatial distribution of soil macrofauna was mainly affected by the type of tree used in charcoal making (Table 5.4). The average number of earthworms in charcoal-making spots rich in *Z. gilletii* PyOM (118.5 individuals m^{-2}) was more than twice the number recorded in spots rich in *C. megalocarpus* PyOM (47.2 individuals m^{-2}) (Table 5.5). While the number of earthworms in spots rich in *Z. gilletii* PyOM significantly increased with increasing distance from the centre of the spots, there was no significant spatial differences found in spots

rich in *C. megalocarpus* PyOM. Notably, the differences observed in the number of earthworms in spots rich in *Z. gilletii* PyOM can be attributed to endogeic earthworms which were dominant. There were no significant spatial distribution differences in epigeic earthworms. Higher number of centipedes were also found to be associated with *Z. gilletii* charcoal-making spots (14.0 individuals m^{-2}). This was twice the number recorded in spots rich in *C. megalocarpus* PyOM (7.0 individuals m^{-2}). Notably, the numbers decreased with increasing distance from the centre of spots rich in *Z. gilletii* PyOM. On the other hand, although beetles, termites and crickets were significantly higher in spots rich in *C. megalocarpus* PyOM, there were no spatial differences in their numbers (Table 5.5). Ants, earwigs, millipedes and spiders were not significantly different across the spots.

Table 5.4: Summary of *p*-values generated from fitting soil macrofauna as a function of source of PyOM and zone of sampling using generalised linear mixed models (GLMM) (n=5).

Soil fauna group	***p*-value**		
	Species	**Zone**	**Species*Zone**
Ants	0.348	0.608	0.911
Beetles	**0.047**	0.269	0.809
Termites	**0.036**	0.147	0.273
Crickets	**0.017**	0.326	0.639
Earwigs	1.000	0.608	0.092
Woodlice	0.092	0.792	0.123
True bugs	**0.004**	0.392	0.100
Earthworms	**<0.001**	0.947	**0.047**
Centipedes	**0.041**	0.680	**0.050**
Millipedes	0.865	0. 365	0.840
Spiders	0.308	0.227	0.770

Table 5.5: Soil macrofauna abundance (mean number of individuals m^{-2} ± SE) as influenced by charcoal-making spots (n=5).

Taxa	Family	Common name	*Croton megalocarpus*					*Zanthoxylum gilletii*				
			Sampling zone									
			W	X	Y	Z	Mean†	W	X	Y	Z	Mean†
Insects												
Hymenoptera	Formicidae	Ants	4.7 (2.0)^{a}	4.7 (3.4)^{a}	6.0 (4.2)^{a}	6.7 (2.7)^{a}	4.5 (4.3)^{A}	0.0^{a}	2.0 (2.0)^{a}	2.0 (2.0)^{a}	2.0 (2.0)^{a}	4.5 (4.3)^{A}
Coleoptera	(Total number)	Beetles	26.7 (5.0)^{a}	28.7 (7.0)^{a}	24.7 (8.6)^{a}	42.0 (8.0)^{a}	**30.6 (3.4)^{A}**	12.0 (4.0)^{a}	28.0 (2.0)^{a}	14.0 (4.7)^{a}	26.5 (10.9)^{a}	**30.6 (3.4)^{A}**
	Carabidae		0.0^{a}	0.0^{a}	0.0^{a}	2.7 (0.6)^{a}	**0.7 (0.3)^{B}**	2.0 (0.3)^{a}	4.0 (0.5)^{a}	0.0^{a}	0.0^{a}	**0.7 (0.3)^{B}**
	Curculionidae		0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.0^{B}**	0.0^{a}	4.0 (1.2)^{a}	0.0^{a}	10.0 (6.2)^{a}	**0.0^{B}**
	Elateridae		0.7 (0.2)^{a}	1.3 (0.6)^{a}	0.7 (0.6)^{a}	5.3 (1.2)^{a}	2.0 (0.9)^{A}	2.0 (0.6)^{a}	4.0 (0.3)^{a}	2.0 (0.2)^{a}	0.0^{a}	2.0 (0.9)^{A}
	Scarabaeidae		15.3 (3.2)^{a}	9.3 (4.6)^{a}	12.7 (5.6)^{a}	21.3 (5.2)^{a}	**14.7 (2.7)^{A}**	4.0 (2.6)^{a}	4.0 (1.3)^{a}	4.0 (2.1)^{a}	10.2 (3.0)^{a}	**14.7 (2.7)^{A}**
	Staphylinidae		10.7 (1.3)^{a}	18.0 (1.9)^{a}	11.3 (2.3)^{a}	12.7 (2.1)^{a}	13.2 (1.9)^{A}	10.0 (1.1)^{a}	10.0 (1.8)^{a}	8.0 (2.0)^{a}	12.0 (2.9)^{a}	13.2 (1.9)^{A}
	Tenebrionidae		0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{A}	0.0^{a}	2.0 (0.6)^{a}	0.0^{a}	0.0^{a}	0.0^{A}
Isoptera	Termitidae	Termites	8.7 (4.2)^{a}	33.3 (11.3)^{a}	10.7 (5.7)^{a}	25.3 (5.5)^{a}	**19.5 (3.9)^{A}**	8.0 (6.0)^{a}	6.0 (4.0)^{a}	12.0 (6.6)^{a}	0.0^{a}	**19.5 (3.9)^{A}**
Orthoptera	Gryllidae	Crickets	1.3 (0.1)^{a}	0.7 (0.1)^{a}	2.0 (0.2)^{a}	2.0 (0.1)^{a}	**1.5 (0.5)^{A}**	0.0^{a}	0.0^{a}	1.0 (0.1)^{a}	0.0^{a}	**1.5 (0.5)^{A}**
Dermaptera	Forficulidae	Earwigs	0.7 (0.1)^{a}	0.7 (0.1)^{a}	0.7 (0.1)^{a}	0.0^{a}	0.5 (0.3)^{A}	0.0^{a}	0.0^{a}	0.0^{a}	2.0 (0.3)^{a}	0.5 (0.3)^{A}
Hemiptera	Coreidae	True bugs	0.0^{a}	0.0^{a}	0.0^{a}	2.0 (0.3)^{a}	**0.5 (0.3)^{B}**	2.0 (0.5)^{a}	8.0 (3.1)^{a}	2.0 (0.2)^{a}	0.0^{a}	**0.5 (0.3)^{B}**
Isopoda	Porcellionidae	Woodlice	0.0^{a}	0.0^{a}	0.0^{a}	0.7 (0.3)^{a}	0.2 (0.2)^{A}	2.0 (1.3)^{a}	0.0^{a}	2.0 (1.0)^{a}	0.0^{a}	0.2 (0.2)^{A}
Earthworms‡												
Oligochaeta	(Total number)	Earthworms	55.9 (24.8)^{a}	47.4 (16.3)^{a}	44.7 (15.7)^{a}	40.6 (17.3)^{a}	**47.2 (7.6)^{B}**	**68.0 (18.7)^{c}**	**86.0 (12.3)^{c}**	**130.0 (14.8)^{b}**	**190.0 (16.3)^{a}**	**47.2 (7.6)^{B}**
	(Epigeic)		16.6 (4.9)^{a}	14.7 (7.0)^{a}	22.7 (8.1)^{a}	20.6 (10.3)^{a}	18.7 (3.2)^{A}	12.0 (4.7)^{a}	22.0 (6.4)^{a}	26.0 (9.1)^{a}	20.0 (5.1)^{a}	18.7 (3.2)^{A}
	(Endogeic)		39.3 (23.7)^{a}	32.7 (14.8)^{a}	22.0 (9.2)^{a}	20.0 (8.6)^{a}	**28.5 (6.4)^{B}**	**56.0 (14.9)^{c}**	**64.0 (19.8)^{c}**	**104.0 (10.3)^{b}**	**170.0 (13.6)^{a}**	**28.5 (6.4)^{B}**
Myriapods												
Chilopoda	Scolopendridae	Centipedes	8.7 (3.4)^{a}	9.3 (4.8)^{a}	2.0 (1.5)^{a}	7.3 (2.3)^{a}	**6.8 (1.6)^{B}**	**20.0 (6.1)^{a}**	**18.0 (7.2)^{a}**	**12.0 (5.9)^{ab}**	**6.0 (4.2)^{b}**	**6.8 (1.6)^{B}**
Diplopoda	Trigoniulidae	Millipedes	1.3 (0.9)^{a}	5.3 (2.1)^{a}	2.7 (1.1)^{a}	4.7 (3.4)^{a}	3.3 (1.1)^{A}	2.0 (2.0)^{a}	2.0 (2.0)^{a}	2.0 (2.0)^{a}	6.0 (2.9)^{a}	3.3 (1.1)^{A}
Arachnids												
Araneae	Araneidae	Spiders	2.0 (1.8)^{a}	2.7 (2.2)^{a}	4.0 (1.6)^{a}	3.3 (2.2)^{a}	3.0 (1.0)^{A}	0.0^{a}	2.0 (1.5)^{a}	0.0^{a}	4.0 (4.0)^{a}	3.0 (1.0)^{A}

† The mean gives an aggregate effect of the tree used in charcoal making. ‡ Earthworms were further separated into ecological groups: epigeic, endogeic and anecic groups. In this study, there were no anecic groups recovered. Within rows, means in bold and followed by different letters in superscript are significantly different at $p < 0.05$ (n=5). Uppercase letters indicate the differences based on tree species used in charcoal making while lowercase letters indicate the differences within the sampling zones. Means were separated based on Tukey's honest significant difference (HSD) test.

5.4 Discussion

5.4.1 Effects of in-field charcoal production and PyOM on soil chemical properties

It is likely that in-field charcoal production generated significant amounts of PyOM that contributed to the high soil pH and PyC at the centre of the spots where charcoal was produced (zone W). Changes in soil pH as a result of increased concentration of pyrolysed materials are frequently reported (Glaser et al., 2002; Ameloot et al., 2013). These changes could be brought about by, but not limited to, presence of negatively charged functional groups such as phenolic, carboxyl and hydroxyl, and high ash content in the pyrolysed material (Chintala et al., 2014). The negative charges in the functional groups can bind H^+, and thus potentially affect soil pH. In addition to the PyOM, ash could have contributed to the observed differences in soil pH. During charcoal preparation, sealing of the traditional earth-mound kilns is often not uniform and air leaks may occur and lead to complete burning of some of the charcoal (FAO, 1987) therefore increasing the concentration of ash in such spots. The production of compounds such as oxides, hydroxides and carbonates in the ash could also bind H^+ ions from the soil solution and therefore contribute to increased soil pH. The mode of charcoal removal from these traditional kilns is usually accomplished by raking charcoal radially towards the outside of the kiln. This is a typical practice that facilitates extinguishing fire from all of the charcoal pieces to avoid re-ignition. In order to retrieve the charcoal that might have been buried in the process of opening the kiln, the mixture of PyOM, ash and burned soil are further spread out. Such a phenomenon could have contributed to the spread of PyOM and ash, and therefore the observed trends in pH and PyC from the centre of charcoal-making spots towards the outside.

Besides changing soil pH and PyC, PyOM and ash could also have contributed to the observed trends in soil P and K. Since pyrolysis mainly leads to losses of C, N, O and H, nutrients that volatilize at greater temperatures such as P, K and other metals in the wood are expected to remain in PyOM (Enders et al., 2012). Higher concentration of P and K are thus expected to be found at the centre of the spots where the kilns were located. It is important to note that while leaves from the trees harvested for charcoal making are locally used as a thin interface between the wood being carbonised and the soil which is used to seal the kiln, their contribution to the nutrients in and around the kiln is likely to be small. The practice of raking the charcoal during retrieval from the kilns mentioned earlier, could have also contributed to the spread of PyOM, ash and burned soil and thus the observed progressive decline in P and K from the centre towards the outside of PyOM-rich spots. Several studies have reported similar results. For instance, Chidumayo (1994a) reported that carbonisation of wood in miombo woodland in Zambia using traditional earth kilns resulted in higher soil pH, P and K. Similarly, Oguntunde et al. (2004) in Ghana reported that soil pH, P, Ca and Mg were higher in charcoal production sites compared to the adjacent soil. Nevertheless, the feedstock plays an important role in determining the amounts of nutrients returned into the soil in such cases. For instance, wood with higher amounts of non-volatile nutrients will be expected to produce PyOM with higher concentrations of these nutrients. Physiological differences among trees influence their ability to retain nutrients in the wood (Chidumayo 1994b), hence the type of tree used in charcoal making will greatly affect the amounts of nutrients in PyOM and their concentration in these spots. It is therefore likely that higher amounts of soil available P in *Z. gilletii* spots could have resulted from higher concentrations of P in the wood of this tree, as indicated by the quality characteristics of the PyOM. However, it should be noted that differences in production practices could affect soil properties of the abandoned charcoal kilns. Thus, other

differences between the charcoal-making spots instead of, or in addition to, chemical quality attributes of individual tree species may cause the observed differences in soil properties. For instance, considerable amounts of C and N are lost from the soil in and around the kiln in the process of charcoal production likely through direct heat (Ketterings and Bigham 2000; Knicker 2007) or operations during kiln construction (digging, loading and unloading). This could, to some extent, explain the observed variation in concentration of these elements.

5.4.2 Effects of charcoal production on soil macrofauna abundance and distribution

In this study, high concentration of PyOM (as indicated by the higher PyC) in the charcoal-making spots had contrasting effects on different soil macrofauna groups. Among these, earthworms, which are known to rely heavily on soil organic matter as a source of energy or to feed on the microbes growing on this substrate or their metabolites (Shan et al., 2010, 2013), showed the clearest trends. The low soil C, and even more the low non-PyC contents (likely more important than PyC as an energy source for soil biota) could have made the soil in charcoal-making spots a less desirable substrate for earthworms. In *C. megalocarpus* spots where total C and non-PyC was significantly higher than in *Z. gilletii* spots, the presumably negative effects of PyOM appeared to be lower, given that the abundance of earthworms was not significantly different between the four sampling zones. Of the two ecological groups of earthworms found in this study, the endogeic group, which ingests substantial amounts of organic matter and mineral soil were the most affected and this was especially conspicuous in *Z. gilletii* charcoal-making spots. In a study by Topoliantz and Ponge (2003) where the authors were looking at the response of earthworms (*Pontoscolex corethrurus*) to charcoal application, it was reported that the burrows made by the earthworms in the soil + charcoal treatment could have been created as the earthworms pushed charcoal particles

aside, perhaps, in search of charcoal-free soil. However, although PyOM could be a less desirable substrate as an energy source, it has been proposed that earthworms could selectively ingest it for other purposes. For instance, Lehmann et al. (2011) suggested that earthworms can ingest biochar particles to help in grinding food in their gizzard, a function similar to that of sand. In addition, its ingestion may benefit earthworms indirectly by enhancing production of earthworm's digestive enzymes from microbial communities or for its detoxifying and liming properties (Topoliantz and Ponge, 2003). Therefore, if all conditions are held constant, the quality of PyOM could be an important determinant of the earthworms' preference for such a material. In this study, if the earthworms were ingesting PyOM, then it is likely that they preferred the PyOM from *Z. gilletii* tree over that from *C. megalocarpus* given the significant differences in earthworm abundance recorded between the soils affected by PyOM made from these two tree species. The quality of PyOM can also be measured by the concentration of toxic substances such as polycyclic aromatic hydrocarbons (PAHs), dioxins, among other compounds (Hale et al., 2012). Though it is unlikely that these toxic compounds could have had a significant influence on the current population of soil macrofauna given the relatively long period of time the PyOM had stayed in the field before sampling was conducted, I cannot rule out such a possibility. The differences observed in earthworm abundance could also be attributed to the influence of PyOM on soil conditions. For instance, studies have reported changes in soil chemical and physical properties as a result of PyOM accumulation from charcoal production (Oguntunde et al., 2004; Coomes and Miltner 2016). In the current study, progressive decrease in pH towards outside of the charcoal-making spots may have accounted for the observed earthworm trends. Additionally PyOM/biochar has also been demonstrated to alter soil tensile strength and bulk density, which can affect the hydrodynamics and gas transport in the soil (Lehmann et al., 2011; Masiello et al., 2015). In other

studies, application of PyOM/biochar has been shown to affect soil albedo, thus possibly affecting soil temperature and moisture (Castracani et al., 2015). Although soil moisture and temperature were not measured spots, I believe that the variation in these parameters may have also contributed to the differences observed in earthworm abundance.

In contrast to earthworms, a significant number of centipedes was recorded in charcoal-making spots, particularly those rich in *Z. gilletii* PyOM. Apart from food, habitat provision has been reported to play a big role in determining the abundance of soil organisms. Pyrolysed materials such as PyOM provide niches for soil microfauna (protozoa, tardigrades and nematodes) and mesofauna (mites and collembola) to access resources and thrive (Lehmann et al., 2011). Since centipedes are known to be predators, this could suggest that their high numbers in charcoal-making spots could have, perhaps, been a consequence of increased prey abundance. There was no spatial variation or definite patterns in abundance of ants, beetles, termites, crickets, earwigs, millipedes and spiders. This could be due to the fact that these groups of macrofauna are relatively mobile, and therefore may not have been directly affected by PyOM.

5.5 Conclusion

The study has shown that soils in the charcoal-making spots were rich in PyC, P and K, which all progressively decreased with increasing distance from the centre of the spots. However, total C and N and non-PyC progressively increased with distance from the centre of the spots. All soil macrofauna studied (except centipedes) were lower in charcoal-making spots, perhaps due to negative effects of the charcoal production process on them. One reason may be that PyC, which was higher in these spots, was too recalcitrant to support soil microbial growth, and therefore reducing the abundance of soil macrofauna such as earthworms which feed on microbes growing

on such substrates and/or their metabolites. Therefore, assessments in agricultural landscapes dominated by charcoal production need to consider the differential effects of *in situ* production of charcoal in contributing to a mosaic of soil conditions influencing soil macrofauna abundance and distribution. Further research is needed to assess short-term vs. long-term effects of *in situ* charcoal production on ecological functions driven by soil macrofauna in such a mosaic of soil conditions and thus the potential effects of such activities on socio-economic welfare smallholder farmers in Africa and other regions where charcoal making is prevalent.

CHAPTER SIX

Effects of pyrogenic organic matter (PyOM) on casting activity of earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae)

Authors: Solomon Kamau [a, b*], Edmundo Barrios [b], Nancy K. Karanja [a], Fredrick O. Ayuke [a], Johannes Lehmann [c, d]

[a] Department of Land Resource Management and Agricultural Technology, College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053, Nairobi, Kenya.

[b] World Agroforestry Centre, United Nations Avenue, P.O. Box 30677, Nairobi, Kenya.

[c] Soil and Crop Science, Cornell University, Ithaca, NY 14853, USA.

[d] Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14853, USA

[*]Corresponding author:

E-mail address: solkam08@gmail.com (S. Kamau)

To be submitted

Abstract

Conversion of tropical forests to cultivated farms often results in intense and rapid changes in soil properties due to either rapid loss of soil organic matter (SOM) or conversion of native SOM into pyrogenic organic matter (PyOM) through slash-and-burn or chop-and-char during forest clearance. However, little is known about changes in soil macrofauna activity that occur when large amounts of PyOM/biochar are added into the soil. A thirty-day mesocosm study was conducted to assess effects of PyOM derived from two trees common in South Nandi, *Croton megalocarpus* and *Zanthoxylum gilletii*, on the activity of a geophagous earthworm, *Pontoscolex corethrurus*. A portion of the PyOM was washed with acetone and 2M HCl, to remove volatile matter and ash/mineral contents, respectively. Each of the PyOM type was mixed with soil at a rate equivalent to 5, 10 and 25 Mg PyOM ha^{-1}. Casts weight was recorded and C and N content of the casts and bulk soil were analysed at the end. Casts dry weight was more affected by amounts than the type of PyOM. The highest mean cast weights (188.1 g and 176.5 g) were recorded in mesocosm that received 5 Mg ha^{-1} of *C. megalocarpus* and *Z. gilletii* PyOM, respectively. Notably, the weight decreased with increasing PyOM amounts with a 4% and 15% reduction in soil with *C. megalocarpus* PyOM and a 6% and 8% reduction in soil with *Z. gilletii* PyOM for the mesocosms which received 10 and 25 Mg ha^{-1} of PyOM, respectively. Similar trends were observed in mesocosms with PyOM washed with the acid. However, cast weight in mesocosms which received PyOM washed with acetone was not significantly different among the different application rates. Lower cast production in mesocosms with a higher concentration of PyOM shows that the substrate could have been unpalatable to the earthworms. Such outcomes could have implications in soil ecosystems if mass application of PyOM/biochar was to be implemented.

Keywords: Carbon; Earthworm casts; Nitrogen; *Pontoscolex corethrurus*

6.1 Introduction

Conversion of tropical forests to cultivated lands often results in intense and rapid changes in soil, and these are likely to affect soil macrofauna abundance and distribution patterns (Beare et al., 1997; Decaëns et al., 2004). These changes are linked to either rapid loss of soil organic matter (SOM) or conversion of native SOM into more recalcitrant forms, through burning during forest clearance. The most common mode of forest clearance is through slash and burn as well as charcoal making, a process which leaves large amounts of charcoal, often referred to as pyrogenic organic matter (PyOM). Charcoal making, usually by traditional earth-mound kilns, is a common practice in most agroecosystems across Africa. These kilns are usually abandoned after charcoal making, a practice which probably creates areas rich in PyOM and such areas may become 'hotspots' of favourable or unfavourable conditions thus bringing about changes in the abundance, diversity and distribution of soil macrofauna.

Given their profound influence of their activities on soil processes and functions, soil macrofauna are an essential part of soil ecosystem and thus, what affects them positively or negatively may have far reaching effects on soil productivity (Barrios, 2007; Mbau et al., 2015). In other occasions, crop residues and agroforestry by-products are deliberately converted into PyOM/biochar and applied as soil amendments with the aim of improving soil productivity or as a means of sequestering C into the soil (Lehmann et al., 2006). Addition of large amounts of PyOM or replacement of the native soil organic matter with PyOM, could alter the carbon substrates and nutrients available for soil macrofauna through a cascade of effects within the soil food web. Thus, soil changes that may occur after forest conversion to cultivated land and/or addition of PyOM can cause disappearance of forest-specific species and establishment of peregrine species that are more adapted to disturbed soils or can withstand effects arising from PyOM additions. For instance,

Pontoscolex corethrurus, a geophagous endogeic earthworm species is said to be highly adapted to tropical cultivated soils over a wide range of soil conditions due to its capacity to consume low-quality organic matter (Topoliantz and Ponge 2005; Ponge et al., 2006). The species has been found to have significant influence on soil structure due to its burrowing and casting activities and thus has been suggested to have played a significant role in formation of *Terra Preta* soils of the Amazon through incorporation of charcoal particles into the soil profile (Ponge et al., 2006). Nonetheless, its casting activity has been shown to promote soil compaction when there is limited or absence of a diverse soil macrofauna community capable of fragmenting the large coalescent casts produced by this species (Barrios et al., 2005). This highlights how dominance of a single soil macrofauna species, as a result of soil management decisions, could affect soil productivity.

With the increasing interest in utilisation of biochar as a soil amendment, there is also an increasing concern about possible presence of organic and inorganic contaminants which could be incorporated into the soil through biochar application (Verheijen et al., 2010). Volatile organic compounds (VOCs) are common organic contaminants often formed during pyrolysis, and thus sometimes end up being accumulated in the PyOM or biochar produced. These compounds are probably formed either by breakdown and/or by rearrangement of the original organic biomass structure (Spokas et al., 2011). For instance, the complex organic compounds are cracked into smaller unstable fragments which may recombine with other free reactive molecules or radicals into more stable but potentially toxic compounds (Hale et al., 2012). Studies have confirmed presence of the most common group of toxic compounds, the polycyclic aromatic hydrocarbons (PAHs), as well as traces of dioxins and furans in biochar (Verheijen et al., 2010; Hale et al., 2012; Domene et al., 2015b). Other potential direct negative effects on soil macrofauna could be brought about by the presence of heavy metal contaminants or due to excessive salinization or liming

effects after application of PyOM or biochar (Domene et al., 2015b). Apart from the direct effects of toxic compounds on soil macrofauna, suppression or stimulation of soil microbes due to the presence of PyOM could indirectly affect soil macrofauna through the food web (McCormack et al., 2013). In spite of such pertinent issues being raised, there is little information on how farm management decisions such as mass application of biochar or the addition of large amounts of PyOM due to charcoal making can affect soil macrofauna abundance and their activities. Of concern is that, extensive and indiscriminate application of biochar without prior information could have irreversible effects on soil biodiversity, and thus soil productivity (Verheijen et al., 2010).

Use of earthworms as a model organisms is vested on the ease of assessing their response to environmental perturbations in tests such as growth, mortality or activity rate as well as reproduction patterns, among other tests (Li et al., 2011). Therefore, the aim of this study was to analyse the casting activity of *P. corethrurus* in PyOM-enriched soil. The PyOM was derived from two tree species; *Croton megalocarpus* and *Zanthoxylum gilletii*, the most common native tree species along the Nandi-Kakamega forest complex. The two types of PyOM were applied either untreated or washed either with acetone or with 2M HCl. The aim of washing with acetone and acid was to remove volatile matter and ash/mineral contents, respectively which could affect the response of earthworms to the presence of PyOM in the soil.

6.2 Materials and methods

6.2.1 The experimental site

The study was conducted at the World Agroforestry Centre's (ICRAF) Soil Ecology Facility in Nairobi, located at Latitude 1° 14' 08" S and Longitude 36° 49' 12" E. At an average altitude of about 1700 m above sea level, mean daily temperature ranges between 11˚C and 25˚C. The soil

used in the experiment was obtained from South Nandi, the same area described in chapter 3. About 200 kg of soil was obtained from each of the three categories of farms which have been cultivated for 10, 16 and 62 years. Except for the duration of cultivation, all the other characteristics of the three catchments were similar (Güereña et al., 2015a). Soil was taken from the upper 10 cm layer; any organic material at the surface was removed prior to excavation. The soil was transported to the experimental site and air-dried mixed and passed through 2 mm sieve.

6.2.2 Pyrogenic organic matter (PyOM) preparation and post-treatment procedure

Pyrogenic organic matter (PyOM) was prepared from trimmed branches of *Croton megalocarpus* and *Zanthoxylum gilletii* trees. These trees are commonly utilised in charcoal making in South Nandi using traditional earth-mound kilns as reported in chapter 5. The branches were separated from the leaves and the wood chopped into lengths of about 2 m. Two portions of land (about 3 m in diameter each) where the kilns were to be located were cleared of any farm residues, levelled and compacted. The chopped wood was placed upright, leaning towards a central pole. Leaves from the trimmed branches were spread over the stack and then covered with soil from around the kiln site. Wood from each tree were pyrolysed separately at temperatures of about 500°C for four days. This was to simulate the charcoal making process as done by the smallholder farmers in Africa, a practice which leaves large amounts of the fine PyOM fragments on such spots. Once ready, the PyOM was ground separately to appropriate size (<2 mm) and divided into three portions. One portion of the PyOM was kept untreated, second portion was washed with acetone while the third was washed with 2M HCl. In each of the treated portions, the PyOM was mixed with the washing agent at a ratio of 1:10 (*w/v*), and the mixture shaken overnight using a reciprocating shaker as described by Güereña et al. (2015b). The PyOM materials were then filtered through Whatman number 42 filter paper. After filtration, the PyOM that has been washed

with acetone was dried overnight at 60°C. The portion that was washed with 2M HCl was further treated with 1N NaOH in order to readjust the PyOM material to its original pH and then filtered. The PyOM material was then washed twice using de-ionised water at a ratio of 3:5 (*w/v*) in order to remove the excess Na^+ and Cl^-. The PyOM was then dried overnight at 60°C. Acid washing was intended to reduce ash and mineral contents.

6.2.3 The experimental procedure

Casting activity of earthworms (*Pontoscolex corethrurus*) was studied using modified mesocosms (0.15 m in diameter and 0.30 m in height). The earthworm species, *P. corethrurus* is an active earthworm and produces large coalescent aggregates that can easily be separated from the rest of the soil, therefore making it suitable indicator for the study of soil biological activity (Pauli et al., 2010). Each of the biochar type (untreated, washed with acetone or with acid) was weighed and mixed with soil corresponding to an application rate of 0, 5, 10 and 25 Mg ha^{-1}. For each of the treatments, 1.5 kg of the soil or soil + PyOM mixtures were placed into the tubes and moistened with water to field capacity through capillary wetting. There were five replication for each treatment. The soil or soil + PyOM mixture was allowed to stabilise for 24 hours after which, two mature *P. corethrurus* (average weight of 1.5±0.03 g) were introduced from the top of mesocosms. Top edges of the mesocosms were then covered with wet muslin cloth to avoid desiccation of earthworms while preventing them from escaping. The experiment was conducted for a period of four weeks. Cast collection was done after every two days and at the end of the study. The casts were oven dried at 105°C and their dry weight recorded. The bulk soil was dried at 60°C awaiting analysis. Casts were ground and passed through 2 mm sieve for C and N analysis.

6.2.4 PyOM and Soil chemical analyses

After drying and grinding, the respective PyOM samples were analysed for the electrical conductivity, pH and major macro-elements C, N (total, NH_4-N andNO_3-N), P, K, Ca and Mg as well as Na and CEC. Polycyclic aromatic hydrocarbons (PAHs) were also analysed from the samples. Electrical conductivity was measured using an electrical conductivity metre while pH was determined using a pH meter with a PyOM-$CaCl_2$ solution ratio of 1:5 (Anderson and Ingram, 1993). Total C and N were determined by FLASH 2000 NC Analyser (ThermoFisher Scientific, Cambridge, UK), extractable NH_4-N and NO_3-N was extracted using 2M potassium chloride (KCl) and determined using steam distillation method (Bremner and Keeney 1965), while K, Na, Ca, Mg and CEC were determined using the compulsive exchange method (Gillman and Sumpter, 1986). Soil parameters measured included: total N, C and pH, whereas C and N were analysed from the casts. Total C and N were determined using NC Analyser. Soil pH was determined using a pH meter with a soil-$CaCl_2$ solution ratio of 1:2.5 (Anderson and Ingram, 1993).

6.2.5 Statistical analysis

The statistical software R, version 3.3.2 (R Core Team 2015) was used in statistical analyses. Due to the fact that surface casting was observed for the first two days only, and that there was no specific patterns between the treatments, these casts were combined with those collected at the end of the experiment. Therefore, all analyses on casts parameters (weight, C and N) are based on the total casts collected. The influence PyOM and earthworms on cast production and C and N dynamics in the casts and bulk soil was tested using generalised linear models. The package lme4 (Bates et al., 2015) was used in the analysis. A general model (where all the factors were included) was first used to test the effects of PyOM type, post-treatment process and application rate and all, two-fold and three-fold, interactions between these factors. Preliminary results from the model

showed no significant interactions between the factors. Further, among the three factors, only rate of PyOM application showed significant influence on the parameters measured. Therefore, the data were reanalysed separately, only taking into consideration the effects of PyOM application rate using one way analysis of variance (ANOVA). Tukey's HSD post-hoc tests were used to separate the means at $\alpha = 0.05$.

6.3 Results

6.3.1 Effects of post-treatment process on PyOM properties

Some chemical characteristics in PyOM from the two trees differed while others did not. The most outstanding difference between the untreated PyOM from the two trees was electrical conductivity (EC), pH, NH_4-N, CEC, Na and exchangeable sodium percentage (ESP) (Table 6.1). The PyOM derived from *Z. gilletii* tree had a higher EC (16.0 dS m^{-1}), NH_4-N content (23.0 mg kg^{-1}), CEC (97.0 cmol (+) kg^{-1}), Na (22.3 g kg^{-1}) and ESP (95%), but lower in pH (2.0) compared to PyOM derived from *C. megalocarpus* tree. Post-treatment of PyOM was only effective on *Z. gilletii* PyOM where washing the biochar with 2M HCl decreased the electrical conductivity increased pH, while NH_4-N, CEC, Na and ESP decreased. However, there was no notable change in chemical quality of PyOM derived from *C. megalocarpus* tree.

Table 6.1: Chemical quality parameters of untreated PyOM and PyOM washed with either 2M HCl or acetone.

Parameter	Units	PyOM treatment type					
		Croton megalocarpus			*Zanthoxylum gilletii*		
		Unwashed PyOM	Acetone-washed PyOM	Acid-washed PyOM	Unwashed PyOM	Acetone-washed PyOM	Acid-washed PyOM
Electrical Conductivity	dS/m	0.41	0.63	0.55	16.00	22.00	0.56
pH ($CaCl_2$)	pH units	8.20	7.90	8.00	2.00	3.80	8.30
Total C	g kg^{-1}	840.0	840.0	810.0	780.0	770.0	810.0
Total N	g kg^{-1}	6.80	7.20	6.20	6.70	5.80	6.50
KCl Extractable NH_4-N	mg kg^{-1}	0.46	1.60	0.30	23.00	5.60	0.88
KCl Extractable NO_3-N	mg kg^{-1}	0.88	1.10	1.40	0.85	1.70	1.50
Water Soluble P	mg kg^{-1}	65.0	66.0	60.0	37.0	51.0	70.0
Exchangeable Cations							
Ca	g kg^{-1}	1.40	1.34	1.70	0.52	1.22	1.50
K	g kg^{-1}	1.99	2.69	3.08	0.39	0.37	3.08
Mg	g kg^{-1}	0.12	0.17	0.21	0.07	0.06	0.22
Na	g kg^{-1}	0.02	0.11	0.01	22.3	27.6	0.02
CEC $_{(effective)}$	cmol (+) kg^{-1}	13.0	15.0	18.0	97.0	130.0	17.0
Exchangeable Ca	% of ECEC	52.0	44.0	47.0	2.70	4.70	43.0
Exchangeable K	% of ECEC	39.0	45.0	43.0	1.00	0.73	46.0
Exchangeable Mg	% of ECEC	8.10	8.80	9.60	0.58	0.37	10.0
Exchangeable Sodium Percentage	% of ECEC	0.70	2.90	0.31	95.0	94.0	0.58

6.3.2 Effects of PyOM on earthworm cast production

Cast weight was affected by amounts rather than the source of PyOM, where the weight generally decreased with increasing amounts of PyOM (Table 6.2). In mesocosms which received untreated *C. megalocarpus* PyOM, cast weight significantly decreased from 188.1 g in lowest PyOM application rate (5 Mg ha^{-1}) to 180.9 g and 160.2 g in mesocosms with an equivalent of 10 and 25 Mg ha^{-1} of the PyOM, respectively. This represented close to 15% decline in cast weight in the highest application rate, compared to a decline of about 4% in mesocosms which received the PyOM at an equivalent rate of 10 Mg ha^{-1}. Similarly, cast weight decreased from 176.5 g in mesocosms with 5 Mg ha^{-1} of untreated *Z. gilletii* PyOM to 165.7 and 163.5 g in mesocosms which received the same PyOM at an equivalent rate of 10 Mg ha^{-1} and 25 Mg ha^{-1}, respectively. This represented about 8% decrease in the highest application rate (25 Mg ha^{-1}) and 6% in mesocosms with an equivalent rate of 10 Mg ha^{-1} *Z. gilletii* PyOM. Although there were no significant differences in cast weight in mesocosms which received different rates of acetone-washed PyOM from either of tree species, major differences were recorded in PyOM washed with HCl. For instance, in *C. megalocarpus* derived PyOM, the cast weight decreased from 182.2 g in the lowest PyOM application rate (5 Mg ha^{-1}) to 177.5 g and 162.5 g in mesocosms which received *C. megalocarpus* PyOM at an equivalent rate of 10 and 25 Mg ha^{-1}, respectively. This represented an 11% decrease in cast weight in the highest application rate and 3% in mesocosms which received PyOM at an equivalent rate of 10 Mg ha^{-1}. In *Z. gilletii* derived PyOM, the cast weight decreased from 185.7 g in the lowest PyOM application rate (5 Mg ha^{-1}) to 169.3 g and 135.9 g in mesocosms which received *Z. gilletii* PyOM at an equivalent rate of 10 and 25 Mg ha^{-1}, respectively. This represented a decrease of about 30% in mesocosms with highest application rate (25 Mg ha^{-1}) and about 10% in mesocosms with an equivalent rate of 10 Mg ha^{-1} of *Z. gilletii* PyOM. Cast weight

in mesocosms with no PyOM additions was not significantly different from that in lower PyOM application rates (5 Mg ha^{-1}), regardless of the type of PyOM and PyOM post-treatment process.

6.3.3 Effects of PyOM on soil and cast C and N dynamics

Similar to the cast weight, C and N dynamics in casts and bulk soil was affected by amounts, rather than the source of PyOM or the PyOM post-treatment method (Table 6.2). As expected, the C content in casts increased with increasing amount of PyOM, regardless of the type of PyOM and post-treatment process. Notably however, only casts produced in mesocosms with the highest PyOM amounts (25 Mg ha^{-1}) had significantly higher C content. In mesocosms with untreated *Z. gilletii* PyOM, for instance, C content in casts increased from 43.5 mg kg^{-1} in the lowest application rate, to 48.7 mg kg^{-1} in the highest application rate, which was a 12% increase. The C content in all the casts produced in mesocosms with untreated *C. megalocarpus* PyOM were not significantly different. Difference in C content between the highest and lowest application rate in mesocosms treated with acetone-washed *C. megalocarpus* PyOM was about 15%, whereas those treated with *Z. gilletii* PyOM was about 17%. In mesocosms where acid-washed PyOM was applied, only those which received *Z. gilletii* PyOM showed significant differences. In this case, C content increased from 41.5 mg g^{-1} in mesocosms with the lowest application rate, to 46.3 mg g^{-1} in mesocosms with the highest amounts, which was a 12% change. In all the cases, there was no significant differences in C content in mesocosms with 5 Mg ha^{-1} and 10 Mg ha^{-1} PyOM. The C content in bulk soil followed the same trend as C in the casts. However, there were no significant differences between C content in the casts and the bulk soil. The content of N showed no major changes both in casts and bulk soil. Generally, C/N ratio and soil pH increased with the increasing amounts of PyOM.

Table 6.2: Cast weight and their C and N content as affected by the untreated PyOM and PyOM washed with either Acetone or HCl.

Type of amendment	Source of PyOM	PyOM application rate	Parameters measured from the casts			Parameters measured from the bulk soil			
			Dry weight (g)	C (mg g^{-1})	N (mg g^{-1})	C (mg g^{-1})	N (mg g^{-1})	Soil pH	C/N ratio
Control (- earthworms)		0 Mg ha^{-1}	-	-	-	39.94^{f}	3.38^{a}	5.57^{abc}	11.84^{e}
Control (+ earthworms)		0 Mg ha^{-1}	180.7^{a}	41.87^{cdef}	3.46^{a}	41.48^{ef}	3.45^{a}	5.54^{abc}	12.01^{e}
Unwashed PyOM	*Croton megalocarpus*	5 Mg ha^{-1}	188.1^{a}	43.12^{bcdef}	3.29^{a}	41.01^{ef}	3.22^{ab}	5.55^{abc}	12.74^{de}
		10 Mg ha^{-1}	180.9^{a}	43.52^{bcdef}	3.37^{a}	43.32^{cdef}	3.26^{ab}	5.63^{a}	13.31^{de}
		25 Mg ha^{-1}	160.2^{b}	45.66^{abcd}	3.27^{a}	47.76^{abc}	2.55^{c}	5.63^{a}	18.76^{a}
	Zanthoxylum gilletii	5 Mg ha^{-1}	176.5^{a}	43.54^{bcdef}	3.45^{a}	42.90^{def}	3.35^{a}	5.51^{c}	12.79^{de}
		10 Mg ha^{-1}	165.7^{b}	45.42^{abcd}	3.47^{a}	45.59^{abcde}	3.42^{a}	5.54^{abc}	13.36^{de}
		25 Mg ha^{-1}	163.5^{b}	48.73^{a}	3.46^{a}	49.21^{a}	3.17^{ab}	5.60^{abc}	15.59^{bc}
PyOM washed with Acetone	*Croton megalocarpus*	5 Mg ha^{-1}	180.7^{a}	41.90^{cdef}	3.36^{a}	39.86^{f}	3.12^{ab}	5.58^{abc}	12.85^{de}
		10 Mg ha^{-1}	179.7^{a}	42.75^{bcdef}	3.34^{a}	44.08^{bcdef}	3.29^{ab}	5.58^{abc}	13.37^{de}
		25 Mg ha^{-1}	175.2^{ab}	48.24^{a}	3.45^{a}	48.42^{ab}	2.83^{bc}	5.62^{abc}	17.25^{ab}
	Zanthoxylum gilletii	5 Mg ha^{-1}	168.7^{ab}	39.87^{f}	3.30^{a}	44.72^{abcde}	3.48^{a}	5.54^{abc}	12.84^{de}
		10 Mg ha^{-1}	178.0^{a}	43.45^{bcdef}	3.38^{a}	45.45^{abcde}	3.40^{a}	5.54^{abc}	13.42^{de}
		25 Mg ha^{-1}	175.3^{ab}	46.39^{ab}	3.36^{a}	48.76^{a}	3.40^{a}	5.55^{abc}	14.33^{ce}
PyOM washed with HCl	*Croton megalocarpus*	5 Mg ha^{-1}	182.2^{a}	40.90^{ef}	3.35^{a}	41.89^{ef}	3.28^{ab}	5.51^{c}	12.78^{de}
		10 Mg ha^{-1}	177.5^{a}	41.59^{def}	3.32^{a}	44.71^{abcde}	3.30^{ab}	5.54^{abc}	13.54^{de}
		25 Mg ha^{-1}	162.5^{b}	45.29^{abcde}	3.36^{a}	46.84^{abcd}	2.94^{abc}	5.53^{abc}	15.80^{bc}
	Zanthoxylum gilletii	5 Mg ha^{-1}	185.7^{a}	41.49^{def}	3.32^{a}	43.47^{cdef}	3.40^{a}	5.52^{bc}	12.79^{de}
		10 Mg ha^{-1}	169.3^{ab}	43.32^{bcdef}	3.39^{a}	44.82^{abcde}	3.44^{a}	5.54^{abc}	13.02^{de}
		25 Mg ha^{-1}	135.9^{c}	46.25^{abc}	3.38^{a}	49.10^{a}	3.12^{ab}	5.60^{abc}	15.90^{bc}
***p*-value**			**0.002**	**<0.001**	0.075	**<0.001**	**0.050**	**0.002**	**0.001**

Within a row, means followed by the different superscript letters are significantly different at $p<0.05$.

6.4 Discussion

6.4.1 Effects of PyOM on earthworm cast production

The few studies which have investigated effects of PyOM/biochar/charcoal effects on earthworm have reported mixed results, with the response of earthworms towards these amendments being affected greatly by the ecological grouping. Among earthworms' ecological grouping, the endogeics, which are known to ingest substantial amount of organic matter and mineral component are the most affected. Given that *P. corethrurus* is an endogeic earthworm, probably addition of more recalcitrant SOM in the form of PyOM could have made soils with higher concentration of PyOM less desirable substrates. Such changes could be attributed to alteration of soil nutrient release patterns and carbon availability (Lehmann et al., 2011; Domene et al., 2014). In the current study for example, increasing PyOM amounts could have caused immobilisation of nutrients as indicated by the high C/N ratio in mesocosms with higher PyOM amounts. A decrease in available nutrients could trigger a change in abundance and diversity of soil microbiota through a cascade of effects in the food web (Domene et al., 2014), thus affecting earthworms' response to the newly added PyOM. For instance, in chapter 5 of this thesis, it was noted that *Nematogenia lacuum*, also an endogeic earthworm species, significantly decreased with increasing concentration of PyOM. Though the authors were looking at abundance rather than the activity of earthworms, the decreasing abundance with increasing PyOM concentration could be an indicator that PyOM was exerting negative effects on the earthworms. In this case, earthworms were perhaps responding by moving away from the centre of charcoal-making spots where PyOM concentration highest. Conversely, given that earthworms in the current study were more restricted of choices, the most probable way of avoiding the presumably unpalatable PyOM would have been by reducing the rate of consumption of the soil + PyOM substrate. This could perhaps explain the observed lower cast weight in mesocosms with a higher concentration of PyOM. The suggestion corroborates

findings of Topoliantz and Ponge (2003) who reported that *P. corethrurus* created a higher burrow volume relative to the casts produced in soil + charcoal treatment which the authors speculated could have been as a result of the earthworms pushing aside the charcoal particles rather than ingesting the substrate.

Post-treatment of PyOM seem to have little influence on the response of earthworms. For instance, despite significant reduction in Na and ESP and an increase of *Z. gilletii* PyOM's pH, cast production in mesocosms which received the PyOM showed the same trends as untreated PyOM. This shows that the response of earthworms to PyOM was not necessarily affected by the pH or mineral contents of PyOM. Apart from nourishment or effects of toxic elements, habitat suitability can also influence the response of earthworms towards addition of PyOM. For instance, Topoliantz and Ponge (2005) reported that amelioration of soil pH after addition of charcoal could have made the soil + charcoal mixture to be a better substrate to live in than soil alone. The authors reported that earthworms are highly chemosensitive and that a decrease in soil pH after addition of charcoal could have complemented the buffering properties of the earthworms' external mucus. However this study showed no major changes in soil pH after addition of PyOM. Although the influence of soil pH cannot be discounted, I cannot decisively state that pH played a significant role in affecting earthworm activity.

6.4.2 Effects of PyOM and earthworms on C and N dynamics

Selective ingestion of mineral and organic particles by earthworms has been shown to have major effects on C and N dynamics of the casts or cast-derived micro-aggregates (Zhang and Schrader, 1993; Bossuyt et al., 2005; Fonte et al., 2007). Thus in most cases, studies have shown significantly higher C content in the casts than the bulk soil. However, results from this study showed no notable difference in C or N content between earthworm casts and the bulk soil, thus contradicting numerous studies which have shown increased C content in the

earthworm casts. For instance in a grass/legume management system, Guggenberger et al. (1996) reported that organic C in *Martiodrilus* sp. (an endogeic earthworm species) casts was more than double that of the bulk soil. Decaëns et al. (1999) also reported similar findings where earthworm (*Martiodrilus carimaguensis*) casts had 1.5–1.9 times higher C and 1.4–1.6 times higher N than the adjacent bulk soil. Despite working from two different management systems (native savannah and man-made pastures) the authors reported that the trends were consistent in both systems. However, given that these two studies were done under field conditions, such results as observed in the current study can be expected. Mesocosms being quite artificial, could have not presented the earthworms with many options like in the natural system and thus could have led to the observed trends. The insignificant differences in C and N between casts and the bulk soil perhaps shows that the earthworms did not necessarily select the particles to ingest, but rather ingested the soil + PyOM as a whole. Nonetheless, the increasing C content with increasing amounts of PyOM shows that the earthworms were able to successfully incorporate C into these biogenic structures. This can be an important process given that mass application of biochar as a soil amendment is hinged on anticipation that soil organisms will incorporate this amendment into the soil. Topoliantz and Ponge (2003, 2005) suggested that the endogeic earthworm *P. corethrurus* can be an important organism in incorporating charcoal in slash-and-burn systems, and thus could have been responsible for the formation of the ancient C and nutrient-rich Amazonian Dark Earths. Further, since *P. corethrurus* produce large coalescent casts that protect soil organic matter from further microbial degradation, this could be an important characteristic in enhancing physical and chemical properties of the soil over long periods of time. Its extensive burrowing and casting activities could also become an important factor in incorporation of the biochar within the soil profile. Post-treatment of the PyOM with acid or acetone did not to affect the total C content,

and thus this could explain why there were no significant effects of post-treatment on cast or bulk soil C content.

6.5 Conclusion

This study has shown that increasing the concentration of PyOM in soil led to a decreased earthworm cast production, an indicator that earthworms could have been avoiding consumption of soil with high amounts of PyOM. Further, C and N from the casts and bulk soil were not significantly different, perhaps indicating that earthworms did not necessarily select particles to ingest, but rather ingested the substrate as a whole. Post-treatment of PyOM seems to have less effects on its chemical properties, except in *Z. gilletii* PyOM where pH increased whereas Na and ESP decreased significantly. Nonetheless, these changes seems to have had little effects on earthworms' response towards PyOM application and thus the observed insignificant differences in cast production or C and N content of the casts. Future studies can look at C and N dynamics of the cast, taking into consideration the cast ageing process.

CHAPTER SEVEN

Short-term effects of biochar on soil chemical properties and soil fauna abundance and diversity in a humid tropical highland Nitisol

Authors: Solomon Kamau[1*], Nancy K. Karanja[1], Fredrick O. Ayuke[1], Edmundo Barrios[2], Johannes Lehmann[3, 4]

[1] Department of Land Resource Management and Agricultural Technology, College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053-00625, Nairobi.

[2] World Agroforestry Centre, United Nations Avenue, P.O. Box 30677-00100, Nairobi.

[3] Soil and Crop Science, Cornell University, Ithaca, NY 14853, USA.

[4] Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14853, USA

[*]Corresponding author:

E-mail address: solkam08@gmail.com (S. Kamau)

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Abstract

Use of inorganic fertilisers in many smallholder cropping systems in tropical Africa is becoming unsustainable due to increasing unresponsiveness of the soils resulting from increased degradation. The situation is worsened by the declining soil organic matter due to continuous cropping, thus impacting negatively on soil biota. Biochar application has been suggested as a promising soil amendment in reversing such trends. A field trial was conducted to evaluate the potential of biochar made from *Prosopis juliflora* biomass and blended with fertilisers on soil chemical properties and soil fauna abundance and diversity in a nutrient deficient Nitisol in the Central Highlands of Kenya over a period of 4 seasons. Treatments comprised: i) biochar alone applied at 5 Mg or 10 Mg ha^{-1}, ii) inorganic fertilisers; Di-Ammonium Phosphate (DAP) at a rate of 75 kg ha^{-1} (13.5 kg N; 15 kg P), Urea at a rate of 100 kg ha^{-1} (46 kg N ha^{-1}) and NPK (23:23:0) at a rate of 150 kg ha^{-1} (34.5 kg N; 15 kg P), and iii) fertiliser + biochar blends of DAP, Urea and NPK either at a ratio of 9:1 (10% biochar) or 4:1 (20%). A control treatment was included where no inputs were applied. Treatments were applied in the first two seasons, while the last two were used to assess residual treatment effects. Maize was the test crop. Soil for chemical analysis was collected at six weeks after crop emergence. Soil macrofauna were obtained using soil monolith method while soil for nematodes analysis was collected using soil auger at eighth week after crop emergence. Total C and N were significantly higher in plots which received biochar or fertiliser + biochar, which recorded more than 15.0 g kg^{-1} C and 1.9 g kg^{-1} N compared to 9.4 g C and 0.9 g kg^{-1} N in control plots. However, there were no significant differences between plots treated with biochar alone or with the fertiliser + biochar blends. Available P, exchangeable K and inorganic N (NO_3^-, and NH_4^+) follow the same trend as C and N. High amounts of biochar appeared to attract higher number of earthworms. Notably, plots with 10 Mg biochar ha^{-1} recorded higher number of earthworms with 206 individuals m^{-2} than plots with 5 Mg biochar ha^{-1} with 105

individuals m^{-2} and no-input with 97 individuals m^{-2}. Conversely, nematodes were negatively affected by biochar particularly the free living bacterivorous nematodes. This study shows that biochar can play a significant role in enhancing soil fertility which may affect the patterns of soil fauna abundance and diversity, thus ultimately affecting soil productivity.

Keywords: Biochar; Soil macrofauna; Soil nematodes; Soil organic carbon

7.1 Introduction

Maintaining high levels of productivity in many cropping systems in tropical Africa is a major challenge given that these systems are reliant on low external organic or inorganic inputs, despite being under continuous cultivation for several decades (Mbau et al., 2015). This has led to poor crop yields and degradation of the soils. One factor that is said to be a major driver of soil degradation is the declining levels of soil organic matter (Ayuke et al., 2011b). Though use of organic residues such as green manure and animal wastes including farm and agro-industrial organic wastes has been proposed as one of the ways of restoring SOM content, intensive cultivation in tropics accelerates its loss due to the high temperature (Ayuke, 2010). Thus the organic residues available for such applications could be limiting due to their alternative use as fuel and animal feeds, especially in dry regions of sub-Saharan Africa (Mbau, 2012). Biochar (a carbon-rich product of pyrolysis) has been suggested as a promising soil amendment which could reduce the rate of SOM loss due to its considerably high recalcitrance and stability to microbial attack (Lehmann et al., 2006; Chan et al., 2008). In addition, it has been suggested as a potential amendment for improving soil fertility. This can be one way of converting available organic biomasses into a slow-degrading product, thus reducing the need for repeated application over short periods of time. Nevertheless, the fertility potential of a given biochar is greatly affected by production conditions and feedstock material. For instance, under similar production conditions, biochar made from woody materials is expected to have very low fertility value compared to that derived from manure (Waters et al., 2011). In cases where woody material are used, there may be a need to supplement the biochar with either organic or inorganic fertiliser sources. Fertiliser + biochar blends may therefore be an option for consideration to the proponents of biochar in the future.

Besides effects on chemical properties, biochar has been shown to cause significant shifts in soil microbial biomass, abundance and diversity (Warnock et al., 2007; O'Neill et al., 2009 Lehmann et al., 2011). This could be linked to alteration in soil nutrient release and carbon availability dynamics, changes in habitat properties such as soil pH and bioavailability of toxic elements, or physical protection of microbes by biochar (Lehmann et al., 2011). Recent studies have also given mixed results on biochar application to soil invertebrates, some showing negative while others positive responses (Verheijen et al., 2010). Despite the growing interest in utilisation of biochar as a soil amendment, there are very few studies which have shown systematic effects of biochar and/or fertiliser + biochar blends on soil fertility changes and how such changes could affect soil fauna and crop productivity.

The aim of this study was to evaluate effects of biochar and fertiliser + biochar blends on soil chemical properties, soil macrofauna in a nutrient deficient soil. It was hypothesised that: i) soil chemical properties will change as a result of biochar application, and ii) soil macro/mesofauna abundance would increase with increased amounts of biochar, but that the magnitude of these effects would be modulated by type of inorganic fertiliser.

7.2 Materials and methods

7.2.1 Description of the study site

The study was conducted at the University of Nairobi's Upper Kabete Field Station, located about 10 km Northwest of Nairobi City at latitude 1° 15' S and longitude 36° 41' E, with an elevation of approximately 1900 m above sea level. The area is classified as the upper sub–humid midland (UM_2) agro–ecological zone (Jaetzold et al., 2005), receiving an average annual rainfall of about 1000 mm in a bimodal rainfall pattern. Approximately 600 mm of the rainfall is received between March–May often locally referred to as "long-rains" and 400 mm between October–December called "short-rains". Temperatures are fairly constant throughout the year,

with minimum and maximum mean temperature average of 14 °C and 24 °C, respectively. Soils are predominantly eutric Nitisols characterized by deep red coloration, are highly weathered, but are well–drained, dark red to dark reddish–brown and very deep. They are among the most productive soils of the humid tropics due to their good physical and chemical properties, good aeration and high water holding capacity. They are moderately acidic and have moderate to low inherent soil fertility (Jaetzold et al., 2005).

7.2.2 Chemical characterisation of the biochar

Biochar used in this study was obtained from Cummins Cogeneration (Kenya), a private electricity producing company, which uses *Prosopis juliflora* tree as a raw material in its operations. Biochar is one of the by-products from the power generation process. Fresh biochar was obtained from the company in sealed bags. Before its application in the trials site or making the biochar-fertiliser blends, the biochar was homogenised and about 50 g sample was collected for chemical analysis. The sample was air dried until a constant weight, fine-ground and stored in bags awaiting analysis. The samples were analysed for pH and macro-elements (C, N, P, K, Ca and Mg). The pH was determined in water using a 1:5 biochar/water ratio. Total C and N were determined by FLASH 2000 NC Analyser (ThermoFisher Scientific, Cambridge, UK) while P, K, Ca and Mg were extracted through a closed-vessel microwave-assisted digestion system (Miller, 1998) and determined using inductively coupled plasma atomic emission spectroscopy (Isaac and Johnson, 1998).

7.2.3 Experimental design and treatment combinations

The farm used in the study had been under maize crop for three consecutive years with no organic or inorganic inputs added during this period. The farm was divided into three blocks. Further, the blocks were divided into plots measuring 6 m by 5 m and the treatments were

randomly allocated in each of the blocks. Treatments combinations comprised: i) biochar only ii) three fertiliser types, Di-Ammonium Phosphate (DAP – 18:46:0), Urea (46:0:0) and NPK (23:23:0) and iii) fertiliser + biochar blends. Biochar was applied at a rate of 5 Mg ha^{-1} or 10 Mg ha^{-1}. The DAP was applied at the rates commonly used by smallholder farmers of 75 kg ha^{-1} (13.5 kg N; 15 kg P), urea at a rate of 100 kg ha^{-1} (46 kg N ha^{-1}) and NPK at 150 kg ha^{-1} (34.5 kg N; 15 kg P). Two ratios were made for each of the three fertilisers; either 9:1 (fertiliser : biochar), equivalent to 10% biochar in the blend, or 4:1 (20%). A no-input control treatment was included. The study was done for four consecutive seasons, from the onset of short rains in 2014 (October) to the end of long rains in 2016 (August). The treatments were applied in the first two consecutive seasons; no inputs were applied in the last two seasons.

7.2.4 Soil sampling, preparation and analysis

In each of the four seasons, soil samples were randomly taken from four points from each plot using soil augers to a depth of 20 cm at the sixth week after crop emergence. The soil was thoroughly mixed to make one composite sample for the analysis. The soil properties analysed included; soil pH, total C, total and available N, available P and exchangeable K. Soil pH was determined in water using a 1:2.5 soil/solution ratio. Available N (NO_3^- and NH_4^+) was extracted using 2M potassium chloride (KCl) and determined using steam distillation method (Bremner and Keeney 1965). Total C and N were determined by FLASH 2000 NC Analyser (ThermoFisher Scientific, Cambridge, UK). Available P and exchangeable K were extracted by Mehlich-3 procedure (Mehlich 1984) and measured using an Inductively Coupled Plasma Atomic Emission Spectrophotometer (Isaac and Johnson, 1998).

7.2.5 Soil fauna sampling

Collection of soil macrofauna was done using soil monolith (0.25 by 0.25 by 0.30 m), randomly excavated within the plots following the standard Tropical Soil Biology and Fertility Programme (TSBF) sampling protocol (Anderson and Ingram, 1993). Sampling was done once each season at eight weeks after onset of the rain season. The soil samples were placed in trays to facilitate hand-sorting of soil macrofauna. All soil macrofauna were first placed in 75% ethanol and at the end of the sampling exercise, the macrofauna (except earthworms) were transferred into fresh ethanol and sealed in vials. Earthworms were transferred into 4% formaldehyde for preservation. The preservative solution was replaced when coloration change was observed. Soil macrofauna were identified at least to genera or species. The soil macrofauna abundance was calculated as number of individuals per square meters (individuals m^{-2}). Sampling for nematodes was done using a soil auger at six points within each plot to a depth of 20 cm. The six cores were mixed thoroughly and a sub-sample derived from them. Extraction of nematodes was done using Baerman pan technique (Forge and Kimpinski, 2007) followed by identification and enumeration. Nematodes were identified to genera and the abundance reported as numbers per 100 grams of soil (numbers 100 g^{-1} soil).

7.2.6 Statistical analysis and data management

Soil macrofauna and soil chemical properties data was subjected to Analyses of Variance (ANOVA) using R statistical software, version 3.3.2 (R-Core Team 2015). Soil fauna data was modelled using generalised linear mixed models as a function of treatments, using the package lme4 in R (Bates et al., 2015). Given that there was a considerable proportion of zero values in fauna data, negative binomial regression was chosen as an extension of the Poisson distribution. The best fitting models were chosen based on the lowest Akaike Information Criterion (AIC). A preliminary analysis was done including seasons as a factor and whenever no seasonal differences were detected, the data from the four seasons was averaged for a single mean. Separation of means was conducted using Tukey's HSD test (p=0.05).

7.3 Results

7.3.1 Chemical characteristics of the biochar and soil before experiment

The soils before the experiment had a pH of 5.5 (Table 7.1). The parameters measured; available P (0.1 mg g^{-1}), exchangeable K (0.6 mg g^{-1}), total C and N (5.0 mg g^{-1} and 0.9 mg g^{-1}, respectively), NH_4-N and NO_3-N (8.0 mg kg^{-1} and 12.0 mg kg^{-1}, respectively) were all low. The biochar was found to have relatively low N, P and Mg contents with values of 14.0 mg, 4.2 mg and 6.5 mg g^{-1}, respectively compared to K and Ca, with values of 112.6 mg and 113.7 mg g^{-1}, respectively. The bulk of the char was C, recording a value of 637.8 mg g^{-1}. Due to the high C and low N and P contents, the C/N and C/P ratios were relatively high with values of 45:1 and 150:1, respectively. The biochar was found to have high pH (8.6).

Table 7.1: Initial chemical characteristics of soil in the study site and biochar

Parameter	**soil**	**biohar**
$pH_{(water)}$	5.5	8.6
Total C (mg g^{-1})	5.0	637.8
Total N (mg g^{-1})	0.9	14.0
NO_3^- (mg kg^{-1})	12.0	-
NH_4^+ (mg kg^{-1})	8.0	-
P (mg g^{-1})	0.1	4.2
K (mg g^{-1})	0.6	112.6
Ca (mg g^{-1})	3.6	113.7
Mg (mg g^{-1})	0.5	4.2
C/N	6:1	45:1
C/P	833:1	150:1

7.3.2 Effects of biochar and fertiliser + biochar blends on soil chemical properties

All soil chemical parameters measured (except pH) were significantly affected by the treatments in the first two seasons (Table 7.2). Total C and N were significantly low in no-input control plots (9.4 g and 0.9 g kg^{-1}, respectively) compared to other treatments which recorded more than 15.0 g kg^{-1} C and 1.9 g kg^{-1} N. This was about 50% higher C content and more than double the amount of N in control plots. However, there were no significant differences between plots treated with biochar alone or with the fertiliser + biochar blends. Available P and exchangeable K and inorganic N (NO_3^-, NH_4^+) also followed the same trend as C and N. In the last two seasons where no inputs were applied, only NO_3^- showed significant differences between the treatments. Plots treated with fertiliser +10% biochar were significantly richer in NO_3^- than the other treatments, with 24.0 g, 26.8 g and 27.2 g kg^{-1} recorded in plots which received DAP + 10% biochar, urea + 10% biochar and NPK + 10% biochar, respectively. There were no significant differences in plots treated with either fertiliser or fertiliser + 20% biochar. Control plots recorded lowest NO_3^- content.

Table 7.2: Soil chemical properties as affected by the biochar and fertiliser + biochar blends.

Parameter	Treatments												*p*-value
	Biochar alone			Fertiliser alone			Fertiliser + 10% Biochar			Fertiliser + 20% Biochar			
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	
Season 1 and 2													
$pH_{(water)}$	5.3 (0.1)^{a}	5.1 (0.3)^{a}	5.3 (0.2)^{a}	5.4 (0.1)^{a}	5.4 (0.1)^{a}	5.1 (0.1)^{a}	5.3 (0.1)^{a}	5.1 (0.2)^{a}	5.2 (0.1)^{a}	5.6 (0.1)^{a}	5.4 (0.1)^{a}	5.2 (0.0)^{a}	0.305
Total C (g kg^{-1})	**9.4 (2.6)^{b}**	**19.3 (2.0)^{ab}**	**19.5 (0.1)^{ab}**	**16.9 (3.5)^{ab}**	**15.1 (2.8)^{ab}**	**15.2 (0.9)^{ab}**	**15.1 (0.9)^{ab}**	**20.2 (0.8)^{ab}**	**22.5 (0.7)^{ab}**	**18.3 (4.4)^{ab}**	**15.0 (4.1)^{ab}**	**26.6 (4.3)^{a}**	**0.002****
Total N (g kg^{-1})	**0.9 (0.1)^{b}**	**1.9 (0.4)^{a}**	**2.3 (0.1)^{a}**	**2.3 (0.1)^{a}**	**2.5 (0.1)^{a}**	**1.9 (0.2)^{a}**	**2.3 (0.2)^{a}**	**2.4 (0.2)^{a}**	**2.1 (0.2)^{a}**	**2.3 (0.0)^{a}**	**2.3 (0.1)^{a}**	**2.3 (0.2)^{a}**	**<0.001*****
NO_3^- – N (mg kg^{-1})	**11.4 (1.9)^{c}**	**37.9 (6.8)^{ab}**	**43.7 (4.1)^{ab}**	**41.2 (7.4)^{ab}**	**35.1 (2.3)^{ab}**	**28.9 (2.4)^{b}**	**41.0 (3.5)^{ab}**	**60.3 (5.4)^{a}**	**49.8 (4.4)^{a}**	**34.9 (5.6)^{b}**	**33.3 (6.1)^{b}**	**51.3 (0.6)^{a}**	**0.014***
NH_4^+ – N (mg kg^{-1})	**32.2 (0.4)^{c}**	**71.8 (7.2)^{ab}**	**73.8 (8.4)^{ab}**	**77.9 (11.8)^{a}**	**63.4 (1.0)^{ab}**	**59.0 (6.3)^{b}**	**81.7 (4.6)^{a}**	**74.4 (15.9)^{ab}**	**62.9 (8.8)^{ab}**	**74.7 (2.2)^{ab}**	**69.1 (6.3)^{ab}**	**80.4 (9.6)^{a}**	**0.007****
Avail. P (mg kg^{-1})	**11.0 (0.2)^{c}**	**36.9 (1.2)^{ab}**	**33.4 (3.6)^{ab}**	**37.6 (0.4)^{ab}**	**33.0 (1.3)^{ab}**	**42.2 (5.6)^{a}**	**39.7 (3.7)^{a}**	**29.4 (2.2)^{ab}**	**35.5 (2.1)^{ab}**	**49.8 (1.9)^{a}**	**24.7 (1.4)^{b}**	**35.6 (5.8)^{ab}**	**0.001****
Exc. K (g kg^{-1})	**0.4 (0.0)^{c}**	**0.7 (0.0)^{b}**	**0.9 (0.0)^{ab}**	**1.0 (0.2)^{a}**	**0.8 (0.1)^{ab}**	**0.9 (0.0)^{ab}**	**0.9 (0.2)^{ab}**	**0.9 (0.1)^{ab}**	**0.9 (0.2)^{ab}**	**1.0 (0.1)^{a}**	**0.9 (0.0)^{ab}**	**1.0 (0.1)^{a}**	**0.006****
Season 3 and 4													
$pH_{(water)}$	5.3 (0.2)^{a}	5.3 (0.1)^{a}	5.5 (0.1)^{a}	5.2 (0.2)^{a}	5.0 (0.1)^{a}	5.2 (0.1)^{a}	5.2 (0.0)^{a}	5.2 (0.1)^{a}	5.2 (0.0)^{a}	5.4 (0.3)^{a}	5.1 (0.2)^{a}	5.1 (0.4)^{a}	0.578
Total C (g kg^{-1})	23.4 (2.5)^{a}	30.7 (0.6)^{a}	29.9 (4.6)^{a}	30.2 (3.8)^{a}	30.4 (3.3)^{a}	30.4 (4.8)^{a}	29.7 (6.8)^{a}	29.8 (5.0)^{a}	27.9 (3.3)^{a}	28.4 (3.0)^{a}	28.8 (5.9)^{a}	31.8 (7.1)^{a}	0.409
Total N (g kg^{-1})	1.1 (0.2)^{a}	1.4 (0.4)^{a}	1.7 (0.1)^{a}	1.7 (0.3)^{a}	1.7 (0.4)^{a}	1.3 (0.3)^{a}	1.7 (0.1)^{a}	1.9 (0.3)^{a}	2.1 (0.2)^{a}	2.0 (0.2)^{a}	1.7 (0.2)^{a}	1.6 (0.0)^{a}	0.085
NO_3^- – N (mg kg^{-1})	**10.7 (3.9)^{e}**	**20.9 (1.8)^{bcd}**	**18.3 (1.5)^{d}**	**20.1 (1.9)^{cd}**	**20.9 (1.7)^{bcd}**	**19.7 (1.8)^{cd}**	**24.0 (1.9)^{ab}**	**26.8 (3.5)^{a}**	**27.2 (3.5)^{a}**	**18.9 (1.6)^{d}**	**22.4 (1.2)^{bc}**	**20.9 (2.4)^{bcd}**	**0.021***
NH_4^+ – N (mg kg^{-1})	32.6 (2.8)^{a}	27.8 (2.0)^{a}	29.9 (2.3)^{a}	32.3 (2.5)^{a}	29.7 (5.7)^{a}	35.5 (2.4)^{a}	38.8 (4.6)^{a}	40.5 (5.8)^{a}	28.2 (6.8)^{a}	34.0 (1.3)^{a}	31.8 (3.2)^{a}	31.7 (2.6)^{a}	0.059
Avail. P (mg kg^{-1})	17.6 (1.0)^{a}	11.3 (0.8)^{a}	17.4 (3.1)^{a}	13.8 (1.3)^{a}	18.4 (2.6)^{a}	18.0 (1.2)^{a}	18.8 (1.8)^{a}	17.4 (4.2)^{a}	13.8 (1.3)^{a}	17.3 (3.1)^{a}	14.9 (1.2)^{a}	17.2 (2.6)^{a}	0.234
Exc. K (g kg^{-1})	0.6 (0.1)^{a}	0.6 (0.1)^{a}	0.6 (0.1)^{a}	0.6 (0.1)^{a}	0.5 (0.1)^{a}	0.7 (0.0)^{a}	0.6 (0.0)^{a}	0.6 (0.0)^{a}	0.6 (0.1)^{a}	0.5 (0.0)^{a}	0.7 (0.1)^{a}	0.5 (0.1)^{a}	0.873

Abbreviations: Av.=available; Exc.=exchangeable; T1 = Control (no organic or inorganic inputs); T2 = 5 Mg ha^{-1} biochar; T3 = 10 Mg ha^{-1} biochar; T4 = DAP; T5 = Urea; T6 = NPK; T7 = DAP + 10% biochar; T8 = Urea + 10% biochar; T9 = NPK + 10% biochar; T10 = DAP + 20% biochar; T11 = Urea + 20% biochar; T12 = NPK + 20% biochar. Within a row, means followed by the different superscript letters are significantly different at $p<0.05$. Significant values: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

7.3.3 Effects of biochar and fertiliser + biochar blends on soil macrofauna

Of the eleven soil macrofauna recovered from the study site, only earthworms, showed significant differences across the treatments (Table 7.3). However, the response to the treatments showed different trends. For instance, high amounts of biochar appeared to attract earthworms. Notably, plots which received biochar at a rate of 10 Mg ha^{-1} recorded the highest number of earthworms (207 individuals m^{-2}), which were significantly different from plots treated with 5 Mg ha^{-1} of the biochar (105 individuals m^{-2}) or no-input control (97 individuals m^{-2}). On the other hand, use of inorganic fertiliser, especially Urea and NPK, appear to have had negative effects on earthworms. For instance, no earthworms were recovered in plots where either Urea alone or with 10% biochar were applied. However, plots with Urea + 20% biochar recorded relatively high number of earthworms compared to the other treatments. The response of earthworms to NPK fertiliser application showed a similar trend to that of Urea. The number of earthworms increased with increasing amounts of biochar, from the lowest of 36 individuals m^{-2} in plots with NPK alone to the highest of 73 individuals m^{-2} in plots with NPK + 20% biochar. The fertiliser, DAP (with and without biochar) did not show any specific trends. No significant differences or specific trends were observed with the other soil macrofauna groups.

Table 7.3: Soil macrofauna abundance (mean number of individuals m^{-2} ± SE) as affected by biochar and fertiliser + biochar blends over the four seasons.

Parameter	Treatments												*p*-value
	Biochar alone			Fertiliser alone			10% Biochar + Fertiliser			20% Biochar + Fertiliser			
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	
Earthworms	**97 (20)^{b}**	**105 (12)^{b}**	**207 (16)^{a}**	**61 (16)^{bc}**	**0 (0)^{d}**	**36 (16)^{c}**	**73 (28)^{bc}**	**0 (0)^{d}**	**49 (10)^{c}**	**61 (14)^{bc}**	**73 (12)^{bc}**	**73 (10)^{bc}**	**0.026***
Beetles	235 (42)^{a}	139 (34)^{a}	160 (42)^{a}	96 (52)^{a}	85 (34)^{a}	136 (36)^{a}	267 (42)^{a}	96 (18)^{a}	245 (60)^{a}	203 (30)^{a}	107 (26)^{a}	213 (34)^{a}	0.074
Centipedes	16 (16)^{a}	27 (26)^{a}	21 (14)^{a}	16 (10)^{a}	85 (35)^{a}	11 (10)^{a}	16 (10)^{a}	64 (38)^{a}	37 (20)^{a}	37 (37)^{a}	11 (6)^{a}	0 (0)^{a}	0.452
Millipedes	37 (20)^{a}	9 (6)^{a}	28 (10)^{a}	9 (9)^{a}	28 (14)^{a}	19 (10)^{a}	75 (28)^{a}	19 (10)^{a}	56 (20)^{a}	9 (9)^{a}	9 (6)^{a}	46 (20)^{a}	0.655
Termites	68 (62)^{a}	0 (0)^{a}	77 (48)^{a}	0 (0)^{a}	222 (69)^{a}	26 (24)^{a}	77 (56)^{a}	0 (0)^{a}	77 (51)^{a}	265 (140)^{a}	146 (131)^{a}	145 (78)^{a}	0.521
Ants	266 (46)^{a}	226 (82)^{a}	133 (46)^{a}	13 (6)^{a}	93 (66)^{a}	66 (30)^{a}	120 (90)^{a}	147 (91)^{a}	80 (10)^{a}	306 (120)^{a}	146 (108)^{a}	292 (64)^{a}	0.718
Cockroaches	16 (16)^{a}	5 (4)^{a}	0 (0)^{a}	0 (0)^{a}	27 (14)^{a}	0 (0)^{a}	0 (0)^{a}	5 (4)^{a}	11 (7)^{a}	0 (0)^{a}	16 (10)^{a}	32 (32)^{a}	0.369
Spiders	5 (5)^{a}	5 (5)^{a}	16 (10)^{a}	53 (46)^{a}	43 (36)^{a}	32 (10)^{a}	0 (0)^{a}	5 (5)^{a}	5 (5)^{a}	16 (10)^{a}	5 (5)^{a}	0 (0)^{a}	0.233
Field crickets	5 (5)^{a}	11 (6)^{a}	0 (0)^{a}	21 (14)^{a}	11 (6)^{a}	0 (0)^{a}	0 (0)^{a}	5 (5)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0.134
True bugs	0 (0)^{a}	11 (11)^{a}	11 (11)^{a}	11 (11)^{a}	21 (21)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	21 (10)^{a}	0 (0)^{a}	11 (10)^{a}	0.415
Pseudoscorpions	5 (5)^{a}	0 (1)^{a}	16 (10)^{a}	5 (5)^{a}	5 (5)^{a}	5 (5)^{a}	0 (0)^{a}	5 (5)^{a}	37 (18)^{a}	16 (10)^{a}	11 (10)^{a}	5 (5)^{a}	0.124

T1 = Control (no organic or inorganic inputs); T2 = 5 Mg ha^{-1} biochar; T3 = 10 Mg ha^{-1} biochar; T4 = DAP; T5 = Urea; T6 = NPK; T7 = DAP + 10% biochar; T8 = Urea + 10% biochar; T9 = NPK + 10% biochar; T10 = DAP + 20% biochar; T11 = Urea + 20% biochar; T12 = NPK + 20% biochar. Within a row, means followed by the different superscript letters are significantly different at $p<0.05$. Significant values: $^{*}p < 0.05$.

7.3.4 Effects of biochar and fertiliser blends on soil macrofauna diversity

The diversity and taxonomic richness varied across different treatments (Table 7.4). Generally, soils amended with either biochar or fertiliser + biochar blends (except urea and its biochar blends) had higher taxonomic richness than the fertiliser alone. For instance, the taxonomic richness of plots treated with biochar (either at 5 Mg or 10 Mg biochar ha^{-1}) and fertiliser + biochar blends had a taxonomic richness greater than 7 species compared to about 3 species in plots treated with either DAP or NPK. Plots treated with urea or with its blends with biochar showed a contrary trend with higher taxonomic richness (7 species) on plots treated with urea alone compared to urea + 10% biochar (4 species) or urea + 20% biochar (5 species). Generally, no-input control plots had a taxonomic richness greater than that of fertiliser alone (except urea), but lower than that of biochar or fertiliser + biochar (except urea + biochar). Soil macrofauna diversity tended to follow a similar trend as taxonomic richness.

Table 7.4: Soil macrofauna species distribution, taxonomic richness and diversity as affected by the treatments over the four seasons.

Soil macrofauna description				Treatments											
				Biochar alone			Fertiliser alone			10% Biochar + Fertiliser			20% Biochar + Fertiliser		
Macrofauna order	Family	Common name	Genera/Species	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Hymenoptera	Formicidae	Ants	*Bothroponera sp.*	133	173	133	13	66	66	66	147	53	279	146	279
			Euponera sp.	40	40	0	0	13	0	53	0	27	27	0	13
			Tetramorium sp.	93	0	0	0	0	0	0	0	0	0	0	0
	Chalcididae		NI†	0	13	0	0	13	0	0	0	0	0	0	0
Coleoptera	Carabidae	Beetles	*Harpalus sp.*	0	0	0	0	0	0	0	0	11	11	11	0
	Curculionidae		*Hypothenemus sp.*	0	0	11	0	0	0	0	0	0	0	0	0
			Sitophilus sp.	21	21	11	0	0	0	11	11	11	0	21	21
	Elateridae‡		NI†	0	32	11	0	32	0	0	0	0	11	0	21
	Staphylinidae		*Leptacinus sp.*	11	11	11	0	0	0	11	0	0	0	0	11
			Philonthus sp.	11	11	0	0	0	0	21	0	11	21	0	21
	Scarabaeidae		*Aphodius lividus*	192	64	117	96	53	139	224	85	213	160	75	139
Isoptera	Termitidae	Termites	*Microtermes sp.*	0	0	0	0	0	0	0	0	0	0	0	137
			Odontotermes sp.	68	0	77	0	222	26	77	0	77	265	146	9
Orthoptera	Gryllidae	Field crickets	*Gryllus bimaculatas*	5	11	0	21	11	0	0	5	0	0	0	0
Blattodea	Ectobiidae	Cockroaches	*Blattella sp.*	16	5	0	0	27	0	0	5	11	0	16	32
Hemiptera	Coreidae	True bugs	*Anoplocnemis sp.*	0	11	11	11	21	0	0	0	0	21	0	11
Oligochaeta	Acathodrilidae	Earthworms	*Dichogaster affinis*	0	0	85	0	0	0	12	0	12	0	0	0
			Dichogaster bolaui	36	81	49	61	0	12	0	0	0	12	49	12
	Eudrilidae		*Polytoreutus annulatus*	0	0	0	0	0	0	0	0	24	0	0	0
	Ocnerodrilidae		*Nematogenia lacuum*	61	24	49	0	0	24	61	0	12	49	24	61
			Gordiodrilus wemanus	0	0	24	0	0	0	0	0	0	0	0	0
Scolopendromorpha	Scolopendridae	Centipedes	NI†	16	27	21	16	85	11	16	64	37	37	11	0
Spirobolida	Pachybolidae	Millipedes	*Epibolus pulchripes*	0	0	0	0	9	0	0	0	0	0	0	37
	Trigoniulidae		*Trigoniulus sp.*	37	9	28	9	19	19	75	19	56	9	9	9
Araneae	Araneidae	Spiders	*Araneus sp.*	5	5	16	53	43	32	0	5	5	16	5	0
Pseudoscorpiones	NI†	Pseudoscorpions	NI†	5	0	16	5	5	5	0	5	37	16	11	5
Species richness (*S*) (***p* < 0.001**)				5.83^{abcd}	7.00^{abc}	7.33^{abc}	2.83^{d}	6.83^{abc}	3.83^{cd}	5.33^{abcd}	4.17^{bcd}	7.67^{ab}	8.17^{a}	5.00^{abcd}	6.67^{abc}
Shannon diversity index (H') (***p* < 0.001**)				1.45^{abc}	1.58^{ab}	1.67^{ab}	0.77^{c}	1.56^{ab}	1.14^{abc}	1.31^{abc}	0.91^{bc}	1.74^{a}	1.66^{ab}	1.06^{abc}	1.36^{abc}

T1 = Control (no organic or inorganic inputs); T2 = 5 Mg ha^{-1} biochar; T3 = 10 Mg ha^{-1} biochar; T4 = DAP; T5 = Urea; T6 = NPK; T7 = DAP + 10% biochar; T8 = Urea + 10% biochar; T9 = NPK + 10% biochar; T10 = DAP + 20% biochar; T11 = Urea + 20% biochar; T12 = NPK + 20% biochar. Means of species richness or diversity index followed by the different superscript letters are significantly different at $p<0.05$. † The soil macrofauna could not be identified beyond the family level.

7.3.5 Effects of biochar and fertiliser + biochar blends on nematodes

Unlike earthworms which appeared to be attracted by biochar, nematodes seem to have been negatively affected by the same amendment. The most dominant functional group in this study was the bacterivorous nematodes, which were fivefold more abundant than the other trophic groups. Three free-living nematode genera namely, *Eucephalobus*, *Rhabditis* and *Primastolaimus*, in the bacterivorous group and one genus, *Aphelenchus*, in the fungivorous group made the bulk of the total nematodes counts. Nonetheless, the trends in response to treatment application were more conspicuous in bacterivorous nematodes (Table 7.5). The population of the three bacterivorous nematodes, *Eucephalobus*, *Rhabditis* and *Primastolaimus*, in the no-input control plots was at least eight times that of the biochar treated plots and at least three times above the plots treated with fertiliser or fertiliser + biochar. Thus, blending biochar with inorganic fertiliser seems to have reduced the effects of biochar alone. Almost all nematodes of the genus *Wilsonema* were recovered in the control plots, with none recorded in plots treated with either biochar or fertiliser + biochar. In the fungivorous group, only *Aphelenchus* genus was significantly different among the treatments, with the highest counts (56 nematodes 100 g^{-1} soil) recorded in the control plots. However, there were no specific trends observed in this genera with the other treatments. The omnivorous nematodes, especially the genus *Discolaimoides*, were significantly low in all the plots treated with fertiliser + 20% biochar, regardless of the fertiliser type. Only *Trichodorus* and *Tylenchus* genera from the herbivorous nematodes showed significant differences among the treatments. In this, *Trichodorus* nematodes were more numerous in control plots (22 nematodes 100 g^{-1} soil) whereas all other treatments, except two, recorded less than 10 nematodes 100 g^{-1} soil. *Tylenchus* genus did not show any specific trends as did *Mononchus*, the only genus recovered in the predator group.

Table 7.5: Soil nematode genera (numbers 100 g^{-1} soil ± SE) as affected by biochar and fertiliser + biochar blends over the four seasons.

Trophic group	Genera	Treatment type												*p*-value
		Biochar alone			Fertiliser alone			10% Biochar + Fertiliser			20% Biochar + Fertiliser			
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	
Bacteriovores	*Acrobeles*	0^{a}	4 (2)^{a}	3 (2)^{a}	6 (6)^{a}	2 (2)^{a}	4 (4)^{a}	3 (2)^{a}	5 (4)^{a}	1 (1)^{a}	0^{a}	10 (8)^{a}	2 (2)^{a}	0.663
	Cephalobus	8 (6)^{a}	17 (10)^{a}	12 (10)^{a}	28 (10)^{a}	27 (18)^{a}	2 (2)^{a}	0^{a}	0^{a}	10 (4)^{a}	16 (14)^{a}	20 (6)^{a}	26 (13)^{a}	0.277
	Eucephalobus	**163 (54)^{a}**	**9 (3)^{c}**	**3 (1)^{d}**	**37 (12)^{b}**	**41 (13)^{b}**	**19 (6)^{c}**	**51 (17)^{b}**	**50 (16)^{b}**	**14 (4)^{c}**	**15 (5)^{c}**	**50 (16)^{b}**	**24 (8)^{bc}**	**<0.001*****
	Primastolaimus	**68 (20)^{a}**	**16 (2)^{c}**	**16 (3)^{c}**	**31 (2)^{b}**	**19 (5)^{c}**	**23 (4)^{c}**	**22 (4)^{c}**	**14 (2)^{c}**	**18 (5)^{c}**	**12 (3)^{c}**	**23 (7) ^{bc}**	**13 (5)^{c}**	**0.014***
	Rhabditis	**82 (17)^{a}**	**13 (3)^{c}**	**14 (4)^{c}**	**53 (20)^{ab}**	**31 (10)^{b}**	**31 (8)^{b}**	**50 (20)^{ab}**	**53 (12)^{ab}**	**44 (4)^{b}**	**20 (4)^{bc}**	**58 (18)^{ab}**	**52 (25)^{ab}**	**0.033***
	Wilsonema	**13 (4)^{a}**	**0^{c}**	**0^{c}**	**5 (2)^{b}**	**0^{c}**	**1 (1)^{c}**	**0^{c}**	**0^{c}**	**0^{c}**	**0^{c}**	**0^{c}**	**0^{c}**	**0.005****
	Total bacteriovores	**334^{a}**	**49^{e}**	**48^{e}**	**160^{b}**	**117^{cd}**	**80^{d}**	**126^{c}**	**122^{cd}**	**87^{d}**	**63de**	**161^{b}**	**117^{cd}**	**0.015***
Fungivores	*Aphelenchus*	**56 (11)^{a}**	**41 (9)^{ab}**	**12 (4)^{c}**	**28 (6)^{b}**	**14 (4)^{c}**	**26 (10)^{bc}**	**48 (6)^{a}**	**19 (6)^{bc}**	**38 (4)^{ab}**	**47 (5)^{a}**	**41 (12)^{ab}**	**16 (9)^{bc}**	**0.042***
	Aphelenchoides	0^{a}	11 (4)^{a}	7 (4)^{a}	11 (8)^{a}	8 (4)^{a}	18(6)^{a}	15 (6)^{a}	7 (2)^{a}	10 (4)^{a}	20 (8)^{a}	19 (18)^{a}	0^{a}	0.241
	Ditylenchus	0^{a}	0^{a}	0^{a}	4 (4)^{a}	0^{a}	0^{a}	0^{a}	2 (2)^{a}	0^{a}	2 (2)^{a}	0^{a}	2 (2)^{a}	0.071
	Total fungivores	56^{a}	52^{a}	19^{a}	43^{a}	22^{a}	44^{a}	63^{a}	28^{a}	48^{a}	69^{a}	60^{a}	18^{a}	0.324
Plant parasitic (Herbivores)	*Criconema*	4 (2)^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.257
	Filenchus	14 (4)^{a}	22 (3)^{a}	26 (61)^{a}	37 (11)^{a}	27 (3)^{a}	26 (5)^{a}	36 (11)^{a}	44 (11)^{a}	10 (1)^{a}	15 (2)^{a}	45 (16)^{a}	17 (2)^{a}	0.395
	Helicotylenchus	4 (2)^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2 (2)^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2 (2)^{a}	0.17
	Meloidogyne	0^{a}	2 (2)^{a}	0^{a}	5 (4)^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3 (2)^{a}	1 (1)^{a}	2 (2)^{a}	0^{a}	0.142
	Pratylenchus	6 (6)^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.257
	Scutellonema	3 (2)^{a}	9 (4)^{a}	5 (4)^{a}	10 (8)^{a}	2 (2)^{a}	2 (2)^{a}	4 (2)^{a}	24 (13)^{a}	0^{a}	3 (2)^{a}	11 (8)^{a}	7 (4)^{a}	0.457
	Trichodorus	**22 (2)^{a}**	**1 (1)^{c}**	**4 (2)^{b}**	**5 (2)^{b}**	**0^{c}**	**1 (1)^{c}**	**6 (2)^{b}**	**12 (3)^{ab}**	**3 (1)^{b}**	**3 (1)^{b}**	**14 (7)^{ab}**	**0^{c}**	**0.009****
	Tylenchorynchus	4 (2)^{a}	0^{a}	0^{a}	4 (2)^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.325
	Tylenchus	**0^{c}**	**0^{c}**	**1 (1)^{c}**	**18 (7)^{b}**	**0^{c}**	**14 (7)^{b}**	**0^{c}**	**22 (10) ^{ab}**	**2 (1)^{c}**	**42 (19)^{a}**	**0^{c}**	**0^{c}**	**0.050***
	Total herbivores	57^{a}	34^{a}	36^{a}	79^{a}	29^{a}	45^{a}	46^{a}	102^{a}	18^{a}	64^{a}	72^{a}	26^{a}	0.227
Omnivores	*Discolaimoides*	**12 (5)^{b}**	**9 (1)^{c}**	**18 (8)^{b}**	**17 (3)^{b}**	**10 (2)^{c}**	**13 (7)^{b}**	**40 (10)^{a}**	**3 (2)^{d}**	**14 (2)^{b}**	**1 (1)^{d}**	**1 (1)^{d}**	**0^{d}**	**0.024***
	Dorylaimodes	2 (2)^{a}	0^{a}	0^{a}	0^{a}	7 (6)^{a}	2 (2)^{a}	4 (4)^{a}	0^{a}	7 (6)^{a}	0^{a}	0^{a}	7 (2)^{a}	0.19
	Labronema	**0^{b}**	**1 (1)^{b}**	**4 (1)^{b}**	**3 (1)^{b}**	**1 (1)^{b}**	**12 (5)^{a}**	**0^{b}**	**13 (2)^{a}**	**2 (1)^{b}**	**3 (1)^{b}**	**0^{b}**	**0^{b}**	**0.021***
	Total omnivores	**14^{c}**	**10^{c}**	**12^{c}**	**20^{c}**	**18^{c}**	**27^{b}**	**44^{a}**	**16^{c}**	**23^{bc}**	**4^{d}**	**1^{d}**	**7^{d}**	**0.011***
Predators	*Mononchus*	4 (4)^{a}	1 (1)^{a}	0^{a}	1 (1)^{a}	1 (1)^{a}	0^{a}	4 (4)^{a}	0^{a}	0^{a}	0^{a}	18 (10)^{a}	2 (2)^{a}	0.245
	Total predators	4^{a}	1^{a}	0^{a}	1^{a}	1^{a}	0^{a}	4^{a}	0^{a}	0^{a}	0^{a}	18^{a}	2^{a}	0.245

T1 = Control (no organic or inorganic inputs); T2 = 5 Mg ha^{-1} biochar; T3 = 10 Mg ha^{-1} biochar; T4 = DAP; T5 = Urea; T6 = NPK; T7 = DAP + 10% biochar; T8 = Urea + 10% biochar; T9 = NPK + 10% biochar; T10 = DAP + 20% biochar; T11 = Urea + 20% biochar; T12 = NPK + 20% biochar. Within a row, means followed by the different superscript letters are significantly different at $p<0.05$. Significant values: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

7.3.6 Correlation of soil fauna with soil chemical properties

Of the two dominant soil macrofauna selected for the correlation analysis, only earthworms showed significant correlation with the soil chemical properties (Table 7.6). The earthworms correlated significantly but negatively with C and C/N ratio. There were no significant correlation between beetles and the soil chemical parameters measured. Among the nematodes groups, only the free-living bacterivorous and fungivorous groups showed significant correlations. In this, bacterivorous nematodes correlated significantly and negatively with C, N and P while the fungivorous nematodesp correlated negatively with P only; no significant correlation with all the other soil chemical parameters was observed. The total nematode abundance followed similar trend to the bacterivores.

Table 7.6: Pearson's correlation coefficients between soil fauna and soil chemical properties.

Soil fauna group		Soil chemical characteristics							
		pH	C	N	P	K	C/N	NO_3^-	NH_4^+
Macrofauna	Earthworms	0.10	**-0.32***	0.13	0.02	0.08	**-0.41****	0.23	0.30
	Beetles	-0.12	-0.19	-0.20	0.03	0.00	0.06	-0.11	-0.06
Mesofauna	Bacteriovores	0.02	**-0.39***	**-0.44****	**-0.44****	-0.26	0.31	-0.24	-0.09
	Fungivores	0.01	-0.19	-0.11	**-0.37***	0.18	0.04	-0.25	-0.11
	Plant parasitic	0.03	-0.26	-0.04	-0.19	-0.16	-0.10	-0.06	-0.02
	Omnivores	-0.30	-0.07	-0.03	0.02	0.13	-0.02	0.20	0.12
	Predators	0.01	0.01	0.04	-0.27	-0.10	0.03	-0.20	-0.04
	Total nematodes	-0.01	**-0.37***	**-0.32***	**-0.43****	-0.16	0.18	-0.21	-0.08

7.4 Discussion

7.4.1 Effects of biochar and fertiliser + biochar blends on soil chemical properties

Biochar application as a soil amendment has been shown to affect soil chemical properties either directly through addition of macro and micro-elements (Glaser et al., 2002; Lehmann et al., 2003) or indirectly through changes in soil pH and reducing aluminium saturation (Van Zwieten et al., 2010). Higher concentration of nutrients are thus expected to increase after addition of biochar. This concurs with the results obtained in this study, where addition of biochar resulted in an increased concentration of all the soil nutrient elements measured compared to the no-input control. Other studies have also reported similar findings. For instance, Novak et al. (2009) reported that application of pecan-shell biochar increased soil organic C, P, K and Ca whereas Lehmann et al. (2003) reported an increase in P, K, Ca and Mg after charcoal addition. Gaskin et al. (2010) on the other hand reported higher K, Ca and Mg after application of peanut-hull biochar. Notably however, in this study, soil pH in biochar treated plots did not change despite the very high pH of the biochar. In addition, there were no significant differences in nutrient elements between plots treated with either biochar, fertiliser or fertiliser + biochar. This shows that the biochar was either supplying almost an equivalent amount of these nutrients as the fertiliser or the crop uptake from plots treated with fertiliser or the blends was higher. The latter seems a more convincing explanation given the differences in grain yield as noted earlier in the results section (data not presented). Nevertheless, the nutrient changes lasted for a season since there were no significant differences in all the nutrient elements (except NO_3^-) after stopping application of inputs. This could perhaps show that *P. juliflora* biochar was not effective in retaining nutrients, thus an indication that the contribution of biochar towards sustainability of soil fertility cannot be generalised. However, other benefits of biochar such as improved water holding capacity, soil structure improvement, as it has been suggested by several studies and which were not measured in this study cannot be

underestimated. In addition, seasonal variability could have influenced the observed results in the current study. Studies on long-term effects of biochar application are therefore needed since its continuous application for long periods of time may give different outcomes.

7.4.2 Effects of biochar and fertiliser + biochar blends on soil fauna

Results from this study indicate that only earthworms showed preference of biochar amended soils which concurs with other studies that have reported similar findings. For instance, Van Zwieten et al. (2010) reported that earthworms preferred soil (Ferrosol) amended with biochar over unamended control soil. The response of earthworms and other soil macrofauna to biochar application may have been influenced by short-term release of organic molecules from freshly added biochar. In their meta-analysis for example, Lehmann et al. (2011) indicated that a portion of C in biochar is readily available for utilisation, which may encourage proliferation of soil microbes. Soil macrofauna such as earthworms could then benefit from such microbes or their metabolites. Other studies have suggested that earthworms may ingest biochar particles to benefit from its liming and detoxifying properties (Topoliantz and Ponge 2003). Such mechanisms can explain the observed increase in earthworms' abundance in biochar amended plots. Apart from direct effects of biochar on food substrate availability, earthworms could have been attracted by amelioration of the physical properties. For instance, it has been suggested that biochar can improve soil porosity and aeration, and thus temperature and moisture regimes in the soil (McCormack et al., 2013). Though these were not measured, I cannot rule out possibility of their contribution to the observed differences in earthworm abundance. Lack of significant differences in all the other soil macrofauna groups could be due to the fact that many of these are highly mobile and may not rely directly on biochar as food substrate. Nonetheless, the higher diversity and taxonomic richness in plots treated with biochar compared to the fertiliser alone could indicate that soil treated with biochar was

providing a better environment to thrive than that treated with fertilisers. This could, perhaps, be the reason why blending the fertilisers with biochar eliminated the possible negative effects that could have been brought about by inorganic fertilisers, as indicated by the higher taxonomic richness compared to fertiliser alone.

Contrary to earthworms, nematodes seems to have been impacted negatively by the biochar. It has been suggested that biochar addition may enhance population of microbial biomass due to the presence of a certain proportion of C which is immediately available after its application (Lehmann et al., 2011). Thus increased microbial biomass production can be expected to trigger a series of soil activities which may affect soil micro and macrofauna as well as higher organisms in the food web. In this study, the general decline in nematode numbers associated with biochar application could indicate that the biochar either increased predators of the nematodes or this amendment was exerting negative effects on them. Studies have shown that incorporation of organic inputs attracts numerous organisms, some of which could be natural enemies to nematodes such as nematophagous fungi (*Arthrobotrys brochopaga* and *A. oligospora*), collembolans and tardigrades which could suppress the population of nematodes (Wang and McSorley, 2005; Akhtar and Malik, 2000). Alternatively, biochar could influence nematodes through release of compounds which may be toxic to them. For instance, Hale et al. (2012) reported that there is an array of potentially toxic organic compounds that are produced during pyrolysis which could affect the response of soil biota to biochar application. Thus, presence of toxic compounds within biochar can override increased food resource base that could be triggered by application of biochar, hence discouraging proliferation of nematodes.
Though not many studies have looked at the effects of biochar on a broad range of nematode functional groups, the few available have given mixed results. For instance, my results contradict the findings from Zhang et al. (2013) who reported significantly higher fungivorous nematodes on plots treated with large amounts of wheat-straw biochar (12 and 48 Mg ha^{-1}) than

in plots with no amendment (control plots). However in the same study, the authors reported that plant parasitic nematodes significantly decreased in these plots, whereas bacterivorous and omnivores-predators were not affected compared to the control plots. Rahman et al. (2014), on the other hand reported that, while application of poultry-litter biochar reduced plant parasitic nematodes by over eightfold, there were no significant effects on free-living nematodes. These results could indicate that the type of biochar (thus its properties) could play an important role in regulating the way nematodes respond to biochar application.

7.5 Conclusions

Results of this study have shown that earthworms were being attracted by biochar whereas nematodes decreased in biochar treated plots. One reason of increase in earthworm abundance may be that biochar was supporting higher microbial growth which benefits them as they are known to feed on microbes growing on such substrates and/or their metabolites. On the other hand, a decrease in nematode numbers could have been caused by attraction of their natural enemies which may suppress their population or the biochar could have been releasing compounds that may be toxic to them. The results also show the potential of biochar to be used as an organic amendment. This can be an important step towards converting the shrub, which has been termed a noxious weed, into a valuable soil amendment for improving productivity of soils that are severely deficient in N and P and low in organic matter. Blending fertilisers with biochar however, seems not to have had much effects in terms of soil nutrient retention as it has been proposed by several studies. Nonetheless, further research with long-term application of biochar could be of great benefit in expounding their impacts, on soil fertility and soil fauna, since seasonal variations could affect the observed results at short-term scales.

CHAPTER EIGHT

General discussion, conclusion and recommendations

8.1 Soil macrofauna abundance as affected by the presence of trees

Smallholder farmers often grow trees with annual crops for food, forage, wood, charcoal, among other products. Trees are known to modify conditions beneath the canopy through shading, root turnover and litter inputs which significantly influence soil moisture, temperature, carbon substrate availability and nutrient regimes. However, there is limited knowledge on the magnitude and pattern of their influence on soil macrofauna abundance in agricultural landscapes particularly in tropical Africa. Chapter 3 has demonstrated that tree species can play a major role in shaping the spatial distribution patterns of soil macrofauna. Earthworms and termites, which are known to feed on soil organic matter showed the most distinctive trends. Earthworms, for instance, were significantly attracted by the presence of indigenous tree species, *Z. gilletii*. On the other hand, termites were attracted to the exotic tree, *E. grandis* whose litter was found to be of lower quality compared to that from indigenous trees. Such differential observations could be due to feeding preference of soil macrofauna groups based on the quality of litter and root biomass. For instance, earthworms are known to selectively ingest soil organic and mineral particles, hence higher numbers presence under the canopy of *Z. gilletii* compared to that of either *C. megalocarpus* or *E. grandis*, as it was demonstrated in chapter 3. Termites are known to produce a variety of enzymes which interact with the gut microflora that facilitate digestion of low quality materials such as woody materials (Lavelle, 1997). Thus their notably higher numbers under the canopy of *E. grandis* can be expected. In chapter 3, it was also evident that soil macrofauna abundance in all the groups increased with increasing duration of cultivation. This supports the importance of trees as 'resource islands' where soil fertility and environmental conditions are limiting. Thus, integrating a diverse tree cover can play a key role in maintaining soil biodiversity, which is especially important in

sustaining ecological services in tropical agroecosystems, majority of which are categorised as low-input systems.

8.2 Soil aggregate stability and C storage under trees is driven by ecosystem engineers

Soil organic matter (SOM) content and soil structure stability are key indicators of soil quality and thus critical components in defining sustainable land use. Trees could be important in shaping not only the abundance and diversity of soil fauna, as has been established in chapter 3, but could also have significant influence on SOM and soil structure through litter fall and root turnover and through the activities of soil fauna under these trees. Earthworms, particularly the endogeic species, *Nematogenia lacuum* were involved in shaping soil aggregation as reported in chapter 4. The weight of micro-aggregates was higher under the canopy of *Z. gilletii* trees, a trend which was observed to be similar to that of the earthworm species *N. lacuum*. *Nematogenia lacuum* is a small sized species that excretes small pellets that could have resulted in formation of micro-aggregates fractions. This earthworm species could also have significant effect on C recovered from the aggregates. Since the lowest micro-aggregates C was obtained from soils under the canopy of *Z. gilletii* tree where earthworm species *N. lacuum* was found in large numbers, this shows that fragmentation of macro-aggregates to micro-aggregates could have led to losses in soil C content. Such results gives an indication that the choice of trees to be planted in the farms could be critical in shaping, not only soil biodiversity, but also SOM dynamics and soil aggregation process.

8.3 Effects of PyOM/biochar on soil macrofauna abundance and activity

Conversion of trees to charcoal removes beneficial effects of the trees while at the same time creates new environmental conditions due to increased concentration of pyrogenic organic matter (PyOM) in the soil. However, there is limited knowledge on how such PyOM additions into the soil, affect the abundance and distribution patterns of soil macrofauna. It has been

hypothesised that applying large amounts of biochar as an amendment can reduce the rate of soil degradation and improve soil fertility (Verheijen et al., 2010). Chapter 5, 6 and 7 have presented results that show contrasting effects of PyOM/biochar on some soil fauna groups. In chapter five for instance, it was found that high concentration of PyOM derived from *Z. gilletii* had negative effects on soil macrofauna, particularly the endogeic earthworm *N. lacuum* in the soil from the field study. This was confirmed in a mesocosm study with PyOM from the same tree species (Chapter 6). Weight of biogenics (casts) from endogeic earthworm, *Pontoscolex corethrurus*, were reduced significantly by increasing the amounts of PyOM applied in the mesocosms. Endogeic earthworms ingest large amounts of organic matter and mineral soil, thus more responsive to the quality of organic and/or inorganic inputs applied to the soil. The reduction in number of earthworms (*N. lacuum*) in the charcoal-making spots and casts of the earthworms (*P. corethrurus*) in the mesocosm study, confirms that high concentration of PyOM induced negative effects on the earthworms. In chapter seven however, biochar obtained from *Prosopis juliflora* appeared to have some ameliorating effects, given the higher number of earthworms recorded in plots treated with this biochar. One reason for such an observation may be that biochar prepared from *P. juliflora* could have been of higher quality compared to PyOM from *C. megalocarpus* and *Z. gilletii* trees. The ratio C/N, frequently used as a measure of organic resource quality was low in *P. juliflora* biochar, whereas C/P ratio was at least four times higher in *C. megalocarpus* and *Z. gilletii* PyOM than *P. julifrora* biochar. Thus the PyOM from charcoal-making spots could have been too recalcitrant to support microbial growth, thus reducing the abundance and activity of these two endogeic earthworms which are known to feed on microbes growing on such substrates and/or their metabolites. However, nematodes appear to have been negatively affected by *P. julifrora* biochar. This could result from two major factors: i) the biochar could have perhaps increased the number of competitors/antagonists/predators of the nematodes or, ii) the biochar could have induced toxic effects to the nematodes. Nematodes are important drivers of soil nutrient cycles through grazing of microbes, thus helps in unlocking nutrients that could otherwise be unavailable to

crops. It is often suggested that variations in the abundance of different trophic groups of nematodes is closely associated with soil management applications, thus may be an indicator of changes occurring in soil food web structure (Forge et al., 2013; Zhang et al., 2013). Thus, the sensitivity of earthworms and nematodes to addition of organic inputs makes these organisms important indicators of soil quality (Karanja et al., 2010).

8.4 Conclusion and recommendations

The information gathered from this work is a starting point towards understanding the spatial influence of biochar on soil fauna. The study shows that soil macrofauna studied responded differently to soil degradation and tree species, thus highlighting complexity of the soil ecosystem. Since soil fauna are important drivers of soil ecosystem functions, such effects could positively or negatively impact on soil productivity. However, dominance specific macrofauna groups may have detrimental effects. For instance, the higher number of earthworm species, *Nematogenia lacuum* under *Z. gilletii* tree, which can be linked to the low aggregate-C observed under this tree is an indicator of such detrimental effects. Thus, promoting tree diversity within the farms can be key in maintaining a healthy soil ecosystem.

Charcoal-making spots were rich in soil nutrients, P and K, thus indicating the great role biochar application can play in supplying the two nutrients. However, the lower number of soil macrofauna in these spots shows the negative effects of the same amendment. Thus, assessments in agricultural landscapes need to consider the differential effects of biochar in contributing to a mosaic of soil conditions, influencing soil macrofauna abundance and distribution. This is especially important in tropical Africa where mass application of biochar is being proposed to address the increasing rate of soil degradation.

The higher NO_3-N in plots with fertiliser+biochar blends, two seasons after the last application shows the great potential biochar has in promoting nutrient use efficiency. Further research is needed to assess the potential of long-term biochar application on soil C and impact on soil biodiversity, mitigation of greenhouse gas emission and soil rehabilitation and productivity.

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APPENDIX

Supplementary tables for paper 2 (To be submitted as: Soil aggregation and C storage along a soil degradation gradient as affected by dominant tree species)

Table S1: Earthworms species distribution as affected by the three tree species in the study site. Source Kamau et al. (2017).

Soil macrofauna description			***Croton megalocarpus***					***Eucalyptus grandis***					***Zanthoxylum gilletii***				
			Sampling zone														
Family	Ecological group	Genera/Species	**A**	**B**	**C**	**D**	**Mean**	**A**	**B**	**C**	**D**	**Mean**	**A**	**B**	**C**	**D**	**Mean**
Termites																	
Termitidae	G II (FWLG)†	*Microtermes sp.*	65.8^{a}	40.1^{a}	73.4^{a}	49.3^{a}	57.2^{A}	240.0^{a}	29.3^{a}	42.6^{a}	80.0^{a}	97.9^{A}	26.7^{a}	102.9^{a}	44.0^{a}	17.4^{a}	47.8^{A}
Earthworms																	
Acanthodrilidae	Epigeic	*Dichogaster affinis*	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{A}	1.3^{a}	7.1^{a}	0.4^{a}	2.2^{a}	2.8^{A}	**0.0^{b}**	**2.2^{ab}**	**0.0^{b}**	**5.8^{a}**	2.0^{A}
		Dichogaster bolaui	6.2^{a}	6.7^{a}	0.4^{a}	6.7^{a}	5.0^{A}	3.6^{a}	2.7^{a}	1.3^{a}	1.8^{a}	2.3^{A}	0.4^{a}	1.3^{a}	6.2^{a}	2.6^{a}	2.6^{A}
		Dichogaster modiglianii	0.4^{a}	0.9^{a}	0.0^{a}	0.9^{a}	**0.5^{B}**	2.2^{a}	0.9^{a}	7.1^{a}	2.7^{a}	**3.2^{A}**	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.0^{B}**
		Dichogaster saliens	3.6^{a}	0.0^{a}	0.4^{a}	0.0^{a}	1.0^{A}	1.3^{a}	1.3^{a}	1.3^{a}	1.8^{a}	1.4^{A}	6.2^{a}	2.2^{a}	0.0^{a}	0.0^{a}	2.1^{A}
Eudrilidae		*Eminoscolex violaceus*	9.8^{a}	6.2^{a}	11.1^{a}	3.6^{a}	**7.7^{A}**	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	**0.0^{B}**	0.0^{a}	0.0^{a}	0.4^{a}	0.4^{a}	**0.2^{B}**
		Polytoreutus annulatus	4.9^{a}	1.3^{a}	5.3^{a}	2.7^{a}	**3.6^{A}**	2.7^{a}	0.9^{a}	1.3^{a}	3.1^{a}	**2.0^{AB}**	0.4^{a}	0.0^{a}	0.4^{a}	0.9^{a}	**0.4^{B}**
		Stuhlmannia sp.	0.4^{a}	0.9^{a}	0.0^{a}	0.0^{a}	0.3^{A}	0.0^{a}	0.9^{a}	0.0^{a}	0.0^{a}	0.2^{A}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{A}
Total epigeic earthworms			25.3^{a}	16.0^{a}	17.3^{a}	13.8^{a}	**18.1^{A}**	11.1^{a}	13.8^{a}	11.6^{a}	11.6^{a}	**12.0^{AB}**	7.1^{a}	5.8^{a}	7.1^{a}	9.8^{a}	**7.4^{B}**
Ocnerodrilidae	Endogeic	*Gordiodrilus wemanus*	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{A}	1.3^{a}	2.2^{a}	1.8^{a}	0.9^{a}	1.5^{A}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{A}
		Nematogenia lacuum	72.5^{a}	39.6^{a}	34.3^{a}	57.8^{a}	**51.1^{B}**	47.2^{a}	57.3^{a}	66.2^{a}	49.7^{a}	**55.2^{B}**	**172.9^{a}**	**178.2^{a}**	**195.6^{a}**	**108.4^{b}**	**163.8^{A}**
Total endogeic earthworms			72.5^{a}	39.6^{a}	34.3^{a}	57.8^{a}	**51.1^{B}**	48.5^{a}	59.5^{a}	68.0^{a}	50.6^{a}	**56.7^{B}**	**172.9^{a}**	**178.2^{a}**	**195.6^{a}**	**108.4^{b}**	**163.8^{A}**
Total earthworm counts			97.8^{a}	55.6^{a}	51.6^{a}	71.6^{a}	**69.2^{B}**	59.6^{a}	73.3^{a}	79.6^{a}	62.2^{a}	**68.7^{B}**	**180.0^{a}**	**184.0^{a}**	**202.7^{a}**	**118.2^{b}**	**171.2^{A}**

Within rows, means followed by different lower case letters in superscript are significantly different at $p < 0.05$. † adopted from Ayuke et al. (2011a). Abbreviations: G II = Group two, W = wood, L = leaf litter, F = fungus grower, G = dead/dry grass.