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Effect of organic amendment on soil carbon dynamics in agricultural ecosystems

(農地土壌生態系における有機物投入が土壌炭素動態に及ぼす影響)

北海道大学　大学院農学院

共生基盤学専攻　博士後期課程

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

Effect of organic amendment on soil carbon dynamics in agricultural ecosystems

By

Toru Hamamoto

Soil carbon (C) contents and dynamics are important in the maintenance of health soils. Organic amendments (e.g. crop residue and organic manure) are known as materials to increase soil C. The degradation of organic amendments and physical disturbance of soils, performed by the fauna, can alter soil microbial community, and thus influence rates of the C cycles. However, little is known about the interaction between different soil biological communities (i.e. soil fauna and microbes) regarding soil C dynamics. Hence the objectives of this research were (1) to investigate the effects of different types of earthworms on CO_2 emissions and microbial biomass during organic material decomposition, (2) to determine the effects of organic amendments on above-ground ecosystems in two agricultural soils in Zambia and (3) to quantify the interaction between soil C dynamics and microbial community changes after organic amendments in two agricultural soils in Zambia.

This first experiment measured the changes in CO_2 emissions and soil microbial biomass during barley decomposition with and without earthworms (*Metaphire Hilgendorfi* and *Eisenia Fetida*). After 32 days incubation, *M. hilgendorfi* had a potential to accumulate microbial biomass carbon (MBC) and nitrate-N, compared to *E. fetida*. The result suggested that the interaction between soil microbes and earthworm is influenced by earthworm species, consequently influencing the soil C and N dynamics.

The second experiment investigated the changes aboveground ecosystems after organic amendments (e.g. cattle manure, poultry manure etc.) in two different soils in Zambia. We

conducted two field experiments using different organic amendments in sandy loam soils and loamy sand soils in Zambia. A split-plot design was used with crop type (cassava, maize, soybean and control (bare) as the main plot and soil amendment (chemical fertilizer, cattle manure, poultry manure, maize residue, and control) as the subplot factors. The results showed that the total number of soil fauna in each site was totally different; we found around 1000/200 individuals at sandy loam soils/loamy sand soils. Organic amendments stimulate soil fauna abundance. For crop production, the organic amendments had positive effects on crop yields in both soils. Based on the results, organic amendments largely contribute to stimulate soil fauna abundance with the increase in nutrient cycles in sandy loam soils, while organic amendments act as nutrient source for crop production in loamy sand soils.

Finally, the third experiment focused on the influence of the organic amendments on C dynamics and soil microbes in C depleted agricultural soils in Zambia (same treatments of second experiments). The results indicate that in the loamy sand soil, organic amendments altered the microbial activity but did not have a major impact regarding the C sequestration in the soil. Contrastingly, the effects of the organic amendments on CO_2 emissions and microbial activities in the sandy loam soil were unclear. Factors such as soil texture and moisture ranges controlled the impacts of organic amendments on soil C cycle and bacterial communities. These studies indicate that the response of organic amendments is markedly influenced by soil biological community. Those different response consequently influenced soil C dynamics and agricultural production. To maintain/increase soil C in agricultural systems, the factors affecting the soil biological community have to be taken into account.

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Chapter 1

Introduction

1.1 Background

This thesis reports the outcomes of a PhD research project where the overall theme of the study was to identify the effects of organic amendments on CO_2 emissions and soil biological community in agricultural soils. For the global C cycle, any activities that favour the decomposition and mineralization of organic material, with consequent C emission, have risks of increasing atmospheric CO_2 levels (Lamb et al., 2016).

Indigenous organisms in soil (e.g. soil fauna and microbes) and their interactions play a major role in organic matter decomposition, but also nutrient cycling with impacts on soil fertility (Barrios, 2007; Obriot et al., 2016). Soil organisms also contribute to aggregate stability, are determinant for soil structure, improving water regulation and decreasing soil erosion (Bardgett 2005; Kibblewhite et al., 2008). These reflect the complexity of the belowground terrestrial ecosystems and linkages with soil function and ecosystem services (Bünemann et al., 2018). The decomposition is one of the major biological processes including the physical breakdown and biochemical transformation of complex materials into simpler organic and inorganic materials (Juma, 1999). Especially, soil fauna (e.g. earthworm), which is decomposers of organic matter, contribute to C dynamics through physical, chemical and microbiological properties (Jouquet et al. 2006; Lavelle et al., 2006).

In agricultural ecosystems, organic amendments are well known agricultural method for maintaining and improving soil quality (Bhattacharyya et al., 2001) by improving soil organic C (SOC) content (Bandyopadhyay et al., 2010; Li et al., 2018). The soil C also improves biological community, activity, and functions in soil, and increased biomass production and soil

C sequestration (Rivers et al., 2016; Jin et al., 2018; Mangalassery et al., 2019). Therefore, regular organic matter applications to soils can be a way of restoring soil organic matter (SOM) content and contributing to C storage in agricultural soils (Peltre et al., 2012).

Soils of sub-Saharan Africa are fragile to degradation and climate change due to low soil C contents, low plant available water, and weak soil structure (Lal, 2019). Researchers have been studying agricultural practices such as conservation agriculture (Kassam et al., 2019) to achieve adaptation and mitigation of climate change, improve degraded soils, and restore soil health, through soil C sequestration (e.g. Thierfelder et al., 2015). Despite these advances in both basic and applied research, however, agricultural productivity in sub-Saharan Africa has stagnated since 1960s for cereals at 1–1.5 Mg ha^{-1} and for grain legumes at 0.2–0.5 Mg ha^{-1} (Lal, 2017, 2019).

Additionally, information on soil microbiology activities and the diversity of microbial communities are seldom reported in relation to crop production systems in sub-Saharan Africa (Wood et al., 2015; Hamamoto et al., 2018). Equally, little information on soil C stocks and dynamics related to soil biota in modern farming systems are missing. In most of sub-Saharan Africa, SOC contents are less than 1.1% (range of C in soil), which is the critical limit for agricultural production (Aune and Lal, 1997; Lal, 2004).

1.2 Research Objectives

The objectives of this project were to:

- Investigate the effects of two types of earthworms on CO_2 emissions and microbial biomass during organic material decomposition; Chapter 3.
- Determine the effects of organic amendments on aboveground ecosystems, including crop yields and soil faunal communities in two agricultural soils in Zambia; Chapter 4.

- Quantify the interaction between soil C dynamics and microbial community changes after organic amendment applications in two agricultural soils in Zambia; Chapter 5.

1.3 Thesis structure

This thesis is divided into 6 chapters. The first and second chapters provide a general introduction and a review of the relevant literatures, respectively. The following three chapters (3, 4, and 5) contain short introduction, materials and methods, results and discussion sections. Finally, chapter six provides an overall discussion, summary and recommendations for future work. In brief each chapter contains the following:

Chapter 1 This chapter briefly introduces information on effects of organic amendments in agricultural soils and the global importance of these effects.

Chapter 2 This chapter is a literature review that describes the current knowledge of organic amendments in agricultural soils. It has been suggested that organic amendments improve soil C contents. However, how organic source and soil biology interact, and consequently their interaction affect C cycle has not been well investigated. There is still a lack of knowledge of the organic amendments with respect to soil biological activity in agricultural soils and associated CO_2 emissions. This review discusses these aspects and states a case for the research performed.

Chapter 3 This chapter reports a laboratory experiment that focused on CO_2 emissions and microbial biomass following earthworm application. The experiment was performed using two different earthworms.

Chapter 4 This chapter describes the effects of organic amendments on aboveground agricultural ecosystems in Zambian soils. The field experiment was performed to reveal how organic amendments affect soil fauna community and crop yields in different two sites in Zambia.

Chapter 5 This chapter discusses the effects of organic amendments on CO_2 emissions, decomposition rate, and soil microbial community structure in different two sites in Zambia. The fertilizer treatments of the field experiment are same treatments of Chapter 4.

Chapter 6 This chapter summarizes the major findings reported in this thesis and contains recommendations for future research.

Chapter 2

Literature Review

2.1 Introduction

Soil carbon (C) is one of the most important components in soils due to their multiple functions. Soil C content is ranged from approximately 50% to 60% of soil organic matter (SOM), which stores nutrients for plants, improves soil structural stability to enhance soil fertility, and thus improves the food security (Fig. 2.1, Carter, 2002; Pribyl, 2010; Trivedi et al., 2018). Soil C alters the physical, chemical, and biological properties of the soils (Rice, 2005).

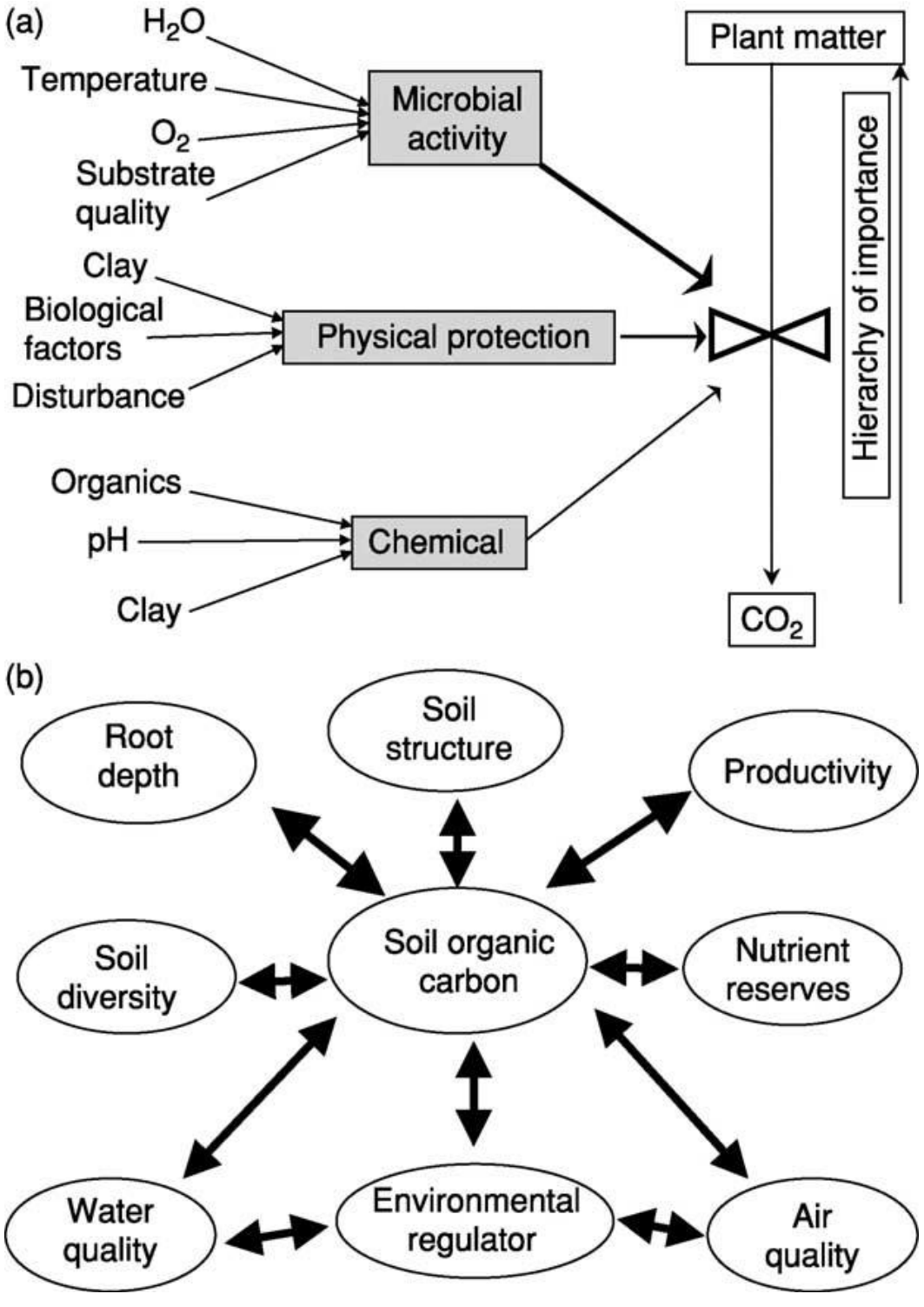

Figure 2.1. (a) Regulation of carbon stabilization in soil; (b) relationship between soil organic C and soil, water, and air quality (Source: Rice, 2005).

Globally, C storage capacity of soils is much larger compared with the pools of C in the atmosphere and vegetation. The amount of soil organic C (SOC) stored estimated at 2,440 Gt C to 2 m depth, compared to atmospheric carbon dioxide (CO_2, 750 Gt C, Batjes, 1996; Conijn and Lesschen, 2015). This means that a slight soil C fluctuation has a significant impact on CO_2 concentration in the air (Schlesinger, 1977). Thus, there is now a large and growing interest in knowing the size of soil C pool and its sequestration potential against CO_2 increase. The '4 per mille Soils for Food Security and Climate' (http://4p1000.org) was launched at the 21st Conference of the Parties to the United Nations Framework Convention on Climate Change (COP21, November 30 to December 11, 2015), with the objective to increase global SOM stocks by 4 per 1000 (or 0.4%) per year as a compensation for the global greenhouse gas emissions by anthropogenic sources and food security improvement (Lal, 2016; Chabbi et al., 2017; Dignac et al., 2017; Minasny et al., 2017; Corbeels et al., 2019). This concept simply consists of the globally ratio of the anthropogenic C emissions (9.3 Gt C yr^{-1}) to the SOC stock (2400 Gt C, Minasny et al., 2017). Therefore, the increase in 0.4% per year of global SOC stocks would completely offset the global anthropogenic CO_2 emissions.

The CO_2 emissions from soils is related to many biotic and abiotic factors. Biotic factors include residue quality, activity of soil fauna and microbes (Oades, 1988; Cortez, 1998). Soil fauna and microbes have a direct implication on decomposition (Oades, 1988; Cortez, 1998; Aira et al., 2008; Kuzyakov and Blagodatskaya, 2015). Biota related to decomposition often classified by size groups that include microflora and micro-, meso- and macro-fauna. Although the soil fauna has been shown to have profound impacts on soil ecosystems and to regulate many important soil processes, the key steps in the major elemental cycles are ultimately conducted by soil microorganisms.

Earthworms are one of the macro-fauna and widely known as a contributor for soil C stock, although they also release CO_2 (Lubbers et al., 2013). The earthworm activity (e.g. casting) can alter soil microbial community and thus influence rates of the C cycles following the organic

amendments in agricultural soils (Fonte and Six, 2010; Gómez-Brandón et al., 2012; Massey et al., 2013). Abiotic factors mainly correspond to soil climate (temperature and moisture) and physio-chemical characteristics (texture and clay mineralogy). These factors have a critical influence on organic matter decomposition and accumulation in soil.

Previous studies on the amount of soil C in the world showed that some agricultural ecosystems have the contents that are below critical limits, particularly in sub-Saharan Africa (Stockmann et al., 2015; Hamamoto et al., 2018), although there are previous reports on soil C increase in other region due to improved management such as organic amendments (e.g. Steiner et al., 2007). Although the studies on soil organic amendments are clearly needed in sub-Saharan Africa, currently there are few studies have been performed to investigate the effect organic amendments on soil biological community.

In this literature review, the current understanding of the mechanisms of decomposition in agricultural ecosystems and the various effects of the organic amendments in sub-Sharan Africa are reviewed.

2.2 Roles of soil C in agricultural ecosystems

2.2.1 Soil C and CO_2 emissions in agricultural ecosystems

The CO_2 emissions account for about 75% of the total greenhouse effect and are greatly affected by the agricultural sector, which contributes to about 10 to 12% of total global anthropogenic emissions of greenhouse gases (5.0 to 6.1 Gt CO_2-eq yr^{-1}) (IPCC, 2007, 2014). Soil C losses by CO_2 emissions are generally promoted by agricultural management (e.g. tillage), due to the enhanced microbial degradation (La Scala et al., 2006; Mangalassery et al., 2014). Agricultural soils are estimated to have historically lost > 50 Gt C globally (Lal, 2003; Janzen, 2004, 2006).

The efforts have to be made to minimize this loss of important soil C. Previous studies estimated > 50% of the SOM they contained before agricultural conversion can be re-captured

(Lal et al., 2004; Ogle et al., 2005). Increase in soil C often can be achieved by applying C such as organic materials (e.g. manure and plant residue) in agricultural ecosystems (Powlson et al., 2012; Paustian et al., 2016). SOM also acts as a source of C as well as energy for microorganisms and contributes to soil microbial biomass production; a long-lived C pool in soil (Jin et al., 2018).

Environmental factors such as climate, soil type and texture, and hydrology may also influence soil biological community, and thus soil C and CO_2 emissions (Fig. 2.2, Bünemann et al., 2018). Particularly in tropical or semi-arid region, macro-fauna (e.g. termites) and microbes have also a great impact on the decomposition of organic amendments (Mando and Brussard,1999; Powlson et al., 2001; Esse et al., 2001; Ouédraogo et al., 2004, 2007).

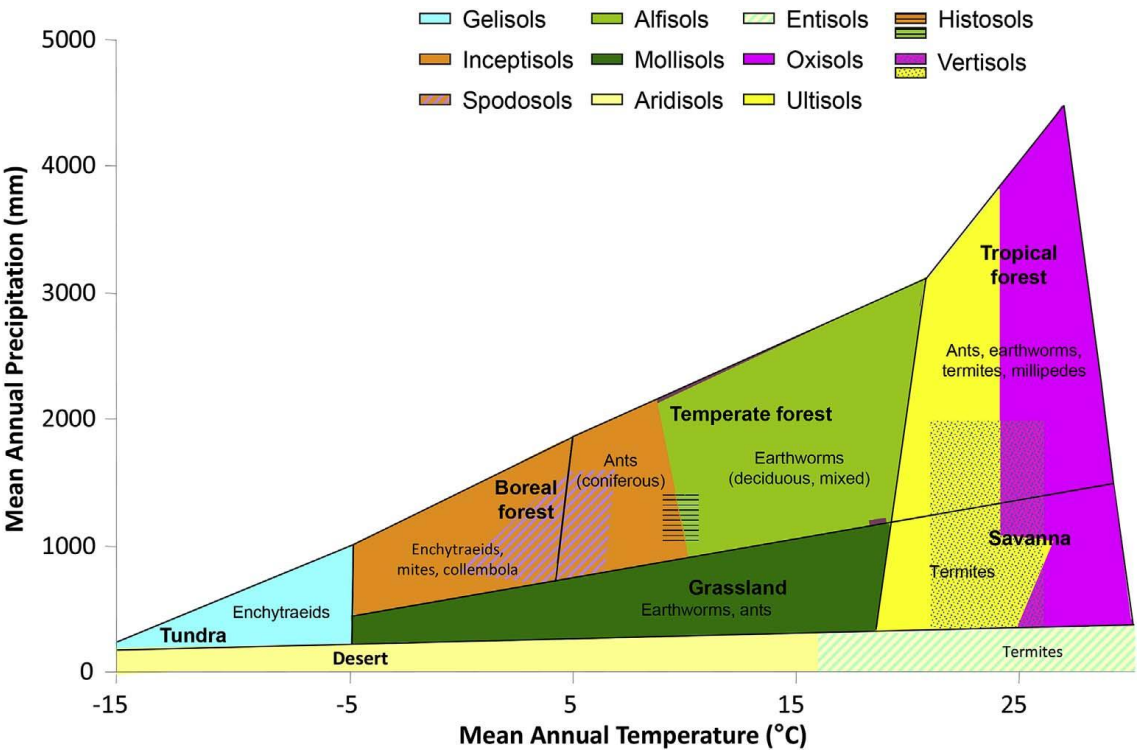

Figure 2.2. Abiotic and biotic factors constituting soil quality in the soils of the world (Source: Bünemann et al., 2018)

2.2.2 Organic matter dynamics by soil fauna and microbes

Organic matter is normally actively decomposed by biomes in soils and its C is respired as CO_2. Respiration is a series of metabolic processes that break down organic C containing compounds to produce energy, water, and CO_2 (Luo and Zhou, 2006). Net soil CO_2 emission is largely derived from plant root respiration and heterotrophic respiration of SOM decomposition (Raich and Schlesinger, 1992). The C substrates in soils are derived from organic material and these can be divided into fresh residue, living organisms, dissolved organic matter, particulate organic matter, inert organic matter (e.g. charcoal), and well decomposed material known as humus (Baldock and Skjemstad, 2000). The SOM pools derived directly from partially decomposed organic materials typically consist of <15% of total SOM (Gregorich et al., 2006; Grandy and Robertson, 2007). Immediately after the C input, soil fauna and microbes consume them as energy source (Ayuke et al., 2011). This section of the literature review outlines each of the biological degradation and stabilization process of organic materials.

2.2.2.1 Decomposition

Decomposition includes not only the CO_2 emissions (mineralization) but also assimilation and immobilization. The biological process includes the physical breakdown and biochemical transformation of complex materials into simpler organic and inorganic materials (Juma, 1999). Soil fauna have multiple effects on decomposition, which is the first step of the decomposition (Coleman, 2008; Grandy et al., 2016). Soil fauna plays a key role in physical breakdown of organic amendments (Ouédraogo et al., 2004) and significantly affect soil physical properties (Tian et al., 1997; Mando et al., 1996; Kooistra and Pulleman, 2010).

Among the soil fauna, earthworms play a key role in organic matter decomposition and C cycling (Blouin et al., 2013; Bossuyt et al., 2005). Earthworms directly affect the decomposition in soils by modifying the organic matter and microbes because they feed on both mineral and organic matter in soils and mix and release them through the earthworms' gut passage (casting,

Monroy et al., 2008; Aira et al., 2009). Moreover, earthworms increase microbial diversity because of interaction with mucus and casts (Thakuria et al., 2010), and because of soil physical changes by aeration (Aira and Domínguez, 2011). However, the impacts of earthworms on nutrient dynamics depend on earthworm species (e.g. Enami et al., 2001; Caravaca et al., 2005). This indicates that how organic source and soil faunal interact and their interaction affect decomposition has not been well investigated (Ouédraogo et al., 2004). Thus, there is still a lack of knowledge of the impact of soil fauna community on soils, although fauna-induced features are found in any soil types and their community can determine the soil physical, biological, and chemical processes (Kooistra and Pulleman, 2010).

2.2.2.2 Mineralization

Mineralization is a biological process in which organic substances are converted to inorganic substances (CO_2 and water) by soil microbes. However, this is not totally microbial process because invertebrate fauna plays important roles to: (i) re-distribute organic materials over various time and spatial scales, (ii) enhance the microbial turnover rate, and (iii) alter microbial biomass and abundance by both creating or removing appropriate conditions for their various activities (Woods et al., 1982; Verhoef amd Brussaard, 1990; Bardgett et al., 1998; Filser et al., 2016). Particularly, earthworm activity has positive effects on C mineralization (Coq et al., 2007; Lubbers et al., 2017). The activity of earthworm creates heterogeneous conditions in the soils because of the mucus secreted from the earthworm and casting activity (Subler and Kirssch, 1998). Subler and Kirssch (1998) reported that consequences of such heterogeneity may alter overall rates of soil processes, including organic matter decomposition, mineralization, and denitrification. Particularly, the soil near the holes created by earthworm activities often show improved biological activity, thus stimulated soil microbes, compared to bulk soils (Kuzyakov and Blagodatskaya, 2015). These zones earthworms created are referred to as the “drilosphere” and often stimulate activity of both other soil organisms and plant root (Brwon et al., 2000).

2.2.2.3 Assimilation and immobilization

Living microbial biomass was often estimated as minimal < 5% of SOM (Liang et al., 2011, 2017). Assimilation and immobilization are the production of inorganic compounds from organic compounds by universal functions of soil microbes. Generally, with increasing SOM content, the potential of immobilization increases, and the quality of the C source in the soil is also an important factor controlling immobilization rates (Chen et al., 2014). This indicates that soil microbes regulate nutrient cycling by effecting the decomposition processes that influence the release and retention of nutrients (Beare et al., 1997). Furthermore, the soil microbial biomass represents a dynamic pool of soil C that functions as a source of plant nutrient (Singh et al., 1989).

2.2.2.4 Soil aggregation and soil C

The SOM improves aggregation of soil particles. Improved aggregation results in better soil structure, allowing for movement of air and water through the soil as well as better plant root growth. More stable soil structure results in less soil erosion, which retains nutrients on the land and protects water quality. Higher levels of SOC or higher proportion of large aggregates reduce the bulk density, thus providing an improved rooting environment. Moreover, many studies report that the excrements dejected by soil fauna had remarkably higher in SOM concentration than the surrounding soil (Henrot and Brussaard 1997).

2.2.2.5 Priming effect

Factors controlling decomposition are mainly temperature, substrate availability and the size of the microbial biomass pool (Wieder et al., 2013). The input of organic materials in soils potentially changes the turnover of native SOM and induces priming effects (Kuzyakov et al., 2000; Blagodatskaya et al., 2007). Increasing microbial activity enhance microbial turnover and consequently increase the release of CO_2. Blagodatskaya and Kuzyakov (2008) proposed two types of priming effects: i) apparent priming which is due to the increased turnover of microbial

biomass, and ii) real priming which is a consequence of the SOM mineralization. It has been suggested that apparent priming effect can represent the accelerated turnover of microbes without an increase in microbial biomass (Blagodatskaya et al., 2007). Regarding soil C sequestration and the priming effects, Fontaine and Barot (2005) and Fontaine et al. (2011) reported that nutrient mining from SOM increases when organic materials with lower nutrient availability are applied. Such priming may induce a negative C balance; the applied of C decreases the soil C contents (Fontaine et al., 2004).

2.2.3 Abiotic factors controlling decomposition

The components of soil C cycle discussed above involve many biological processes and these are influenced by various abiotic factors. These factors include substrate quantity and availability, soil moisture, soil texture, and soil C contents.

2.2.3.1 Substrate quality and availability

Regarding the organic materials' characteristics, C/N ratio is largely important property because it can determine nutrient dynamics in the soil (Baggs et al., 2003; Al-Kaisi and Yin, 2005; Garcia-Ruiz and Baggs, 2007). In general, organic amendments with lower C/N ratio (< 30) increase mineralization (Alexander, 1977; Chen et al., 2014). Chen et al. (2015) also showed their N and neutral detergent fibre content are factors controlling decomposition. In addition to crop residue, manure is key to improve productivity as well as soil physical properties, particularly in degraded fields because of its multiple nutrients, soil pH, and large amount of SOM (Ouédraogo et al., 2001; Gandah et al., 2003).

2.2.3.2 Soil moisture

The soil moisture is important factor controlling gas emissions for the substrate supply for soil microorganisms (Schindlbacher et al., 2004), and influences gas diffusivity (Smith et al., 2003). For example, drought stress decrease soil metabolic activity and CO_2 emissions (Ma et al.,

2016), whereas higher water content (water filled pore space of > 60%) also decreases the emissions due to limited gas diffusion (Kiese and Butterbach-Bahl, 2002). Thus, water infiltration is one of the most important factors controlling CO_2 emissions and SOM stabilization in growth rates of the microbes (Yuste et al., 2007; Feiziene et al., 2011). Additionally, the interaction among soil moisture, texture and CO_2 emission rates, and a relatively stronger correlation in higher clay content, compared to lower clay content (Mapanda et al., 2010). In field-scale studies, the seasonal course of soil gas emissions usually reflects seasonal development of soil temperature and soil moisture (Schaufler et al., 2010). However, if soil moisture becomes limiting, CO_2 fluxes are suppressed even with high soil temperatures (e.g. summer season) (Davidson et al., 1998; Garten et al., 2009).

2.2.3.3 Soil texture

Soil textures are classified by the fractions of sand, silt, and clay. Soils with relatively higher clay contents tend to store soil C more effectively when compared to the soils with relatively lower clay contents (Schimel et al., 1985a,b; Spain, 1990; Amato and Ladd, 1992). More specifically, previous studies showed that the amounts of C in African soils are positively related to the amount of clay and silt (Giller et al., 1997; Sakala et al., 2000). Sugihara et al. (2012) also indicated that the efficiency of soil C stock form organic amendments was lower in sandy soils (less than 10%) than clay soils (approximately 15%) in sub-Sahara Africa. This is because sandy soils are particularly difficult to manage as they easily lose nutrients by leaching and erosion due to poor aggregation (Burt et al., 2001). Also, the factors controlling immobilization derived from organic materials were differently affected by soil texture (Mellado-Vázquez et al., 2019).

2.2.3.4 Soil C contents

The CO_2 emissions were positively related to the amount of C in soils, in general (Caravaca and Roldán, 2003). Additionally, it has been reported that particularly in the Southern hemisphere

bacterial diversity and soil C contents were strongly correlated, compared to Northern hemisphere (Delgado-Baquerizo et al., 2016). Therefore, microbial activities related to C dynamics is relatively higher and have less potential to store soil C compared to other regions. Indeed, in sub-Saharan Africa, large areas are regarded as C-depleted soil regions (with SOC contents < 1.1%) (Nyamangara, 2002; Mapanda et al., 2011; Hamamoto et al., 2018).

2.2.4 Potential effects of biodiversity on agricultural ecosystems

As explained above, soil C cycling is influenced by soil biomes. In fact, biodiversity is positively related to soil fertility and plant productivity (Delgado-Baquerizo et al., 2017). Thus, a stimulus of soil organisms, including any biological groups (e.g. fauna and microflora) can enhance nutrient cycling and modify soil structure (Altieri, 1999; Grandy et al., 2016).

In agricultural soils, seasonal patterns in soil biological communities are highly complicated, with yearly fluctuation (Yeates et al., 2000), and the effects of land use and crop type (Lauber et al., 2013). Previous studies show that soil microbial community become more complex and stable due to increase in the food chain length with time, even though less is known about timescale belowground community changes (Neutel et al., 2002; Bardgett and van der Putten, 2014). Thus, increase in species have no effect of ecosystem function related to soil C cycling, after a certain level of richness has been reached (Asymptotic form, Fig. 2.3). As a result, the belowground community changes are important for ecosystem functioning (Bardgett and van der Putten, 2014).

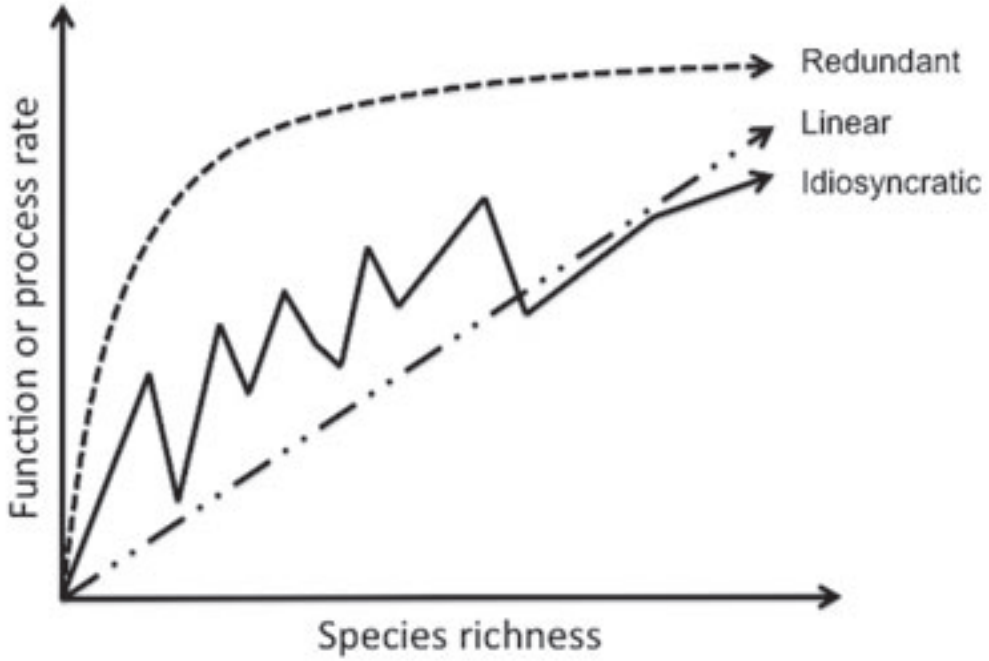

Figure 2.3. Three commonly accepted forms that a positive relationship between species richness and ecosystem functioning or process rates might take. The linear relationship would occur if each species possesses unique traits that enhance the process rate or function in question. The redundant, or asymptomatic, relationship would occur if species show functional redundancy; that is, multiple species share a trait that enhances process rates or functioning. Thus, the chance of adding a species possessing a trait not already found in the community becomes progressively smaller as species richness increases. The idiosyncratic relationship would occur if species with similar traits differ in their ability to enhance the function or process rate in question, or if biotic interactions enhance (e.g. facilitation) or inhibit (e.g. competition) functioning or process rates. In this case the community composition is more important than species richness *per se*. (source: Nielsen et al., 2011)

2.3 Organic amendments in C depleted soils in sub-Saharan Africa

2.3.1 The importance of organic amendments on soil C

Organic amendment can be used to increase soil carbon (C) and nitrogen (N), which are the indicators of agricultural production (Luo et al., 2010; Aparma et al., 2014; Li et al., 2017). Thus, the use of organic amendments is potentially an alternative practice to conventional agriculture. Murphy et al. (2007) showed that organic materials are decomposed in the soil, and then the continuous release of nutrients can sustain the microbes for relatively longer time, when compared inorganic fertilizers. Recycling of agricultural organic wastes has therefore been historically performed in agricultural systems and this method is receiving heightened attentions even for the modern farming because of the necessity of maintaining soil productivity with cost-effective manners (Rasmussen et al., 1998; Fan et al., 2012; Misselbrook et al., 2012). In addition, increase in soil C due to organic amendments may highly remove net CO_2 from the atmosphere (Paustian et al., 2016) and consequently reduce the global warming potential (Järveoja et al., 2016).

At the same time, the utilization of organic amendments in low organic C soils may expect the greatest benefits from their application, compared to relatively fertile soils (van Zwieten, 2018). Thus, the use of organic amendments is widely promoted in Africa. According to FAOSTAT (http://www.fao.org/economic/ess/environment/data/livestock-manure/en/), the largest share of total livestock manure N inputs to soils were in Asia (35%) and Africa (25%) in 2017. Additionally, over the ten-year period 1961-2017, total livestock manure N inputs grew significantly in Africa (+3.9% yr^{-1}), compared to Asia (+1.1% yr^{-1}). Despite those results, mature crop biomass (maize) without cobs is typically only 2.5 t C ha^{-1} in this region (Sugihara et al., 2010). The continental average grain yield in sub-Saharan Africa has been stagnated since 1960s (Lal, 2016, 2017). Thus, more understanding of the effects of organic amendments on soils in sub-Saharan Africa is required.

A large amount of organic amendment is often required to achieve the soil C increase (an increase of 0.07% to 0.43% per year was achieved by applying equivalent to approximately 35 t fresh manure ha^{-1} $year^{-1}$) (Poulton et al., 2018). Therefore, most of C derived from organic input is lost from soils due to the soil biological decomposition of organic amendments, although organic amendments contribute continuous soil C increase. Additionally, in tropical region, previous studies show the significant increase in soil fertility and soil C contents with large amount (> 17 t ha^{-1} $year^{-1}$) of organic material supply (Kaihura et al., 1999; Zingore et al., 2008).

Because the amount of organic materials used in agricultural soils is less than the amount required to steadily increase soil C, the soils have experienced a massive decline in soil C (Agbenin and Goladi, 1997; Solomon et al., 2002; Lemenih et al., 2005; Zingore et al., 2005; Kintché et al., 2010; Moebius-Clune et al., 2011). Although, currently only about 10% of the global SOC stock in Africa are currently estimated (between 133 and 184 Gt for the 0–100 cm soil layer), the amount of C stock is depending on the ecoregion (Henry et al., 2009; Scharlemann et al., 2014) and the conversion of the natural ecosystems to agricultural ecosystems often results in the decline of soil C. This is one of the reasons behind the low yields in Africa (Chianu et al., 2012).

Thus, the maintenance and improvement of cropland productivity is extremely challenging since soil C has continuously declined in Africa (Kamau et al., 2019). This decline is often because of lower rate use of soil amendments than recommended, particularly in smallholder farms (Murage et al., 2000; Corbeels et al., 2019). The result of extremely low (on average ≤10 kg N ha^{-1} yr^{-1}) fertilizer application rates in sub-Saharan Africa (Liu et al., 2010), induce a higher crop uptake than the nutrient input from fertilizer (Zhou et al., 2014). Also, some smallholder farmers in Africa generally remove the remaining crop residue after harvest or before cultivation (Murage et al., 2000; Sugihara et al., 2010). Moreover, particularly in sub-Sahara Africa, many studies have reported the rapid decomposition of both of SOM or applied

organic matter due to the climate and the activeness of macro- and micro- fauna in this region (Powlson et al., 2001; Mazzilli et al., 2014; Lal, 2008; Martinsen et al., 2017).

Increased formation of macroaggregates with the application of organic materials and manures have been also reported by Mikha and Rice (2004) and Ghosh et al. (2016). Under organic amendments, greater increase (> 38%) in proportion of large macroaggregates compared to chemical fertilizer treatments (Mikha and Rice, 2004; Mangalassery et al., 2019). The different farming practices such as fertilization influence macroaggregates more than microaggregates (Ashagrie et al., 2007). However, those aggregate improvements are due to continues application more than over 5 years. Thus, it seems to be difficult to predict how organic amendments dynamic and interact inside soils, and consequently influence soil C.

2.3.2 The effects of organic amendments on agricultural ecosystems

The components of soil C cycle discussed above involve many biological processes and these are influenced by various abiotic factors. These factors include substrate quantity and availability, soil moisture, soil texture, and soil C contents.

2.3.2.1 Crop production

The importance of SOC in regulating crop production has been well established (Lal, 2004). The meta-analysis showed that organic amendments (only or with chemical fertilizer) can increase SOC, and consequently provide higher crop yields, compared to only mineral fertilization, although performance of crop production depends on crop species, organic amendment types, edaphic properties, and climatic conditions (Fig. 2.4, Luo et al., 2018). Cereal crops such as wheat, maize, and barley production particularly influence significant effect on organic amendments, possibly due to synchronized their nutrient demands with the slow release of nutrients from organic amendments (Seufert et al., 2012; Crews and Peoples, 2005; Luo et al., 2018). Regarding negative effects of climate on crop production, however, it is still unclear

to how much organic amendments counteract that due to their positive effect on soil biological condition (Agegnehu et al., 2016; Blanchet et al., 2016; Luo et al., 2018). Currently, agricultural ecosystems in African countries are considered poor resilience and most vulnerable to climate changes (Slingo et al., 2005).

Additionally, in many cases, African small-scale farmers' yields for cereals rarely exceed 0.5 to 1.5 t ha^{-1} while a potential of 5–8 t ha^{-1} is attained in research station trials and on commercial farms (Bationo et al., 2006; Tittonell and Giller, 2013). This means that fertilizer input and appropriate management can theoretically increase crop yield by > 90%. The reasons for lower crop productivity are facts such as soil fertility decline, insufficient and inappropriate fertilizer application, unreliable rainfall and a variable climate, lack of improved cultivars, and labour constraints (Liu et al., 2010; Thiefelder et al., 2015). These negative factors reflect soil C degradation as well in this region (Bationo and Buerkert, 2001; Bationo et al., 2007).

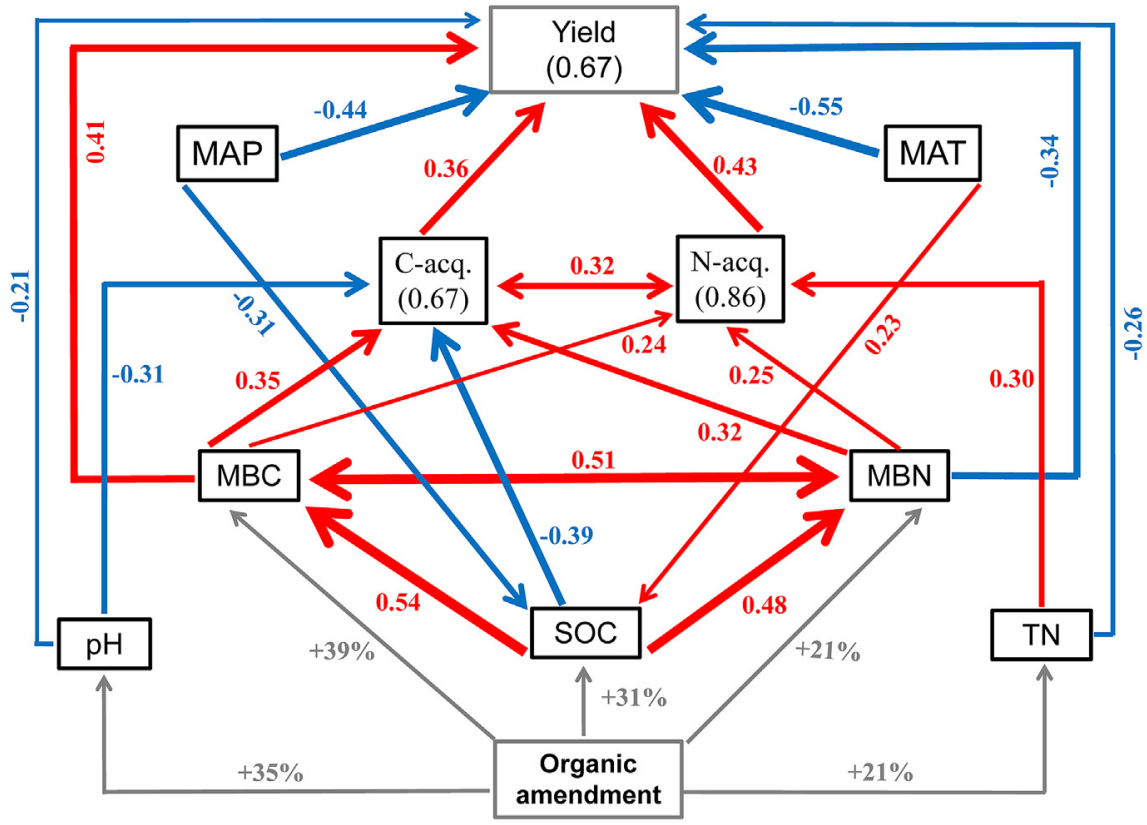

Figure 2.4. Structural equation model (SEM) showing the direct and indirect effects of climatic (MAP and MAT), soil physicochemical factors (SOC, TN, and pH), microbial biomass (MB) pools and soil enzyme activities on crop yields under organic amendments. The red and blue arrows represent significantly positive ($p < 0.05$) and negative ($p < 0.05$) relationships, respectively. The grey arrows show the effects of organic amendment on soil physiochemical properties and MB pools (TN, SOC, pH, MBC, and MBN) - the percentage next to grey arrows denote the relative effect of organic to mineral-only fertilization on a given variable. Numbers next to red and blue arrows denote for path coefficients between different variables and the thickness of the arrows represent the magnitude of these effects. Figure abbreviations denote for: soil organic carbon (SOC), soil total nitrogen (TN), C-acquisition enzyme activity (C-acq), N-acquisition enzyme activity (N-acq), mean annual temperature (MAT), mean annual precipitation (MAP), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN). (Source: Luo et al., 2018)

2.3.2.2 CO_2 emissions

Organic amendments increase soil C, but also increase CO_2 emissions. The amount of CO_2 emissions derived from organic amendments is dependent on many factors such as the level of applied organic amendments, the quantity of C already in the soils, and soil moisture as described above. In sub-Saharan Africa, CO_2 emissions increased from approximately 6 t C ha^{-1} to 12 t C ha^{-1} for four years, when plant residue was applied (25 t C ha^{-1}) (Sugihara et al., 2012). This result indicates that there was the large amount (9 t C ha^{-1}) of missing C, possibly due to C leaching and decomposition due to macro-fauna, as well as CO_2 emissions. Therefore, the efficiency of C accumulation was relatively low, although there is a possibility of using land management to increase C sequestration in dry tropical croplands.

2.3.2.3 Soil fauna community

For the persistence of soil faunal diversity and biomass, the maintenance of SOM through integrated soil fertility management is important (Ayuke et al., 2011). With addition of C via organic amendments stimulated the soil heterotrophic indigenous biological abundance, diversity and activities, compared to the impacts of mineral fertilizers (García-Gil et al., 2000). Lavelle *et al*. (2006) reported that the presence of soil fauna promoted the decomposition of organic matter and soil C stock process. Thus, ground dwelling and soil invertebrates represent biological indicators for monitoring soils (Ritz et al., 2009) and more than 80% of soil fauna consists of arthropods (Culliney, 2013). Pitfall traps for these invertebrates have been widely used for environmental investigation and are a well-designed technique. Also, their diversity on cultivated soils has been known as a good indicator of the effect of human activity on soil biodiversity, because of their sensitive to environmental changes (Lawes et al., 2005; Hendrickx et al., 2007).

In sub-Sahara African ecosystems, the decomposition of organic amendments can be relatively faster, compared to the global average, due to regional climate and the activeness of soil fauna,

and it makes difficult to steadily increase soil C (Powlson et al., 2001; Mazzilli et al., 2014; Lal, 2008; Martinsen et al., 2017). Ouédraogo et al. (2004) reported that around 90% of applied organic materials was decomposed by macro-fauna during within 3-month in Burkina Faso. Ouédraogo et al. (2007) also reported that, in semi-arid Africa, the organic amendments promote the decomposition by soil fauna, thus the interaction between the organic amendment types/amounts and soil fauna activities need to be further studied to improve soil C efficiently.

2.3.2.4 Soil microbial community

Addition of organic matter can increase soil microbial biomass, and their effect is larger than mineral-only fertilization (Wang, 2007; Smukler et al., 2008; Kallenbach and Grandy, 2011; Chen et al., 2017; Luo et al., 2018).

Regarding microbial processes following the application of organic amendments to soils, crop N uptake and N release from the organic amendments by the activities of soil microbes can synchronize due to mineralization (Singh et al., 2007). In a dry tropical agricultural ecosystem, microbial biomass and activity are also widely changed by dry and rainy season mainly due to soil moisture changes. Soil microbes act as a nutrient source (i.e. mineralization) during the rainy season and as a sink (i.e. immobilization) in the dry season (Singh et al., 1989; Srivastava, 1992; Singh et al., 2007; Tripathi and Singh, 2007; Sugihara et al., 2010). Thus, plant residue amendments early in the rainy season increased microbial biomass, and consequently contribute crop nutrient from soil microbes (Sugihara et al., 2010).

Organic amendments were shown to build soil C and soil microbial biomass, especially over the long term, but there is still a lack of information on the role of organic amendments in modifying microbial functionality, and how this in turn influences crop productivity (e.g. nutrient cycling and storage, or development of soil resilience to environmental stressors) (van Zwieten, 2018).

2.3.2.5 Soil fauna and microbial interaction

While the direct impact of soil macro-fauna on soil C cycling is notable, it has been proposed that soil faunal contribution to soil process is greatly due to their interactions with the soil microbial community (Grandy et al., 2016; Trap et al., 2016). The presence of soil macro-fauna alters both microbial biomass and diversity following microbial community changes, as well as increased functions of the degradation of organic matter such as phenol oxidase and glucosidase activities (Bray et al., 2019). Soil faunal feeding activities such as gut passage and fecal material deposition can modify microbial community and have been shown to enhance their activity particularly during early stages of degradation, likely due to the enrichment of litter with microbes and creation of decomposition "hotspots" (Hanlon and Anderson, 1980; Nechitaylo et al., 2010; Wickings and Grandy, 2011; Kuzyakov and Blagodatskaya et al., 2015). Therefore, soil fauna has a potential for positive priming effect on SOC due to stimulation of microbial activity (Bardgett et al., 1998; Ouédraogo et al., 2007). However, the current limitation is to predict the direction and magnitude of microbial responses to faunal activity under variable scenarios of climate and land-use (e.g. Fig. 2.5, Grandy et al., 2016). This variation is likely to have important consequences for soil C dynamics.

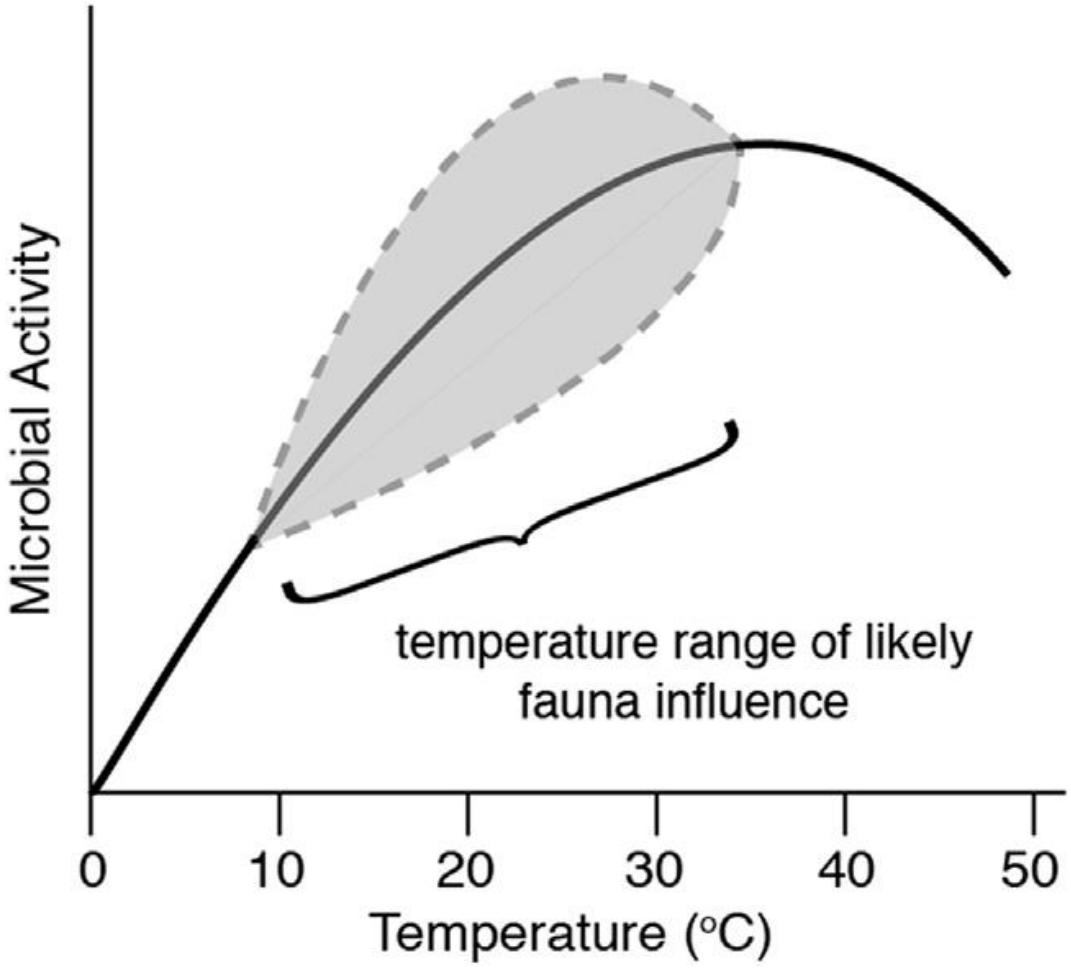

Figure 2.5. Potential fauna effects on microbial activity in response to temperature. Microbial activity increases with temperature up to their temperature maximum (here using 35°C only as an example). Fauna may have a narrower temperature range of activity than microbes (here generalized as 10-30°C), and over this range of activity fauna may either decrease or increase microbial activity (represented by hashed lines and shading, highlighting the potential variation in microbial activity due to fauna). (Source: Grandy et al., 2016)

2.3.3 The combined effects of organic amendments and inorganic fertilizer on crop yield and soil biology

Organic fertilizer alone usually results in relatively lower productivity levels than chemical fertilizer (Trewavas, 2001; Seufert et al., 2012). Considering the nutrient supply for soils and crops, Ge et al. (2010) stated that the combined use of organic materials with inorganic fertilizers is important to establish sustainable agricultural systems. Many previous reports showed that the combined use of organic materials and inorganic fertilizers has been gaining increasing recognition as a feasible and practical approach in boosting crop yields in the short term, while enhancing SOM in the long term, when compared to the sole application of mineral or organic manure (Palm et al., 2001; Vanlauwe et al., 2001; Dawe et al., 2003; Bandyopadhyay et al., 2010; Diacono and Montemurro, 2010; Chivenge et al., 2011; Liang et al., 2012; Yan et al., 2012; Kumar et al., 2017).

Although organic materials are rarely produced in enough quantities on African farms to meet the nutritional needs of crops, they are still useful nutrient sources (Sánchez, 2010). Janssen (2011) compared the long-term effects of organic amendments with chemical fertilizer application in sub-Saharan Africa and showed that organic amendments are critically important to maintain the crop production from such relatively poor soils. Also, Kamaa et al. (2011) found that the continuous combination with organic and inorganic fertilizers in Kenya increased soil bacterial diversity, compared to single use organic or inorganic fertilizer. Chemical fertilizer often promotes organic matter decomposition (Goyal et al., 1999; Gnankambary et al., 2008). Thus, the combined use of organic and chemical fertilizer might be favour condition for continuous soil improvement in this region.

2.4 Research recommendation

Organic amendments in agricultural soils has a potential to increase soil C, although a very complex interaction of soil biological processes and environmental factors control soil C

dynamics. These complex interactions are required to be investigated to improve our knowledge of the biological response of CO_2 emissions after organic amendments.

Firstly, the influence of earthworms, which is one of the largest contributors of soil C stock, on soil C dynamics during organic material decomposition must be studied. It is known that differences in earthworm species composition affect microbial biomass and nutrient retention in soils (Greiner et al., 2012; Groffman et al., 2015; de Menezes et al., 2018; Gómez-Brandón et al., 2018). Better understanding of the impact of earthworm species' differences on C and N dynamics in agricultural soils will provide basic information to evaluate the efficacy of organic amendments to maintain soil health.

In agricultural ecosystems in sub-Saharan Africa, although organic amendments have largely potential benefits, evidence on the impacts of organic agriculture (including organic amendments), particularly on soil fertility and biodiversity, still is scarce and inconclusive (Kamau et al., 2019). An understanding of the soil microbiology and interactions with its effect and soil nutrient dynamics are especially needed now, because soil microbial communities often indicated functional redundancy and have a strongly influence on soil C cycling. Therefore, the mechanisms how organic amendments relate soil C dynamics via microbial response are required in improve sustainable agricultural production in sub-Saharan Africa.

To further improve our understanding of the interaction among organic amendments and crop production in such degraded soils, further studies on aboveground agricultural ecosystems and are critically important. Few studies investigated the effects of organic amendments on aboveground arthropod abundance in this region, although agricultural ecosystems are strongly related to soil faunal community (Bationo et al., 2007; Osler and Sommerkorn, 2007; Ayuke et al., 2011; Kamau et al., 2019). Thus, studies of aboveground ecosystems' response to organic amendments such as crop production and soil fauna community are needed to increase our knowledge of the semi-arid tropical agricultural ecosystems.

Chapter 3

The role of different earthworm species (*Metaphire hilgendorfi* and *Eisenia fetida*) on CO_2 emissions and microbial biomass during barley decomposition

A manuscript from this study has been accepted for publication in Sustainability: Hamamoto, T., Uchida, Y. The role of different earthworm species (Metaphire hilgendorfi and Eisenia fetida) on CO2 emissions and microbial biomass during barley decomposition.

3.1 Abstract

Earthworms are commonly known as essential modifiers of soil carbon (C) and nitrogen (N) cycles, but the effects of their species on nutrient cycles and interaction with soil microbial activities during the decomposition of organic materials remain unclear. We conducted an incubation experiment to investigate the effect of two different epigeic earthworms (*M. hilgendorfi* and *E. fetida*) on C and N concentrations and related enzyme activities in agricultural soils with added barley residues (ground barley powder). To achieve this, four treatments were included; (1) *M. hilgendorfi* and barley, (2) *E. fetida* and barley, (3) barley without earthworms, and (4) without earthworms and without barley. After 32 days incubation, we measured soil pH, inorganic N, microbial biomass C (MBC), water or hot-water soluble C, and soil enzyme activities. We also measured CO_2 emissions during the incubation. Our results indicated the earthworm activity in soils had no effect on the cumulative CO2 emissions. However, *M. hilgendorfi* had a potential to accumulate MBC (2.9 g kg^{-1} soil) and nitrate-N (39 mg kg^{-1} soil), compared to *E. fetida* (2.5 g kg^{-1} soil and 14 mg kg^{-1} soil, respectively). In conclusion, the interaction between soil microbes and earthworm is influenced by earthworm species, consequently influencing the soil C and N dynamics.

3.2 Introduction

Organic amendment can be used to increase soil carbon (C) and nitrogen (N), which are the indicators of agricultural production (Luo et al., 2010; Li et al., 2017). The degradation of organic matter and physical disturbance of soils, performed by the earthworms, can alter soil microbial community, and thus influence rates of the C and N cycles following the organic amendment (Fonte and Six, 2010; Gómez-Brandón et al., 2012; Massey et al., 2013; Griffiths et al., 2018). Barley is one of the major crop species in the world and it is important to fully understand how the C from barley cycles in agricultural soils. Thus, a study will be required to understand interactions among earthworm and soil C dynamics, particularly using barley as organic materials.

Differences in earthworm species composition affect microbial biomass and N retention in soils because of their different feeding strategies (Aira et al., 2008; Sampedoro and Domínguez, 2008; Aira and Domínguez, 2011; Groffman et al., 2015). Epigeic earthworms are litter dwellers and inhabit the surface level of soils. Thus, these earthworms enhance decomposition rates of their habitats interacting with microorganisms and other organisms (Aira et al., 2008; Sampedoro and Domínguez, 2008; Aira and Domínguez, 2011). Better understanding of the impact of epigeic earthworms on C and N dynamics in agricultural soils will provide basic information to evaluate the efficacy of organic amendments to maintain soil health.

Uchida et al. (2004) reported that *Metaphire hilgendorfi*, which is one of the dominant species in Hokkaido, Japan, ingests litter and soil faster and more efficiently compared to other epigeic species. This species was also reported to decrease the biomass of both gram-positive and gram-negative bacteria in soils (Chang et al., 2016). In contrast, Enami et al. (2001) investigated a microbial response by the presence of *M. hilgendorfi*, and found that the gram-negative bacteria increased in the soils. Another earthworm type, *Eisenia fetida* (one of the major epigeic species), had no effect on water-soluble C in soils (Caravaca et al., 2005), while Zhang et al.

(2000) and Aira et al. (2007) reported that *E. fetida* have the potential to improve C mineralization and increase microbial activity related to decomposition. These contradictory results might be due to the lack of information about the interaction among earthworms, soil C dynamics and soil microbial activities.

To assess the changes in soil microbes, the measurements of soil microbial enzyme activities are commonly used, regarding the decomposition of organic matter and nutrient cycles (Tao et al., 2009). Those enzymatic activities, which are particularly related to N dynamics, are strongly correlated with the addition of organic C (D'Haene et al., 2003; Jin et al., 2010). However, studies observing the interaction between the earthworm species and soil N enzyme activities are few (Tao et al., 2009; Wu et al., 2012; Hoang et al., 2016).

Therefore, we conducted an incubation study and our aims were to investigate the role of the two earthworm species on soil C and N dynamics, and microbial changes with organic materials application. We hypothesized that the earthworm species change the patterns of soil C and N with the presence of organic matter: *M. Hilgendorfi* increases soil microbial biomass and thus have more potential to immobilize C to soils, while *E. fetida* promotes soil C mineralization.

3.3 Materials and Methods

3.3.1 Soil Characteristics and Earthworms

We conducted an incubation experiment using an agricultural soil, plant residues (finely ground barley (*Hordeum vulgare*) grain) from a private company (ITOEN Ltd., Tokyo, Japan) and two different earthworm species. The soil (0–5 cm depth) was collected from the experimental maize (*Zea mays*) farm of Hokkaido University, Sapporo, Japan (43°04′31.6″N, 141°20′03.4″E, 13 m above sea level) in August 2017. At the sampling, the soil moisture was 68.9 ± 0.6% as gravimetric and the bulk density was 0.48 ± 0.03 g cm^{-3} (n = 5). Soils were air-dried and sieved to 2 mm. The total C and N in soils were 42.3 ± 1.2 g kg^{-1} soil and 3.60 ± 0.29 g kg^{-1} soil,

respectively (n = 3). C and N concentrations of the barley were 44.9 ± 0.3% and 2.0 ± 0.2%, respectively (n = 3). Total C and N content of the samples were determined using an elemental analyser (2400 Series; PerkinElmer Inc., MA., USA). Two types of earthworms were used; one was *M. hilgendorfi* sampled from the forest in Hokkaido University in August 2017 and the other was *E. fetida* obtained from a private company (Iwase farm, Ibaraki, Japan).

3.3.2 Experimental Design

The soils were packed into 2 L glass bottles (12.2 cm diameter, 5 cm depth and 573 g soil bottle^{-1}). The bulk density of the soil in the bottles was 1.0 g cm^{-3}. Soil moisture (gravimetric) was maintained at 30% (bottle weight basis) during the incubation period, which is optimum soil moisture content for an earthworm species *Lumbricus terrestris* (Berry and Jordan, 2001). The weight of each bottle was measured every day and distilled water was added to replace the evaporated water. Before the application of the barley and the earthworms, the bottles with soils were pre-incubated for one week to allow passing the bacterial flush induced by drying–rewetting of soils and/or soil physical disturbance by sieving (Van Gestel et al., 1993; Franzluebbers et al., 2000; Datta et al., 2014; Vidal et al., 2017).

Then, four earthworms (one of the two species) and the barley grains (5 g) were added to each bottle (equivalent of 342 individuals m^{-2}). Before the earthworms were added to the bottles, the feed remaining within each earthworm was emptied by placing the earthworms on an agar plate with water (> 12 h). This was performed to minimize the addition of C to soils through earthworms. Immediately before the application, the average living mass of *M. hilgendorfi* and *E. fetida* was 6.70 ± 0.18 and 1.14 ± 0.04 g per bottle (573 and 96 g m^{-2}), respectively. The earthworm density was determined in accordance to the review by Fründ et al. (2010), showing that the typical earthworm density (all species concerned) in temperate region was 10–1000 individuals m^{-2}. Vidal et al. (2017) determined 25 mg of dry matter g^{-1} fresh weight day^{-1} as a good compromise to favour earthworm survival. Thus, we applied the same quantity; 5 g of

barley (2.25 g C kg^{-1} soil) was added to each bottle. The treatments were (1) *M. hilgendorfi* and barley; M, (2) *E. fetida* and barley; E, (3) barley without earthworms; B, and (4) without earthworms and without barley (Control); C, with three replicates. Those four treatments were incubated for 32 days to investigate soil C dynamics when the earthworms are actively decomposing the organic materials. The length of the incubation period was determined since there were no significant differences in CO_2 emissions after day 20 followed by the commencement of the incubation.

3.3.3 Measurements of Soil Chemical Properties

After the 32 days incubation, the soils were sampled from the surface (0–2 cm depth) excluding barley residue and mixed for further analysis. For the determination of the inorganic-N concentrations (ammonium-N; NH_4^+-N, nitrate-N; NO_3^--N), 5 g samples were extracted with 25 mL of 10% KCl. After 30 min shaking, the extractant was filtered through filter paper. Then, inorganic-N concentrations in soil samples were measured using a colorimetric method with a flow injection analyser (AQLA-700; Aqualab, Tokyo, Japan) as described previously (Hamamoto and Uchida, 2015). The water-soluble C (WSC) and hot water-soluble C (HWSC) were measured using a modified previously reported method (Uchida et al., 2012). Sub-samples of 3 g each were placed in centrifuge bottles and shaken with 30 mL of distilled water at 20°C for 1 h. The samples were then centrifuged for 30 min at 4000 rpm, the supernatant was filtered through filter paper (Grade 5C, < 5 mm; Advantec, Tokyo, Japan) and the amount of soluble organic C was determined using a total C analyser (TOC 5000A; Shimadzu, Japan). This was the WSC fraction of the soil organic C. A further 30 mL of distilled water was added to the sediments remaining in the centrifuge tubes after the supernatants (WSC) were decanted and the tubes were capped and left for 16 h in a hot-water bath at 80°C. The samples were then centrifuged, filtered, and analysed for soluble organic C concentrations using the same methods as for the WSC analysis. This was the HWSC fraction of the soil organic C.

The microbial biomass C (MBC) was measured using a modified chloroform fumigation method of Vance et al. (1987) and Toda and Uchida (2017). Two samples of 2 g each were taken from each soil; one was immediately extracted with 10 mL of 0.5 M K_2SO_4 and filtered through filter paper, whereas the other 2 g samples were fumigated under chloroform for 48 h using an evacuated desiccator and then extracted in the same way. The extracts were stored in a freezer until C analysis. At that point, extracts were diluted at 1:10 with water and organic C was determined in 25 mL aliquots of the diluted samples using a total organic C analyser (TOC-5000A; Shimadzu, Kyoto, Japan). The amount of organic C in the non-fumigated samples was subtracted from the amount of organic C in the fumigated samples; then, values were multiplied by 2.64 to convert them to MBC.

3.3.4 Measurements of Soil Enzyme Activities

Urease activity (UA) and nitrification enzyme activity (NEA) were measured using modified previously reported methods (Kandeler and Gerber, 1988; Ito et al., 2008; Mogi et al., 2017). For UA, 5 g soil sample taken from each bottle was mixed with 10 mL of 0.08 mol L^{-1} aqueous urea solution. After incubation for 2 h, 50 mL solution (1M KCl and 0.01M HCl) was added, and the mixtures shaken for 30 min. The resulting suspensions were filtered, and the filtrates analysed as described above in the soil N measurement section. For NEA, a 5 g soil sample taken from each bottle was placed in a 50 mL polyethylene bottle and incubated for 4 days at room temperature (25°C) after the addition of 0.2 mg N g^{-1} soil of ammonium sulphate. NO_3^--N concentrations were measured calorimetrically as explained above in the soil N measurement section. NEA was estimated based on the difference between the NO_3^--N concentration in the fresh soils before the commencement of the incubation and NO_3^--N concentration in the soils after the 4 days incubation. Denitrification enzyme activity (DEA) was estimated by measuring the increases in N_2O concentrations during a soil incubation using an acetylene block technique. The method was modified based on Mogi et al. (2017). A 3 g soil sample taken from each bottle was incubated in a 100 mL vial with 3 mL of a solution with excess amounts of C and N

substrates (2 g C L^{-1} as glucose and 200 mg N L^{-1} as KNO_3). The air in the headspace was completely replaced by N_2 gas, and then 10% of the N_2 gas was replaced with acetylene. After 2 h incubation, 30 mL of headspace gas was sampled. Sampled N_2O gases were measured by gas chromatograph (GC-2014; Shimadzu Co., Japan).

Substrate-induced respiration rate (SIR) and specific growth rate (SGR) were measured for each of the soil samples, the equivalent of 50 g dry soil in glass bottles (0.9 L). Each soil sample was amended with glucose at a rate of 1 mg C g^{-1} soil. After amendment, each glass bottle was sealed by lid with tubes, which allowed air to pass from outside to the gas analyser (Isotopic CO_2 Analyser; Los Gatos Research, CA, USA) with a Multi-port Inlet Unit (MIU). To minimize the effect of any contamination (e.g., human respiration), the ambient air was continuously pumped from outside using two air pumps during analysis (non-noise W1000; Japan Pet Design, Tokyo, Japan). Excess ambient air allowed to pass outside to avoid the accumulation of respired CO_2 in the bottles. The air flow was controlled by a purge-meter to avoid the accumulation of respired CO_2 in the bottles (P-710; Tokyo Keiso, Tokyo, Japan). The flow rate of inflowing air was set at approximately 50 mL $minutes^{-1}$, and the outflowing air was regulated as 35 mL $minutes^{-1}$. Each bottle was measured for five minutes and switched to the next bottle by MIU after measurement. Thus, CO_2 emission from each bottle was measured every 70 minutes (12 samples and 2 times of ambient air) and continuously analysed up to 15 h. SIR was calculated by average of CO_2 emission rate for 3.5 to 4.6 h after glucose amendment. The SIR phase was followed by an exponential growth phase (with a specific growth rate, SGR), which continued until the substrate availability in the soil water solution becomes limiting. SGR was calculated by linear regression after logarithmic transformation of the CO_2 emission rate.

3.3.5 Measurements of Soil Respiration

Soil respiration rates were monitored throughout the incubation period for 32 days and measured using a nondispersive infrared gas analysis sensor (C12329-01; Hamamatsu Photonics

K.K., Shizuoka, Japan) equipped with an air-pump (EAP-01; AS ONE, Osaka, Japan). Each incubation bottle was sealed by lid with two tubes (inlet and outlet), which allowed air to circulate in the bottle headspace and the gas analysis sensor. Silicon rubber was used to seal the gap within the circulation loop. The soil respiration concentration was calculated based on the increase of CO_2 concentrations in the bottle headspace over 30 minutes.

3.3.6 Statistical analysis

The data was analysed using one-way analysis of variance (ANOVA), while the time course of CO_2 emission was analysed using a mixed model for repeated measurements. Tukey's test was performed for the analysis of significant differences for each treatment. CO_2 emissions were log-transformed ($\log_{10}$(flux)), to achieve normal distribution, prior to analysis. To compare the effects of CO_2 emission on earthworm and barley application, control treatment value was excluded from other treatments. After that, the data were analysed using a mixed model for repeated measurements to investigate earthworm and barley amendments. All statistical analyses were performed in R ver. 3.4.1 with a threshold *P*-value of 0.05.

3.4 Results

3.4.1 Soil nutrient status after soil incubation with earthworms

Barley and earthworm application significantly influenced soil chemical properties compared to soils without barley (Control). Soil pH in water was increased by barley application, while such an increase was not observed when barley was applied with *M. hilgendorfi* (Fig. 3.1a). At the same timing, the amount of NO_3^--N in the E and B treatments were significantly lower than that in the M and Control treatment (Fig. 3.1b). The WSC concentration in soils was increased by the addition of the barley, particularly in E and B treatment (Fig. 3.1c). There was no effect of B treatment on MBC compared to Control treatment (Fig. 3.1d). Contrastingly, M treatment resulted in the highest MBC concentrations (2.9 ± 0.2 g kg $^{-1}$ soil), whereas E treatment showed

the lowest MBC concentrations (2.5 ± 0.1 g kg $^{-1}$ soil). For SGR, there was a significantly lower growth rate when applied barley (Fig. 3.1e).

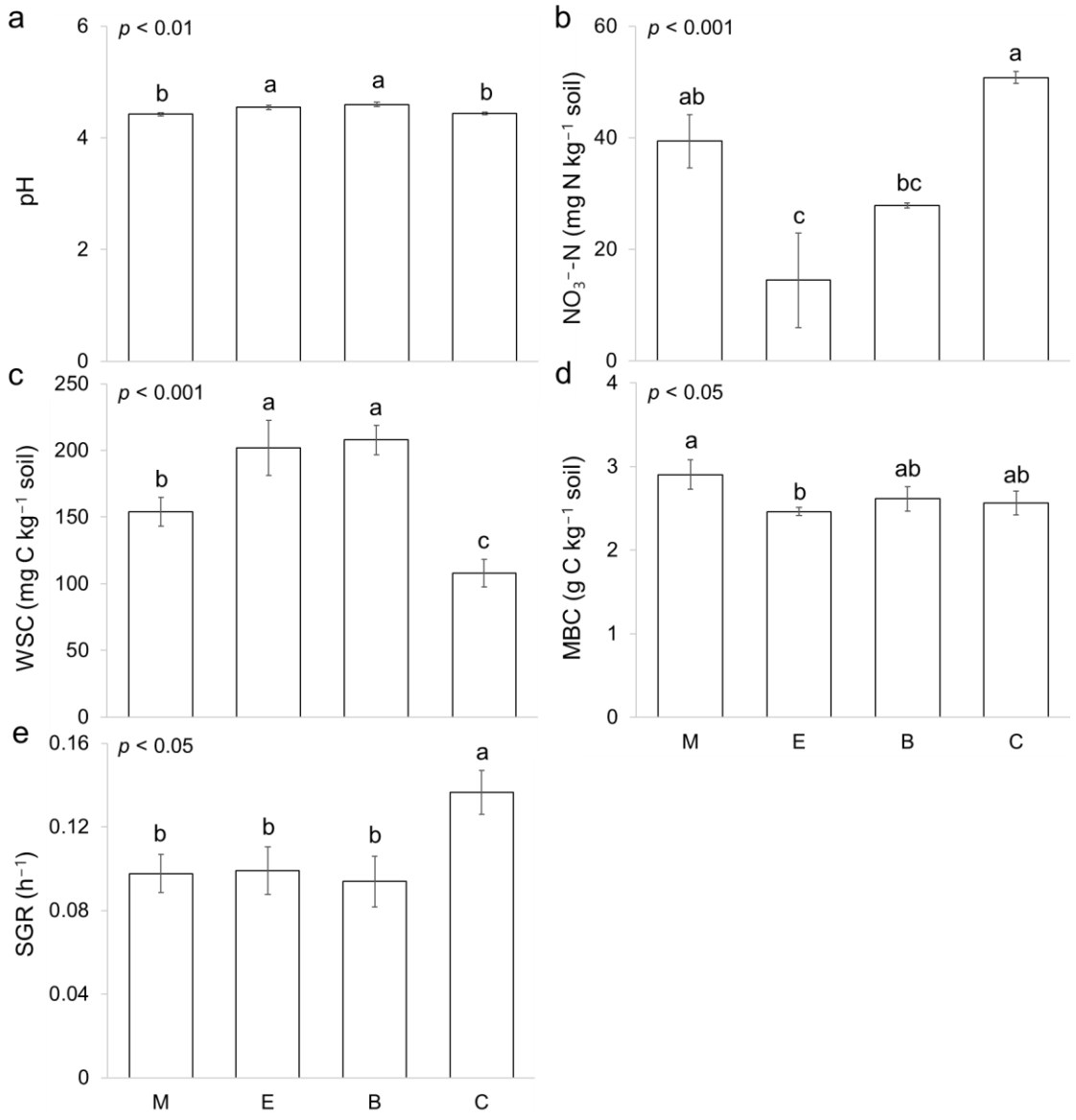

Figure 3.1. Soil pH (a), amount of soil nitrate (NO_3^--N, b), water soluble carbon (WSC, c), microbial biomass carbon (MBC, d), and specific growth rate (SGR, e) under different treatments. The treatments include *M. hilgendorfi* and barley; M, *E. fetida* and barley; E, barley without earthworms; B, and without earthworms and without barley (Control); C. Level of significance was determined by one-way analysis of variance (ANOVA). Different letters indicate significant differences between treatments ($p < 0.05$). Error bars represent standard deviation (n = 3).

3.4.2 Microbial activity and soil respiration

There were no significant differences among the treatments regarding soil enzyme activities (e.g., UA, NEA and DEA) and N and C related characteristics (Table 3.1). The cumulative soil CO_2 emissions were significantly higher with barley ($p < 0.05$), compared to without barley (Fig. 3.2). Regarding the time course changes in soil CO_2 emissions, no significant differences among barley application were seen (Fig. 3.3). The soil respiration of barley-only treatment was lower at the beginning (day 0), and CO_2 emissions rate increased and peaked at day 8 and decreased towards the end of incubation.

Table 3. 1. The non-significant (p > 0.05) effects of earthworms on soils. Level of significance was determined by one-way ANOVA. The treatments include *M. hilgendorfi* and barley; M, *E. fetida* and barley; E, barley without earthworms; B, and without earthworms and without barley (Control); C. Error values represent standard deviations (n = 3).

	M	**E**	**B**	**C**
NH_4^+-N (mg N kg^{-1} soil)	24.1 ± 10.6	14.7 ± 9.2	21.0 ± 2.9	20.0 ± 1.7
TN (g N kg^{-1} soil)	5.43 ± 0.25	5.28 ± 0.37	4.52 ± 1.29	5.58 ± 0.26
HWSC (mg C kg^{-1} soil)	815 ± 24	867 ± 123	800 ± 8	843 ± 68
TC (g C kg^{-1} soil)	45.8 ± 6.29	39.6 ± 0.37	39.4 ± 0.2	40.7 ± 0.08
CN ratio	8.49 ± 1.54	7.53 ± 0.45	9.69 ± 3.39	7.31 ± 0.34
UA (mg NH_4^+-N kg^{-1} soil h^{-1})	7.61 ± 6.82	23.5 ± 4.04	19.5 ± 10.11	17.5 ± 9.31
NEA (µg NO_3^--N kg^{-1} soil h^{-1})	194 ± 42	233 ± 59	91 ± 58	121 ± 41
DEA (µg N_2O-N kg^{-1} soil h^{-1})	158 ± 97	85 ± 7	93 ± 18	66 ± 16
SIR (mg CO_2 kg^{-1} soil h^{-1})	3.34 ± 0.73	3.14 ± 0.44	3.50 ± 0.60	2.15 ± 0.15

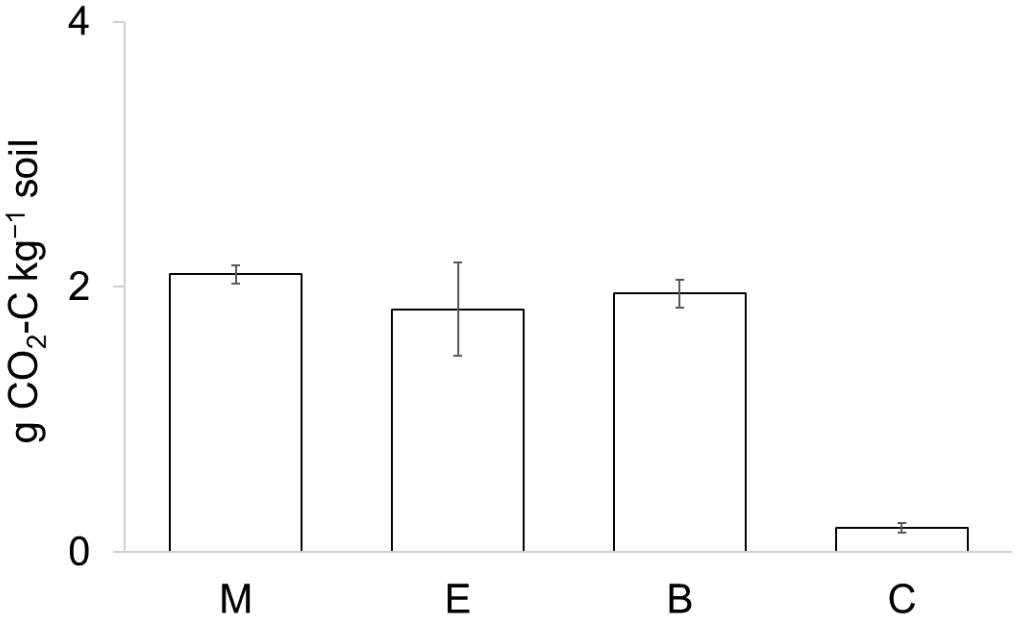

Figure 3.2. Cumulative amount of CO_2-C evolved from soils throughout the experiment. The treatments include *M. hilgendorfi* and barley; M, *E. fetida* and barley; E, barley without earthworms; B, and without earthworms and without barley (Control); C. Error bars represent standard deviation (n = 3).

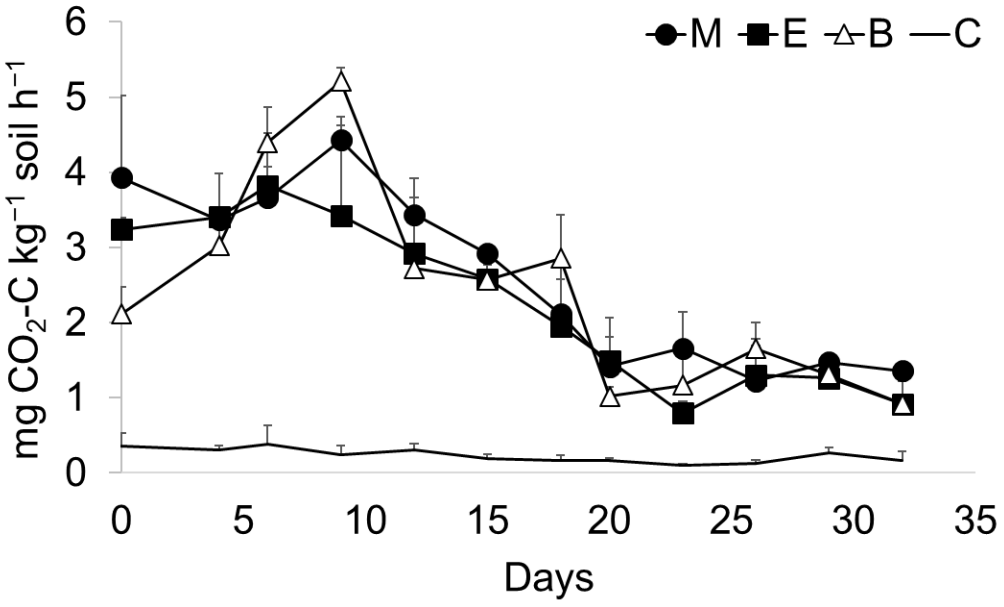

Figure 3.3. The time course for CO_2 emissions throughout the experiment. Circles represent the soils with barley and *M. hilgendorfi* (M) whereas squares represent the soils with barley and *E. fetida* (E). Triangle and line-only depict soils with and without barley (B and C), respectively. Error bar represents one standard deviation (n = 3).

3.5 Discussion

3.5.1 Effects of earthworms on soil C contents

Organic amendments increased the WSC concentration, compared to the soils without barley treatments. Bastida et al. (2012) reported that the increase of WSC could be due to mineralization processes. In addition, a previous study reported that soil pH was increased by barley application because of enhanced C mineralization (Khalil et al., 2005). However, in our study, *M. hilgendorfi* suppressed the effect of barley application on the increase in WSC and soil pH when compared to *E. fetida*. Contrastingly, for MBC, the *M. hilgendorfi* treatment showed the highest values within all the treatments. These facts suggest that with the presence of *M. hilgendorfi*, C derived from barley tends to be immobilized and remained in soils as MBC. Thus, *M. hilgendorfi* had a positive impact regarding the increase in soil microbes after the addition of organic materials compared to the soils with barley and the soils with barley and *E. fetida*. Similarly, Enami et al. (2001) observed a significant increase in amounts of total and bacterial phospholipid-derived fatty acids due to the presence of *M. hilgendorfi* with rice straw. In contrast, a previous study reported that *E. fetida* decreased MBC (Aira et al., 2002). Ruz-Jerez et al. (1992) suggested that *E. fetida* might become the competitors against microbes for carbonaceous substrates as energy and this might be a reason for the decreased MBC. Another study also suggested that *Lumbricus* species (same family as *E. fetida*) selectively consume soil fractions with a high concentration of microbes (Hendriksen, 1990). According to a previous study, those two earthworms are distributed in similar agricultural ecosystems and in a similar density (Nakamura, 1988). We also note that the earthworm distribution depends on agricultural management systems (e.g., mulch), and the application of organic materials often positively impacts the activity of earthworms. Thus, the information regarding these differences between the earthworm species can be used to establish strategies to efficiently increase soil C using agricultural residues.

3.5.2 Effects of earthworms on soil N dynamics

M. hilgendorfi increased the amount of NO_3^--N in soils in this study, compared to the soils with *E. fetida*. To support this, Kawaguchi et al. (2011) observed that the cast of *M. hilgendorfi* became hotspots of nitrification. We also visually observed more holes within the soils with *M. hilgendorfi* compared to *E. fetida*, although we did not measure the physical changes in the soils during the incubation. The aeration of the soils (physical changes in the soils) could be a reason for the stimulation of N mineralization and nitrification. Greiner et al. (2012) also found that *M. hilgendorfi* improved the soil aggregate formation, resulting in N mineralization. In contrast to *M. hilgendorfi*, our studies showed a lower NO_3^--N concentration the soil with barley and *E. fetida*. This result may suggest the gaseous loss of NO_3^--N during organic matter decomposition. Also, there is a possibility of the acceleration of N assimilation by *E. fetida*, although further studies are needed to confirm this (Hartenstein and Hartenstein, 1981; Mitchell et al., 1982).

Overall, the earthworms influenced the substrate availability and microbial biomass, but not the other measured "potentials" of microbial enzyme activities related to N cycles (e.g., nitrification and denitrification potentials). One of the reasons for this might be because our study was conducted only for 32 days. A longer incubation period might accumulate more casts by earthworms and further influence the potentials of soil microbial activities. *M. hilgendorfi* may have a long-term effect of microbial activities because the amount of those casts, which become a major source of soil aggregates formation, will reach more than 20 t ha^{-1} $year^{-1}$ (Caravaca et al., 2005; Kawaguchi et al., 2011). Additionally, the activity of earthworm creates heterogeneous conditions in the soils because of the mucus secreted from the earthworm and casting activity (Subler and Kirsch, 1998). The soil near the holes created by earthworm activities often show improved biological activity, thus stimulated soil microbes, compared to bulk soils (Kuzyakov and Blagodatskaya, 2005). Because our study was an incubation study with a relatively smaller amount of soil, we sampled soils and then homogenised for each

analysis. Thus, this might have diluted the impacts of the earthworm compared to the control soils, particularly enzyme activities.

Our incubation study suggested that even when we applied the same type of organic matter into soils, its impacts on soil microbes differ depending on the earthworm species. Thus, further study will be required to understand interaction with earthworms and nutrient cycles at the field condition (e.g., earthworm populations) (Kruck et al., 2006).

3.5.3 Effects of earthworms on CO_2 emissions

The current study also indicated that the earthworm activity in soils had no effect on the cumulative CO_2 emissions for 32 days. A previous quantitative review reported that the earthworm presence increased soil CO_2 emissions by 33% on average, but the results also stated that the data from the short-term (< 30 days) studies were markedly variable (Lubbers et al., 2013; Lubbers et al., 2017). Also, we did not observe clear differences in CO_2 emissions between samples with and without earthworms. This might depend on the amount of soil C. A similar study using *E. fetida* showed no significant effect on soil CO_2 emissions from soils (total C = 1.71%) (Zhu et al., 2018). On the other hand, Caravaca et al. (2005) reported that CO_2 emissions were increased by earthworms in soils with depleted C condition (total C = 0.32%). The CO_2 emissions were positively related to the amount of C in soils, in general (Caravaca and Roldán, 2003). Thus, CO_2 emissions derived from earthworms might be negligible particularly in soils with higher C contents. In our study, the C contents of the soil was 4.23%. Thus, in our soils, soil microbes were active both with and without the presence of earthworm, actively emitting CO_2.

3.6 Conclusion

This study reports that two different epigeic earthworms (*M. hilgendorfi* and *E. fetida*) with the presence of barley residue differently influenced soil C and N dynamics. The soils with *M.*

hilgendorfi and barley accumulated MBC and NO_3^--N more efficiently compared to the soils with *E. fetida* and barley. In contrast, no significant differences were observed between the soils with different earthworms regarding the soil enzyme activities. Future research on the effect of earthworm on the C and N cycle, then consequent microbial community change under different soil C contents, is recommended to fully understand soil C accumulation in the field scale. Particularly, the microbial mechanisms to sequester C in soils (e.g., cell wall production) and their interaction with the presence of earthworms will be an interesting topic to be investigated.

Chapter 4

The effect of organic amendments on soil fauna community and crop production in Zambia

4.1 Abstract

In sub-Saharan Africa, the use of organic amendments (e.g. cattle manure, maize residue etc.) is being widely prompted. However, crop production in sub-Saharan Africa has been stagnated. The positive impact of organic amendments on nutrient cycling and crop yields is also influenced by soil biology. Surface-dwelling soil macro-fauna particularly represent biological indicators for soil nutrient cycling. Thus, basic information is needed at the field level, regarding the impacts of organic amendments on crops and soil faunal communities. We conducted a field experiment using different organic amendments under different cropping systems (cassava, maize and soybean) in sandy loam soils and loamy sand soils in Zambia. The results showed that the total number of soil fauna in each site was totally different; we found around 1000/200 individuals at sandy loam soils/loamy sand soils. Organic amendments showed the higher crop yields in loamy sand soils, while the crop production was generally higher in sandy loam soils, compared to loamy sand soils. These results indicate that organic amendments largely contribute to stimulate soil fauna abundance with the increase in nutrient cycles in sandy loam soils, while organic amendments act as nutrient source for crop production in loamy sand soils. This information is critically important and can help to establish site specific strategies to deal with how to use limited organic materials.

4.2 Introduction

Most of the small-scale farmers in sub-Saharan Africa are unable to access the required levels of inorganic fertilizers due to their high costs (Sanchez, 2015). Thus, the combined effects of organic amendments and inorganic fertilizer on aboveground agroecosystems need to be further studied in southern Africa to improve the efficiency of fertilizer use and to establish sustainable agricultural systems. At present, the use of organic amendments, such as animal waste products, is being widely promoted in Africa. Total livestock manure N use in agricultural systems has been increasing in Africa over the last decades (+3.9% yr^{-1}, FAOSTAT http://www.fao.org/economic/ess/environment/data/livestock-manure/en/, accessed Dec 2019). Despite these facts, cereal grain yields are typically only 1 t ha^{-1} in this region (Sanchez, 2015), and the continental average grain yield in sub-Saharan Africa has been stagnated since 1960s (Lal, 2017, 2019). Thus, the crop use efficiency of organic fertilizers as well as of chemical fertilizers needs to be further studied in African region. The organic amendments often improve the nutrient and moisture holding capacity of the soils, thus improve the crop use efficiency of chemical fertilizers (Ge et al., 2010; Janssen, 2011).

The positive impact of organic amendments on nutrient cycling and crop yields is influenced by soil biology (Miyazawa et al., 2002; Ouédraogo et al., 2006; Rousseau et al., 2013; Roy et al., 2018). For example, the role of soil fauna in the decomposition processes of the organic amendments is widely recognized, particularly for complex organic materials such as crop residues, whereby macro-fauna increases the surface area of the organic materials before stimulating the activities of smaller soil decomposers by breaking the organic materials into smaller particles (Tian et al., 1995; Lavelle et al. 2006; Ouédraogo et al., 2004; Karanja et al., 2006). Thus, soil fauna community can be indictors of crop production and soil fertility due to their high responsiveness to environmental condition (Rousseau et al., 2013; Bünemann et al., 2018).

Surface-dwelling soil macro-fauna represent biological indicators for monitoring soils (Ritz et al., 2009) and more than 80% of soil fauna consists of arthropods (Culliney, 2013). Pitfall traps for these invertebrates have been widely used for environmental investigation and are a well-designed technique. Also, their diversity has been known as a good indicator of the effect of human activity on soil biodiversity, because of their sensitive to environmental changes (Hendrickx et al., 2007; Lawes et al., 2005). Some previous study without using pitfall trap methods reported that some macro-fauna (e.g. millipedes) were not observed due to thier higher mobility (Tian et al., 1993). Also, Ouédraogo et al. (2004) used a litterbag method and reported that a strong correlation between organic material mass loss and the dynamics of termites, although there was no significant correlation between remaining organic resource and other soil fauna in agricultural soils in semi-arid West Africa. Thus, pitfall trap method is potentially a favour method to investigate the effect organic amendments on such surface-dwelling soil macro-fauna in aboveground ecosystems.

The organic amendments can also sequester C in soils, although the magnitudes of the C sequestration depend on the soils' inherent characteristics (based on texture, mineralogy, etc.) (Muñoz-Rojas et al., 2015; Farina et al., 2017). For example, clay minerals bind to organic matter and improve the capacity of soils to sequester C. Additionally, soil faunal communities are influenced by soil texture characteristics, for example, Rousseau et al. (2013) reported that soil macro-fauna abundance and diversity are negatively related to sand contents in soils. Plant variability is also one of the controlling factors of crawling soil fauna community (Honek, 1988; Thomas et al., 2006). Thus, the interactions among soil texture and other physical characteristics, soil biological characteristics and plant types (crop variety) may strongly control the C cycling and nutrient releases from organic amendments.

In sub-Saharan Africa, there is an uncertainty regarding the interaction between soil faunal community and organic amendments. Basic information is needed at the field level, regarding the impacts of organic amendments on different crops in different soils, as well as on soil faunal

communities. This information is critically important and can help to establish site specific strategies to deal with how to use limited organic materials. Thus, we conducted a field scale study and our aims were to investigate the effect of organic amendments on aboveground biomass in two difference agricultural soils in Zambia. We hypothesized that the organic amendments increase soil fauna community and consequently improve crop yield, while their impacts are limited in soils which is less soil faunal abundance.

4.3 Materials and Methods

4.3.1 Description of field study site

We conducted field experiments from December 2017 to April 2018 at two sites (Lusaka and Kabwe), with different soil textures and chemical properties (Table 4.1). Lusaka site is located at agricultural experimental field of International Institute of Tropical Agriculture (14°23'44.6"S, 28°29'39.9"E). Kabwe site is located within the agricultural experimental field of Zambian Agricultural Institute (15°18'09.4"S, 28°18'17.6"E). Zambia is divided into three major agro-ecological zones, based on total annual rainfall received in a unimodal pattern between October and April (e.g. region I, IIa and IIb, and III). According to those zones, both of Lusaka and Kabwe sites are in the region IIb (annual rainfall of between 800 and 1200 mm). However, the rainfall was lower at Lusaka site (232.6 mm), than at Kabwe site (734.4 mm), during this experimental period (From December 2017 to April 2018).

Table 4.1. Soil chemical and physical properties at the Lusaka (sandy loam soils) and Kabwe (loamy sand soils) sites prior to the study.

	Lusaka	Kabwe
pH_KCl	5.0	4.0
P (mg kg^{-1} soil)	6.7	32.7
K (mg kg^{-1} soil)	98.3	52.7
Na (mg kg^{-1} soil)	4.3	3.3
Ca (mg kg^{-1} soil)	852.0	91.7
Mg (mg kg^{-1} soil)	260.7	20.7
CEC (cmolc kg^{-1} soil)	6.7	1.4
Total C (%)	1.42	0.51
Total N (%)	0.08	0.10
Clay (%)	19	12
Silt (%)	19	12
Sand (%)	63	76

Table 4.2. Nutrient contents of each organic material. CM: cattle manure amendment, PM: poultry manure amendment, and MR: maize residue amendment.

	CM	PM	MR
C (%)	21.1	65.6	13.9
N (%)	0.9	1.9	0.3
P (%)	0.1	0.1	0.1
K (%)	2.5	0.8	1.2

Table 4.3. Amounts of fertilizer application at 5 different treatments. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

	CF	CM	PM	MR	NF
C (kg ha^{-1})	0	2500	2500	2500	0
N (kg ha^{-1})	108	108	108	108	0
P (kg ha^{-1})	40	12.4	3.2	63.3	0
K (kg ha^{-1})	20	300.8	32.3	233.3	0

4.3.2 Experimental design

The experiment was laid out in a randomized block design using three replicate plots per soil amendments with un planted 1 m wide strip separated (Fig. 4.1). Within cassava (*Manihot esculenta*), maize (*Zea mays*), and soybean (*Glycine max*) planting and bare (no crop) plots, subplots with five soil amendments in each two sites were established:

(1) Chemical fertilizer-treated plot; hereafter referred to as 'CF'

(2) Cattle manure-treated plot; hereafter referred to as 'CM'

(3) Poultry manure-treated plot; hereafter referred to as 'PM'

(4) Maize residue-treated plot; hereafter referred to as 'MR'

(5) Control (nothing applied to the soil); hereafter referred to as 'NF' plots.

Each plot had 4.5 m × 3 m, 3 m × 3 m, and 2.5 m × 3 m, in cassava, maize, and soybean plots, respectively. Cassava/maize/soybean was planted with 0.75 m × 0.75 m/0.75 m × 0.5 m/0.5 m × 0.25 m line and row spacing, respectively. Each Crop was planted on 12 December 2017 in the Lusaka sites and on 18 December 2017 in the Kabwe site. The amount of amendments was adjusted to a certain C input (2.5 t C ha^{-1}). For CF and MR, 200 kg ha^{-1} of D-compound (10-20-10-NPK), and urea was applied to adjust to the same N contents (Table 4.2 and 4.3). Maize straw and leaves were cut into 10-cm pieces before the application, for the MF plots. The organic materials were applied 3 days before planting and incorporated into the soil (15 cm depth) using hand hoes, whereas the chemical fertilizers were applied just after planting. Insecticides mainly against for termites and worms were also used to prevent crop damages during crop growing season.

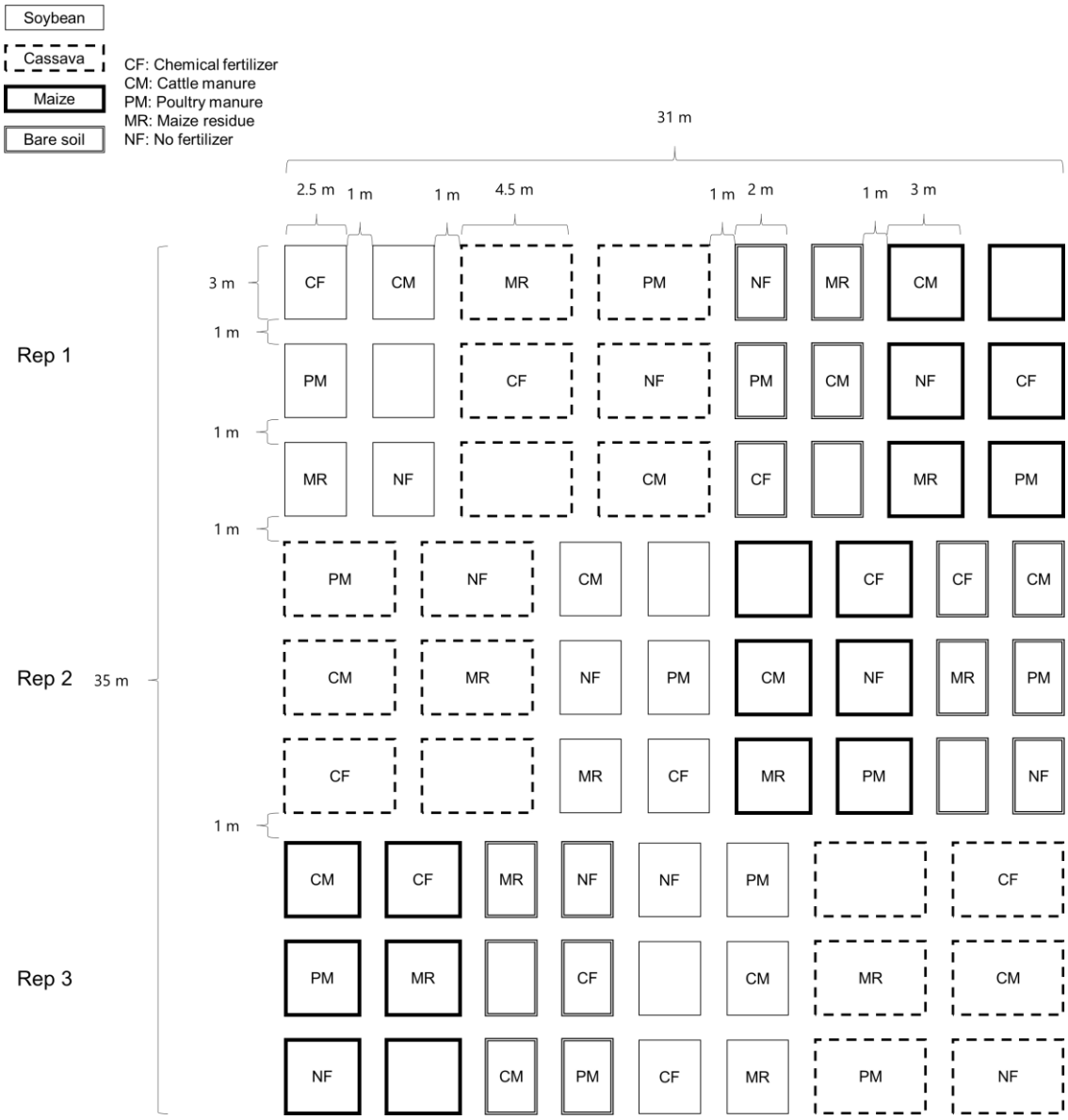

Figure 4.1. Field experiment plot layout in the both Lusaka and Kabwe sites. The dotted line: cassava cropping, the bold line: maize cropping, the solid line: soybean cropping, and the double line: no cropping (bare soil). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

4.3.3 Soil fauna sampling

Soil fauna was collected by pitfall traps every two weeks as same timing of soil sampling after planting up to April 2018 (total 13 sampling times). A plastic cup (500 mL volume, 8 cm diameter and 14 cm deep) was placed at the center of each plot. The traps did not contain any reagents to select specific arthropods. Four drain holes were opened at the bottom of the trap, and the height of the trap was same as the ground surface. Plastic boards were installed above the surface of the traps due to rain clearance. The trapped arthropods were stored by 70% ethanol. They were counted and identified by their external morphology.

4.3.4 Plant height and SPAD measurements

The growth survey (plant height and chlorophyll content) was conducted at 45, 65, and 85 days after planting. The average of three plant heights was used as an observation for each plot. Chlorophyll content of cassava was measured using a sixth leaf from the top of each plant by using SPAD-502 chlorophyll meter (Minolta Camera Co., Japan). The SPAD value of the maize and the soybean plants was measured for the third leaf from the top of each plant. The average of the three readings from three separate plants were used for each plot.

4.3.5 Plant sampling and analysis

To determine the biomass production, cassava was harvested at July 2018, and maize and soybean were harvested at April 2018. The central 2 rows soybean, 10 maize, and 5 cassava from each plot were harvested. The plant material was divided into each part (e.g. leaves, stems, cobs, and grain), chopped into small pieces, and dried one week in a greenhouse. For cassava, representative sample of known weight (approximately 1 kg) in each plot was taken and then oven dried at 70°C until attaining constant weight. For maize and soybean, all samples were oven dried using the same procedure as described above.

4.3.6 Statistical analysis

All statistical analyses were performed using R ver. 3.6.1. For the soil moisture, crop height, and SPAD value, two-way repeated-measures analysis of variance (RM-ANOVA) was used to determine the effects of organic amendments throughout the experimental period. Tukey's test was performed for the analysis of significant differences for each treatment. Soil fauna community analysis used a permutational multivariate analysis of variance (PerMANOVA) and the BrayCurtis distance based on 999 permutations of the raw data. was analysed using one-way analysis of variance (ANOVA). Pearson correlation analysis was used to identify the correlation between grain yield and SPAD value or between crop biomass and SPAD value. All data are presented as mean ± s.d..

4.4 Results

4.4.1 Soil moisture

Soil gravimetric moisture at Lusaka site was generally higher when compared to Kabwe site (Fig. 4.2). Soil moisture at Kabwe site decreased more rapidly compared to Lusaka site and often showed the values less than 5% during the experimental period. At both Lusaka and Kabwe site, two-way RM-ANOVA showed both significant differences of organic amendments and the sampling timings on soil moisture during the experimental period. The soil moisture measured in CM and MR plots was higher than the other treatments at both sites, when averaged across the experimental period. Also, the decrease rates of soil moisture contents when there were no major rains (e.g. day 10 to 42) in CM and MR plots were more moderate compared to the other treatments.

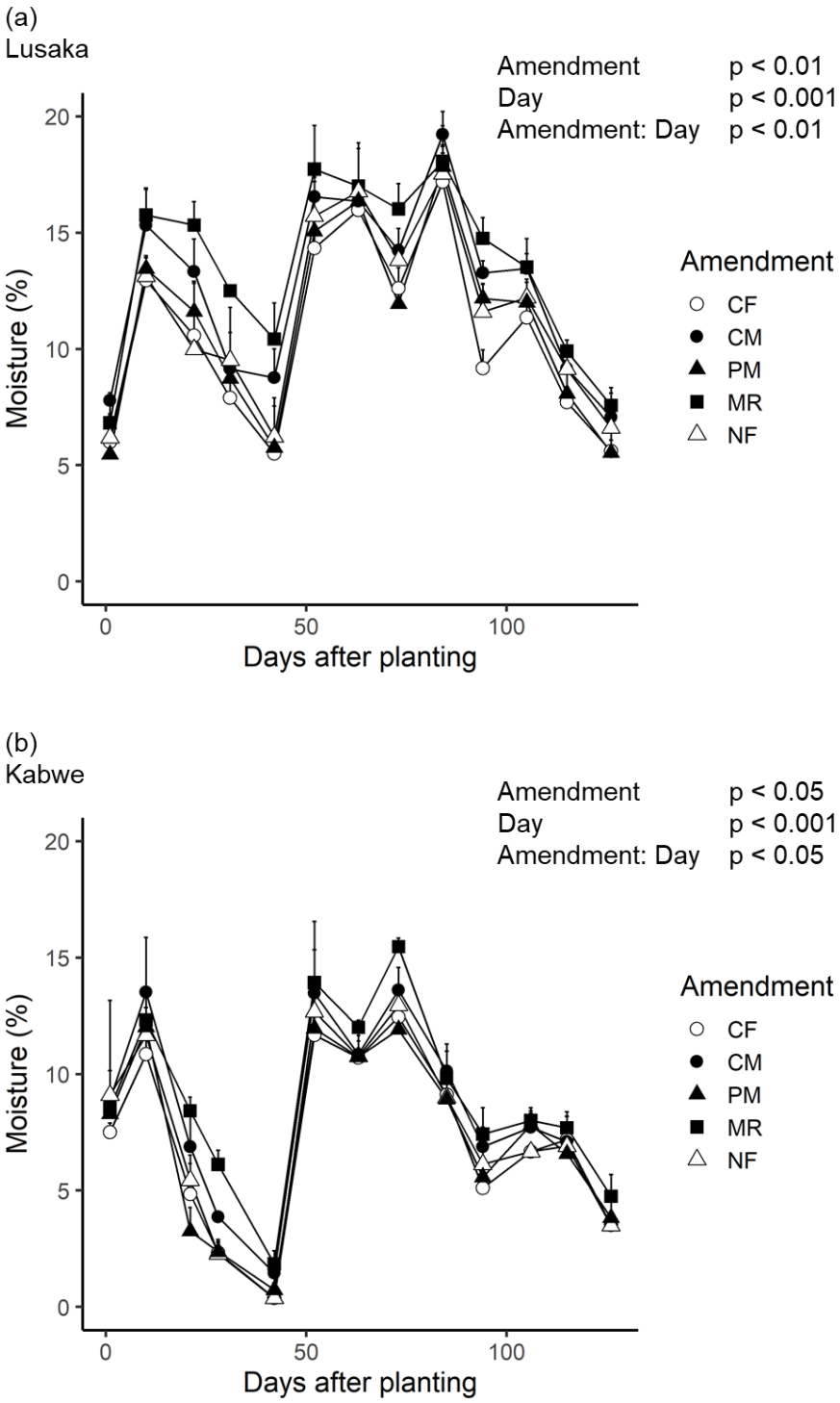

Figure 4.2. The time course changes of the soil gravimetric moisture at (a) Lusaka site and (b) Kabwe site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

4.4.2 Soil faunal community and abundance

We collected 944 individual soil fauna in total at Lusaka site, while there were 150 individual soil fauna at Kabwe site. around 90% of soil fauna was classified as *Araneae* (Spider), *Coleoptera* (Beetle), *Dermaptera* (Earwig), *Orthoptera* (Grasshopper), *Spirobolida* (Millipede) at both sites (Fig. 4.3 and 4.4). Most of *Coleoptera* was *Scarabaeoidea* (Earth-boring dung beetle) or *Tenebrionidae* (Darkling beetle). The other soil macro-fauna counted were *Chilopoda* (Centipede), *Hemiptera* (True bug), *Isopoda* (Wood louse), *Mantodea* (Mantis), and *Solifugae* (Camel spiders).

Because of very low abundance of soil fauna at Kabwe site, we conducted statistical analysis such as PerMANOVA only at Lusaka site. Fertilizer treatments ($p < 0.01$) and crop types ($p < 0.05$) had a significant effect on the soil faunal community structure, according to PerMANOVA.

There was a significantly higher proportion of soil fauna in organic amendments (particularly, CM and MR treatments) than that in the other fertilizer treatments, when averaged across crop types ($p< 0.05$, Fig. 4.3). Within the individual soil fauna group, the significant effect of fertilizer treatments was observed at *Araneae*, *Coleoptera*, *Orthoptera*, and *Spirobolida* (Fig. 4.5). *Araneae* significantly increased MR treatments, when compared to CF and CM treatments. *Coleoptera* significantly increased in MR treatments, but only in soybean fields. *Orthoptera* was significantly lower abundance in PM treatments, while MR treamtents was higher proportion. *Spirobolida* was lower in CF treatments, when compared to other treatments. Although there was no significant effect of fertilizer treatments and crop types on the abundance of *Dermaptera*, there was a higher proportion in CF treatments than that in the other fertilizer treatments (data not shown).

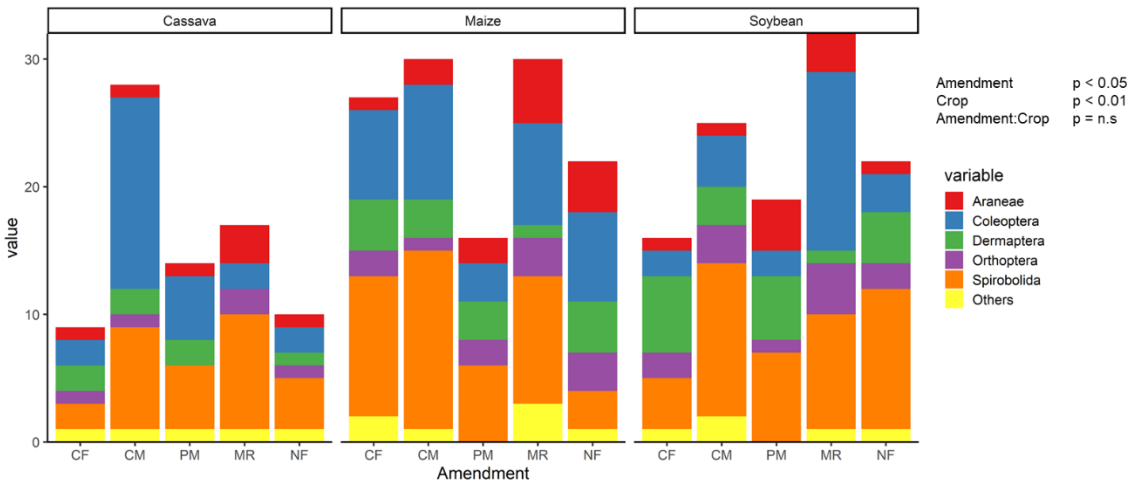

Figure 4.3. Numbers of individuals of soil fauna in Lusaka site. Level of significance was determined by and two-way ANOVA. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

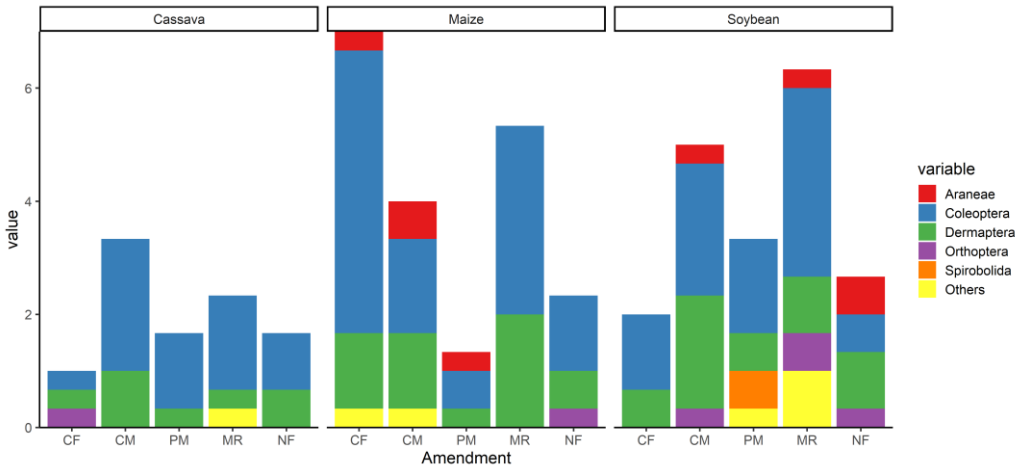

Figure 4.4. Numbers of individuals of soil fauna in Kabwe site. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

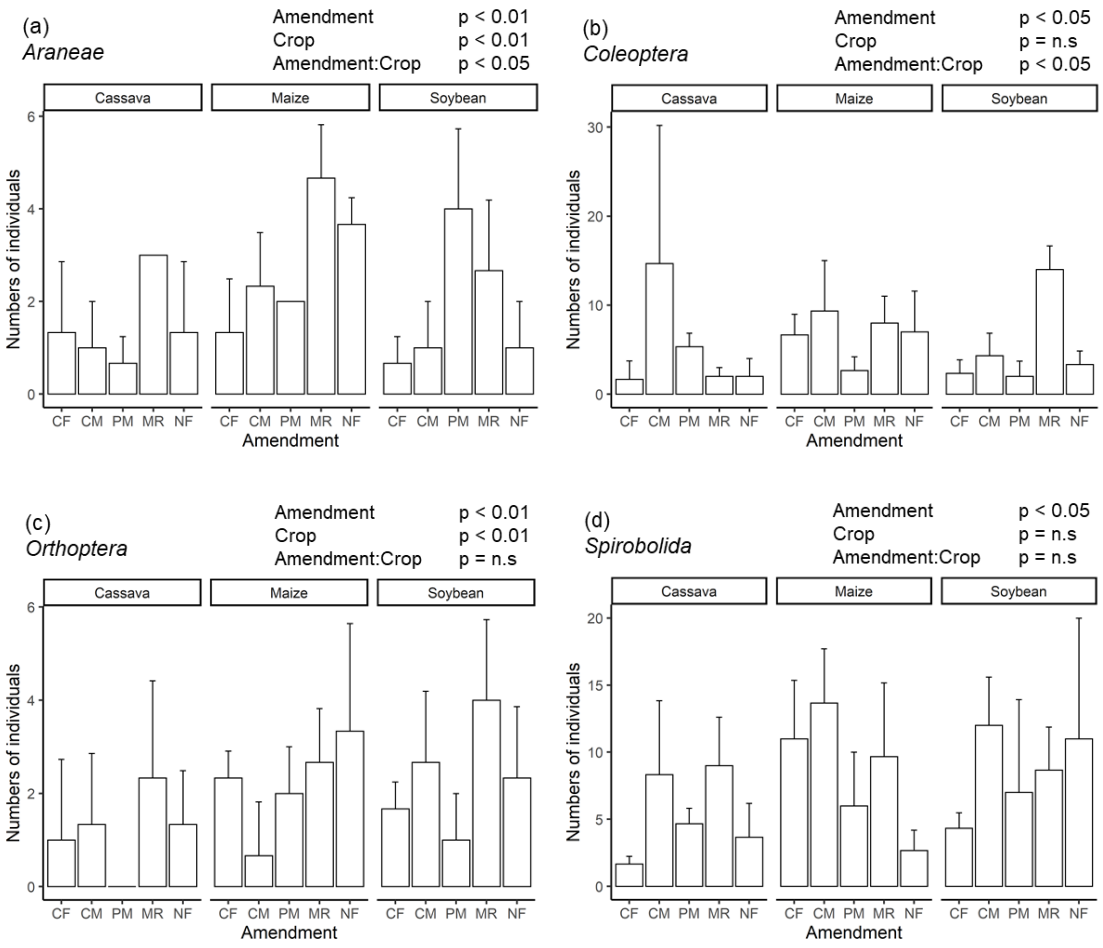

Figure 4.5. The significant effect of organic amendmetns on individual soil fauna abundacnce. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

4.4.3 Crop productivity

Crop height at Lusaka site was generally higher than at Kabwe site. Maize and soybean growth had significant effects of fertilizer treatments, although there was site dependent.

At Lusaka site, the CF treatments were significantly lower soybean growth throughout experimental period ($p < 0.05$, Table 4.4 and Fig. 4.6). For the soybean, higher SPAD value in the early and late stages were shown (i.e. v-line).

At Kabwe site, cassava height was no differences between fertilizer treatments, while some significant differences of SPAD value were shown (Table 4.4 and Fig. 4.7). The MR treatments showed higher value 43 days after planting, whereas the CF treatments showed relatively higher SAPD value after that days. For the maize, there were significant interaction effects ($p < 0.05$) between sampling timings and fertilizer treatments. The CF treatments showed higher maize growth only 64 days after planting. The CF treatments also showed higher SPAD value than organic amendments during experimental period. The SPAD value in organic amendments were ranged within the values from CF to NF treatments. Although there were no significant effects of fertilizer treatments on soybean growth, CF treatments showed higher soybean SPAD value.

Table 4.4. The level of significance for crop height at each measurement day. Levels of significance (n.s., *, ** and * represent $p \geq 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) using one-way ANOVA.**

Site	Day	Cassava		Maize		Soybean	
		Crop height	SPAD value	Crop height	SPAD value	Crop height	SPAD value
Lusaka	44	n.s.	n.s.	n.s.	n.s.	**	*
	64	n.s.	n.s.	n.s.	n.s.	**	*
	85	n.s.	n.s.	n.s.	n.s.	*	n.s.
	106	n.s.	n.s.	NA	n.s.	NA	NA
Kabwe	43	n.s.	*	n.s.	***	n.s.	n.s.
	64	*	*	*	*	n.s.	*
	86	n.s.	n.s.	n.s.	*	n.s.	n.s.
	106	n.s.	**	NA	ns	NA	NA

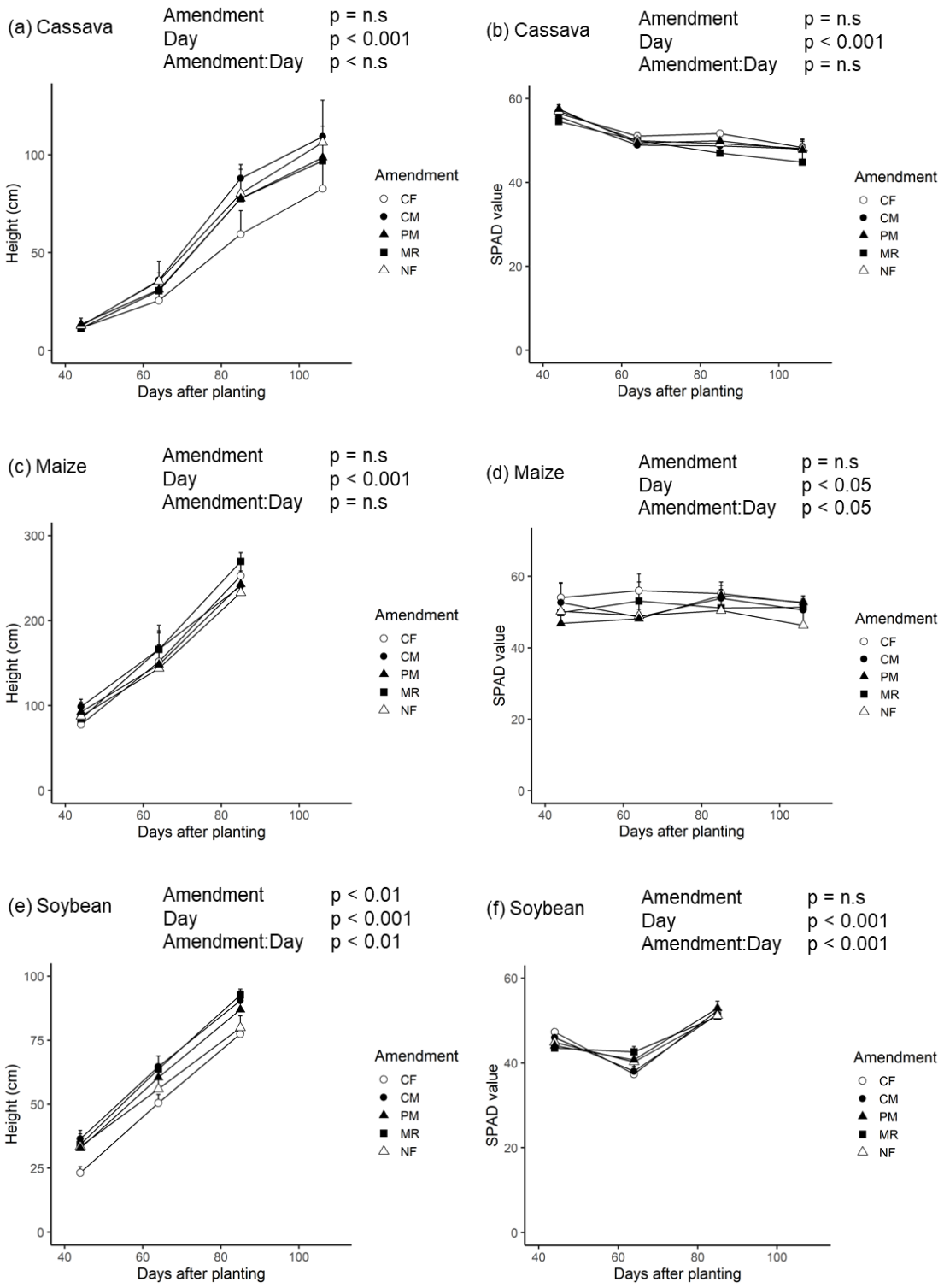

Figure 4.6. The time course changes of the crop growth and SPAD value at (a and b) cassava, (b) maize, and (c) soybean at Lusaka site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

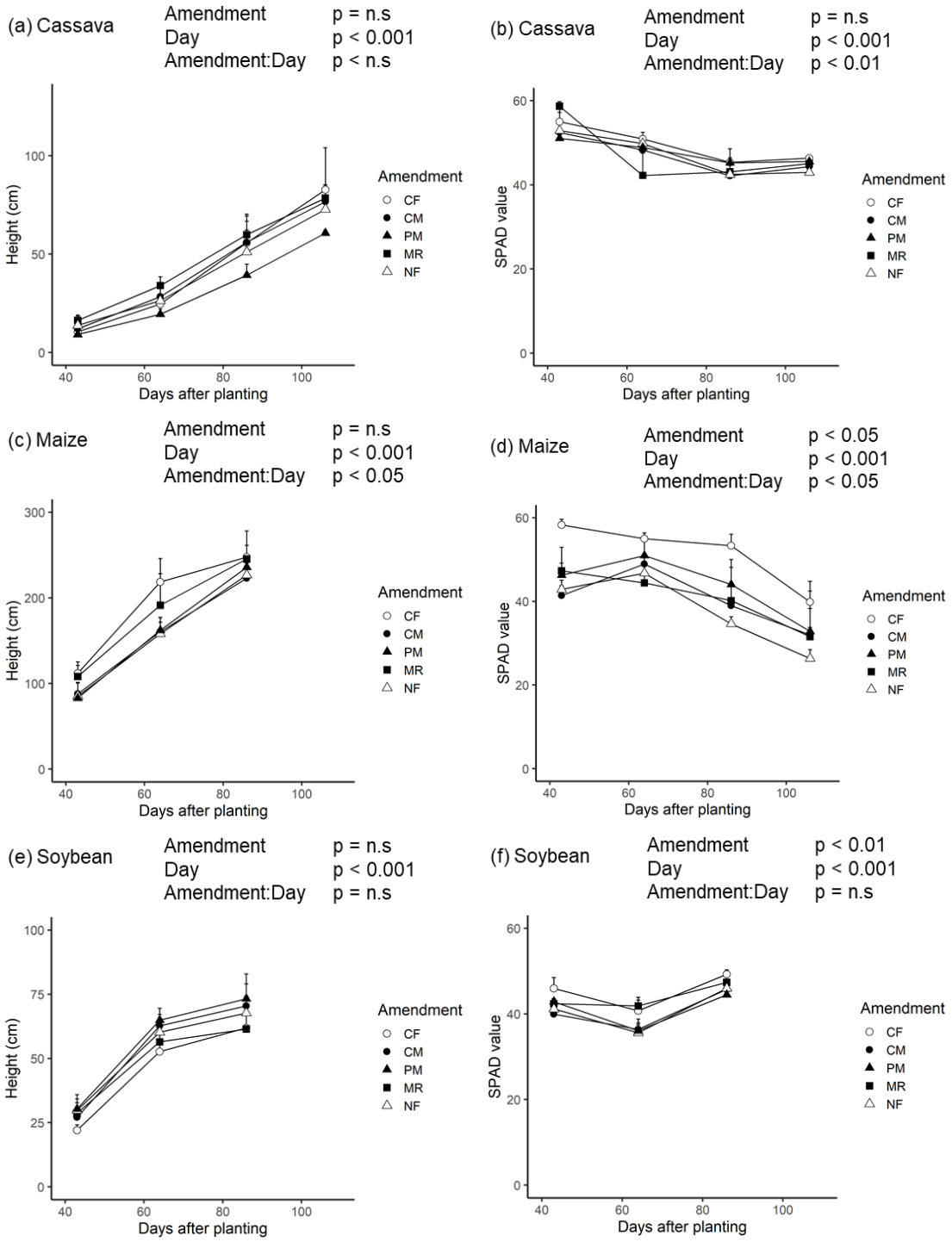

Figure 4.7. The time course changes of the crop growth and SPAD value at (a and b) cassava, (b) maize, and (c) soybean at Kabwe site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

Crop yields were generally higher at Lusaka site, compared to Kabwe sites except the cassava yields (Table 4.5). At Lusaka site, organic amendments had no significant effects on the cassava and maize yields. The soybean yield from MR treatments (2.56 t ha^{-1}) showed relatively higher values compared to the PM and NF treatments (1.60 t ha^{-1} and 1.84 t ha^{-1}, respectively), although there were no significant differences among their straw yield.

At Kabwe site, The effects of fertilizer treatments depended on crop types and fertilizer materials. For cassava production, CM treatments showed the significant higher yield (15.0 t ha^{-1}), compared to PM treatments (9.98 t ha^{-1}). Additionally, CM and PM treatments showed the higher soybean yields (1.90 t ha^{-1} and 2.11 t ha^{-1}, respectively), compared to CF treatments (1.17 t ha^{-1}). In contrast, maize yield in CF treatments was higher (7.85 t ha^{-1}) than CM and NF treatments (4.05 t ha^{-1} and 2.79 t ha^{-1}, respectively).

For the relationship between cassava production and growth analysis, aboveground biomass had significantly correlated with plant height at Kabwe site, while no significant relationship was shown at Lusaka site (Table 4.6 and 4.7). Maize grain yield at Lusaka site, there was significant and positive correlation with both plant height and SPAD value on 64 days after planting, although such correlation with either plant height or SPAD value was observed at other sampling times (e.g. day 44 and day 85). Similarly, both plant height and SPAD value had significantly positive correlation with maize grain yield at Kabwe site but particularly on 43 and 86 days after planting. Contrastingly, there was no significant correlation for soybean production at Lusaka site. At Kabwe site, soybean grain yield had positively correlated with plant height, while negatively correlated with SPAD value.

Table 4.5. Crop yield, straw, and total biomass of cassava, maize, and soybean at Lusaka and Kabwe sites. Difference lowercase letters indicate a significant difference between fertilizer treatments. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

Biomass (t ha^{-1})	Amendment	Lusaka			Kabwe		
		Cassava	Maize	Soybean	Cassava	Maize	Soybean
Grain	CF	10.1 ± 2.78	7.76 ± 1.11	2.29 ± 0.07 ab	12.9 ± 2.06 ab	7.85 ± 0.23 a	1.17 ± 0.25 b
	CM	9.08 ± 1.66	7.85 ± 1.85	2.19 ± 0.30 ab	15.0 ± 0.37 a	4.05 ± 1.22 b	1.90 ± 0.12 a
	PM	11.2 ± 2.30	6.93 ± 0.79	1.60 ± 0.08 b	9.98 ± 1.65 b	4.34 ± 0.77 ab	2.11 ± 0.13 a
	MR	9.81 ± 2.35	8.61 ± 1.20	2.56 ± 0.22 a	12.5 ± 1.22 ab	6.13 ± 1.77 ab	1.62 ± 0.20 ab
	NF	5.42 ± 0.24	6.64 ± 2.00	1.84 ± 0.34 ab	10.6 ± 1.72 ab	2.79 ± 0.64 b	1.74 ± 0.23 ab
Straw	CF	8.58 ± 0.44	8.29 ± 0.95	2.44 ± 0.29	7.74 ± 1.48	8.79 ± 0.34 a	1.87 ± 0.15 b
	CM	9.02 ± 0.90	7.05 ± 0.36	2.15 ± 0.27	8.85 ± 0.74	5.38 ± 0.99 bc	2.19 ± 0.21 ab
	PM	9.18 ± 1.44	6.27 ± 0.34	1.66 ± 0.13	5.79 ± 0.16	5.70 ± 0.74 bc	2.53 ± 0.12 a
	MR	8.92 ± 2.58	9.02 ± 1.16	2.44 ± 0.37	7.37 ± 1.27	7.46 ± 1.24 ab	2.07 ± 0.24 ab
	NF	6.63 ± 1.52	5.92 ± 1.43	1.70 ± 0.28	5.87 ± 1.55	4.74 ± 0.37 c	1.95 ± 0.06 b
Total	CF	18.7 ± 3.01	16.1 ± 2.06	4.74 ± 0.23 ab	20.6 ± 2.93 ab	16.6 ± 0.12 a	3.04 ± 0.39 b
	CM	18.1 ± 2.50	14.9 ± 1.51	4.34 ± 0.56 ab	23.9 ± 1.12 a	9.43 ± 2.21 b	4.09 ± 0.31 a
	PM	20.4 ± 1.93	13.2 ± 1.12	3.27 ± 0.19 b	15.8 ± 1.80 b	10.1 ± 1.50 b	4.63 ± 0.24 a
	MR	18.7 ± 4.92	17.6 ± 2.35	5.00 ± 0.60 a	19.9 ± 2.49 ab	13.6 ± 3.00 ab	3.68 ± 0.33 ab
	NF	12.0 ± 1.57	12.6 ± 3.44	3.54 ± 0.55 ab	16.5 ± 3.02 ab	7.53 ± 1.01 b	3.68 ± 0.28 ab

Table 4.6. Pearson's correlation analysis between crop growth parameters (height and SPAD value) and crop biomass at Lusaka site. Levels of significance (n.s., *, ** and * represent p ≥ 0.05, p < 0.05, p < 0.01 and p < 0.001, respectively) using one-way ANOVA.**

Site	Crop	Day	Yield		Straw		Total	
			Crop height	SPAD value	Crop height	SPAD value	Crop height	SPAD value
Lusaka	Cassava	44	−0.12	−0.16	0.15	0.04	0.02	−0.13
		64	−0.34	0.05	0.05	−0.18	−0.21	0.04
		85	−0.18	0.23	0.3	0.03	0.01	0.17
		106	−0.22	0.15	0.39	0.19	0.02	0.18
	Maize	44	0.49	0.75**	0.06	0.5	0.3	0.68**
		64	0.92***	0.63*	0.6*	0.79***	0.83***	0.76***
		85	0.61*	0.45	0.74**	0.47	0.73**	0.5
		106	NA	0.33	NA	0.55*	NA	0.47
	Soybean	44	−0.03	0.01	−0.11	0.17	−0.07	0.08
		64	0.08	0.06	0.06	0.08	0.07	0.07
		85	0.19	−0.11	0.13	−0.15	0.16	−0.14

Table 4.7. Pearson correlation analysis between Crop height and SPAD value and crop biomass at Kabwe site.

Site	Crop	Day	Yield		Straw		Total	
			Crop height	SPAD value	Crop height	SPAD value	Crop height	SPAD value
Kabwe	Cassava	43	0.03	0.08	0.29	0.24	0.14	0.15
		64	0.32	0.03	0.54*	0.16	0.43	0.09
		86	0.38	−0.33	0.68**	−0.13	0.53*	−0.27
		106	0.28	0.06	0.65**	0.15	0.46	0.1
	Maize	43	0.8***	0.9***	0.83***	0.92***	0.82***	0.91***
		64	0.81***	0.49	0.84***	0.47	0.83***	0.49
		86	0.63*	0.86***	0.67**	0.84***	0.65**	0.86***
		106	NA	0.83***	NA	0.75**	NA	0.79***
	Soybean	43	0.56*	−0.55*	0.28	−0.07	0.47	−0.37
		64	0.65**	−0.65**	0.66**	−0.33	0.70**	−0.55*
		86	0.38	−0.48	0.4	−0.29	0.42	0.42

Additionally, positive correlations between maize grain yield and the abundance of *Dermaptera* at Lusaka site (Fig. 4.8). No significant correlation between other crop biomass and other soil fauna was noted.

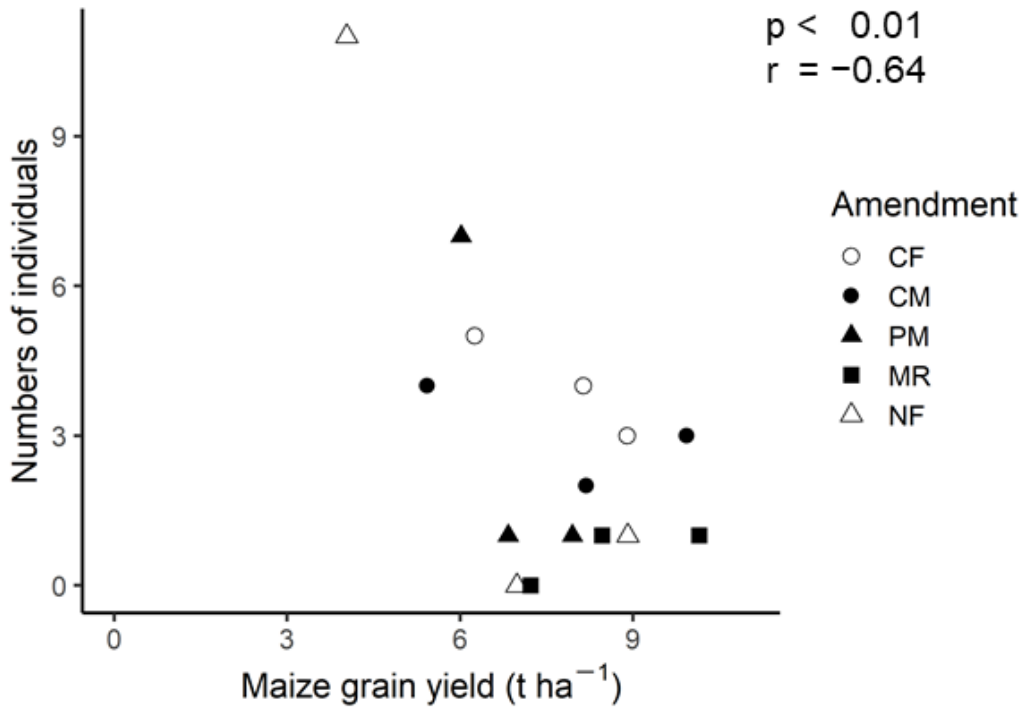

Figure 4.8. Relationship between abundance of *Dermaptera* and maize grain yield in Lusaka site. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

4.5 Discussion

4.5.1 Effects of organic amendments on soil fauna community and their decomposition contribution

A relatively higher soil fauna abundance was observed in Lusaka site (sandy loam soils), compared to Kabwe site (loamy sand soils). Kamau et al. (2019) reported significant differences of soil fauna abundance due to biophysical condition. They showed that the lower soil fauna abundance in regions with the less soil erosion and the lower annual precipitation, which is against the current study. Hemerik and Brussaard (2002) observed that the period of no fertilizer treatment positively correlates to the abundance of soil macro-fauna. Another previous study showed that that soil macro-fauna abundance and sand contents in soils are negatively related (Rousseau et al., 2013). Thus, the current study showed that soil fauna abundance might be largely affected by soil condition and texture rather than climatic condition (i.e. rainfall).

The current study also showed that the organic amendments at Lusaka site generally increased the abundance of soil macro-fauna. CM and MR treatments showed increases in the abundance of dominant soil macro-fauna group including *Araneae*, *Coleoptera*, *Orthptera*, and *Spirobolida*. Decomposition process of organic materials incorporated into soil is relatively faster, compared to the organic materials applied on soil surfaces (Karanja et al., 2006).

Additionally, Ouédraogo et al. (2007) reported that, in semi-arid Africa, the combined effect of among organic amendments and the presence of soil fauna (e.g. termites, earthworm, Coleoptera, and Myriapoda) accelerated the decomposition rate, and consequently increased crop yield. Thus, despite the short duration of this study, we suggest that there was a large soil fauna contribution to promote decomposition with increased soil fauna abundance at Lusaka site.

We also observed the significant differences of crop types on soil fauna abundance. Especially in cassava plots, when vegetation cover is dense, reduced solar radiation lead to low

temperatures on the bare ground of the fields. Thus, such micro-climatic condition changes might affect soil fauna abundance.

We note that the exclusion of macro-fauna potentially leading to an underestimation of crop residue breakdown rates (e.g. termites, Ouédraogo et al., 2004). Thus, further study will be required because such soil fauna are large impacts on the decomposition process in this region (Bünemann et al., 2018).

4.5.2 Effects of organic amendments on soil moisture, crop growth and SPAD value

The higher soil gravimetric moisture content in sandy loam soils at Lusaka site than in loamy sand soils at Kabwe site was largely reflecting differences in the water holding capacities of the soils, although there was the gap of amount of rainfall between sites during experimental period. Soil moisture increased due to organic amendments during the experimental period at both Lusaka and Kabwe sites. This indicates that organic amendments improved the soil water holding capacity. Previous studies also showed that organic amendments improved water availability and the resistance to penetration, compared to inorganic fertilizer amendments (Obi and Ebo, 1995; Agegnehu et al., 2016; Liu et al., 2018). Thus, the organic amendments might induce changes in aboveground ecosystems not only by providing substrates but also through the changes in moisture availability.

Although our study showed that organic amendments increased soil moisture status, the effect of organic amendments on crop growth were dependent on crop types and sites. Obi and Ebo (1995) reported that the positive effects of the heights of maize on organic amendments in Nigerian soils were highly significant, which was not accordance with our findings. This might be because the current study applied two times higher inorganic N into soils, compared to the previous report. Also, water limitation reduces maize growth due to limited N uptake needs of the plant and accessibility of soil N for crop uptake (Buljovcic and Engels, 2001; Gonzalez-

Dugo et al., 2010; Nasielski et al., 2019). However, current study showed that soil moisture was recovered by more than 10% after 52 days after planting, when compared to before that days. This indicates that maize crops can uptake from soil N as well as inorganic N fertilizer due to their higher availability. Thus, temporal higher maize growth was observed in CF treatments at Kabwe site, although there were no significant differences with fertilizer treatments at the end of experiment.

Soybean height with CF treatments was lower than the other treatments at Lusaka site. McAndrews et al. (2006) also found positive effects of organic amendments on soybean growth compared to urea application. Probably, this was because organic materials' ability to release nutrients more slowly compared to chemical fertilizer (Seufert et al., 2012; Crews and Peoples, 2005; Luo et al., 2018). The soybean growth at Kabwe site similar trend with Lusaka site, but up to 64 days after planting, possibly due to low level of soil moisture.

Contrastingly, crops grown in the plots with organic amendments showed generally lower SPAD value in maize and soybean at both sites, compared to single use of chemical fertilizer treatments. Previous studies showed the strong and positive relationships between the leaf N contents and SPAD value observed from multiple crop types including cassava, maize and soybean (Wood et al., 1993; Bullockand Anderson, 1998;Fritschi and Ray, 2007; Tagoe et al., 2008; Kanto et al., 2012). Thus, our result indicates that single use of inorganic fertilizer application increased crop N status due to higher N use efficiency in single use of chemical fertilizer.

A limitation of current experiment is that the effect of combination with organic and chemical fertilizer application could have been underestimated because urea is generally applied at mid-term growth stage (e.g. McAndrews et al., 2006; Sileshi and Mafongoya, 2006). Early urea application timing, which was performed in the current study, significant decreases maize plant height, compared to mid-term application (Amanullah et al., 2009). However, our study focused

on different fertilization with same amount C and N supply since less unknown the effect of availability of different organic materials for plants when applied same C and N contents. Additional studies will be needed to determine the relationship between plant growth and combined use of organic materials and chemical fertilizer with different application timings.

4.5.4 Interactions between organic amendments and soil fauna on crop performance

At Lusaka site, for cassava and maize yield, no significant differences with nutrient supply derived from organic and inorganic fertilizer were shown due to rapid soil faunal decomposition as described above. In contrast, soybean yield showed higher MR treatments at Lusaka site compared to other fertilizer treatments. MR treatments have a potential to increased water availability as described above. Also, soybean is well known that soybean productivity easily reduces erratic precipitation and limited ground water, compared to other crops (Mera et al., 2006; Le et al., 2012; Wijewardana et al., 2018, 2019). Thus, improved soil moisture retention by maize residue significantly increased soybean yield due to lower precipitation at Lusaka site.

At Kabwe site, CF treatments showed highest maize grain yields, with positive correlation with plant height and SPAD value, particularly early stages. Additionally, maize grain yield differences among fertilizer treatments was similar to the amount of applied urea N (CF > MR > PM > CM = NF). Thus, inorganic N application increased maize grain yield more effectively compared to the organic nutrient sources, in the current study, in Kabwe. Contrastingly, cassava tuber yield and soybean grain yield were relatively higher in CM treatments in Kabwe. This result indicates that slow release of organic nutrient derived from mainly microbial decomposition process improved their productivity due to poor nutrient capacity in soils and poor soil fauna abundance at Kabwe site. Also, soybean grain yield was positively correlated with plant height, but negatively correlated with SPAD value on day 43 and 64 after planting. Their negative relationship was not consistent with previous studies (Martín et al., 2005;

Shafagh-Kolvanagh et al., 2008). However, Dong et al. (2019) reported water stress in particularly early growth stage increased SPAD value. Thus, our soybean results show the overcompensation in the SPAD value derived from severe water stress particularly in early growth period due to erratic precipitation and poor soil moisture retention at Kabwe site.

Regarding the relationship with soil fauna abundance and crop production, the abundance of *Dermaptera* was negatively correlated maize grain yield. *Dermaptera* is recognized as predators as well as maize pests, whicih prefer cultivated soils, compared to undisturbed (i.e. no-tillage) soils (Bailey, 2007). In addition, we also observed higher relative abundance of *Dermaptera* on CF treatments. Thus, soil fauna community changes following organic amendments might be indicators of crop production (e.g. prevent from such pests), although further research will be required.

4.6 Conclusion

We conducted a field experiment to investigate the effect of organic amendments on aboveground agricultural ecosystems in two different soils in Zambia. The organic amendments increased soil fauna abundance only in the sandy loam soils, particularly cattle manure and maize residue amendments. These findings indicate that in sandy loam soils, organic amendments contribute a favor crop condition with improved decomposition rates by increasing soil fauna abundance. In loamy sand soils, contrastingly, interaction with organic amendments and soil fauna community is unclear, although organic amendments improve crop production. Further study is required to determine the interaction with the organic amendments and soil fauna community to maintain continuous food security in this area.

Chapter 5

The impacts of organic amendments on bacterial community structures and carbon cycle in two contrasting Zambian agricultural soils

5.1 Abstract

In sub-Saharan Africa, efforts have been made to increase its soil carbon (C) contents in agricultural systems, due to severe soil degradation related to soil C depletion. The C can be added to soils as organic amendments and the derived-C is utilized by microbes. Thus, the information between soil organic amendments and soil microbial community structures is important in this area. We conducted a field experiment using different organic amendments (e.g. cattle manure, maize residue etc.) in a sandy loam soil and a loamy sand soil in Zambia. Changes in soil carbon dioxide (CO_2) emissions, bacterial abundance and community structures were monitored for 126 days. The organic amendments increased CO_2 emissions, bacterial abundance and diversity only in the loamy sand soil, particularly cattle manure and maize residue amendments. These findings indicate that in the loamy sand soil, organic amendments altered microbial activity but might not have a major impact regarding the C sequestration in the soil. Contrastingly, abiotic factors controlled the impacts of organic amendments on microbial activities in sandy loam soils. Site, soil type and climate specific strategies are needed to deal with the issues of soil C depletion in semi-arid region.

5.2 Introduction

Carbon (C) in agricultural soils is positively correlated to their productivity (Lal, 2004; Kamaa et al., 2011; Luo et al., 2018). This fact is critically important in sub-Saharan Africa, soil C

depletion is observed in wide area within agricultural systems. The extensive area in sub-Saharan Africa is observed with soil C contents <1.1%, which is critical limit for agricultural productivity in this region (Nyamangara, 2002; Lal, 2004; Mapanda et al., 2011; Hamamoto et al., 2018). Particularly in southern Africa, soils are fragile due to cultivation, with soil C ranging from 0.5% to 2.5% (Lal and Stewart, 2000; Sakala et al., 2000; Zingore et al. 2008; Swamepoel et al., 2016). Thus, previous studies on the impact of agricultural management on soil C has been investigated in this region (Sakala et al., 2000; Chivenge et al., 2007; Thierfelder et al., 2013). Organic amendments (e.g. organic manure and crop residue) are known as materials to maintain or increase soil C (Aparna et al., 2014), while it has been suggested that combined use of organic amendments and chemical fertilizer (e.g. urea) is important to maintain the crops' fertilizer use efficiency (Ge et al., 2010; Janssen, 2011).

However, in sub-Saharan Africa, the rapid decomposition of SOM and organic amendments due to high temperature and the activeness of macro- and micro-fauna is making it difficult to steadily increase soil C (Powlson et al., 2001; Ouédraogo et al., 2004, 2007; Lal, 2008; Mazzilli et al., 2014; Martinsen et al., 2017). Also, there are significant effects of organic amendments on soil microbial community structure. Soil microbes provide us important life supporting functions, including nitrogen (N) and C cycling, thus it is important to maintain their abundance and diversities (Schloter et al., 2018). Generally, combined use of organic amendments and chemical fertilizer increases those parameters (Zhong et al., 2010), although only a few studies have been conducted to observe the diversities of soil microbes in agricultural soils in sub-Saharan Africa (Wood et al., 2015; De la Cruz-Barrón et al., 2017; Hamamoto et al., 2018). Several reports stated that more information is required regarding soil microbes in crop production systems in sub-Saharan Africa to understand the basics of soil functions in this region (Lagerlöf et al., 2014; Wood et al., 2015; Wild, 2016). Thus, effects of organic amendments on soil biology in order to increase soil C has to be further studied in this region.

The efficiencies of the organic amendments to improve soil C contents are partly controlled by soil inherent characteristics (based on texture, mineralogy, etc.) and soil types (Muñoz-Rojas et al., 2015; Farina et al., 2017). For example, soils with relatively higher clay contents tend to store soil C more effectively when compared to the soils with relatively higher sand contents (Schimel et al., 1985a,b; Spain, 1990; Amato and Ladd, 1992; Gentile et al., 2010). Generally, the amounts of C in African soils are positively related to the amount of clay and silt, at the continent scale (Giller et al., 1997; Sakala et al., 2000; Swanepoel et al., 2016). However, the detailed studies focusing on the different organic amendments in different soils have not been well-studied in sub-Saharan Africa. The information from this type of studies is critically important to establish site specific strategies to deal with the issue of C depletion in this area.

To understand the link between the organic amendments and soil C cycle, the release of C by microbes (soil CO_2 emissions or soil respiration) and soil microbial community can be studied. This process is important to maintain/improve the microbial functions of the soils, as discussed earlier, because microbes gain energy through the release of C through the decomposition of organic amendments. However, if excess C (from both organic amendments and soil C) is released via microbial CO_2 emissions, the increase of soil C will not be achieved. Many factors contribute to the magnitudes of CO_2 emissions after organic amendments: the types of organic amendments, environmental conditions (e.g. soil moisture; Sugihara et al., 2012), soil characteristics (e.g. the amount of soil C and soil texture; Caravaca and Roldán, 2003), and land management (e.g. plant residue application, fertilizer application, tillage; Chatskikh and Olesen, 2007; Mapanda et al., 2011). However, field observations on the responses of soil CO_2 emissions to organic amendments into sub-Sahara African soils are limited, although some studies showed general trends of CO_2 emissions in this agricultural fields (Mapanda et al., 2010, 2011; Sugihara et al., 2012). The loss of C through the decomposition process can be also assessed using litterbag methods. Studies have recognized that nutritious conditions in soils (e.g. fertilizer application) significantly affects decomposition rates, mostly because of affecting

microbial activity (Kwabiah et al., 1999; Gnankambary et al., 2008). Although decomposition process studies using litterbags has been widely performed, the interactions among organic amendments decomposition and soil microbial communities were not clear.

In this study, we collected data on CO_2 emissions, litterbag decomposition rates, bacterial abundance and taxonomic diversity from two different experimental sites in Zambia. Our main objectives were (1) to investigate the impact of different types of organic amendments on CO_2 emissions and litterbag decomposition, (2) to evaluate the bacterial responses towards different organic amendments in the different soils, and (3) to compare the effect of soil texture on soil C dynamics and bacterial community in dry tropical agroecosystems in Zambia. We hypothesized that organic amendments in sandy soils can quickly alter soil microbial abundance and diversity, but the benefits of the organic amendments on the increase of soil C will be minimum. Our results determine soil microbial community changes and CO_2 emissions in two different agricultural soils in response to organic amendments could provide valuable information for agricultural management for in sub-Sharan Africa.

5.3 Materials and Methods

5.3.1 Description of field study site

We conducted a field experiment from December 2017 to April 2018 at two sites (Lusaka and Kabwe) with different soil textures and chemical properties (Table 5.1). Lusaka site is located within the agricultural experimental field of International Institute of Tropical Agriculture (14°23'44.6"S, 28°29'39.9"E). Kabwe site is located within the agricultural experimental field of Zambian Agricultural Institute (15°18'09.4"S, 28°18'17.6"E). The rainfall was lower at Lusaka site (232.6 mm), than at Kabwe site (734.4 mm), during experimental period (From December 2017 to April 2018) .

Table 5.1. Soil chemical and physical properties at the Lusaka (sandy loam soils) and Kabwe (loamy sand soils) sites prior to the study.

	Lusaka	Kabwe
pH_KCl	5.0	4.0
P (mg kg^{-1} soil)	6.7	32.7
K (mg kg^{-1} soil)	98.3	52.7
Na (mg kg^{-1} soil)	4.3	3.3
Ca (mg kg^{-1} soil)	852.0	91.7
Mg (mg kg^{-1} soil)	260.7	20.7
CEC (cmolc kg^{-1} soil)	6.7	1.4
Total C (%)	1.42	0.51
Total N (%)	0.08	0.10
Clay (%)	19	12
Silt (%)	19	12
Sand (%)	63	76

Table 5.2. Nutrient contents of each organic material. CM: cattle manure amendment, PM: poultry manure amendment, and MR: maize residue amendment.

	CM	PM	MR
C (%)	21.1	65.6	13.9
N (%)	0.9	1.9	0.3
P (%)	0.1	0.1	0.1
K (%)	2.5	0.8	1.2

Table 5.3. Amounts of fertilizer application at 5 different treatments. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

	CF	CM	PM	MR	NF
C (kg ha^{-1})	0	2500	2500	2500	0
N (kg ha^{-1})	108	108	108	108	0
P (kg ha^{-1})	40	12.4	3.2	63.3	0
K (kg ha^{-1})	20	300.8	32.3	233.3	0

5.3.2 Experimental design

For each site, a plot experiment was conducted. There were no plants grown on the plots and the plots had 2 m × 3 m. The experimental design was a randomized complete design with five fertilizer treatments with three replicates:

(1) Chemical fertilizer treatment; hereafter referred to as 'CF'

(2) Cattle manure treatment; hereafter referred to as 'CM'

(3) Poultry manure treatment; hereafter referred to as 'PM'

(4) Maze residue treatment; hereafter referred to as 'MR'

(5) Control (nothing applied to the soil); hereafter referred to as 'NF' treatment.

The amount of amendments adjusted to a certain C input (2.5 t C ha^{-1}). Zingore et al. (2008) compared to effects of the soil texture on manure application in sub-Saharan Africa and reported that 17 t ha^{-1} $year^{-1}$ of organic material supply into soils are required to significantly increase soil organic C. Also, Sugihara et al. (2012) estimated the annual CO_2 emissions as around 2 t C ha^{-1} yr^{-1} in sub-Saharan Africa.

For N basal input in CF and MR, 200 kg ha^{-1} of D-compound (10-20-10-NPK), and urea was applied to adjust same N contents (Table 5.2 and 5.3). Organic materials were applied 3 days before chemical fertilizers were applied.

5.3.3 Soil sampling and moisture analysis

Soils (0 to 5 cm) in bare plots were collected every 2 weeks during crop growing season (from December 2017 to April 2018). For each sample, four points taken inside each bare plot avoiding the plot edge were combined and mixed. The sols moisture content of each soil sample was measured by oven-drying fresh soils at 100°C > 24 hours immediately after soil sampling and reweighing the dried soils.

5.3.4 Measurement of CO_2 emissions

At the same timings for the soil samplings, we measured CO_2 emissions rate at both Lusaka and Kabwe sites in bare plots. The CO_2 emission rates were measured by a closed-chamber system every 2 weeks during experimental period. A portable soil respiration chamber attached a non-dispersive infrared CO_2 analyzer (DIK-0450; DAIKI, Japan) was used. We measured gases at 0 min and 5 min using this chamber. Also, we checked the liner increase in CO_2 concentration in the chamber for 5 min in both Lusaka and Kabwe sites by measuring every 1 min. All field measurements were conducted between 9:00 and 13:00 h.

5.3.5 Measurement of decomposition rate

To evaluate the decomposition rate, 100 μm mesh sized (only permit entrance to microorganisms) litter bags were buried vertically at 10 cm depth in each treatment 23 days after the organic amendments. Each bag was 10 cm × 12 cm and contained 6 g of dried maize residue (chopped within 2 cm length). The distance between the litterbags was > 20 cm. At 30, 60 and 120 days after their incorporation, litter bags were retrieved. The soils were carefully brushed off from the litterbags, and the inside residues were taken out and gently shaken to eliminate the interfusion of soil. The residues were air dried for 48 h, and then dried at 60°C for 48 h. The dried samples were weighed for the remaining mass.

5.3.6 Measurement of bacterial abundance

DNA was extracted from the air-dried soils with modified method according to Sagova-Mareckova et al. (2008) and Miller et al. (1999). The extracted DNA was purified with Agencourt AMPure XP (Beckman Coulter) according to a predetermined protocol. The concentration of the purified DNA was measured with Qubit dsDNA HS Assay Kit (Invitrogen, USA). The purified DNA was then diluted 50 times with nuclease-free water for qPCR. We used Mx3000P/Mx3005P QPCR Systems (Agilent, USA). For primers, 515F/806R (Carini et

al., 2016) were chosen for V4 region of 16S rRNA quantitative analysis. For qPCR, samples were prepared with 15 μL of the KAPA SYBR Fast qPCR kit (Kapa Biosystems, USA) and, 1.2 μL of forward primer, 1.2 μL of 1 reverse primer, 0.18 μL of bovine serum albumin (100 mg mL^{-1}) and 3 μL of DNA extract. Nuclease-free water was added to make up to a final volume of 30 μL. Cycling conditions were 30 s at 95°C, 35 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 1 min, followed by 95°C for 1 min, 55°C for 30 s and 95°C for 30 s. All reactions were run in duplicate and compared to standard curves containing purified E. coli genomic DNA for 16S rRNA gene quantification.

5.3.7 Amplification and sequencing of 16S rRNA in soils

Bacterial community analysis was performed on all DNA samples extracted from soil. The first PCR was conducted using primers same as qPCR to amplify the V4 region of 16S rRNA. For PCR, samples were prepared with 10 mL of AmpliTaq Gold® 360 Master Mix (Applied BiosystemsTM, USA), 0.4 μL of forward primer, 0.4 μL of reverse primer and 1 μL of DNA extract. Nuclease-free water was added to make up to a final volume of 20 μL. The first PCR cycle was 95°C for 10 min, then 25 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. The first PCR products were then purified with Agencourt AMPure XP (Beckman Coulter) according to the given protocol. Using the amplicon obtained from the first PCR procedure, another PCR was performed to make it Ion Torrent sequencing sample-specific. To achieve this, a forward primer of 515F attached with the sequence of Ion Xpress Barcode Adapters Kit (Life Technologies), and reverse primer of 806F attached with the sequence of Ion P1 adaptor (Ion Torrent Life Technologies, USA) were used. The PCR sample contained 10 mL of AmpliTaq Gold® 360 Master Mix (Applied Biosystems, USA), 0.4 mL of forward primer, 0.4 mL of reverse primer and 4 mL of purified first PCR product. Nuclease-free water was added to make up to a final volume of 20 μL. The second PCR cycle was 95°C for 10 min, then 5 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. The second PCR products were purified with the same method as above. The concentration

of purified DNA was measured using Qubit dsDNA HS Assay Kit (Invitrogen, USA). The final length and concentration of the amplicons were confirmed using a Bioanalyzer DNA 1000 Kit (Agilent Technologies, USA). The library was diluted to 50 pM and loaded into the Ion 318 chip (Ion Torrent Life Technologies, USA) using the Ion Chef Instruments (Ion Torrent Life Technologies, USA) with the Ion PGM™ Hi-Q Chef kit.

5.3.8 Analysis of the 16S rRNA based bacterial community structures

The barcoded 16S rRNA gene sequences were de-multiplexed, quality-fltered, and assessed using the Quantitative Insights Into Microbial Ecology (QIIME) workflow (Caporaso et al., 2010). Operational Taxonomic Units (OTUs) were prepared by eliminating all the OTUs that matched the GreenGenes 13_5 reference sequence with 97% similarity. On average, 28,010 reads were mapped per sample for 16S rRNA, ranging from 10,138 to 314,835. Rarefaction was performed and the sequence data were subsampled to 10,000 sequences per sample to ensure fair comparisons between the samples (Gihring et al. 2011; Schöler et al., 2017; Mickan et al., 2019).

5.3.9 Statistical analysis

All statistical analyses were performed using R ver. 3.6.1. For the CO_2 emissions, litterbag decomposition, the relative amounts of soil bacterial DNA, Shannon-Wiener index, species richness, and evenness, two-way repeated-measures analysis of variance (RM-ANOVA) was used to determine the effects of organic amendments throughout the experimental period. The CO2 emission was analysed using one-way analysis of variance (ANOVA). Tukey's test was performed for the analysis of significant differences for each treatment. All data are presented as mean ± s.d..

5.4 Results

5.4.1 CO_2 emissions rate and soil moisture

The CO_2 emission rates at Lusaka site were generally higher during experimental period, when compared to Kabwe site. At Lusaka site, two-way RM-ANOVA showed the significant differences of the sampling timings during the experimental period. The CO_2 emission rates from CM, PM, and MR treatments peaked to 447, 345, and 305 mg CO_2-C m^{-2} h^{-1} on day 52, 84, and 63, respectively (Fig. 5.1a). Contrastingly, at Kabwe site, the effect of fertilizer treatments on CO_2 emissions rate was significant ($p < 0.05$, Fig. 5.1b). The CO_2 emissions peaked at the beginning of the experiment, when averaged across the organic amendment treatments. Also, the CO_2 emission rates from CM treatment on day 10 were higher peak (466 mg CO_2-C m^{-2} h^{-1}). Small peaks of the emissions rate were also observed from day 52 to 63 (139 and 167 mg CO_2-C m^{-2} h^{-1} from CM and MR treatments).

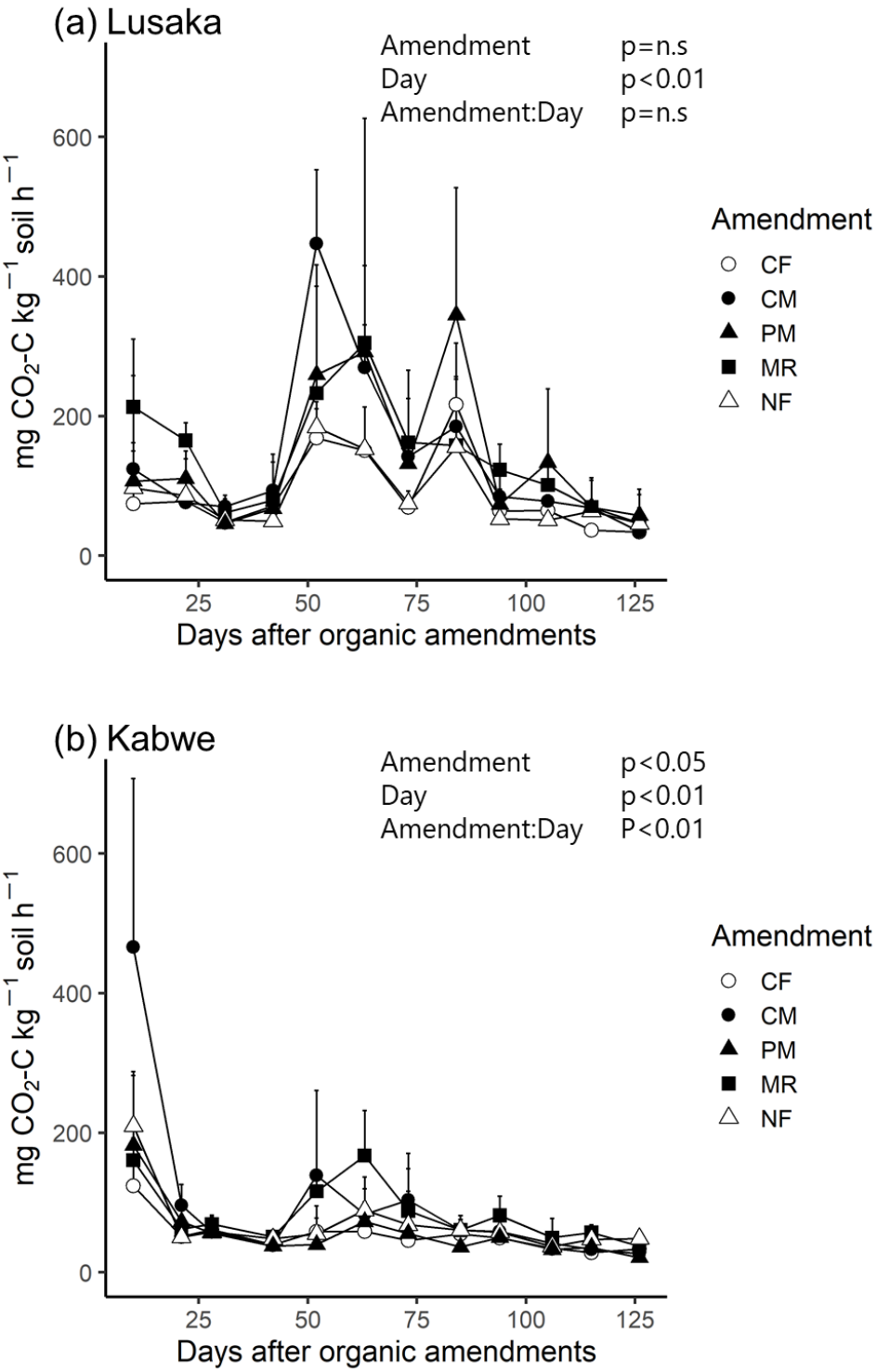

Figure 5.1. The time course changes of the CO_2 emissions rate at (a) Lusaka site and (b) Kabwe site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

Organic amendments did not significantly increase the cumulative emissions at Lusaka, although the cumulative emissions were higher than those at Kabwe, when averaged across the fertilizer treatments; 3.5 and 2.3 t CO_2-C ha^{-1} from Lusaka and Kabwe site, respectively(Fig. 5.2). In contrast, at Kabwe site, there was a significant effect of fertilizer treatments on the cumulative CO_2 emissions ($p < 0.01$). The amount of cumulative CO_2 emissions from organic amendment plots were variable at Kabwe site (from 1.8 to 3.3 t CO_2-C ha^{-1}).

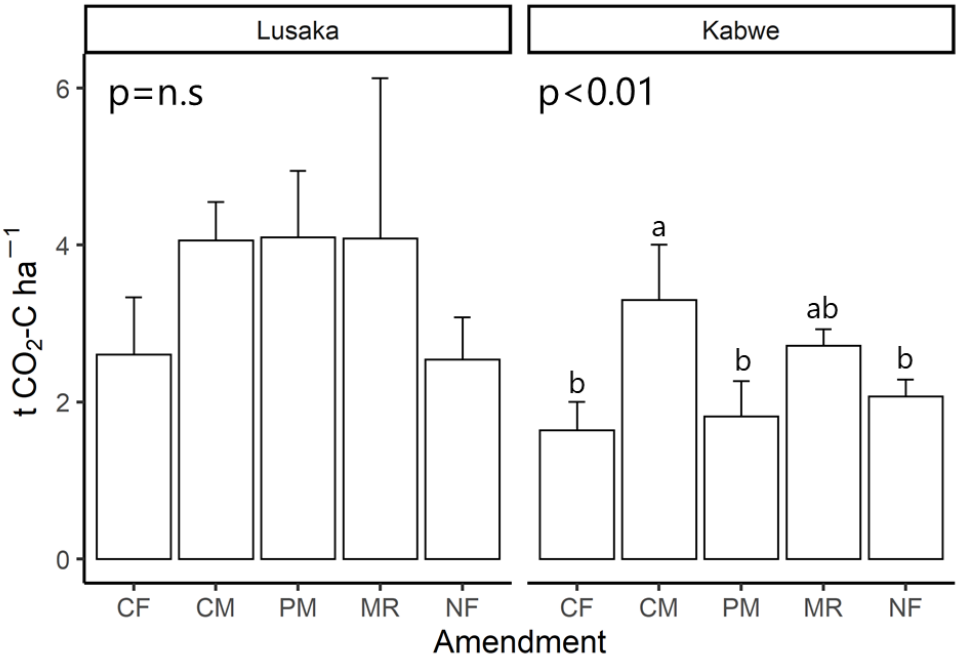

Figure 5.2. The cumulative CO_2 emissions at (a) Lusaka site and (b) Kabwe site. Difference lowercase letters indicate a significant difference between fertilizer treatments CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

The CO_2 emissions rates were positively correlated with soil moisture at Lusaka ($r = 0.59$, $p < 0.001$), and the relationship was stronger than at Kabwe ($r = 0.39$, $p < 0.001$, Fig. 5.3). Soil moisture ranges were higher at Lusaka site. The higher soil moisture content in sandy loam soils at Lusaka site than in loamy sand soils at Kabwe site was largely reflecting differences in the water holding capacities of the soils, rather than the amount of rainfall received at each site during experimental period.

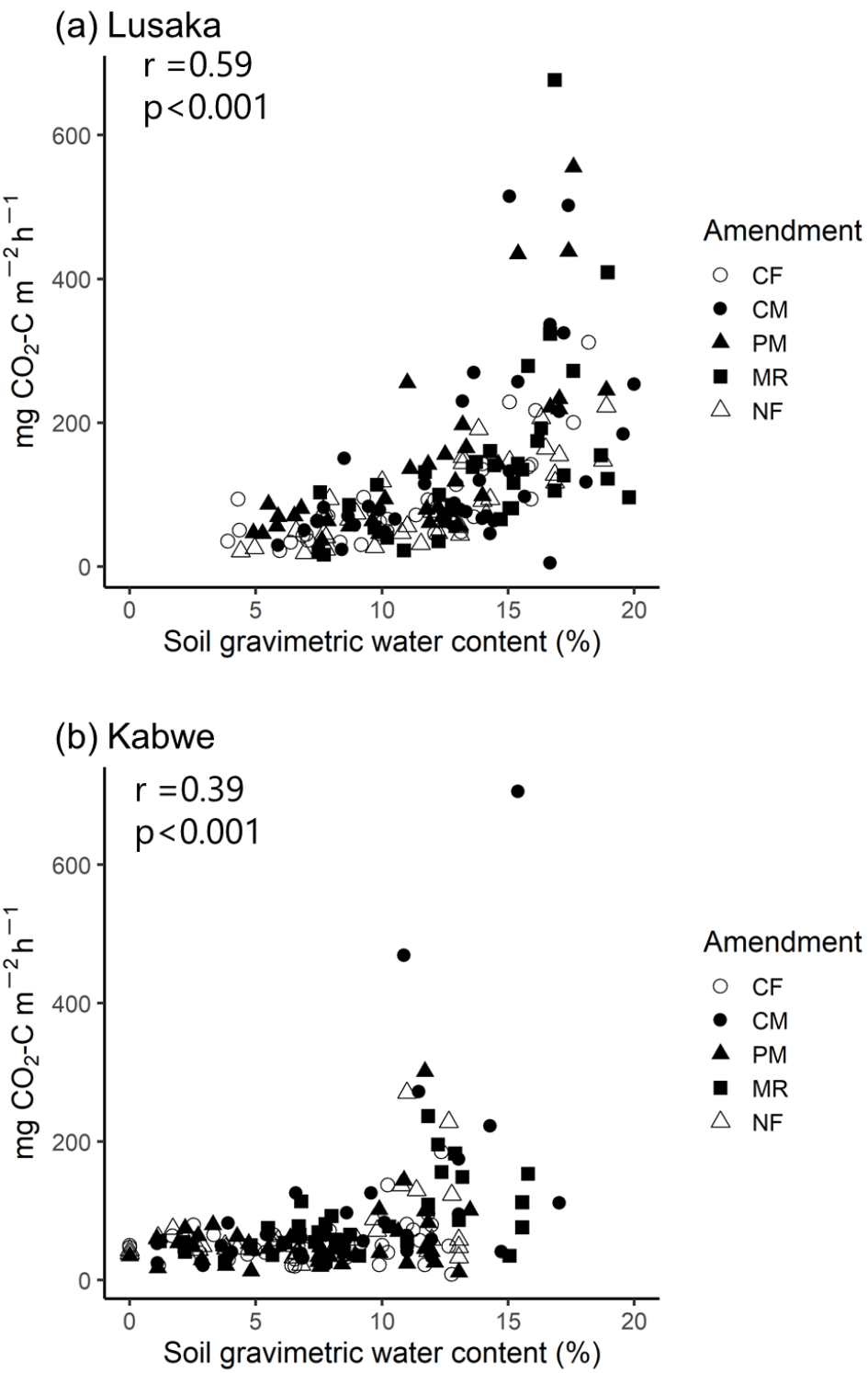

Figure 5.3. Relationship between CO_2 emission rates and soil moisture at (a) Lusaka site and (b) Kabwe site. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

5.4.2 Litter bag decomposition rate

There was a significant effect of fertilizer treatments on litter bag decomposition rate only at Kabwe site ($p < 0.01$, Fig. 5.4). Approximately 60% of the contents in the litterbag was decomposed within 120 days at both sites. On 122 days after buried, 40%, 43%, 46%, 56%, and 67% of litter were remained in MR, NF, CM, PM, and CF treatments, respectively.

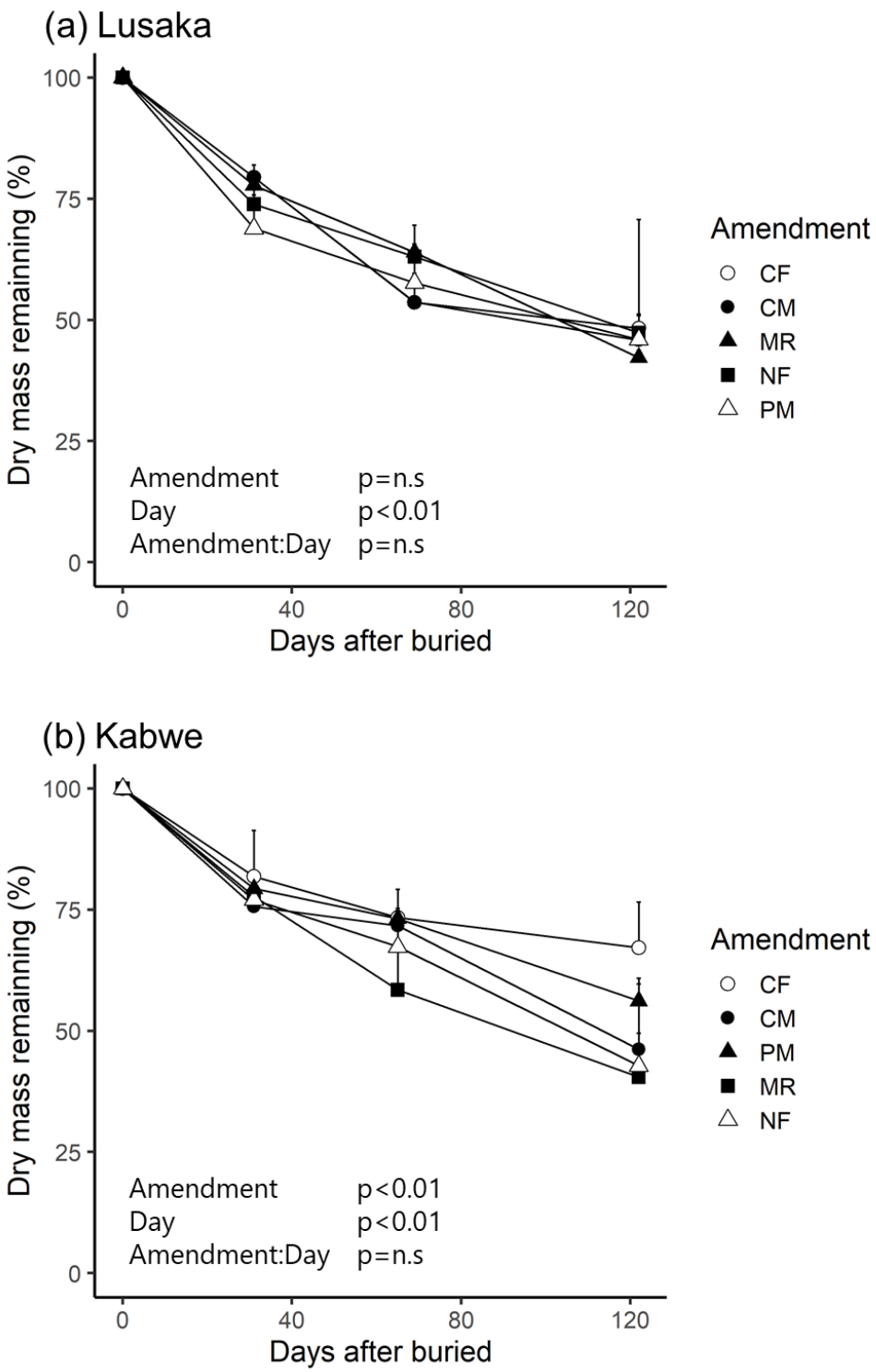

Figure 5.4. The time course changes of litter bag decomposition rate at (a) Lusaka site and (b) Kabwe site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

5.4.3 Soil bacterial abundance

At Lusaka site, the effects of the organic amendment types on the abundance of soil bacteria were unclear (Fig. 5.5a). In contrast, there was a significant interaction between organic amendment types and time course at Kabwe site ($p < 0.05$, Fig. 5.5b). The CM and MR treatments had higher bacterial abundance throughout the experimental period, particularly 52 days onwards after the organic amendment applications. Also, bacterial abundance showed continuous increase within MR treatment during the experimental period.

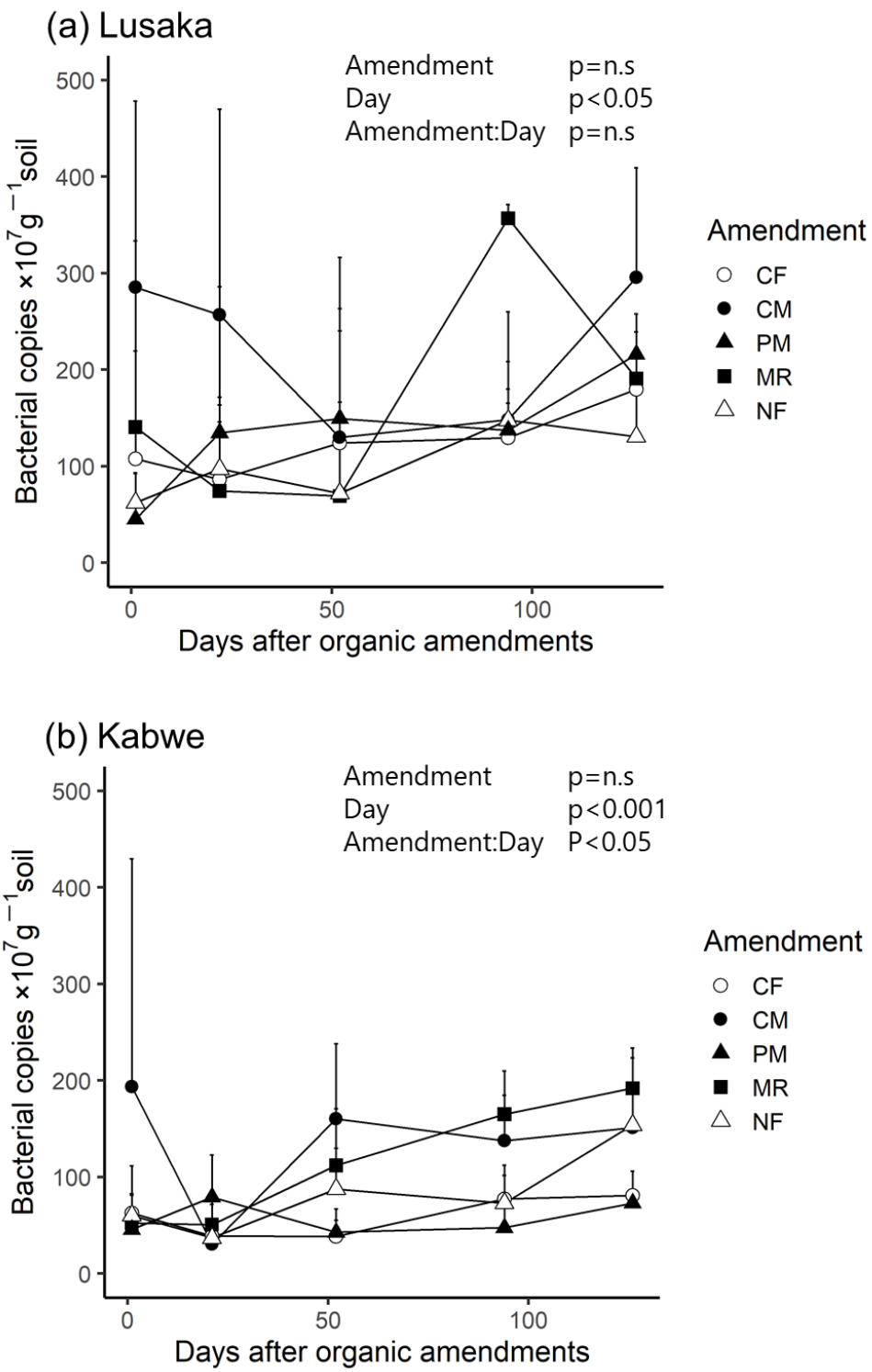

Figure 5.5. The time course changes of bacterial abundance at (a) Lusaka site and (b) Kabwe site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

5.4.4 Bacterial community structures

The microbial taxonomic composition of different fertilizer treatments and days, summarized at the class level, showed a total of 63 phyla and 196 classes (Archaea and Bacteria domains). *Actinobacteria* or *Chloroflexi* were either the first or second largest dominant phyla at both Lusaka and Kabwe sites, when averaged across the treatments (Fig. 5.6 and 5.7). The predominant *Actinobacteria* subgroups (more than 1% of mean relative abundance) were *Actinobacteria* and *Thermoleophilia* at both sites. The predominant *Chloroflexi* subgroups were *Chloroflexi*, *Ellin6529*, *Gitt.GS.136*, *Thermomicrobia*, and *TK10* at Lusaka site. Contrastingly, at Kabwe site, the predominant *Chloroflexi* subgroups were *Chloroflexi*, *Ellin6529*, *Thermomicrobia*, and *TK10*.

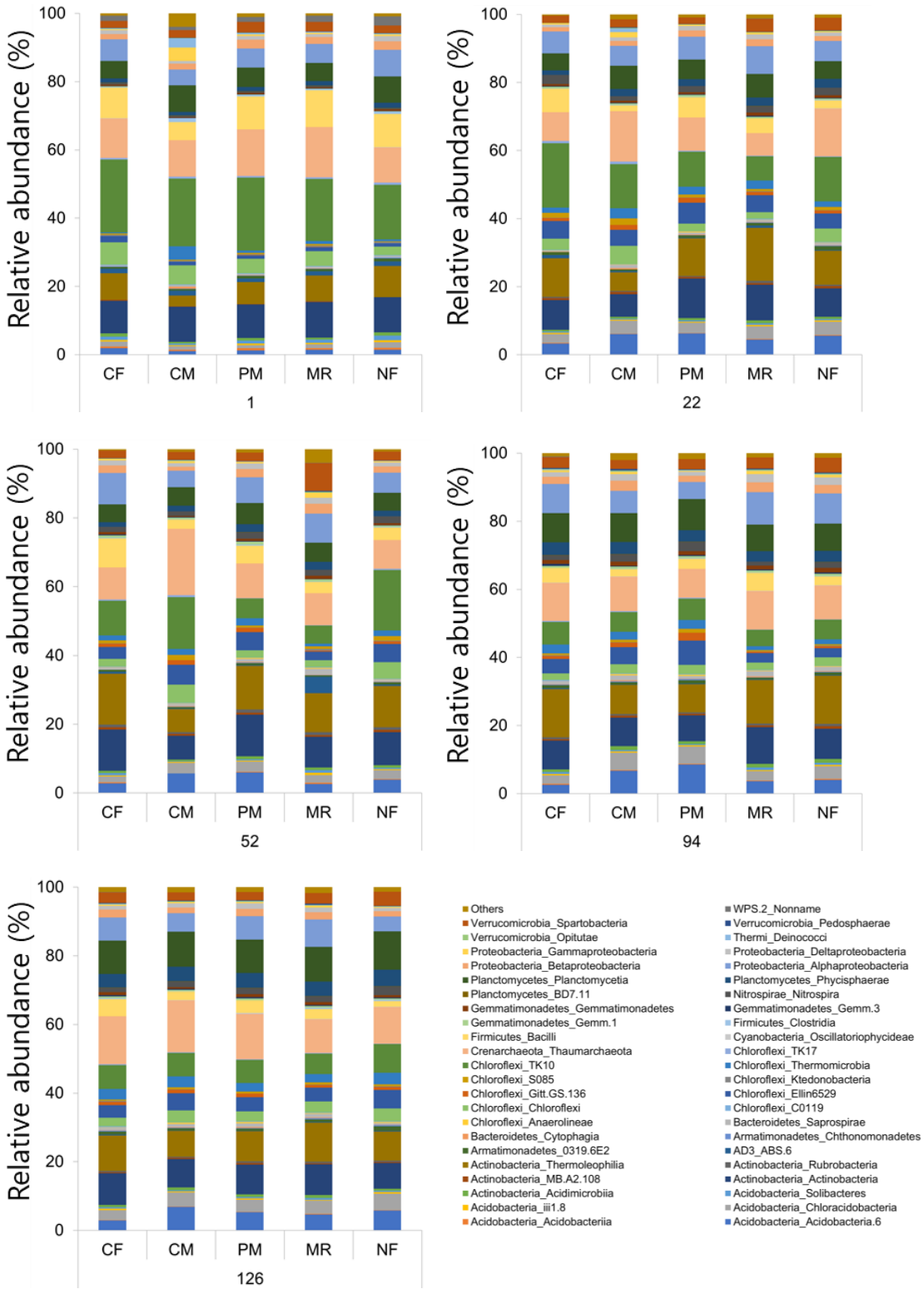

Figure 5.6. The time course changes of relative abundance of soil microbes at Lusaka site. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

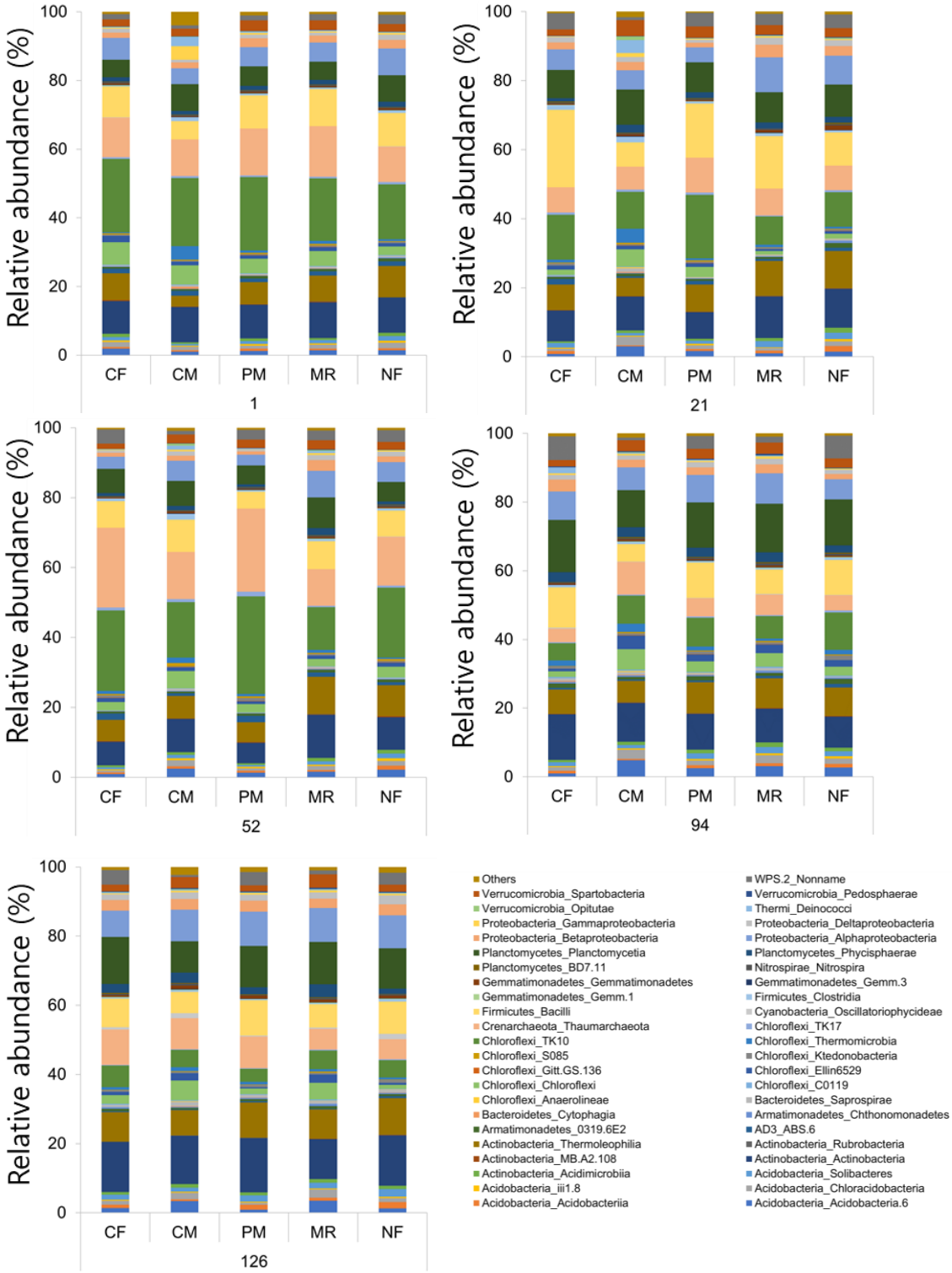

Figure 5.7. The time course changes of the relative abundance of soil microbes at Kabwe site. CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment

The organic amendments had a significant positive effect on soil bacterial diversity at the class level at Kabwe site, but not for the Lusaka site (Fig. 5.8). The soil bacterial diversity indices in CM and MR treatments were higher throughout the experimental period compared to the other treatments, particularly 21 ($p < 0.05$), 52 ($p < 0.05$) and 126 ($p < 0.01$) days after planting.

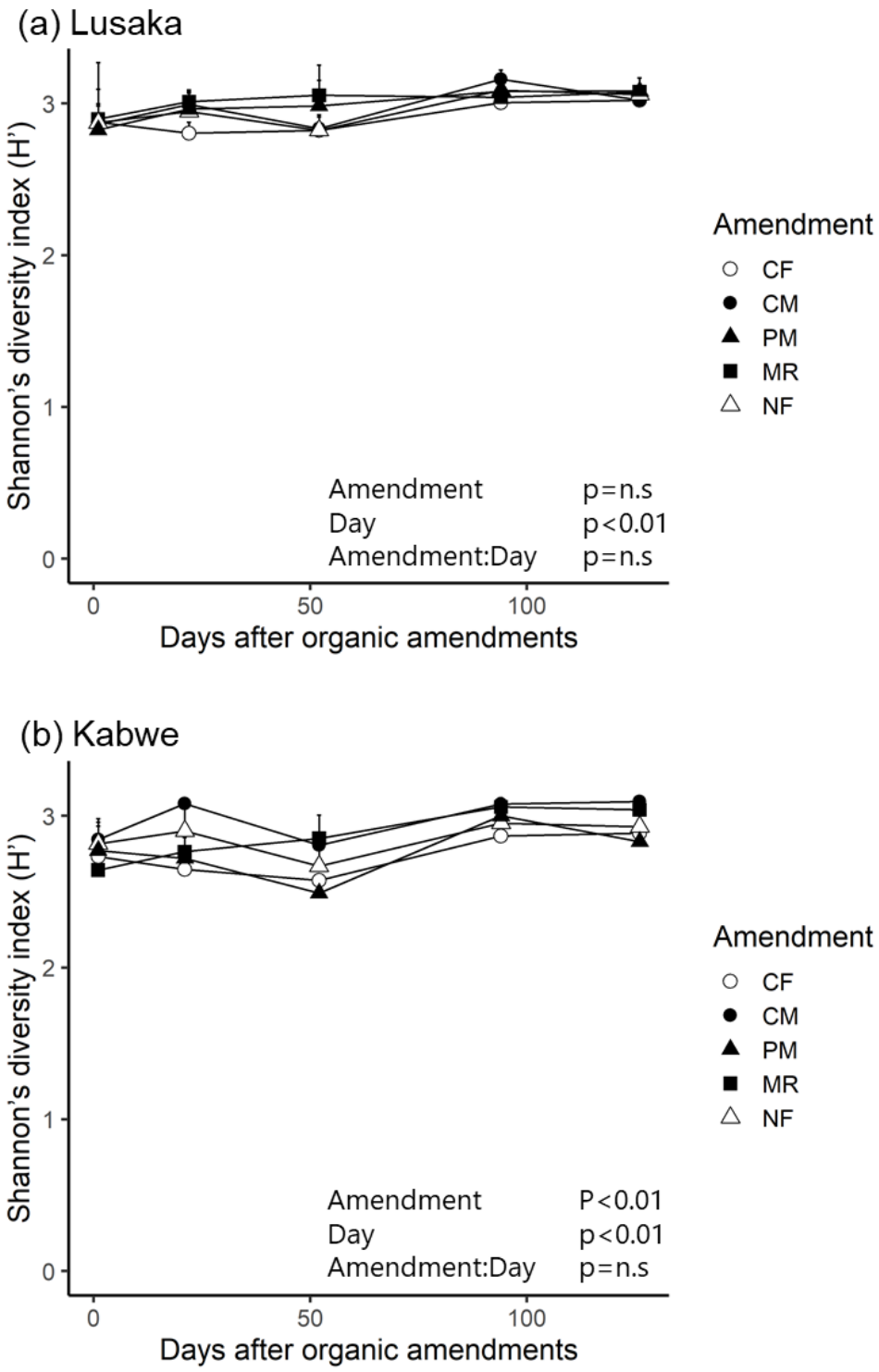

Figure 5.8. The time course changes of bacterial diversity at class level at (a) Lusaka site and (b) Kabwe site. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

Fig. 5.9 shows the richness and evenness at class level at both sites. The results showed the significant increase in richness due to organic amendments at Lusaka site ($p < 0.05$), while there was a significant effect of fertilizer treatments on bacterial evenness at Kabwe site ($p < 0.01$).

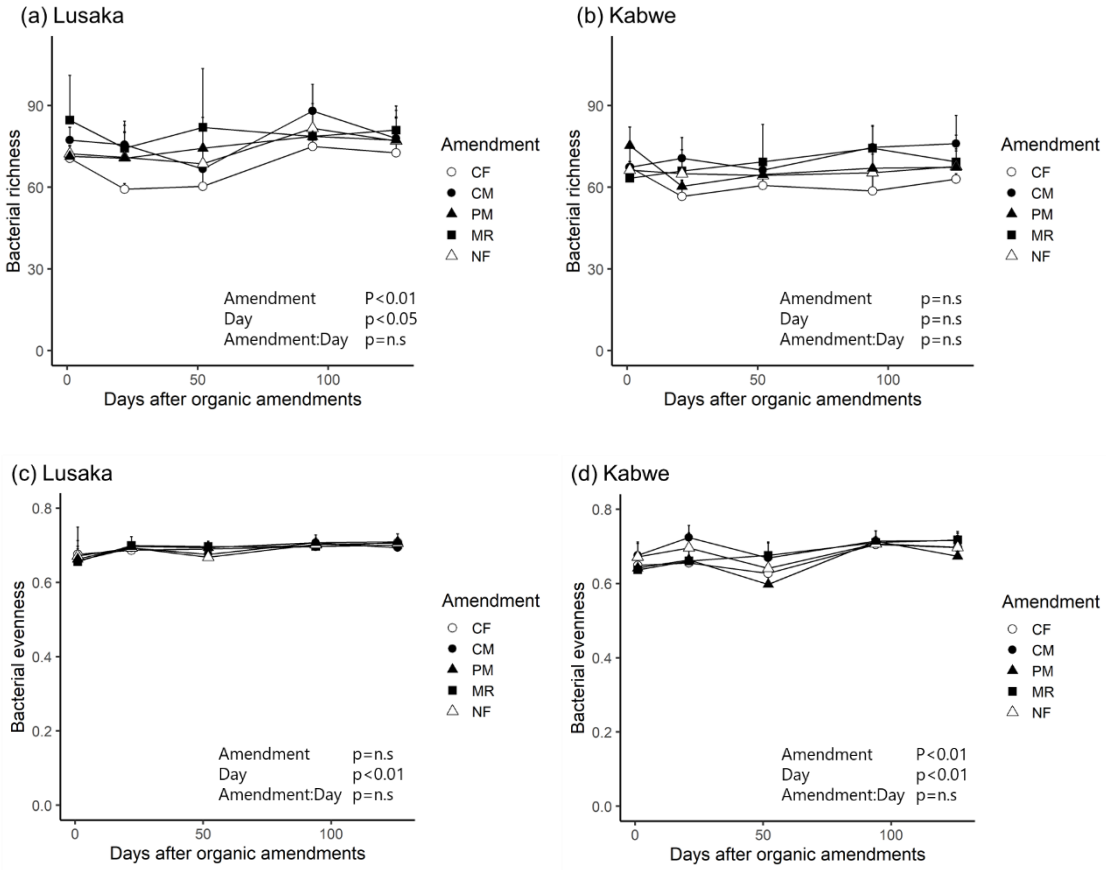

Figure 5.9. The time course changes of (a and b) bacterial richness and (c and d) bacterial evenness at class level at both sites. Level of significance was determined by and two-way RM-ANOVA. Error bars represent standard deviation (n = 3). CF: chemical fertilizer amendment, CM: cattle manure amendment, PM: poultry manure amendment, MR: maize residue amendment, and NF: no fertilizer amendment.

5.5 Discussion

5.5.1 Factors controlling CO_2 emissions from organic amendments

At the Lusaka site, soil CO_2 emission rates were higher throughout the experimental period, compared to the Kabwe site. This was due to higher C availability in the soils at Lusaka, when compared to the Kabwe site. The soils at the Lusaka site also had relatively higher silt and clay contents when compared to the Kabwe site, thus it had a higher capacity to sequester C (Caravaca and Roldán, 2003; Wang et al., 2003; Matus et al., 2007). Clay content is positively correlated with the capacity of soils to sequester C, both through physical protection in aggregates and by chemical stabilization to mineral surfaces (Six et al., 2002; Gentile et al., 2010; Rosenstock et al., 2016).

However, the positive response of CO_2 emissions by organic amendments, compared to the non-amended soils, was not observed at the Lusaka site. Mapanda et al. (2011) also observed that cattle manure with inorganic-N fertilizer did not increase CO_2 emissions in sandy loam soils in Zimbabwe. This might be due to soil moisture limitation. At the Lusaka site, CO_2 emissions were more strongly correlated to soil moisture, compared to the Kabwe site. The positive impact of soil moisture on CO_2 emissions has previously also been observed in African region (Mapanda et al., 2010; Sugihara et al., 2012). Thus, at the Lusaka site, C was available for microbial respiration even for the control site and the microbes actively respire the available C when soil moisture is optimum.

Contrastingly, in Kabwe soils, with relatively higher sand contents, the organic amendments significantly increased CO_2 emission rates. This might be due to low C availability in the control soil (Caravaca and Roldán, 2003) or the relatively lower C immobilization capacity in loamy sand soils (Kabwe) than sandy loam soils (Lusaka) (Vinten et al., 2002). Sugihara et al. (2012) also showed the lower efficiency of soil C stock derived from organic amendments in sandy soils (> 90% of added C was respired), compared to clay soils in sub-Sahara Africa.

Additionally, under tropical conditions, the use of inorganic fertilizer induces a faster decomposition of added organic amendments (Goyal et al., 1999).

At Kabwe site, cumulative CO_2 emissions were markedly variable by the types of organic amendments, even though the added amount of C was the same. For example, the PM treatment did not significantly increase CO_2 emissions, compared to the control, within the Kabwe soils. Poultry manure used in the current research had a relatively higher C concentration than other organic materials. Thus, C might be more recalcitrant in applied PM amendment, compared to others, reducing the decomposition rates and the consequent C emissions. Another reason can be because of the smaller application rate (weight) of PM, compared to other organic amendments. In the current study, the application rates of the organic amendments were adjusted using the amount of C. The bulk weight of organic amendments varied across the treatments, the lowest was the PM among all the treatments (approximately 15, 4.5, 20 t ha^{-1} of CM, PM and MR application rate, respectively). Thus, the relatively smaller gravimetric amount of PM applied reduced the contacts between soil microbes to PM and did not result in the increased CO_2 emission rates, in the Kabwe soil.

5.5.2 Interaction with decomposition rate and organic amendments

Similar to the trend of CO_2 emissions, the decomposition rates, calculated based on the litterbag experiment, were positively influenced by organic amendments in Kabwe, but not in Lusaka. This might be due to soil texture and microbial differences between the two soils. No significant effect of soil texture on litter bag method is also reported previously (Scott et al., 1996; Li et al., 2019), and it may be due to limited contact between clay minerals and organic materials (Baumann et al., 2009). In fact, the current study showed the similar litterbag decomposition rates between NF (control) treatment from the two sites. Thus, the different decomposition trends following different fertilizer treatments at Kabwe site might be due to microbial alteration related to the nutrient inputs to soils (Kwabiah et al., 1999). Particularly in the Kabwe

site, the use of chemical fertilizer without organic amendments (CF) decreased decomposition rates. Similarly, previous studies concluded that the single use of inorganic fertilizer lowered microbial diversity and activities (e.g. dehydrogenase activity) related to decomposition process, compared to combined use of chemical fertilizer and organic amendments (Dhull et al., 2004; Zhong et al., 2010). Thus, we note that organic amendments are critically important to maintain soil microbial ability to decompose organic matter, but this might be relevant only in low C sandy soils, similar to the Kabwe soil.

5.5.3 Effects of organic amendments on soil microbial abundance and community

We observed similar dominant bacteria (*Actinobacteria* and *Chloroflexi*) at both Lusaka and Kabwe sites. Those dominant phyla are called ancient phyla (Battistuzzi and Hedges, 2009) and represent drought stressed community in particularly C depleted soils such as sub-Sharan Africa (Delgado-Baquerizo et al., 2016; Zhou et al., 2016; De la Cruz-Barrón et al., 2017; Santos-Medellín et al., 2017; Hamamoto et al., 2018; Dube et al., 2019).

The increase of bacterial abundance and diversity by the organic amendments was not clear in the Lusaka soil. This result might be due to no significant effect of organic amendments on CO_2 emissions at Lusaka site. However, bacterial richness was increased by organic amendments. Nielsen et al. (2011) reviewed that the increase in species have no effect on ecosystem function related to soil C cycling, after a certain level of richness has been reached (Asymptotic form). Thus, we suggest that this increase in the richness does not show enhanced decomposition process due to soil bacterial community already reached to a certain level of the richness. We also note that soil faunal abundance in the Lusaka site was higher, compared to the Kabwe site by using pitfall traps (data not shown). Soil fauna might have played a key role in organic decomposition (e.g. physical breakdown) in Lusaka (Ouédraogo et al., 2004). This possibility suggests that the applied C in the Lusaka site was less recalcitrant than the Kabwe site, and it is

more susceptible for the loss as CO_2. Thus, the organic amendments might not be expected to be a sole method to improve/maintain soil C at the Lusaka site. Further studies are needed to understand interactions among soil biological community (faunal-microbial interaction) and soil C dynamics after organic amendments.

Contrastingly, cattle manure and maize residue amendments significantly increased the bacterial abundance, diversity, and evenness at the Kabwe site towards the end of experimental period. These results are consistent with a previous study conducted in loamy sand soils, in which the 16S rRNA gene abundance increased in organic farm by 80%, compared to conventional farm (Aparna et al., 2014). Also, Kamaa et al. (2011) found that the continuous combination with organic and inorganic fertilizers in Kenya increased soil bacterial diversity, compared to single use organic or inorganic fertilizer. Organic amendments are decomposed in the soil, and then the continuous release of nutrients from the organic amendments can sustain the microbes for longer time, when compared inorganic fertilizers (Murphy et al., 2007). Thus, the combined use of organic and chemical fertilizer might be favor condition for soil microbial decomposition.

There is another possibility that such increased microbial diversity and abundance improve decomposition rate of added organic materials because the Kabwe soil had a relatively higher sand content. Sandy soils have less ability to protect added C against microbial decomposition, when compared to soils with higher clay contents (Chivenge et al., 2007). Our result from the Kabwe site also showed that the increase in the bacterial abundance was correlated to the alteration in the litterbag decomposition processes (MR ≈ CM ≈ NF > CF ≈ PM). Thus, organic amendments altered soil bacteria community and their community became more suitable to decompose complex organic substances. Previous studies stated that long-term organic amendments in sandy soils in tropical arid region have a potential to increase soil C with increase in the microbial diversity (Dhull et al., 2004; Chivenge et al., 2007; Aparna et al., 2014). Therefore, organic amendments have a large potential to sequester soil C in the Kabwe

site, along with the diversification of the microbiome. However, we emphasize that the rate of C sequestration depends on the capacity of the soil to protected C.

5.6 Conclusion

We conducted a field experiment using organic amendments in a sandy loam soil and a loamy sand soil in Zambia. Changes in soil CO_2 emissions, bacterial abundance and community structures were monitored for 126 days. The organic amendments increased CO_2 emissions, bacterial abundance and diversity only in the loamy sand soil, particularly cattle manure and maize residue amendments. These findings indicate that in the loamy sand soil, organic amendments have a large potential to sequester soil C in the Kabwe site, along with the diversification of the microbiome, although the rate of C sequestration depends on the capacity of the soil to protected C. In sandy loam soils, Contrastingly, abiotic factors controlled the impacts of organic amendments on microbial activities. Further study will be required to determine the efficiencies of the organic amendments to improve soil C contents in this area.

Chapter 6

Synthesis and recommendations for further research

This chapter synthesises my research and identifies emergent opportunities for advancement by further research.

6.1 Overall summary and future work

6.1.1 The role of different earthworm species (*Metaphire hilgendorfi* and *Eisenia fetida*) on CO_2 emissions and microbial biomass during barley decomposition

This study used two different earthworm species (*Metaphire hilgendorfi* and *Eisenia fetida*). The objective of this study was to investigate how those two different earthworms influence CO_2 emissions from soils and soil microbes during the decomposition of an organic material, namely barley. The soils were incubated in the laboratory under a constant moisture status. After 32 days incubation, CO2 emissions were not influenced by the presence of earthworms, when the soils with barley were compared. Thus, the earthworm presence did not alter the soil respiration. However, soils with *M. hilgendorfi* had a significantly higher soil microbial biomass C (MBC, 2.9 g C kg^{-1} soil), but lower water-soluble C (WSC, 154 mg C kg^{-1} soil), compared to soils with *E. fetida* (2.5 g C kg^{-1} soil and 202 mg C kg^{-1} soil for MBC and WSC, respectively). Thus, the earthworm intervention to soils potentially influence C immobilization and mineralization process, depending on earthworm species: *M. Hilgendorfi* have more potential to immobilize C to soils, compared to *E. fetida*. However, this incubation experiment was not able to detect changes microbial enzyme activity related to nitrogen (N) cycle due to those earthworms. The activity of earthworms has a key role in nutrient dynamics but "heterogeneous" (section 2.2.2.2). Particularly, soil microbial growth is stimulated in the drilosphere, and consequently improve the growth of soil fauna (Stromberger et al., 2012). In

our study, soils were homogenized when those parameters are measured. Thus, further experiments will be needed to divide into each part such as bare soil and drilosphere to reveal the effects of earthworms and soil C dynamics.

Additionally, we did not observe significant effect of earthworms on soil CO_2 emissions from soils. There is possibility that CO_2 emissions derived from earthworms are negligible particularly in soils with higher C contents. Thus, in future work, it would be interesting to rerun a similar experiment but include soil C gradients.

6.1.2 The effect of organic amendments on soil fauna community and crop production in Zambia

This study was conducted to investigate the impacts of organic amendments on aboveground agricultural ecosystems in two different soils in Zambia. Chapter 3 showed that the earthworm activity has a potential to increase soil C sequestration. In addition to earthworms, many previous studies indicate that soil fauna diversity use as environmental indicator of agricultural production (section 2.3.2.3). Various soil fauna lives and interacts each other at the field level. However, particularly in sub-Saharan Africa, there is a lack of knowledge regarding the soil faunal community in agricultural soils. Basic information is still required to understand the impacts of agricultural management on soil faunal community. At the present, the use of organic materials is increasing in Africa, although crop yield has stagnated since 1960s. Thus, our field experiment focused on the effects of organic amendments on aboveground ecosystems in Zambia.

It was concluded that the organic amendments improve both crop production and aboveground biodiversity at the Lusaka site, but not Kabwe site. One of the reasons for site-dependent variable effects were soil fauna abundance. The soil fauna abundance was completely different at the Lusaka and Kabwe site; around 5 times higher individuals in Lusaka site than that in Kabwe site. Such differences can largely affect decomposition process. This may also have been

due to the history of land managements. However, the current study does not show how soil fauna gradually degrades abundance due to conversion from natural forest to crop lands, and agricultural method (e.g. tillage). It will be interesting to study changes in the soil fauna community and abundance in terms of agricultural developments and managements.

In tropical or semi-arid region, many previous studies have been shown that termites gave a large impact on the decomposition of organic amendments (section 2.2.1). Additionally, further research needs to investigate other soil fauna such as belowground soil faunal community (e.g. earthworm) as shown above for Chapter 3.

The morphological identification of soil fauna needs to consume time and usually requires significant taxonomic expertise to characterize due to their diversity (e.g. Smith et al., 2008; Yu et al., 2012; Oliverio et al., 2018). Thus, to effectively understand whole soil fauna community, extracted DNA from soils and analysing the DNA by metabarcoding is currently developed, while those techniques have been widely applied to investigate soil microbial communities (e.g. Fløjgaard et al., 2019). Also analyses of stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) is suitable technique to understand trophic interactions of soil fauna (e.g. Pollierer et al., 2009). The combined measurement of $\delta^{15}N$ and $\delta^{13}C$ provided insights into the compartmentalization of the soil fauna food web. The following questions need to be answered: (1) What is the relationship between changed soil fauna community due to organic amendments and C dynamics?; and (2) did organic amendments make more complex food web or temporal increase in soil fauna abundance due to just fresh C source input?

6.1.3 The impacts of organic amendments on bacterial community structures and carbon cycle in two contrasting Zambian agricultural soils

In addition to soil fauna community and crop production, organic amendments stimulate soil microbial activity as outlined above (section 2.3.2.4). In this study, the effect of organic

amendments on soil microbial community and CO_2 emissions in two different soils in Zambia. Given the result of Chapter 4 and 5 would be interesting to enhance our understanding of soil C dynamics when applied organic materials in Zambian soils.

Organic amendments increased CO_2 emissions as well as soil bacterial diversity at the Kabwe site. The data obtained previously in Chapter 4 from the Kabwe site showed that the soil fauna abundance was lower compared to the Lusaka site. The results also showed that the decomposition rate and bacterial abundance were positively related. Therefore, those results indicate the microbial activity is strongly correlated to the decomposition processes in Kabwe. Contrastingly, at Lusaka site, which had relatively higher soil fauna abundance, compared to the Kabwe site, there was no significant effect of organic amendments on soil microbial composition and CO_2 emissions. Thus, the results show that the organic amendments at the Lusaka site potentially stimulate faster C loss due to altered activities of decomposer food web (mainly macro-fauna communities). Future research needs to measure decomposition rates, considering the interactions among soil fauna and soil microbes.

In addition to soil bacteria, fungi are also the main drivers of the soil C dynamics including decomposition and monetarization (Moore et al., 2003). For example, saprotrophs and mycorrhizal species of fungi generally decompose organic matter. Elfstrand et al. (2007) also found higher fungi /bacteria ratios in soils receiving organic materials. Those changes in microbial community structures in turn have important implications for the SOC mineralization.

6.2 General comments

- In Chapter 3, the residues used were straw and old roots. Thus, it was needed to re-run similar experiment considering different types of residue materials. Many of the parameters studied also did not respond significantly, presumably reflecting the limitations in the experimental setup. Also, the soils used in Chapter 3 was relatively

fertile soils when compared to in Chapter 4. Such poor soils or soils along soil C gradients will be required to synchronize all of experiments.

- In Chapter 4 and 5, the experiments conducted in Zambia were constrained by the other soil fauna because I used insecticide against for termites and worms. Also, I focused on aboveground soil fauna but not belowground soil fauna due to tillage management. I collected soil fauna using pitfall traps every two weeks, although generally around one week is the favour sampling interval and yields grater capture rates of arthropods compared whit longer sampling intervals. Future studies need to consider these issues.
- The field experiment in Chapter 4 and 5 performed using two soils and different time scale. However, the soil microbial analysis was conducted using air-dried soil, which did not represent soil condition at the field. Also, in general, most of previous field-scale studies have been shown that long-term (at least more than 1 year) effect of organic amendments. This was because there was no equipment related to such analysis and no well managed lands in our experimental fields in Zambia. Thus, further work can be performed by developing the experiment, to investigate the continuous effect of organic amendments on soil microbial community.

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