# Greening with Black

## Biochar-Soil amendment for low-emission agriculture

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**Greening with Black: Biochar-Soil Amendment for Low-Emission Agriculture**

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# CHAPTER ONE: General Introduction

## 1.1 Climate Change, Agriculture and Food Security

Climate change mitigation is a top priority goal of the 21st century. Whereas climate change affects agriculture through changes in temperature and the amount and variability of rainfall, agriculture affects climate change through alteration in soil cover and emission of greenhouse gasses. This interconnectedness between agriculture and climate change poses a significant global contest when addressing the food security challenge. Ultimately, addressing the food security challenge implies dealing with the feedbacks between climate change and agriculture. Although climate change is predicted to affect food security in many parts of the world, its effect is estimated to be greater in the already vulnerable farming communities in sub-Saharan Africa (SSA). The impact of climate change therefore has wide ramifications on the livelihood to many people. Addressing climate change is therefore a prudent way to ensure livelihood sustainability in Africa.

## 1.2 Agriculture and greenhouse gas emissions

Increase in anthropogenic greenhouse gas (GHG) concentrations between 1951 and 2010 are the most likely cause of rise in mean surface temperature globally (Bindolf *et al.,* 2013). Estimates from the Intergovernmental Panel on Climate Change (IPCC, 2014) show that in 2011, the concentrations of carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) were, respectively, 40%, 150%, and 20%, higher than the amounts before the industrial revolution. The concentrations of these gases have all increased since 1750 due to human activity (IPCC, 2014). Agriculture contributes 24% of total greenhouse gas emissions (Ciais *et al*. 2013), and is the leading contributor (56%) of non-carbon dioxide greenhouse gasses ($CH_4$

and $N_2O$) (IPCC, 2014). The major sources of agricultural emissions are the heavy use of nitrogen fertilizers, cultivation of rice paddies, and emissions from livestock. Whereas agricultural soils contribute to GHG emissions, they can also mitigate climate change through direct and indirect GHG abatement such as sequestration of carbon (C) as soil organic matter (SOM), depending on the management practices.

According to Ciais *et al*. (2013), anthropogenic $N_2O$ emissions are 1.7 to 4.8 TgN ($N_2O$) $yr^{-1}$ from the application of nitrogen fertilizers in agriculture. In highly-weathered low-fertility soils of the humid tropics of sub-Saharan Africa, continuous use of nitrogen fertilization and supplementation with green manure is common practice, and is an important soil management strategy (Mucheru-Muna *et al.,* 2007). However, the use of these amendments risks increasing $N_2O$ emissions due to decomposition of organic matter or the provision of excess mineral nitrogen to support denitrification (Jarecki et al., 2009; Peterson and Sommer, 2011). Therefore, climate-responsive innovations that increase food production while sequestering soil carbon and reducing $N_2O$ are needed to reduce the impact of agriculture on the climate system.

### 1.3 Biochar and greenhouse gas emissions from agricultural soils

Biochar has been proposed as a climate-smart strategy to address the Climate-Agriculture nexus. Several studies have shown that biochar can improve soil fertility (through nutrient additions, improved aeration and moisture storage, reducing leaching of nutrients through leaching, improving nutrient uptake by plants), increase soil carbon sequestration and reduce emission of $CH_4$ and $N_2O$ from agricultural soils (Zhu et al., 2014; Ding et al., 2016). Owing to the wide variability in these properties, the effects of biochar remain inconsistent and therefore inadequate to make robust recommendation for soil management. Whereas some

studies report mitigation of GHGs from soils following biochar amendment, others report increased emissions. Moreover, the mechanisms underpinning the response remains poorly understood. Further investigations are needed to elucidate the biogeochemical mechanisms responsible for observed disparity in emissions responses in biochar amended soils.

## 1.4 Mechanisms and controversy surrounding biochar effects on GHGs in soils

### 1.4.1 Methane

Several studies have shown that biochar can reduce $CH_4$ emission from soils as well as increase oxidation of atmospheric $CH_4$ (Zhang et al., 2010; Yoo and Kang, 2011; Case et al., 2014), suggesting that it can be a good management option to reduce methane concentrations in the atmosphere (Liu *et al*., 2014; Schimmelpfennig *et al.*, 2014). At the same time, several other studies report the opposite, i.e. increased emissions of $CH_4$ from biochar-amended soils (Spokas, 2013; Singla and Inubushi, 2014), thereby raising questions as to whether biochar increases or mitigates atmospheric $CH_4$.

Soil-atmosphere exchange of $CH_4$ in biochar amended soils is determined by the interaction of several factors, including biochar properties (i.e., feedstock, pyrolysis temperature and quantity) and soil properties (soil texture, soil organic matter content, soil pH, and soil moisture) (Lehmann *et al*., 2011; Castaldi *et al*., 2011; Feng *et al*., 2012; Fungo *et al.,* 2014). Some of the mechanisms proposed include: (i) increased diffusivity of the biochar-amended soil together with reduced (buffered) fluctuations in soil moisture (Karhu *et al*., 2011), (ii) adsorption of $NH_3$ thereby lowering the $NH_4^+$ concentrations (Taghizadeh-Toosi *et al*., 2012); (iii) changes in the Archaeal methanogenic or bacterial methanotrophic community and activity (Feng *et al*., 2012; Khan *et al*., 2013).

Given the conflicting information in the scientific literature, it is unclear how the various factors, alone and combined, produce the observed effect. There is a need for an assessment in order to improve our understanding of the dominant factors (van Zwieten *et al.*, 2015). To put the study in context, we highlight the principle processes and organisms responsible for production and consumption in the soil, and how introduction of biochar can affect them.

***$CH_4$ production and consumption in soils***

Atmospheric $CH_4$ results from either biogenic, thermogenic or pyrogenic sources (Ciais *et al*., 2013). Biogenic fluxes arise largely from the biological process in which organic matter decomposition by specialized bacteria under anaerobic conditions (Ciais *et al*., 2013). Two groups of micro-organisms present in the soil perform the two principle processes of methane production (methanogenesis) and consumption (methanotrophism) (Hanson and Hanson, 1996; Frarrel *et al*., 2013a).

Methanogenic *Archaea* (Methane-producing microbes) is a phylogenetically diverse group within several unique features, including acting as cofactors and unusual membrane lipids (Bratin *et al*., 1992; Hanson and Hanson, 1996). Methanogenesis happens only in the absence of oxygen, or where predominantly reducing conditions prevail, with no oxidizing agents such as $Fe^{2+}$, $NO_3^-$, or $SO4^{2-}$. Methanogens are also highly sensitive to oxygen, temperature (about 20–40°C), and pH (4.0–8.0) (Serrano-Silva *et al*., 2014). Methanogens are obligate methanogenic deriving energy from the reduction of more oxidized forms of carbon atoms (such as methanol, acetate or methylamine; Cassidy *et al.,* 1996) to form methane and transform carbon dioxide to methane. Furthermore, methanogens utilize a very narrow range of substrates and some can even fix $N_2$ (Jones, 1998; Frarrell *et al*., 2013b).

Methanotrophs (Methane-oxidizing bacteria) are ubiquitous, phylogenetically and physiologically diverse (Bratin *et al*., 1992; Hanson and Hanson, 1996). They are obligate methanotrophic and cannot use other substrates (Topp and Hanson, 1991). Methane is oxidized to carbon dioxide through the intermediate Using methanol, formaldehyde, and formate sequential providing energy for Methanotrophs using formadehyde and the primary source of carbon and O2 and the terminal electron accepter (Zheng et al., 2014). The enxyme Methane monooxygenase is the responsible catalyst for this reaction (Hornibrook, *et al*., 2009). According to Schnell and King (1995), soils can consume methane as long as they are sufficiently porous to allow diffusion of oxygen to take place. Methanotrophs utilize various aliphatic and aromatic molecules, and are sensitive to many of the same inhibitors (Frarrell *et al*., 2013b).

***Effects of biochar on $CH_4$ in the soil***

Following the addition of biochar to the soil, a myriad of physical-chemical and biological processes acts interactively to determine the amount and rate of methane fluxes at the soil-atmosphere interface. A simplified model of the critical relationships between biochar and $CH_4$ fluxes is represented in Figure 1.1. In brief, the soil environment can be divided into three components; (1) The microbial community – performs a sequence of biological processes resulting in the production or consumption of $CH_4$; (2) The soil matrix (soil-biochar surfaces) – regulates the supply of substrates, toxins, and reaction surfaces for chemical species and microbes; and (3) The soil micro-environment – mainly soil solution and air, controls the movement of ions and molecules within the soil, and gas exchange between the soil and the atmosphere.

Processes affecting $CH_4$ fluxes that can arise from the addition of biochar into the soil include the following: (i) Biochar may contain a number of organic molecules some of which may be utilized by methanogens, including highly specific one such as acetates, alcohols and methylamines (Frarrel *et al*., 2013); (ii) Biochar is thought to offer better protection of microbes against predators because of its pore structure (Saito and Marumoto, 2002; Thies and Rillig, 2009; Lehmann *et al*., 2011); (iii) Biochar may remove organic molecules that could be toxic to microorganisms, such as phenols (De Luca *et al.,* 2009) or produce others such as ethylene (Spokas *et al*., 2010); (iv) Encapsulation of soil organic matter, including microbes, may reduce decomposition and mineralization or protect microbes from predators; (v) Complexation and/or chelation between biochar surfaces (phenolic hydroxyl and acid groups) and essential trace metals can affect the availability and reactivity of methanogenic as well as methanotrophic activities (Baldock and Smernik, 2002; Bolan *et al*., 2012; Glaser *et al*., 2000); (vi) Surface adsorption on biochar may reduce the toxicity of free ammonium to methanotrophs (Berglund *et al*., 2004; Lee *et al*., 2005; Lehmann *et al*., 2006). The observed response in the above-mentioned processes is a function of the quality of biochar, soil properties and the soil micro-environment (Van Zweiten *et al*., 2015).

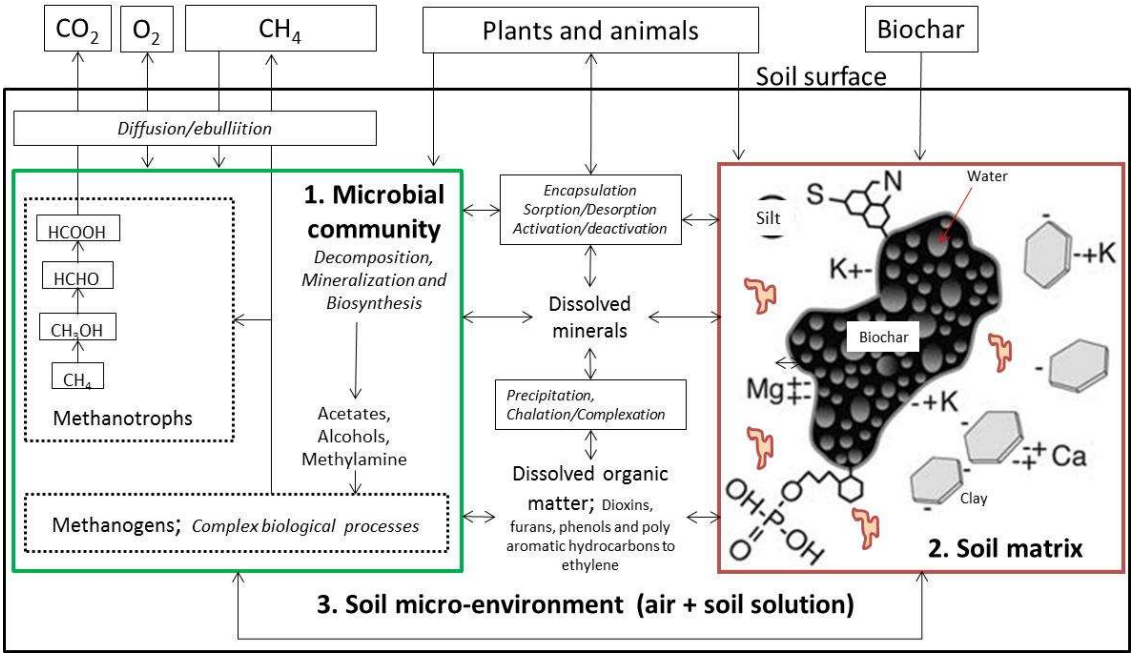



Figure 1.1: Conceptual framework for relationships between biochar and $CH_4$ fluxes in soil

### 1.4.2 Nitrous oxide

*$N_2O$ production in soils*

$N_2O$ originates mainly from agricultural soils through nitrification and denitrification. Nitrification is a two-step process and includes: (i) the oxidation of $NH_3$, and (ii) the oxidation of $NO_2^-$ (Subbarao et al., 2006). Step (i) is rate-determining, and is mediated by autotrophic ammonia–oxidizing prokaryotes while conversion of $NH_3$ to hydroxylamine is catalyzed by ammonia monooxygenase in autotrophic oxidation of $NH_3$ (Francis et al., 2007; Hayatsu et al., 2008). In step (ii), hydroxylamine oxidoreductase oxidizes hydroxylamine to $NO_2^-$. During ammonia oxidation, $N_2O$ is always emitted (Poth and Focht, 1985).

Denitrification involves sequential bacteria-mediated steps that start with reduction of $NO_3^-$ or $NO_2^-$ to NO, then to $N_2O$, and finally to $N_2$ (. Each step is catalyzed by highly specialized reductase enzymes that include, respectively, nitrate reductase, nitrite reductase, nitric oxide

reductase, and nitrous oxide reductase (Lashof and Ahuja, 1990; Kim and Bruce, 2008). The catalytic action of nitrite reductase in reducing $NO_2^-$ to NOx, is crucial in the denitrification process since it results in production of $N_2O$ as a product (Zhang eta al., 2009). The rate of $N_2O$ production in the above step is controlled by several factors, including concentration of ($NO_3^-$ and $NH_4^+$), soil temperature, organic carbon content, and aeration (Farquharson and Baldock, 2008; Kim and Bruce, 2008).

*Effect of biochar on $N_2O$ production*

The mechanisms for reduction of $N_2O$ emission revolve around the following two fundamental principles; (i) inhibition of $N_2O$-forming processes (denitrification, nitrification and nitrifier denitrification), and (ii) Inhibition of the reduction of $N_2O$ to $N_2$ by nitrous oxide reductase ($N_2O$-R) and complexation of nitrous oxide by soil/biochar particles. Theoretically, these processes can take place simultaneously in the soil. Biochar addition to soil can thus influence some of these processes that contribute to the fluxes of $N_2O$ (Fig. 1.2). The potential effects of biochar on $N_2O$ emission are summarized in Table 1.1.

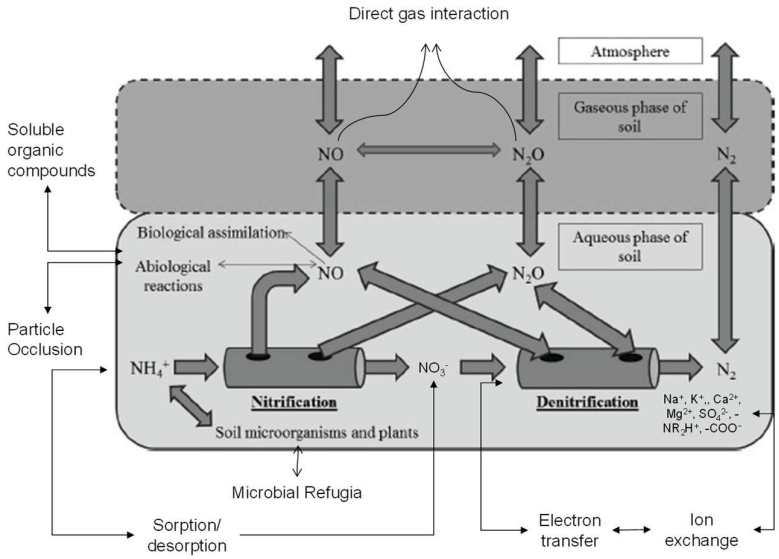



Figure 1.2: Processes hypothetically influencing $N_2O$ emission from soil after addition of biochar

Table 1.1: Processes and mechanisms for the effects of biochar on emission of $N_2O$ in soils

| **Biochemical process** | **Reaction mechanisms** | **Citation** |
|---|---|---|
| Organic N mineralization | • Retention of inorganic N on biochar surfaces through surface adsorption and microbial immobilization | Berglund et al., (2004); Novak et al., (2010); Bruun et al., (2011); Dempster et al. (2011); Knowles et al., (2011); Ippolito et al., (2012); Nelissen et al., (2012), Nelissen et al. (2015); Nelissen et al., (2014) |
| Ammonia volatilization | • Adsorption of $NH_3$ to biochar depending on biochar surface area and CEC of the soil | Lee et al. (2005); Lehmann et al. (2006) |
| | • Adsorption of $NH_4^+$ and $NH_3$ | Van Zwieteng rt al. 2010); Kizito et al. (2014); Wang et al. (2015) |
| Nitrification | • Biochar exerts a direct effect on the activity of ammonia oxidizers and nitrifies by altering $NH_4^+$ availability. | Dempster et al. (2011); Knowles et al., (2011); Ippolito et al., (2012); Nelissen et al., (2014); Nelissen et al. (2015); (Castaldi et al., 2011; Wang et al., 2015; He et al, 2018) |
| | • Biochar-induced changes in soil pH and other physico-chemical properties on the functioning of ammonia oxidizers and nitrifiers at a community level. | Yanai et al. (2007); Wu et al. (2012) |
| Denitrification | • Enhancing $NO_3^-$ immobilization in microbial biomass and uptake by plants, | Cayuela et al. (2013) |
| | • Increasing soil pH | Tryon (1948); Liang et al. (2006); Cheng et al. (2008); Van Zwieteng et al. (2014); Su et al., (2019) |
| | • Promoting soil aeration by reducing bulk density and increasing porosity | Yanai et al. (2007) |
| | • Increasing nosZ gene expression which encodes the nitrous oxide reductase that reduces $N_2O$ to $N_2$ | Ducey et al. (2013); Harter et al. (2014) He et al. (2018) |
| | • Catalyzing electron transfer | Cayuela et al. (2013) |

*Inhibition of $N_2O$ formation*

Some studies (e, g, Deluca *et al*., 2006) have demonstrated that addition of biochar increased the rate of nitrification significantly. However, these authors did not show whether the fluxes of $N_2O$ reduced correspondingly. Although they hypothesize that increased nitrification might have resulted in increased $N_2O$ emission, this process is not necessarily present in the soil. This is because $N_2O$ may not be produced if sufficiently oxic conditions prevail. Van Zwieten *et al*. (2008) showed that the nitrification rate was not affected by the presence of biochar, suggesting that nitrifier activity is not affected by biochar. Being a highly porous material, addition of biochar to the soil will increase pore space and soil aeration and aggregation and water-holding

capacity. Increased aeration does not favour production of $N_2O$. Although there is evidence of an inverse relationship between pH and $N_2O$ emission (Bergaust et al., 2010; Liu et al., 2010; Raut et al., 2012l Su), the responsible mechanism is not understood. These studies postulate that low pH interferes with the assembly of the enzyme $N_2O$-reductase.

*Reduction of $N_2O$ to $N_2$*

Van Zwieten et al. (2008) postulated that the reduction in $N_2O$ emission with biochar application was due to removal of the native soil organic carbon that acts as substrate for heterotrophic denitrifiers. On the other hand, it is also possible that the adsorption of soil organic carbon may enhance its accessibility to microorganisms that flourish on, or around biochar particles. Biochar addition to soil could modify the availability of $NH_4^+$-N, which is also used by some microbes as an energy source instead of $CH_4$ while using $O_2$ as an electron acceptor (Bykova *et al*., 2007). In such cases, the $CH_4$ uptake into the soil will be reduced while allowing more oxygen to be available hence less denitrification.

Biochar contains a number of functional groups that include hydroxyl, methyl, carboxylic and alkene groups (Li eat al., 2013; Stella-Mary et al., 2016). With increasing charring temperature, mass cleavage of O-alkylated groups and anomeric O-C-O carbons occurs prior to the production of fused-ring aromatic structures (Li eat al., 2013; Stella-Mary et al., 2016). The composition of these functional groups may modify the activity of denitrifyers and therefore the amount of $N_2O$ emitted from the soil. The interactions that exist between biochar-biochar surfaces and biochar-soil surfaces may result in the adsorption and/or complexation of some important bio-molecules responsible for $N_2O$ formation or direct interaction with $N_2O$ itself. Enhancing enzymatic reduction of $N_2O$ to $N_2$ by biochar is an alternative mechanism by which

biochar reduces emission of $N_2O$. It is possible that increased pH favours activity of reductase enzymes through improved access to substrates and/or cofactors.

## 1.5 Research problem

Whereas numerous studies have been conducted, the complexity of the interaction among multiple processes, as described in the previous paragraphs, make our understanding of the role of biochar in regulating GHG emissions incomplete, with several contradictions in the observed effects. Furthermore, most studies on biochar have relied on short-term laboratory investigations, raising questions as to the applicability of the findings to practical situations in field settings and the residual effect of biochar. Cognizant of the abovementioned knowledge gaps, this study was set out to investigate the following specific objectives:

## 1.6 Objectives of the study

The overall objective of this study was to improve our understanding of the effect of biochar amendment on processes and mechanisms governing the emission of GHG from a variety of tropical soils. The specific objectives of the study were;

1. To determine the effect of biochar quality (feedstock, production temperature, and biochar pre-treatment activation) on the fluxes of $N_2O$ and $CH_4$
2. To evaluate the role of biochar in nitrogen transformation and associated emission of $N_2O$ and $N_2$
3. To assess the influence of biochar on soil aggregate distribution under field conditions under conventional tillage practices
4. To quantify emission intensity, carbon stocks and crop yield in a low-fertility soil under various management options

5. To analyze the residual effect of biochar on soil mineral nitrogen, $NH_3$ and $N_2O$ in a field soil three years after application of organic and mineral resources

## 1.6 Significance of the study

Results from this study will be helpful in designing soil fertility management practices such as reducing the use of nitrogen fertilizers. Efforts are underway to include biochar in carbon-trading schemes. The information in this thesis can be helpful in providing indications as to the timeframe within which biochar can stay in the soil while providing benefits for farmers. The wide-scale use of biochar as a climate-smart agricultural practice will depend on the comprehensive scientific understanding of the processes it influences after application to agricultural soils. The net magnitude of these processes and their interaction has to be assessed mechanistically and for their practical significance. These processes must be seen to produce practical benefits to farmers in terms of crop yields while maintaining their carbon sequestration and emission reduction potential. The fast-changing climate coupled with the high population growth lays urgency to these investigations since they are likely to provide immediate and long-lasting solutions to the climate change-agriculture-food security nexus.

## 1.7 Study area

This study was conducted using soils from western Kenya, a region where sustainable soil management is extra important because the soils in this region are highly weathered and low in nutrients due to the continuously high temperatures. However, information on appropriate management options is chronically lacking. The farming in western Kenya is representative of many areas in sub-Saharan Africa. That is to say, faming is primarily small-scale (farms averaging 0.3 to 3 hectares in size), with about 75% of total agricultural output produced on rain-fed agricultural lands, and the systems are highly integrated (many enterprises in spatial

mixtures on the same piece of land) (Government of Kenya, 2012). The area receives bimodal rainfall, with long rains occurring from March to June and the short rains from August to November, totaling 1500–1800 mm annually (Fig. 1.3). Long-term mean temperature ranges between 22 and 24 °C. The geology of the survey area consists of rocks and sediments of Precambrian to recent times. The system of rock covers in the area is characterized by granites. The granite is not uniform throughout. It has variations which include syenites, granodiorites, dolerites and rhyolites (GoK, 1984).

Some of the processes that make tropical soils different from temperate ones are as follows: (a) Ionization of water is four times as high as at 10°C, (b) Silica is eight times as soluble, (c) Dissolution proceeds much quicker, (d) Less $CO_2$ penetrates the soil, (e) The hydrolytic power of the solution is much higher, (f) Soil water is less viscous, (g) More water penetrates deeper in the soil (Buring, 1970). Two soil types were used; Acrisol and Feralsols (). All of these soils are characterized by a high content of 1:1 type clays, a presence of highly insoluble minerals such as quartz sand and sesquioxides, and a low cation exchange capacity (CEC).

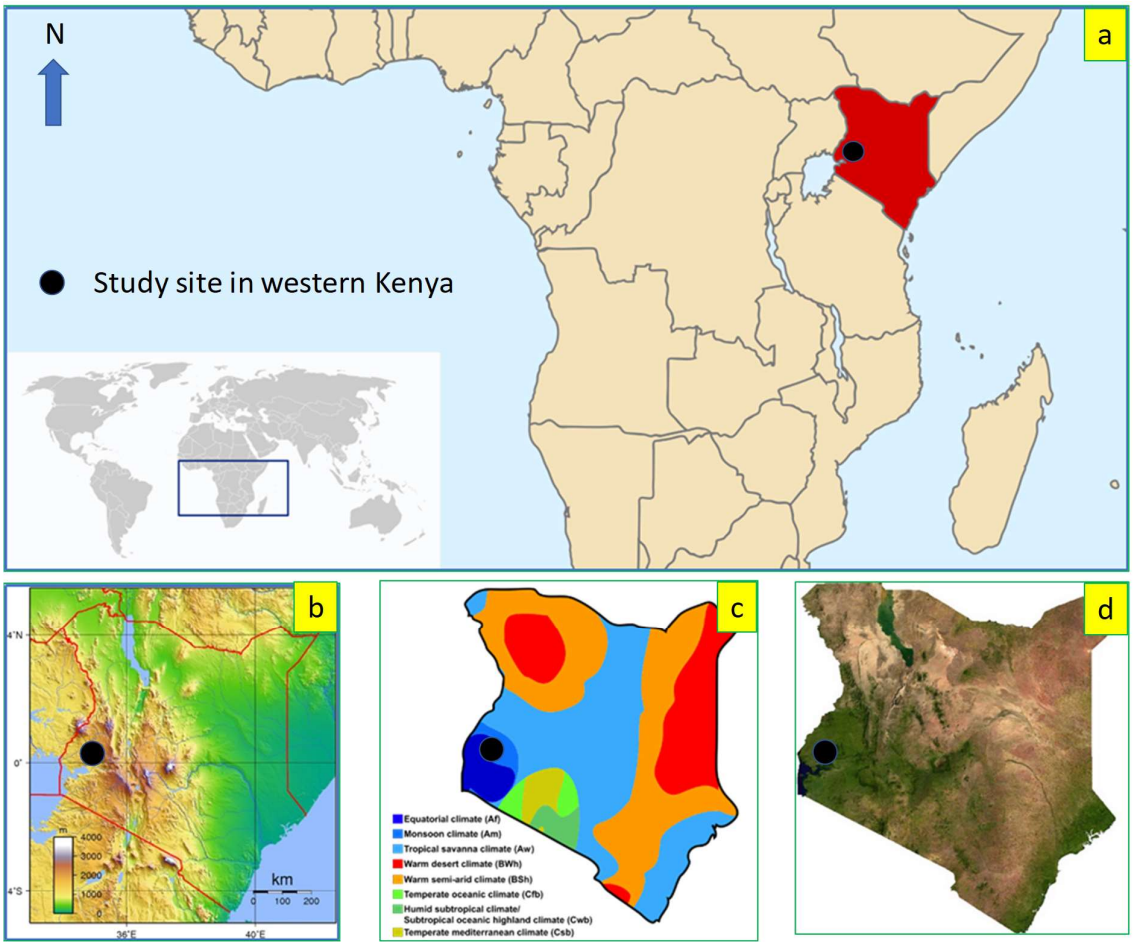



Figure 1.3: Location of the study site in western Kenya; (a) Location of Kenya relative to the world map; (b) Elevation map of Kenya showing the western highlands; (c) The climate zones indicating the Equatorial climate in the study site; (d) A satellite map showing the vegetation structure.

# CHAPTER TWO: $N_2O$ and $CH_4$ emission from soil amended with steam-activated biochar

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Fungo, B., Guerena, D., Thiongo, M., Lehmann, J., Neufeldt, H., and Kalbiz K., 2014. $N_2O$ and $CH_4$ emission from soil amended with steam-activated biochar. *Journal of Plant Nutrition and Soil Science*, *177:* 34–38. https://onlinelibrary.wiley.com/doi/10.1002/jpln.201300495

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## 2.1 Introduction

Biochar has been reported to reduce emission of the potent greenhouse gasses (GHGs), nitrous oxide ($N_2O$) and methane ($CH_4$) from soils (*Singh* et al., 2010; *Cayuela* et al., 2013). Studies on the impact of biochar applications to soil further indicate that biochar quality (e.g. feedstock, pyrolysis conditions) is important but highly variable (*Joseph* et al., 2010). More recently, post-production modifications of biochars have targeted to improve their properties with respect to nutrient retention. *Borchardet al*. (2012) showed that steam activation of biochar increased retention of $NO_3$-N by up to 55% compared to non-activated biochar. Activation by steam is also reported to increase the surface area from <10 $m^2/g$ to approximately 650 $m^2/g$ and pore size up to 14 Å (*Azargohar and Dalai*, 2008) depending on temperature and activation time.

The above process likely removes condensates of volatile compounds and increases biochar's pore space and size, surface area and adsorptive capacity for small molecules such as gasses and common solvents (*Rouquerol* et al*.,* 1999) and thus enhances diffusion of gasses and also the mobility and efficiency of microorganisms. These effects are also thought to alter the surface functional groups that affect the electro-chemical properties of biochar. Steam activation also enhances porosity (*Azargohar and Dalai*, 2008; *Enders* et al., 2012;

*Schimmelpfennig and Glaser*, 2012) of the biochar that should increase soil aeration, which in turn affects the production of $N_2O$ and $CH_4$. However, it has not been directly investigated whether steam activation has also an effect on $N_2O$ and $CH_4$emissions. The objective of this study was to investigate the effect of steam activation of biochar on the emission of $N_2O$ and $CH_4$ from a highly weathered tropical upland soil.

## 2.2 Materials and Methods

### *Experimental design*

The soil for the experiment was collected from Kapsengere on the southern Nandi hills in western Kenya (00' 09' 34''N and 34' 57' 37''E) at an elevation of 1750 m a.s.l. The area receives a mean annual rainfall of 2024 mm with mean annual temperature of 19ºC. The soil is a humic Acrisol that was converted to agriculture around the year 1900 and has a low nutrient content. Soil used for the experiment was collected from a depth of 0.2 m, air-dried and sieved through a 2 mm sieve before being mixed with biochar to an equivalent rate of ~15 t/ha (2.6 kg of dry soil with 36 g of the biochar). Two source materials (maize stover and eucalyptus wood) were used to produce biochar. The feedstock was chopped and ground into 5 mm-sized particles and fed into a 60 L batch pyrolysis unit using Argon as a sweep gas at a flow rate of one litter per minute. For each feedstock, two pyrolysis temperatures (350°C and 550°C) were used to make biochar. The pyrolysis unit was programmed with a ramp temperature rate of 5˚C per min and a maximum temperature dwell time of two hours before cooling. For each grade of biochar made, one part was steam activated (activated biochar) while the other was not (non-activated biochar) making a total of nine treatments (Table 2.1). Steam activation was done by injecting de-ionized water into the kiln after reaching maximim temperature. Water flow rate was 10 mL per minute for two hours.

Table 2.1: Experimental treatments

| Treatment No. | Feed stock | Pyrolysis temperature (°C) | Steam activation |
|---|---|---|---|
| 1 | Maize stover | 350 | Activated |
| 2 | | | Non-activated |
| 3 | | 550 | Activated |
| 4 | | | Non-activated |
| 5 | Eucalyptus wood | 350 | Activated |
| 6 | | | Non-activated |
| 7 | | 550 | Activated |
| 8 | | | Non-activated |
| 9 | No biochar | - | - |

Four replicated plastic pots (0.17 m *d* and 0.15 m *l*) per treatment were prepared and laid out in a completely randomized design in a greenhouse. Due to the inherent P deficiency of the soil, an equivalent of 1 kg / ha of triple super phosphate was added. The moisture content of the pots was maintained at 70% of water holding capacity by periodic weighing and replacement of water lost by evaporation. The mean temperature of the greenhouse ranged from 20°C to 35°C. The experiment was run for 34 days.

***Gas measurements***

Measurements of $N_2O$ and $CH_4$ were conducted using the static chamber method. A cuvette (cylindrical chamber) made from stainless steel (0.15 m *d* and 0.185 m *l*) was fitted with two openings to allow air to flow in and out during measurement. The openings were connected to the inlet and outlet points of a photo-acoustic infrared multi-gas monitor (INNOVA 1312-5, Lumasense Technologies A/S, Ballerup, Denmark) by two 0.7 m-long Teflon tubes as inflow and outflow to the cuvette. The multi-gas monitor was calibrated and set to compensate the cross-interference of gases and water vapor with $NH_3$, $N_2O$ and $CO_2$. The calibration of the gas monitor was done by Lumassence Technologies, Denmark two weeks before the experiment. To overcome the problem of cross-interference, a filter carouse was installed in the monitor and an algorithm that computes the interference $CH_4$ on $N_2O$ was included. Inside the cuvette,

air humidity and temperature were determined by a digital thermo-hygrometer (PCE-313 A, Paper-Consult Engineering Group, Meschede, Germany). This was attached to the cuvette from the outside and only the sensor reached inside the cuvette through a tight screw connector made of PVC. The concentration of the gas in the chamber was determined at time 0, 2, 4 and 6 minutes after chamber closure.

***Soil and biochar analysis***

Elemental analysis was done by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Ciros, CCD, Spectro, Germany) after sample extraction using modified dry-ashing technique (*Enders* et al., 2012). The pH was measured in triplicate in 1:20 soil:deionized water after shaking at 200 rpm for 24 h. Percentage of volatile material, ash content, and elemental (C, H, O, N, and S) content were determined on an oven dry-weight following the ASTM D 3172 and 3176 standard methods. Soil and biochar properties were determined in four replicates. The properties of the soil and biochar used in the experiment are shown in Table 2.2.

***Statistical analysis***

Cumulative gas fluxes were obtained by calculating the area of trapezia under the flux-time curve and summing the results while assuming linear changes in measurements between time intervals. Treatment effects (n=4) were tested using multivariate analysis of variance and individual treatments separated at 5% level of significance using Wilk's lamda. Partial Eta-square (Table 3) indicates the proportion of total variability attributable to the respective factor. The interpretation is similar to that of $R^2$ in univariate regression analysis (*Levine and Hullett,* 2002). The $CO_2$-e of each treatment was calculated as the sum of the individual gas fluxes

multiplied by their corresponding "global worming potential", considering the $CO_2$-equivalent of 298 for $N_2O$ and 25 for $CH_4$ over a 100-year time horizon (see *Zhang* et al., 2012).

Table 2.2: Chemical properties of soil and biochar used in the pot experiment

| Characteristic | Soil | Maize Stover | | | | Eucalyptus | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 350 | | 550 | | 350 | | 550 | |
| | | None | Steam | None | Steam | None | Steam | None | Steam |
| pH | 6.2 | 9.3 | 9.3 | 10.0 | 10.0 | 7.5 | 7.0 | 9.5 | 10.2 |
| P | 108.7 | 1.91 | 2.70 | 3.22 | 2.96 | 0.14 | 0.11 | 0.21 | 0.34 |
| K | 0.82 | 25.40 | 27.78 | 39.70 | 14.53 | 0.88 | 1.09 | 3.44 | 4.44 |
| Ca | 0.57 | 4.84 | 4.34 | 6.57 | 12.05 | 4.72 | 4.49 | 6.03 | 6.59 |
| S | nd | 0.72 | 0.84 | 0.90 | 0.59 | 0.08 | 0.05 | 0.09 | 0.07 |
| Na | nd | 1.09 | 0.63 | 0.45 | 1.17 | 1.68 | 1.91 | 2.05 | 1.95 |
| Mg | 0.11 | 2.55 | 2.58 | 3.29 | 5.62 | 1.12 | 1.29 | 1.88 | 1.94 |
| Fe | 5.29 | 3.02 | 3.31 | 4.86 | 0.26 | 0.00 | 0.42 | 0.06 | 0.11 |
| Al | 6.52 | 4.57 | 5.04 | 7.29 | 0.16 | 0.06 | 0.03 | 0.01 | 0.02 |
| Mn | nd | 0.19 | 0.17 | 0.25 | 0.09 | 0.20 | 0.26 | 0.36 | 0.40 |
| Cu | nd | 0.08 | 0.03 | 0.06 | 0.12 | 0.02 | 0.03 | 0.15 | 0.11 |
| Ti | nd | 0.08 | 0.07 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ni | nd | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Sr | nd | 0.05 | 0.05 | 0.07 | 0.02 | 0.04 | 0.04 | 0.05 | 0.06 |
| B | nd | 0.00 | 0.01 | 0.01 | 0.00 | 0.03 | 0.02 | 0.03 | 0.01 |
| Zn | 14.6 | 0.09 | 0.21 | 0.11 | 0.06 | 0.14 | 0.25 | 0.07 | 0.04 |
| Ba | nd | 0.10 | 0.09 | 0.13 | 0.01 | 0.08 | 0.09 | 0.12 | 0.12 |
| C (%) | nd | 67.1 | 57.5 | 67.7 | 78.97 | 67.1 | 61.6 | 83.2 | 87.7 |
| N (%) | nd | 0.78 | 0.71 | 0.51 | 0.58 | 0.23 | 0.22 | 0.19 | 0.13 |
| O (%) | nd | 14.0 | 26.6 | 14.0 | 6.4 | 27.1 | 32.3 | 10.8 | 7.3 |
| H (%) | nd | 3.51 | 3.63 | 1.94 | 3.86 | 3.84 | 3.71 | 2.37 | 2.45 |
| *Volatile matter (%) | nd | 32.2 | 31.0 | 14.1 | 12.0 | 35.1 | 34.6 | 12.2 | 11.5 |
| Ash (%) | nd | 14.6 | 11.6 | 15.8 | 10.2 | 1.7 | 2.2 | 3.5 | 2.4 |
| Molar C:N | nd | 85.5 | 81.3 | 133.9 | 136.3 | 287.8 | 282.9 | 438.8 | 681.3 |
| Molar H:C | nd | 0.05 | 0.06 | 0.03 | 0.05 | 0.06 | 0.06 | 0.03 | 0.03 |
| Molar O:C | nd | 0.21 | 0.46 | 0.21 | 0.08 | 0.40 | 0.52 | 0.13 | 0.08 |

*Material lost when moisture-free biochar is heated to 950ºC. Units for the elements are in mg g-soil$^{-1}$. nd=Not determined.

## 2.3 Results and Discussion

Overall, biochar addition to soil reduced $N_2O$ but increased emission of $CH_4$ (Fig. 2.1a). All of the activated biochars showed lower emissions of $N_2O$ compared to the non-activated biochar by 10-41%, except the 350°C wood biochar which showed no difference due to activation (Fig. 2.1a). This may be due to removal of sorbed labile carbon from volatile condensates, and

improving diffusion of toxic oxygen (*Case* et al., 2012) thereby allowing for more effective $N_2O$ reductase enzyme and thus lower $N_2O$ emission from soil. The electron shuttle role proposed by *Cayuela* et al. (2013) for biochar's role on $N_2O$ reduction to $N_2$ may be due to organic rather than mineral matter interactions. Further investigation into the complexity and distribution of organic functional groups is necessary to better understand impact on of biochar emission of GHGs.

Activated stover-derived biochar increased $CH_4$ emission by approximately 14-70% but a reverse effect of similar magnitude was observed for activated wood-derived biochar (Fig. 2.1b). There seems to be significant difference in pore structure between stover and wood derived biochar to explain this disparity in $CH_4$ emission following steam activation. Some studies (*Steinbeiss* et al., 2009; *Feng* et al., 2012) have suggested that the continuous porous structure of biochar increases retention of $CH_4$ and thus provide "hot spots" for methanotrophic proliferation. *Spokas* et al. (2011) reported that volatile organic carbon composition of biochar, linked with others, result in both stimulative and inhibitive effects in plant and microbial systems.

A multivariate analysis showed that feedstock, temperature and steam activation of biochar all have significant effect on emission of both $N_2O$ and $CH_4$ (Table 2.3). Presence of biochar could catalyze $N_2O$ reduction to $N_2$ as end-product by acting as an electron shuttle (*Cayuela* et al., 2013). By increasing the pore volume and size (*Azargohar and Dalai*, 2008) and altering surface functional groups, activation of biochar may affect its pH buffer capacity, specific chemical toxins, metal complexes and precipitation products resulting from its addition to soil. All these very likely processes are not clearly understood and thus warrant further investigation. Results further show that feedstock, temperature and activation explained 32, 10 and 56% of

differences in $N_2O$ while 22%, 22% and 21% of variations in missions of $CH_4$ emissions were explained by feedstock, temperature and steam activation, respectively (Table 3). Emission of $N_2O$ is lower by 17% if maize stover biochar is activated compared to Eucalyptus wood biochar, and 3% lower if pyrolysis temperature is 350°C compared to 550°C. $CH_4$ emission is higher by 21% if activated biochar was made from maize stover compared to Eucalyptus wood and 10% lower if the pyrolysis temperature was 350°C compared to 550°C.

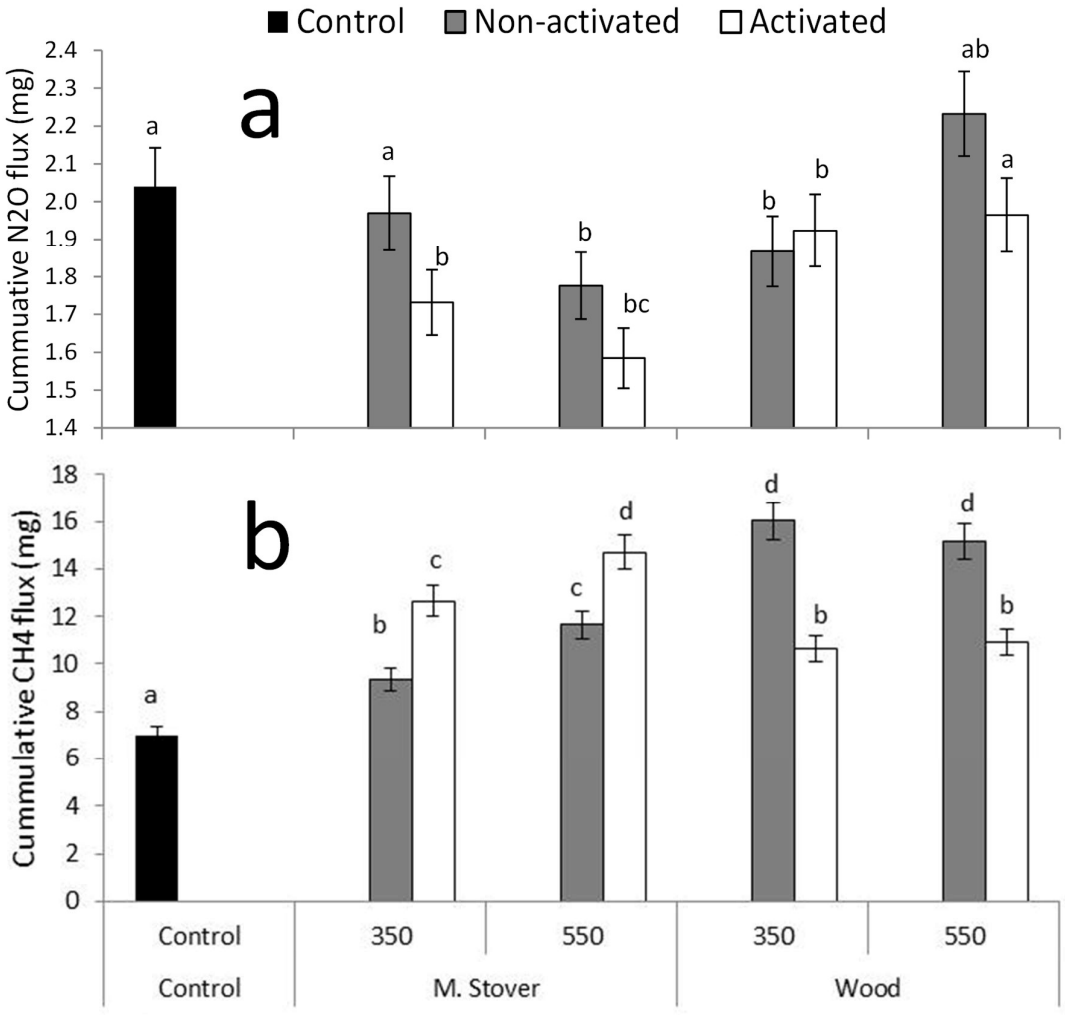



**Fig. 2.1:** Cumulative emissions of nitrous oxide (a) and methane (b) over 34-days and as affected by biochar quality (feedstock, pyrolysis temperature and steam activation). Different

letters indicate significant differences between the treatments at $P<0.05$. Error bars are standard errors (n=4).

Important to note is that steam activation explained a large part (56%) of variation in $N_2O$ emissions, suggesting a crucial role of either surface area, pore size and volume or surface functional groups on $N_2O$ production and/or reduction. Sorption behavior of $N_2O$ on biochar has been reported in earlier studies (*McBaim,* 1926; *Hitoshiet al.,* 2002). $N_2O$, having both N and O heteroatoms, normally serves as a good ligand in metal ions and is expected to have ligand properties somewhat similar to those of NO or $N_2$ (*Dong-Heon*, 2006). Although the $N_2O$ coordination adducts reported in literature are scarce, one notably well-characterized $N_2O$ coordination complex is known. Spectral evidence suggests the existence of $N_2O$ plus $[Ru(NH_3)_5(N_2O)]^{2+}$, which is in equilibrium with $N_2O$ (*Armor and Taube*, 1971). Ruthenium (Ru) is a d-block element like iron and copper, which are known to play important roles in denitrification enzymes. Formation of complexes similar to that observed with Ru may occur in soils, favouring the observed reduction in $N_2O$ but this area requires more research. Feedstock, pyrolysis temperature and activation may all affect the speciation and abundance of metals and therefore requires more research.

Table 2.3: Effect size indices (partial eta-squared) for feedstock, pyrolysis temperature and steam activation on emission of $CH_4$ and $N_2O$ from soil

| Source of variation | Partial eta-squared | |
|---|---|---|
| | $CH_4$ | $N_2O$ |
| Feedstock | 0.22 | 0.32 |
| Pyrolysis temperature | 0.22 | 0.10 |
| Steam activation | 0.21 | 0.56 |
| Error | 0.35 | 0.08 |

Only non-activated biochar made from eucalyptus wood at 550°C showed a significantly higher $CO_2$-e (0.39 kg $CO_2$-e) of the combined effect of $N_2O$ and $CH_4$ emissions from soil

compared to an unamended control (0.31 kg $CO_2$-e; sd=3.48). None of the other treatments differed in their $CO_2$-e from the control.

## 2.4 Conclusions and Recommendations

We conclude that steam-activation of eucalyptus wood-derived biochar enhances biochar's capacity to suppress $CH_4$ and $N_2O$ emission. The effect of steam activation is dependent on feedstock and pyrolysis temperature of the biochar. Further investigation into the pore structure, organic by-products, surface functional groups and especially the surface metal species of biochar following activation will cast more light on responsible mechanisms.

# CHAPTER THREE: Nitrogen turnover and $N_2O/N_2$ ratio of three contrasting tropical soils amended with biochar

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Fungo B., Zhe C., Butterbach-Bahl K., Lehmannn J., Saiz G., Braojos V., Kolar A., Tenywa M., Kalbitz K., Neufeldt H., and Dannenmann M., 2019. Nitrogen turnover and $N_2O/N_2$ ratio of three contrasting tropical soils amended with biochar. *Geoderma 348, 12–20* https://doi.org/10.1016/j.geoderma.2019.04.007

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## 3.1 Introduction

Nitrous oxide ($N_2O$) is a potent Long-Lived Greenhouse Gas (LLGHG), and involved in the destruction of stratospheric ozone (Ciais et al., 2013). Agricultural soils are an important source of atmospheric $N_2O$, with denitrification representing the single most important biochemical process releasing $N_2O$ into the atmosphere (Butterbach-Bahl and Dannenmann, 2011; Harter et al., 2014a, 2014b). Measures for reducing $N_2O$ emission from agricultural soils such as biochar addition are increasingly considered to mitigate the impact of agriculture on climate change.

A number of factors affecting $N_2O$ emission in biochar-amended soils have been investigated, including feedstock, pyrolysis temperature, biochar pre-treatment, soil and biochar pH, soil type and soil moisture regime (Castaldi et al., 2011; Wu et al., 2012; Ameloot et al., 2013; Chen et al., 2017). For example, Yanai et al. (2007) suggested that a pH increase resulting from biochar addition could enhance $N_2O$ reductase activity, thereby increasing the reduction of $N_2O$ to N2in the last step of denitrification. Van Zwieten et al. (2009) hypothesized that metals present on biochar surfaces might act as catalysts in the reductionof $N_2O$ to $N_2$. Physical

adsorption of $N_2O$ and NO on activated coconut charcoal has also been reported (Bagreev et al., 2001; Hitoshi et al., 2002; Cornelissen et al., 2013). Case et al. (2015) found that the suppression of soil $N_2O$ emissions was not due to limitations of inorganic N availability in the soil caused by biochar-induced inorganic N immobilization. Furthermore, direct impacts of biochar on the activity of mineralizing and nitrifying microbes (Lehmann et al., 2011) may also occur but have, so far, hardly been investigated.

Using the $_{15}N$ gas-flux method, Cayuela et al. (2013) observed a consistent reduction of the $N_2O/N_2$ ratio in 15 different soils after amendment with biochar, and proposed that biochar may act as an "electron shuttle", facilitating the last step of denitrification ($N_2O$ to $N_2$). According to Singh et al. (2010), sorption capacity of biochar through oxidative reactions on the biochar surfaces increase the effectiveness of biochar in reducing nitrate leaching, nitrification and $N_2O$ emissions. However, biochar effects on $N_2O$ emissions may also be mediated by its impact on prevailing soil conditions (Karhu et al., 2011; Yu et al., 2011; Case et al., 2012) that can influence the gross nitrogen turnover rates such as ammonification, nitrification, and inorganic N immobilization (Clough and Condron, 2010; Karhu et al., 2011). These conditions in turn exert feedbacks on $N_2O$ formation and consumption.

Knowledge on interactions between biochar addition, gross N turnover rates and soil $N_2O$ emissions is limited. Such detailed processbased understanding of N cycling in biochar-amended soils is important, since the ultimate effect of biochar addition on N gaseous losses could also depend on biochar's direct and/or indirect effect on ammonification, nitrification, microbial inorganic N immobilization, since these processes ultimately provide or remove substrate for denitrification and also impact N gas product ratios (Butterbach-Bahl and Dannenmann, 2011, Butterbach-Bahl et al., 2013). Furthermore, understanding biochar effects

on gross N turnover is generally desirable to understand biochar effects on key soil functions such as fertility and nutrient retention (Clough and Condron, 2010). So far, the influences of biochar on gross N turnover rates and the $N_2O$:$N_2$ emission ratio, have only been considered separately in these earlier studies (Cayuela et al., 2013; Case et al. 2015).

In this study, we provide data collected simultaneously on both the soil microbial gross N transformations as well as $N_2O$ and $N_2$ emissions under the influence of biochar amendment and also measure the dynamics of all the soil mineral N pools. The objective of this study therefore was to provide a mechanistic understanding of biochar effects on the interplay of gross soil N mineralization, nitrification and immobilization as well as denitrification and the $N_2O/N_2$ product ratio. Three mineralogically contrasting tropical agricultural soils were used. We generally expected a coupling of soil gross N turnover (mainly gross nitrification) and $N_2O$ emissions, and that biochar impacts on gross N turnover would thus also affect $N_2O$ emissions. Specifically, we hypothesized that biochar addition to soil would (1) decrease nitrification and soil nitrate availability due to increased immobilization of mineral N; (2) decrease soil $N_2O$ emissions due to reduced total denitrification.

## 3.2 Materials and methods

### *3.2.1 Preparation of the biochar and soils*

The feedstock from eucalyptus wood was chopped and ground into 5 mm-sized particles and fed into a 600 l batch pyrolysis unit using argon as a sweep gas at a flow rate of one liter per minute. The pyrolysis unit was programmed to run with a ramp temperature rate of 5 °C per min, reaching maximum temperature of 550 °C and a dwell time of 2 h at maximum temperature before cooling to room temperature. Three soil types with contrasting characteristics were sampled (0–0.2m topsoil) at the following sites in Western Kenya; (i)

Gambogi (E34° 57′37″and N00°09′34″an Acrisol under cultivation for ~100 years mainly with maize-beans intercropping hereafter, Acrisol-100), (ii) Kechire (E35°0′00'and N0° 4′0″, an Acrisol after approximately 10 years of conversion from tropical high forest to maize cultivation, Acrisol-10), and (iii) Yala (a Ferralsol also under maize-beans cultivation>100 years, Ferralsol-100). The properties of the biochar and soil at each site are presented in Table 1. All the three soils are characterized by high content of 1:1 type clay presence of highly insoluble minerals such as quartz sand and sesquioxides, and low CEC. The organic matter content (Acrisol 10 > Acrisol 100 > Ferralsol 100Yala) and clay content (Kechire<Gambogi<Yala) were the major distinguishing features among the soils. In addition, the presence of iron and aluminum oxides as well as low amounts of available calcium and magnesium ions characterized the Ferralsol.

### *3.2.2 Experimental setup*

The experiment consisted of nine treatments that were derived from the three soils (Acrisol 10, Acrisol 100 and Ferralsol) and three biochar addition rates (0, 2% and 4%w/w). The properties of the three soils are shown in Table 3.1. The pH of the biochar was adjusted to that of the soil using diluted HCl. The pH of the soil-biochar mixture was monitored and correlation between delta-pH (difference between original and final pH of the soil) was not correlated with $N_2O$ emission. Then, air-dry sieved soils (2mm mesh) were rewetted to 40% of water holding capacity (WHC) and incubated at 25 °C for seven days before the start of the experiment to stabilize microbial processes. After the stabilization period, each treatment was prepared by adding the appropriate biochar rate to the bulk soil and mixed thoroughly.

The incubation was performed in two experiments that were run independently but under identical incubation conditions; Experiment 1 was used for 15N isotope labeling as a basis for

the application of the 15N pool dilution technique (as described in more detail by Dannenmann et al., 2010; Dannenmann et al., 2011) to quantify gross N turnover (nitrification, ammonification and NH4+/NO3− - consumption/immobilization, three replicates for each treatment) and the associated N2O emissions (six replicates for each treatment).

Table 3.1: Properties of biochar and soils from three sites in western Kenya, which were used in the incubation experiment

| Soil property | Units | Biochar | Soils | | |
|---|---|---|---|---|---|
| | | | Kechire | Gambogi | Yala |
| pH | | 6.31 | 6.68 | 6.01 | 5.39 |
| EC(S) | uS $m^{-1}$ | 19.6 | 12.2 | 8.80 | 12.5 |
| N | g $kg^{-1}$ | 0.27 | 2.8 | 2.6 | 2.1 |
| P | mg $kg^{-1}$ | 135 | 2.77 | 2.30 | 20.3 |
| K | mg $kg^{-1}$ | 1490 | 263 | 223 | 550 |
| Ca | mg $kg^{-1}$ | 1920 | 2130 | 1950 | 2100 |
| Mg | mg $kg^{-1}$ | 150 | 413 | 312 | 226 |
| Mn | mg $kg^{-1}$ | 188 | 499 | 782 | 600 |
| S | mg $kg^{-1}$ | 36.5 | 7.25 | 14.0 | 10.4 |
| Cu | mg $kg^{-1}$ | 0.77 | 7.58 | 1.97 | 6.85 |
| B | mg $kg^{-1}$ | 1.07 | 1.25 | 0.33 | 0.68 |
| Zn | mg $kg^{-1}$ | 108 | 11.7 | 13.5 | 15.1 |
| Na | mg $kg^{-1}$ | 180 | 16.5 | 15.9 | 20.7 |
| Fe | mg $kg^{-1}$ | 164 | 123 | 67.2 | 192.3 |
| Al | mg $kg^{-1}$ | 559 | 888 | 939 | 895 |
| C.E.C | meq/100g | 18.2 | 21.0 | 16.2 | 15.3 |
| C:N ration | | 3218 | 9.7 | 9.4 | 10.5 |
| SOC | g $kg^{-1}$ | 869 | 27.2 | 24.3 | 19.0 |
| Sand | % | nd | 61.2 | 30.7 | 22 |
| Silt | % | nd | 18.3 | 47.5 | 43 |
| Clay | % | nd | 20.5 | 21.8 | 35 |

*nd = Not determined*

Experiment 2 was deployed using the helium flow soil core method (Butterbach-Bahl et al., 2002; Dannenmann et al., 2008) to simultaneously measure $N_2O$ and $N_2$ in order to determine the $N_2O/N_2$ ratio in soils which did not receive $^{15}N$ additions with two replicates for each treatment. Only two analytical replicates were possible due to limited capacities of the Helium soil core system and the long time needed for gas exchange. However, all $N_2$ flux measurements

were average fluxes from seven simultaneously incubated soil cores so that spatial replication was comparably good.

### *3.2.3 Gross rates of nitrogen turn-over and $N_2O$ production*

Gross rates of ammonification, nitrification and inorganic N consumption were determined using the $^{15}N$ pool dilution technique as described in detail by Dannenmann et al. (2010). Briefly, 200 g samples of air-dry soil were placed in 500 $cm^3$ incubation bottles fitted with rubber caps to allow for air tightness during gas sampling. The bottles were prepared in duplicates to allow for separate enrichment with either $^{15}NO_3^-$ or $^{15}NH_4^+$. After mixing the wet soil with biochar, the moisture content of the treatments was raised to 70% WHC w/w and maintained at that level throughout the experiment by daily weighing and replacing water lost by evaporation. The incubation bottles were placed in the thermostatically–controlled incubator maintained at 25 °C (the average daily soil temperature in western Kenya) throughout the 20-day experimental period. Before destructive soil sampling, the incubation bottles were closed, gas-tight, using the rubber caps, and 10 ml of gas was sampled at 0, 30, 60 and 90 min after closing. The range of $R^2$ values ranged from 0.75 to 0.99%. However, a flux was included in the analysis only if the $R^2$ was>85%. The gas samples were collected using a 20-ml syringe and injected into pre-evacuated 10-ml gas vials.

Analyses of gas samples were done using a gas chromatograph equipped with an Electron Capture Detector (ECD) for $N_2O$ analysis as described in detail by Yao et al. (2010). Nitrous oxide flux was calculated from linear changes of $N_2O$ concentrations in the headspace (Yao et al., 2010). Immediately after gas sampling, the soils in the bottles were enriched with a solution of either $K^{15}NO_3$ or $(^{15}NH_4)_2SO_4$ at 50 atom% enrichment. The Isotopically labelled solution was applied by spraying it onto the soil, accompanied by intensive mixing (Dannenmann et al., 2010). For each sample, half of the soil was extracted 1 h after enrichment ($T_0$) and the second

half was stored in an incubator at 250C for 24 h before the second extraction ($T_1$). Sixty grams of T0 and T1 samples were extracted with 120 ml of 0.5M potassium sulphate ($K_2SO_4$) solution by end-to-end shaking for 60 min. All extracts were filtered through 0.45 µm syringe filters. The diffusion method was used for subsequent trapping $NH_4^+$ or $NO_3^-$ as $NH_3$ on acid traps made of ashless filter paper (Brooks et al. 1989; Dannenmann et al., 2006, 2010). The 14/15N-ratio of the N captured on the dried filter papers was analyzed using an elemental analyzer coupled to a mass spectrometer as described in detail by Guo et al. (2013). Ammonium and nitrate concentrations in extracts were quantified using colorimetric auto-analysis (AQUAfast COD165 Thermoreactor, Thermofisher Scientific, USA) according to the VDLUFA method C 221 (Hoffmann, 1991). Gross rates of ammonification and nitrification were calculated using the equations given by Kirkham and Bartholomew (1954) using $T_0$ and $T_1$ data on N pools and $^{15}N$ enrichment of ammonium and nitrate, respectively.

Furthermore, we calculated gross inorganic $NH_4^+$ and $NO_3^-$ consumption and then estimated immobilization of $NH_4^+$ by subtracting nitrification rates from $NH_4^+$ consumption rates (Davidson, 1992). We do not declare nitrate consumption to resemble biotic and/or abiotic nitrate consumption because other nitrate fates such as denitrification may be substantial in the incubations. Soil pH was determined using a pH meter after shaking a 1:2.5 w/v soil-to-water mixture and allowing it to stand overnight before measurement. Gas flux analyses and determination of gross N turnover were conducted immediately after biochar addition, and after 3, 7, and 20 days in triplicate. Overall, 216 jars with soil were used for this purpose.

### *3.2.4 $N_2$ emissions*

Emission rates of $N_2$ were determined by use of the helium gas flow soil core method (Butterbach-Bahl et al., 2002) with the modified setup for smaller soil samples and better

representation of spatial variability described by Dannenmann et al. (2010). The method is based on the exchange of the soil and headspace atmospheres by a helium-oxygen atmosphere containing only 25 ppm N2in an extremely gas-tight incubation system and the subsequent simultaneous automated detection of $N_2$ concentration changes in the headspace above the cores by use of a pulse discharge helium ionization detector (PDHID) for $N_2$. The general set-up of the system consists of the steering unit, two vessels containing seven soil cores equipped for automated flushing both through the soil cores and headspace, automated sampling and the detection devices and systems. Details of the system and of the conditions for $N_2$ analysis are described by Dannenmann et al. (2011). The soils were pre-treated as described above and placed in the seven cores (0.01m3 volume each) of a single incubation vessel (soil moisture 70% WHC w/w incubation temperature: 25 °C).

After closing the vessels, the soil cores were flushed for 72 h to quantitatively remove $N_2$ from the soil and headspace atmospheres. Subsequently, an artificial headspace atmosphere was created (5 h of flushing with 80% He, 20% $O_2$, 25 ppm $N_2$, 400 ppb $N_2O$) and finally the concentration change of $N_2$ in the two cuvettes was monitored automatically for 8 h on an hourly basis according to Butterbach-Bahl et al. (2002). Every sample gas analysis was accompanied by six automated calibration gas measurements of the gas chromatographs. For each treatment, two replicates (each consisting of combined N gas measurements from seven soil cores) were used. Before starting the measurement, the air-tightness of the system was checked with a parallel set-up containing empty vessels and soil core dummies made of steel; the inherent leakage rate of $N_2$ was<20 $\mu g N_2\text{-}N m^{-2} h^{-1}$.

### *3.2.5 Data analysis*

Calculation of cumulative fluxes during the incubation period was based on linear interpolation between measurements. All biogeochemical N data were expressed on a soil dry weight (sdw) basis. The main effect of biochar presence, biochar rate or soil type was tested using factorial ANOVA after natural log transformation, and individual means were separated by the methods of Least Significant Difference (LSD) at 95% level of confidence. Correlation analysis was used to assess the relationships between soil properties and N transformation processes and gaseous N products.

## 3.3 Results

### 3.3.1 Biochar and $N_2O$ emission

The cumulative $N_2O$ losses over the incubation period followed the order Acrisol10 > Acrisol-100 > Ferralsol (Fig. 3.1), i.e., decreased with decreasing soil organic carbon content (Table 1). The application of biochar reduced cumulative $N_2O$ emission (Fig. 3.1) by 53 to 78% across soils and biochar addition treatments. Increasing the application rate of biochar from 2% to 4%, however, did not significantly reduce cumulative $N_2O$ emissions from any of the three soils (Fig. 3.1). No significant correlations were found between various mineral concentrations in biochar and $N_2O$ emissions.

### 3.3.2 Extractable $NO_3^-$-N and $NH_4^+$-N

Fig. 3.2 illustrates the dynamics of soil $NO_3^-$-N and $NH_4^+$-N concentrations during the 20-day incubation period. All three soils showed comparable initial $NO_3$- concentrations of ca 10 mg N $kg^{-1}$sdw, while initial $NH_4^+$-N concentrations strongly differed across soils with the pattern Acrisol10 > Acrisol100 > Ferralsol, with the latter showing extremely low $NH_4^+$ concentrations. For Acrisol 10and Acrisol 100 soils, $NH_4^+$ concentrations decreased throughout the incubation, while there was a parallel increase in $NO_3^-$ concentrations in the same order of

magnitude (Fig. 3.2). In contrast, the Ferralsol showed no pronounced change in soil mineral N concentrations. Towards the end of the incubation, biochar addition had resulted in increased $NO_3-$ concentrations in Acrisol 10 but decreased $NO_3-$ concentrations in the Acrisol100 (only for the high addition rate) and in the Ferralsol (for both addition rates) (Fig. 3.2). In contrast, soil $NH_4^+$concentrations were significantly reduced by biochar addition, but only for the Acrisol 10.

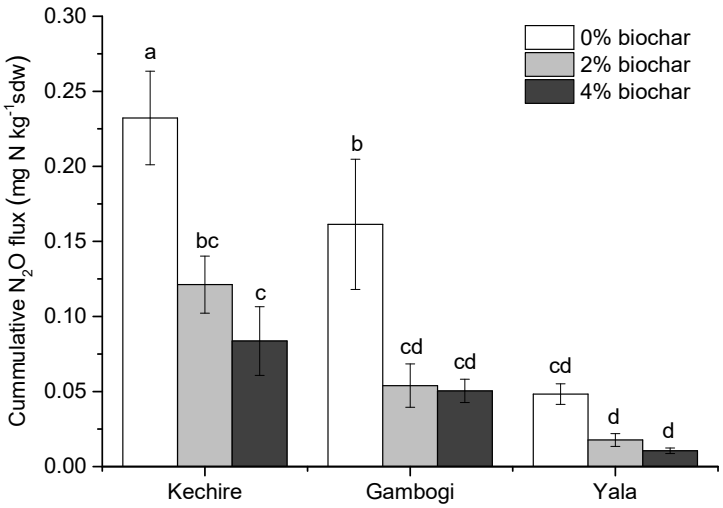



Figure 3.1: Cumulative $N_2O$ fluxes after 20-day incubation from three contrasting tropical agricultural soils after amendment with different quantities of biochar. Error bars are standard errors of the mean (n=6). Different indices indicate significant differences between biochar addition treatments ($P< 0.05$, LSD test).

### 3.3.3 Gross ammonification and nitrification rates

For the Acrisol 10 and Acrisol 100 soils, gross nitrification rates were similar to gross ammonification rates, indicating a nitrate-oriented N cycle. In contrast, the Ferralsol showed gross nitrification rate to be significantly lower than gross ammonification (Fig. 3.3; Table 3.2). Overall, a biochar addition rate of 2% increased ammonification rates of Acrisol 10 (69%) and Ferralsol (639%) soils, with a similar effect of the high application rate of 4% (85% increase

for Acrisol10 and 282% increase for Ferralsol) over the entire incubation period, while no persistent or unidirectional effect was observed for Acrisol100 (Figs. 3.3 and 3.5). With regard to gross nitrification rates, no persistent effects of biochar addition were generally observed over the incubation period (Fig. 3.3). Despite these variable effects, cumulative gross nitrification rates as calculated over the entire incubation period were significantly increased for all three soils at 4% biochar addition but not at 2% biochar addition (Table 3.2, Fig. 3.5).

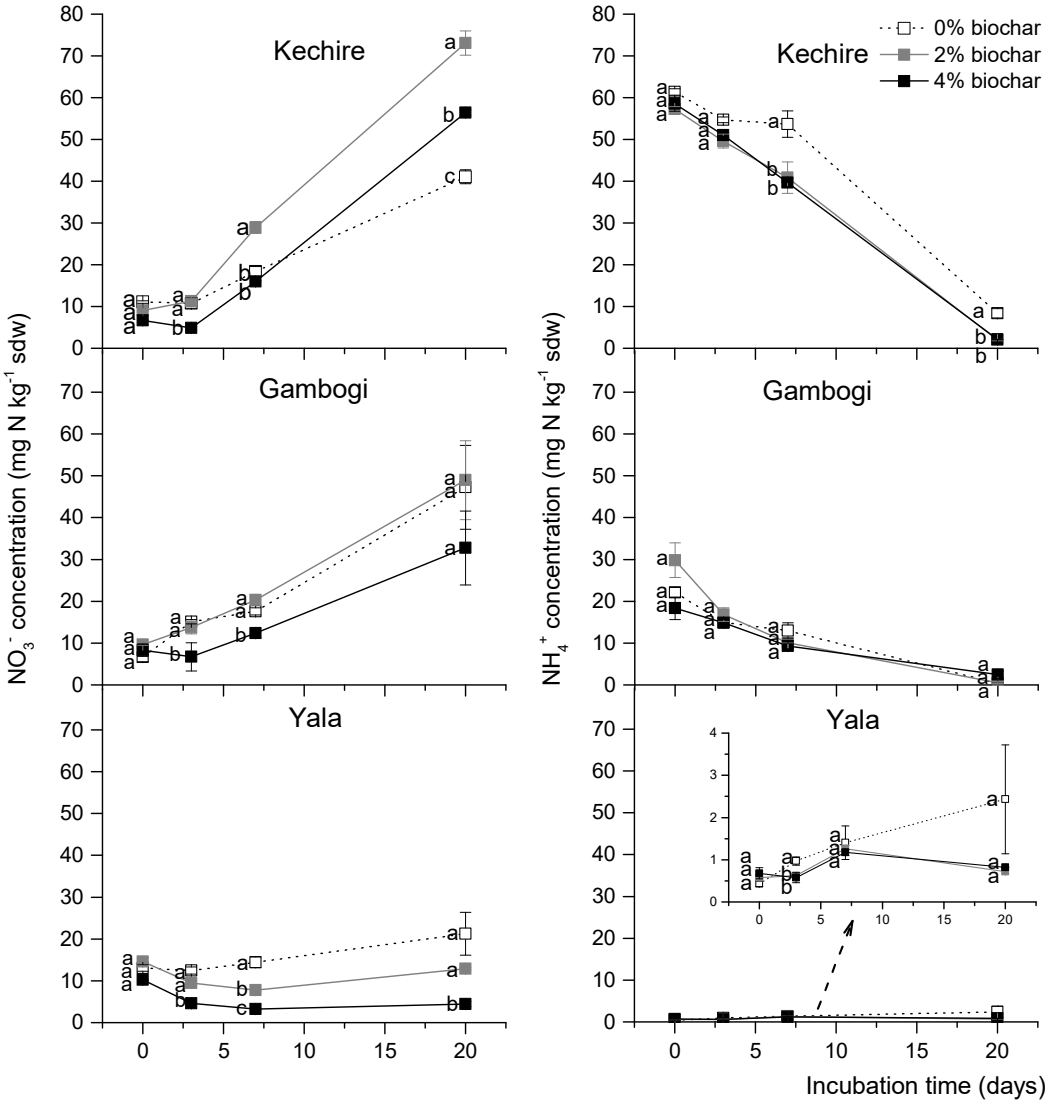



Figure 3.2: Concentrations of $NO_3^-$-N (A) and $NH_4^+$-N (B) during a 20-day incubation in three soils amended with 2% and 4%w/w biochar. Error bars represent standard error, and n=6.

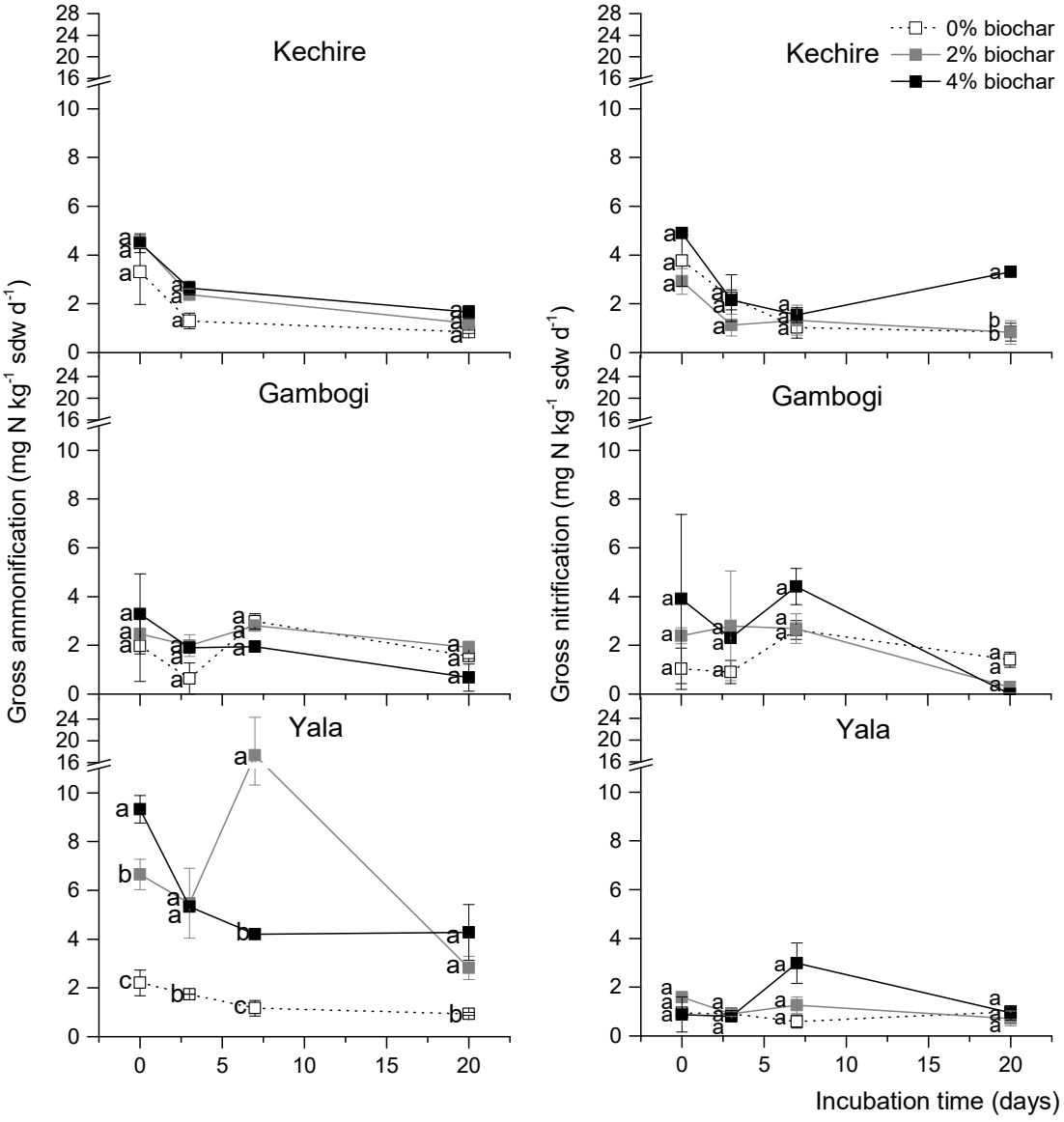



Figure 3.3: Gross ammonification (left panels) and nitrification (right panels) rates during a 20-day incubation in three soils amended with 2% and 4%w/w biochar. Error bars represent standard error, and n=6.

### 3.3.4 Gross $NH_4^+$-N and $NO_3^-$-N consumption rate

Ammonium immobilization rates as calculated from ammonium consumption minus gross nitrification resulted in significantly negative for the Acrisol 10, indicating N dynamics such as heterotrophic nitrification, i.e., a direct oxidation of organic N to $NO_3-$ (Fig. 3.4). Hence, we did not calculate overall mean $NH_4^+$ immobilization fluxes for this soil (Fig. 3.5). Application of biochar increased $NH_4^+$-N immobilization only for the Ferralsol. In contrast, biochar generally increased $NO_3-$consumption for the Acrisol 100, decreased $NO_3-$ consumption for the Acrisol 100 and had no effect on $NO_3^-$ consumption in the Ferralsol (Table 3.2, Figs. 3.4, 3.5).

### 3.3.5 Dinitrogen losses

For dinitrogen losses, only two measurements are available so that from a statistical perspective we were unable to distinguish across soils and treatments. Dinitrogen emissions generally exceeded $N_2O$ emissions by at least an order of magnitude, so that they may represent total denitrification rates very well. Without biochar application, $N_2$ emissions were about an order of magnitude lower for Acrisol 10 compared to Acrisol 100 and Ferralsol (Fig. 3.5, Fig. 3.6). Similar to other results on N turnover, biochar tended to exert variable effects on soil $N_2$ emissions (Fig. 3.5). For the Acrisol 10, a very large increase in $N_2$ emissions was observed with increasing biochar addition (Fig. 3.5, Fig. 3.6). In contrast, for the Acrisol 100, biochar addition did not change $N_2$ emissions. To further complicate the picture, biochar addition decreased $N_2$ emissions from the Ferralsol (Fig. 3.5, Fig. 3.6).

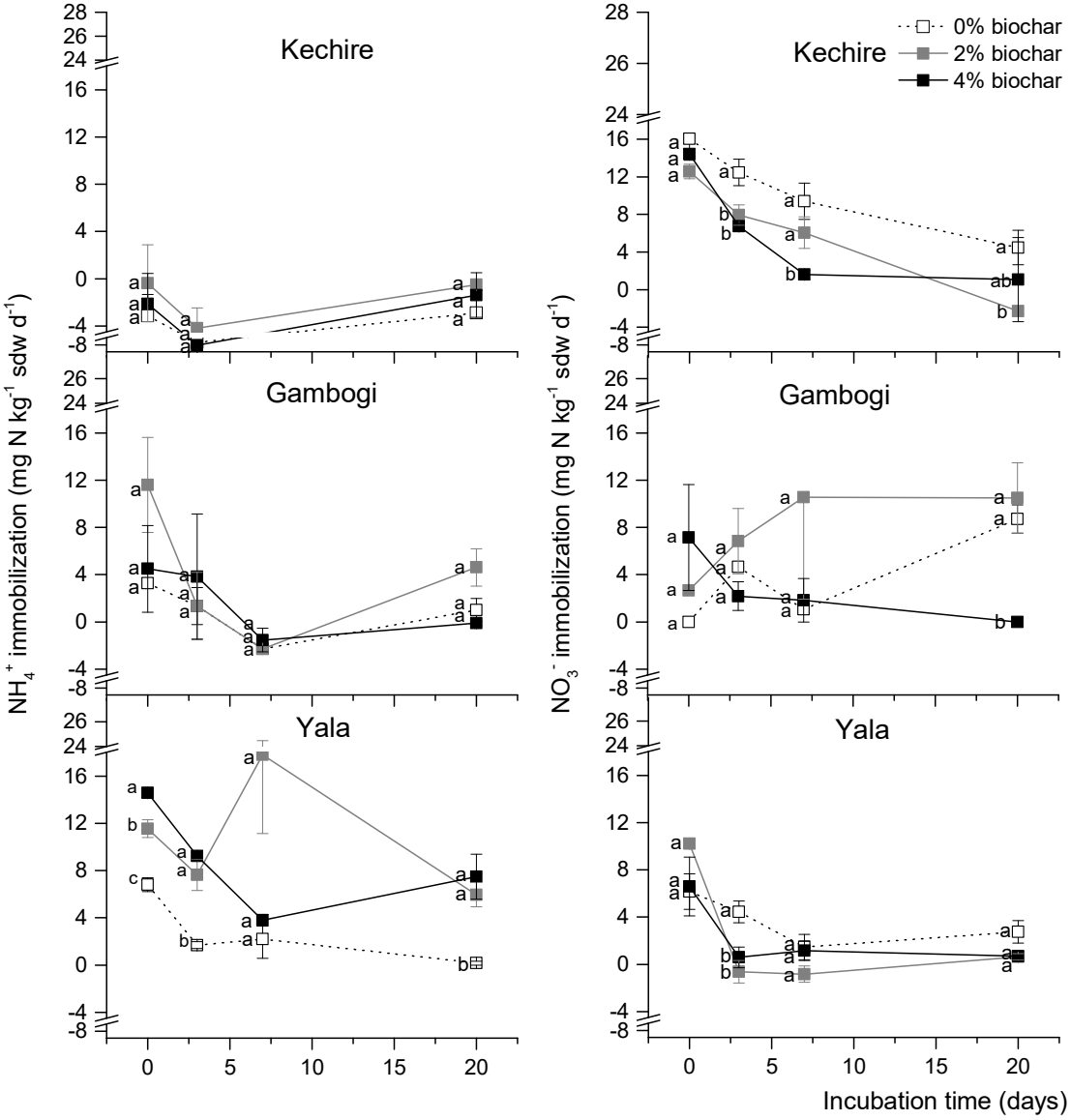



Figure 3.4: Immobilization of $NH_4^+$-N (A) and $NO_3^-$-N (B) during a 20-day incubation of three soils amended with 0%,2% and 4% w/w biochar. Error bars represent standard error of the mean (n=6).

Table 3.2: Cumulative nitrogen transformation over the 20-day incubation in three contrasting soils after amendment with different quantities of biochar (mg N $kg^{-1}$sdw 20 $days^{-1}$) with standard error in brackets. Cumulative $N_2O$ is given in the same unit.

| N process | Soil type | 0% biochar | 2% biochar | 4% biochar |
|---|---|---|---|---|
| Ammonification | Kechire | 27(7)$^{b}$ | 43(4)$^{a}$ | 50(5)$^{a}$ |
| | Gambogi | 42(12)$^{a}$ | 49(7)$^{a}$ | 28(7)$^{b}$ |
| | Yala | 27(7)$^{b}$ | 199(101)$^{a}$ | 103(11)$^{a}$ |
| Nitrification | Kechire | 33(9)$^{b}$ | 27(9)$^{b}$ | 57(6)$^{a}$ |
| | Gambogi | 34(12)$^{a}$ | 37(19)$^{a}$ | 44(7)$^{a}$ |
| | Yala | 17(3)$^{a}$ | 22(7)$^{a}$ | 36(14)a |
| $NH_4^+$-N immobilization | Kechire | n.a. | n.a. | n.a. |
| | Gambogi | 15(15)$^{b}$ | 63(17)$^{a}$ | 23(26)ab |
| | Yala | 39(21)$^{b}$ | 243(90)$^{a}$ | 146(16)a |
| $NO_3^-$-N consumption | Kechire | 187(33)$^{a}$ | 104(30)$^{b}$ | 90(33)b |
| | Gambogi | 49(48)$^{a}$ | 151(99)$^{a}$ | 38(27)a |
| | Yala | 60(27)$^{a}$ | 27(2)$^{a}$ | 32(20)a |
| $N_2O$ fluxes | Kechire | 0.26(0.034)$^{a}$ | 0.12(0.019)$^{ab}$ | 0.08(0.023)$^{b}$ |
| | Gambogi | 0.16(0.043)$^{a}$ | 0.05(0.015)$^{b}$ | 0.05(0.008)$^{b}$ |
| | Yala | 0.05(0.007)$^{a}$ | 0.02(0.004)$^{ab}$ | 0.01(0.002)$^{b}$ |

*Values with similar superscripts are not significantly different*

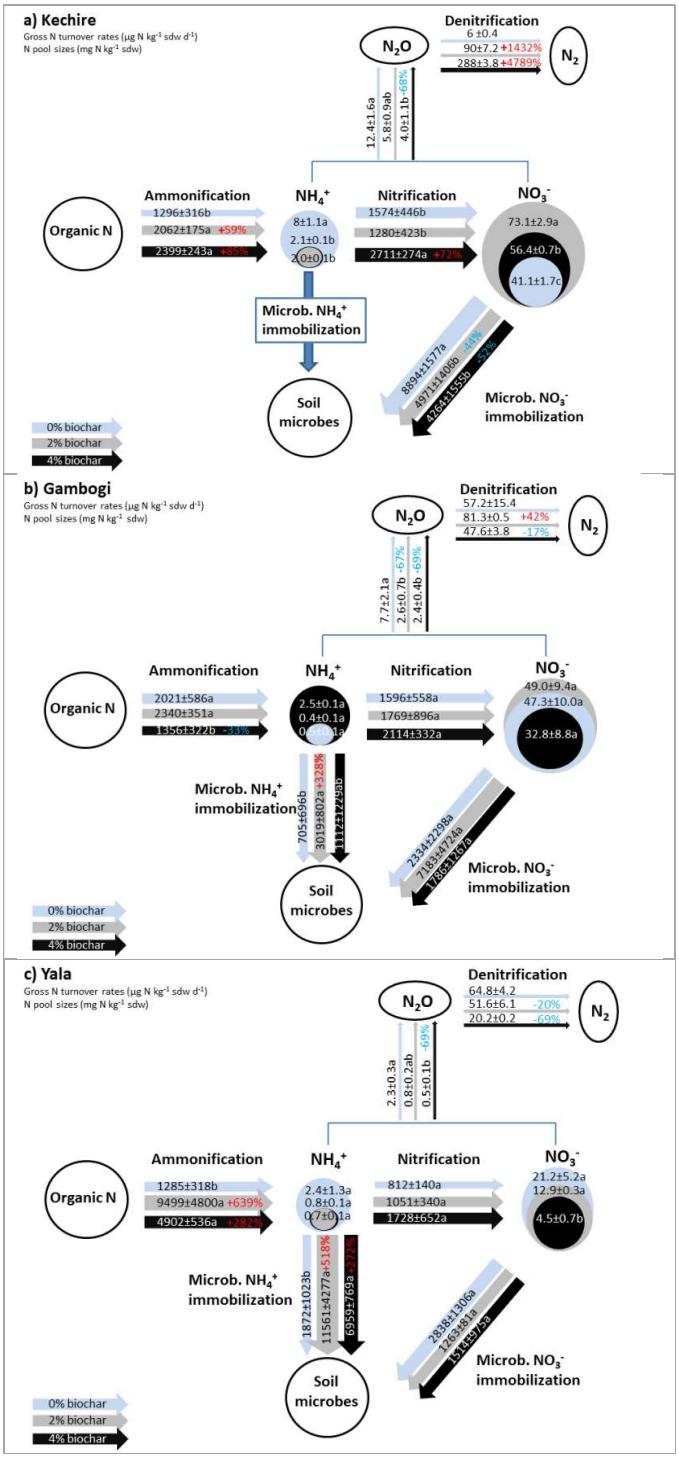



Fig 3.5: Mean gross N turnover rates (µg N $kg^{-1}sdwd^{-1}$) and N pool sizes (mg N $kg^{-1}sdw$) for the three soils and three biochar treatments. Blue: 0% biochar addition (control treatment); Grey: 2% biochar addition; Black: 4% biochar addition. Thickness of process arrows and nitrogen pool signatures is representative for respective turnover rates and pool sizes.

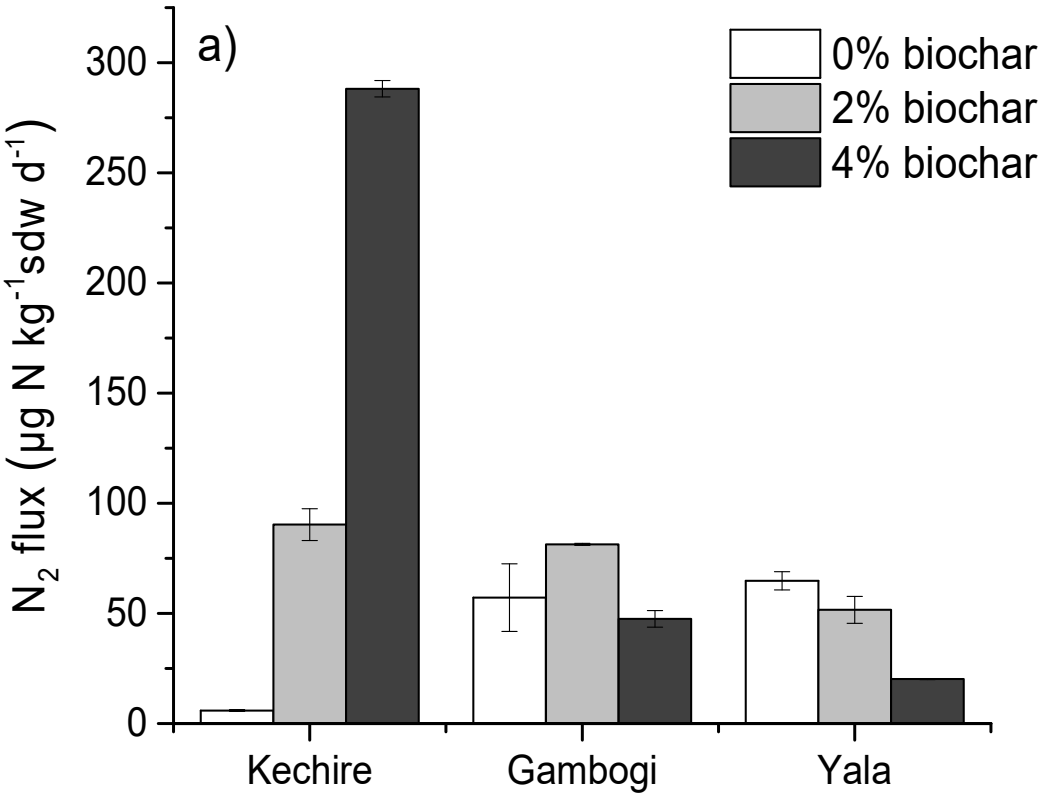



Figure 3.6: Dinitrogen emission rates from the three investigated soils at day 3 of the incubation period as influenced by biochar addition. (n=4)

## 3.4 Discussion

### 3.4.1 Biochar effects on $N_2O$ emission are largely decoupled from biochar effects on soil inorganic N availability and gross N turnover

One important finding of my study was that biochar had a consistent mitigation effect on $N_2O$ emission (ca 70% reduction) independent of the soil type and amount of biochar (Fig. 3.1). It is remarkable that this was observed for all three soils given their different initial $N_2O$ emissions, properties and management history. This is generally consistent with earlier studies reporting that biochar reduced net $N_2O$ emissions at the soil-atmosphere interface, although the mitigation of $N_2O$ emissions in this study was higher than the average effect reported (Case et al., 2012; Saarnio et al., 2013; Cayuela et al., 2013; Cayuela et al., 2015; Hagemann et al.,

2017). Some of the proposed mechanisms underlying $N_2O$ emission reduction include the reduction of mineral N ($NH_4^+$ and $NO_3^-$) availability, thus reducing the availability of N substrates for nitrification and denitrification (Singh et al., 2010). This mechanism relates on the one hand to biochar/soil surface/colloidal chemistry (e.g. pH and redox potential).

On the other hand, through addition of C, also heterotrophic microbial immobilization could increase after biochar addition, thereby also reducing soil mineral N availability. Furthermore, the different redox-active components of biochar directly affect denitrification and its single steps – e.g., through a promotion of nitrate and $N_2O$ reduction via electron donation, a decrease in total denitrification by serving as alternative electron acceptor, or – most universally – by acting as electron shuttle for the nosZ harbouring bacterial community, thereby increasing gross $N_2O$ reduction and net $N_2O$ exchange at the soil-atmosphere interface (Cayuela et al., 2013; Chen et al., 2017). The latter universal process might dominate in my study in view of the consistent $N_2O$ reduction across soils, while biochar effects on soil mineral N availability were inconsistent and multidirectional (Fig. 3.5). Further or associated mechanisms how biochar impacts $N_2O$ reduction in denitrification have been reported and encompass e.g., entrapment in water-saturated soil pores and consequent stimulation of microbial $N_2O$ reduction by classical denitrifiers and atypical $N_2O$ reducers (Harter et al. 2016).

The second important observation of my study is that $N_2O$ emission was not directly coupled to dynamics gross microbial N turnover (ammonification, nitrification and microbial N immobilization). This might reflect that denitrification dominates $N_2O$ emissions with denitrification and in particular the $N_2O$:$N_2$ ratios not directly depending on ammonification and nitrification. A decoupling of denitrification from ammonification and nitrification seems also possible in view of denitrification rates being several orders of magnitude lower than gross

soil N turnover, and due to the different environmental and soil biogeochemical controls (Butterbach-Bahl et al., 2013).

For soil $NH_4^+$ concentrations, there was a persistent and significant trend for reduced concentrations under biochar addition across soils (Fig. 3.5). However, this did not affect gross nitrification as a potential source process for $N_2O$, which was either increased (Acrisol 10 soil) or overall unchanged (Acrisol 100 and Ferralsol). The biochar-induced reduction of soil $N_2O$ emissions was also uncoupled from biochar effects on gross ammonification, which was either increased (Acrisol 10 and Ferralsol) or decreased (Acrisol100soil) by biochar (Fig. 3.5). Consequently, the persistent biochar-induced reduction of $N_2O$ emissions across three different agricultural soils, which had contrasting soil properties, gross N turnover and inorganic N availability, is supporting a rather universal mechanism that is acting during gross $N_2O$ formation and consumption through denitrification such as the "electron shuttle theory" (Cayuela et al., 2013). Sun et al. (2017) showed that biochars were able to rapidly transport electrons not only via surface functional groups but also through the carbon matrix, increasing electron transport in soils.

We have previously shown (Fungo et al., 2014) that steam-activation of biochar increases biochar's capacity to mitigate $N_2O$ emission. This suggests that the "electron shuttle" mechanism is facilitated by the surface chemistry of biochar to reduce activation energy required to cause cleavage of the $N_2O$ molecule to form N2. In fact, Chen et al. (2017) have shown that redox-active components (dissolved aromatic moieties and condensed aromatic structure) decreased total N denitrified because their dominant quinone moieties and electrical conductivity structure served as alternative electron acceptors. Chen et al. (2017) further observed that the redox-active components of biochar accelerated the last step of denitrification

and decreased $N_2O$ emission by 74%–99%. In all cases their study showed a significant increase in organic matter-oxidizing and nitrate-reducing bacteria in the nosZ harbouring bacterial community, which promoted $N_2O$ reduction.

A promotion of $N_2O$ reduction to $N_2$ by biochar should result in increased $N_2$ emissions. The data on $N_2$ emissions available in the current study, however, support this for only the Acrisol 10. This is attributed to the high CEC due to secondary minerals in the Ferralsol compared to the Acrisol. There is needs to note, however, that $N_2$ emissions are usually at least an order of magnitude larger than net $N_2O$ exchange at the soil-atmosphere interface (Fig. 3.5). This means that a small increase in gross $N_2O$ consumption due to biochar addition might hardly change the larger $N_2$ emissions in this study (see also Wen et al., 2016). Although the spatiotemporal resolution of my $N_2$ data preclude firm conclusions, the observed patterns tend to support that – independently of biochar effects on $N_2O$ reduction – there might be further effects of biochar on total denitrification, which again seems to be variable across the soils under investigation.

### 3.4.2 Biochar effects on gross N turnover

Though biochar effects on gross N turnover were variable across soils and biochar addition rate, we observed a remarkably strong stimulation of gross ammonification by a factor of 3–6 induced by biochar addition in the Ferralsol and a stimulation of gross nitrification in the Acrisol 10 soil by 70% at least under 4% addition. Soil physicochemical properties may affect gross N turnover and availability of N via interaction with the minerals (Kizito et al., 2014), physical entrapment of substrates, diffusion in micro-pores (Fidel et al., 2017), and availability of easily mineralizable organic carbon (Lan et al., 2017). Increased nitrification and ammonification following biochar amendment has also been reported in previous studies. The suggested mechanisms include (i) provision of energy for microorganisms to degrade existing SOM through co-metabolism (Clough and Condron, 2010; Anderson et al., 2011; Nelissen et

al., 2012); and (ii) absorbing potential allelochemical inhibitors of microbial metabolic pathways, such as monoterpenes and various polyphenolic compounds that are inhibiting nitrification (Ball et al., 2011).

A stimulation of microorganisms might also be based on the micronutrients such as Ca, Mg, Cu and B that are supplied by biochar. In the case of the Ferralsol, with the high clay content, CEC due to dominance of kaolinite and sesquioxides, low C and N contents and low inorganic N availability, the absorption capacity of clay minerals for available OC and $NH_4^+$ might explain the very low gross N turnover rates in the 0% biochar control treatment compared to the other two soils. Consequently, biochar addition indeed might have stimulated the microbial community by addition of C substrates, as all heterotrophic processes (ammonification, immobilization, denitrification) responded positively to the biochar treatment (Fig. 3.5).

## 3.5 Conclusion and recommendations

Our study demonstrates that biochar consistently reduced $N_2O$ emission in three different agricultural soils of western Kenya. As this effect was decoupled from biochar effects on gross soil N turnover and inorganic N concentrations, it may have been due to a universal mechanism such as the promotion of $N_2O$ reduction within the last step of denitrification, i.e., the "electron shuttle theory". Biochar effects on gross N turnover were, in contrast to those on $N_2O$ emissions, very variable across soils. Despite a large number of analyzed soil parameters, it remained difficult to disentangle the mechanisms of these different biochar effects on gross N turnover, which makes it difficult to predict biochar effects on soil functions related to soil microbial inorganic N production and consumption.

# CHAPTER FOUR: Aggregate size distribution in a biochar-amended tropical Acrisol under conventional hand-hoe tillage

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Fungo B., Lehmann J, Kalbitz K, Thiongo M, Okeyo I, Tenywa M, and Neufeldt H., 2017. Aggregate size distribution in a biochar-amended tropical Acrisol under conventional hand-hoe tillage. *Soil & Tillage Research 165, 190–197*

*http://dx.doi.org/10.1016/j.still.2016.08.012*

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## 4.1 Introduction

Biochar (pyrogenic organic matter) has shown promise for contributing to the triple benefit of improving soil productivity (Biederman and Harpole, 2013; Qian *et al*., 2015), sequestering soil carbon (Lehmann, 2007; Schneider *et al*., 2011; Lorenz and Lal, 2014) and reducing emission of greenhouse gasses (i.e. $CO_2$, $CH_4$ and $N_2O$) in agricultural soils (Cayuela *et al*., 2013; Fungo *et al.,* 2014). According to Woolf *et al.* (2010), sustainable global implementation of biochar projects can potentially off-set 12% (1.8 Pg $CO_2$-$C_e$ per year) of current anthropogenic $CO_2$-C equivalent emissions. However, the rate and scale of soil organic matter (SOM) turnover following biochar amendment depends on complex associations among biochar as well as soil properties (pH, native SOM, texture, mineralogy), agro-ecological conditions (precipitation and temperature), and management interventions such as use of manure and mineral fertilizers, tillage and irrigation.

Soil aggregation is a good indicator of soil quality because it mediates microbial feedbacks of C and N cycling in soils (Kapkiyai *et al*., 1999; Jimenez *et al*., 2011; Demisie *et al*., 2014). Biochar incorporation into soil can improve soil aggregate stability (Liu *et al*., 2014; Zhang *et*

*al*., 2015; Obia *et al*., 2016) by increasing exchangeable cation status of the soil, such as calcium (Enders *et al*., 2012; Jien and Wang, 2013), thereby inhibiting clay dispersion and associated disruption of soil aggregates. Biochar can also affect aggregation by the replacement of $Na^{+}$ and $Mg^{2+}$ in clay and aggregates through adsorption on its surfaces (Kwon and Pignatello, 2005). Under acidic environments such as those in highly weathered soils of the humid tropics, the hydroxyl and carboxylic groups on the oxidized biochar surface could also adsorb clay particles to increase macro-aggregate formation (Jien and Wang, 2013). However, the location of SOC within the aggregates and its chemical characteristics, which affect the rate of its decomposition (Balesdent *et al*., 1998; Christensen, 1996 Luo *et al*., 2014) and thus is sequestration potential, have not received much attention.

The effect of biochar on soil aggregation is disputed (c.f. Busscher *et al*., 2010; Peng *et al*., 2011; Zhang *et al*., 2015). Whereas increase in soil aggregate sizes as a result of increase in SOC when synthetic fertilizers are applied to the soil has been widely reported (Halvorson *et al*., 1999; Plaza-bonilla *et al*., 2012), some evidence of the reverse trend has also been observed (Sainju *et al*., 2003; Khan *et al*., 2007; Le Guillou *et al*., 2011; Plaza-Bonilla *et al*., 2013). Biochar is expected to increase aggregation because it can act as a nucleus of aggregation, similar to other particulate organic matter or microorganisms, especially because biochar increases microbial biomass (Lehmann *et al*., 2011;). Furthermore, increased OM input by roots and microbial mucilage following biochar amendment would increase aggregation (Abiven *et al*., 2015). Hence, it is unclear how N fertilizers in combination with biochar can affect both soil aggregate size distribution and the resultant physical protection of SOC.

When biochar is applied with green manure as *Tithonia diversifolia*, there is likely a greater amount of microbial activity (Li *et al*., 2012) and concomitant production of metabolites which,

through a variety of bonding mechanisms, may contribute to aggregate build-up. Mechanisms of interaction between biochar and the soil matrix that may result in soil stabilization include (1) occlusion in aggregates (Bachmann *et al*., 2008), (2) formation of biochar-cation complexes (interactions with polyvalent cations of soil minerals), or (3) interactions via polyvalent cations with soil mineral surfaces (OM-mineral associations) (von Lützow *et al*., 2007). Thus, biochar can be a binding agent for aggregate formation and stabilization. However, our understanding of these effects on aggregation of soil remains speculative. Understanding the effect of introducing biochar in such a system will aid predicting the long-term effects of these cropping practices on soil quality and C storage.

The objectives of the study were to determine the effect of biochar on (i) size and distribution of soil aggregates, (ii) changes in the content of SOC in different soil fractions, and (iii) relationships among aggregation, SOC, soil respiration ($CO_2$ emission) and biomass production under integrated soil fertility management on an Acrisol of the humid tropics. We hypothesized that under conventional hand-hoe tillage practices, (i) biochar would increase soil aggregation because over time, biochar gets more oxidized (Cheng *et al.,* 2008), so there may be more cation bridges between clay and biochar (increasing its ability to form organo-mineral and Biochar-SOM interactions), (ii) soil aggregation increases with increased amount of easily mineralizable organic matter inputs (such as *T. diversifolia* manures) because of the increased microbial activity and therefore mucilage, but might decrease with addition of mineral N fertilizer (such as urea) because of increased decomposition of easily mineralizable SOM, and (iii) increased soil respiration is related to SOM increases and larger aggregates.

## 4.2 Materials and Methods

### *4.2.1 Study site*

The field experiment was established in September 2012 at Kapsengere on the southern Nandi hills in western Kenya. The sites receive ~2000 mm mean annual rainfall in a bimodal distribution, with two rainy seasons per year (March–July and September–January) with a mean annual temperature of 26°C. Precipitation and air temperature were monitored throughout the experiment with the help of a weather station located near the experimental field. The soil is classified as Typic Kandiudults (USDA, 1999) developed on biotite-gneisses parent material. The natural vegetation is composed of tropical rainforest of Guineo-Congolian species. The trial was conducted for four consecutive maize rainy seasons (September 2012 to August 2014).

### *4.2.2 Preparation of the Biochar and T. diversifolia*

The biochar was produced by chopping and grinding Eucalyptus wood so as to pass through a 2-mm sieve. The sieved material was then pyrolyzed at a ramp of 5°C $min^{-1}$ to a maximum temperature of 550°C and retained for one hour before cooling to room temperature. In the laboratory, the resultant biochar was characterized for pH, surface area, CEC, elemental composition. *T. diversifolia* was prepared by cutting leaves from the field and chopping them into 50-mm lengths, air-dried and ground to pass through a 1-mm sieve before field application. The chopping and grinding was done to ensure uniform application in the field and reduce effects on soil physical properties. The physical and chemical characteristics of the above materials are presented in Table 4.1.

### *4.2.3 Experimental design*

The treatments were selected to represent presence and absence of biochar as well as low and high input of *Tithonia* green manure, with and without mineral nitrogen (N) fertilizer. This arrangement represented a range of conventional management practices of many small-holder farmers in integrated soil fertility management systems. Most farmers in the study area are small scale, resource poor, mixing small quantities of each of these amendments.

Table 4.1: Physical-chemical properties of the soil at start of experiment and the amendments used in the field trial (nd = not determined)

| | Biochar and soil | | Green manure (*T. diversifolia*) | |
|---|---|---|---|---|
| Property | Biochar | Soil | Property | |
| C (g $kg^{-1}$) | 868 | 23.3 | N (mg $g^{-1}$) | 21.5 |
| N (g $kg^{-1}$) | 27 | 21.0 | P (mg $g^{-1}$) | 2.3 |
| pH | 6.31 | 6.01 | K (mg $g^{-1}$) | 43.2 |
| EC (S $mm^{-1}$) | 196 | 88.0 | Ca (mg $g^{-1}$) | 13.6 |
| K (mg $kg^{-1}$) | 1490 | 223 | Mg (mg $g^{-1}$) | 2.6 |
| Ca (mg $kg^{-1}$) | 1920 | 1950 | S (mg $g^{-1}$) | 2.5 |
| Mg (mg $kg^{-1}$) | 150 | 312 | Mn (mg $kg^{-1}$) | 264 |
| Mn (mg $kg^{-1}$) | 188 | 782 | B (mg $kg^{-1}$) | 53.2 |
| S (mg $kg^{-1}$) | 36.5 | 14.0 | Zn (mg $kg^{-1}$) | 89.7 |
| Cu (mg $kg^{-1}$) | 0.77 | 1.97 | Mo (mg $kg^{-1}$) | 1.29 |
| B (mg $kg^{-1}$) | 1.07 | 0.33 | Fe (mg $kg^{-1}$) | 951 |
| Zn (mg $kg^{-1}$) | 108 | 13.5 | Cu (mg $kg^{-1}$) | 11.0 |
| Na (mg $kg^{-1}$) | 180 | 15.9 | Na (mg $kg^{-1}$) | 72.7 |
| Fe (mg $kg^{-1}$) | 164 | 67.2 | | |
| P (mg $kg^{-1}$) | 135 | 9.30 | | |
| Al (mg $kg^{-1}$) | 559 | 939 | | |
| C.E.C (meq 100 $g^{-1}$) | 18.2 | 16.2 | | |
| Silt (%) | nd | 17.5 | | |
| Sand (%) | nd | 10.7 | | |
| Clay (%) | nd | 71.6 | | |

The experiment was laid out in a randomized complete block design with three replicates. The treatments included the following: two levels of biochar (0 and 2.5 t $ha^{-1}$); three levels of green manure applied as *T. diversifolia* (0, 2.5 and 5 t $ha^{-1}$); and two levels of mineral N applied as Urea (0 and 120 kg N $ha^{-1}$) (Table 2). Each treatment was established in a 2 x 2-meter plot

separated by a one-meter distance within and between rows. Due to the inherently low fertility of the soil, 30 kg $ha^{-1}$ of $P_2O_5$ as TSP and 30 kg $ha^{-1}$ of $K_2O$ as Muriate of Potash were applied to each plot.

Table 4.2: Experimental treatments for determining the effect of Biochar, *T. diversifolia* green manure and Urea on soil aggregate distribution and C content in a maize field in western Kenya

| Treatment | Biochar | | Green manure (*T. diversifolia*) | | Mineral N fertilizer (Urea) | |
|---|---|---|---|---|---|---|
| | Rate (t $ha^{-1}$)• | *Code* | Rate (t $ha^{-1}$)¤ | Code | Rate (kg N $ha^{-1}$)* | Code |
| 1 ($B_0T_0U_0$)(Control) | 0.0 | B0 | 0.0 | T0 | 0.0 | U0 |
| 2 ($B_0T_{2.5}U_0$) | 0.0 | B0 | 2.5 | T2.5 | 0.0 | U0 |
| 3 ($B_0T_5U_0$) | 0.0 | B0 | 5.0 | T5 | 0.0 | U0 |
| 4 ($B_0T_0U_{120}$) | 0.0 | B0 | 0.0 | T0 | 120 | U120 |
| 5 ($B_0T_{2.5}U_{120}$) | 0.0 | B0 | 2.5 | T2.5 | 120 | U120 |
| 6 ($B_0T_5U_{120}$) | 0.0 | B0 | 5.0 | T5 | 120 | U120 |
| 7 ($B_{2.5}T_0U_0$) | 2.5 | B2.5 | 0.0 | T0 | 0.0 | U0 |
| 8 ($B_{2.5}T_{2.5}U_0$) | 2.5 | B2.5 | 2.5 | T2.5 | 0.0 | U0 |
| 9 ($B_{2.5}T_5U_0$) | 2.5 | B2.5 | 5.0 | T5 | 0.0 | U0 |
| 10 ($B_{2.5}T_0U_{120}$) | 2.5 | B2.5 | 0.0 | T0 | 120 | U120 |
| 11 ($B_{2.5}T_{2.5}U_{120}$) | 2.5 | B2.5 | 2.5 | T2.5 | 120 | U120 |
| 12 ($B_{2.5}T_5U_{120}$) | 2.5 | B2.5 | 5.0 | T5 | 120 | U120 |

***•One kg of biochar per treated plot; *100 g per treated plot; ¤1 and 2 kg of T. diversifolia, respectively. Biochar C = 86.8%, T. diversifolia C ~ 48%***

### *4.2.4 Management of experiment*

Conventional tillage, where a hand-hoe is used to mix the top 0.10 - 0.15 m of the soil, was used during land preparation at the start of each season, and the two weeding times during each season. Application of biochar was done once at the start of the first season on 3rd October 2012. Constant amounts (2.5 or 5 t $ha^{-1}$, Table 2) of green manure, phosphorus (TSP) and potassium (MoP) were applied to each plot once at the start of each season. Mineral N (urea)

was applied in two splits; 40% at planting and 60% 30 days after planting. The biochar, manure and mineral fertilizer were broadcast on the soil surface by hand and incorporated into the top 0.1 m soil. Two seeds of the maize cultivar HB 513 were planted at a spacing of 0.25 m within and 0.5 m between rows (40 plants per planting hole). Weeding was done at 30 and 50 days after planting then tilled with a hand-hoe to a depth of 0.1 m. Thinning was done during the first weeding to retain one plant per pocket. In total, four consecutive seasons of maize crop were harvested (3rd October 2012 through 17th August 2014).

### *4.2.5 Soil respiration and above ground biomass*

We used data on soil respiration ($CO_2$ evolution at soil surface) and aboveground biomass. Briefly, measurements were conducted using a static closed chamber method. The chamber consisted of a PVC tube (diameter = 0.3 m; height = 0.15 m) transversely divided into two parts to make a base (0.05 m) and a cover (0.1 m). The base was driven into the soil to ~0.02 m below the soil surface. To ensure air-tight conditions, a rubber ring was placed between the base and the cover. A photo-acoustic infrared field gas monitor (INNOVA 1402, Lumasense Technologies A/S, Ballerup, Denmark) was used to analyze the fluxes in the field.

The gas monitor was connected to the chamber by two 0.7 m-long Teflon tubes as gas inlet and outlet. Inside the cuvette, air humidity and temperature were monitored by a digital thermo-hygrometer (PCE-313 A, Paper-Consult Engineering Group, Meschede, Germany) attached to the cover from the outside and only the sensor reached inside the chamber through a rubber screw connector. Two chambers were set up in each plot. For each gas sampling event INNOVA recorded four measurements at 2-minute intervals after closing the chamber. Flux measurements were conducted weekly except during dry periods where bi-monthly measurements were taken. Measurements were taken at similar time during the day (9-11am).

Temperature ranged from 23-28ºC. No significant relationship between temperature and $CO_2$ flux was observed.

### *4.2.6 Soil sampling and analysis*

Composite soil samples were taken from five random locations within each plot from a 0 - 0.15 m depth on 17th August 2014 (24 months after biochar application). Soil cores (*d*=50 mm, *l*=50 mm; *v*=100 $cm^3$) were used to collect samples for bulk density determination. Approximately 200 g of the air-dry, 2-mm sieved soil samples were packed in zip-locks and taken to the laboratory for analysis.

### *4.2.7 Soil fractionation and chemical analysis*

Particle size fractionation procedure was used to determine the mean weight diameter (MWD) as an indicator of soil aggregate distribution. Bulk soil was divided into four size fractions; (i) Large Macro-aggregates (>1000 µm, designated LM); (ii) Small Macro-aggregates (250-1000 µm, designated SM); (iii) Micro-aggregates (250-53 µm, designated M) and (iv) Silt + Clay (< 53 µm, designated S+C). Four sieves corresponding to these size classes were sequentially arranged vertically. Eighty grams of an air-dried soil sample, without disturbing the aggregates was put on the first sieve of the set in a water bucket and was gently moistened for 10 min. After the soil was moistened with water, aggregates were separated by moving the sieve vertically at 30 strokes $min^{-1}$ for 5 min. At the end of wet-sieving, all aggregate-size fractions remaining on each sieve were collected, dried at 60°C, and the sand and aggregates were separated (Wang *et al*., 2012). The mean weight diameter (MWD, mm) was calculated as follows:

$$\text{MWD} = \sum_{i=1}^{n} w_i \,.\, \overline{x_i}$$

where $\overline{x}$ is the average diameter of the openings of the two consecutive sieves, and $w_i$ the weight ratio of aggregates remained on the ith sieve. For the determination of aggregate size distribution, the weight ratio of aggregates of each sieve to the total weight of aggregates was calculated. Then, the C and N content in the various size fractions was determined. Soil $pH_{water}$ was determined with a glass electrode (Soil:Water = 1:5 w/v). Soil organic C and total N were measured with an Elementar Vario max CNS Analyzer (German Elementar Company, 2003). It was assumed that TOC = SOC since these acid soils have negligible amounts of inorganic carbonates.

### *4.2.8 Statistical analysis*

The cumulative $CO_2$ flux for each treatment was derived using a linear Trapezoidal rule with sampling dates as the time intervals. Changes in SOC content were calculated as the difference between values at the beginning and end of the sampling period, as well as subtracted the C addition from biochar and *T. diversifolia*. Biochar-induced differences for each treatment were calculated as the difference between the treatment value and that of the control. Treatment main effects and their interaction on MWD and C content in soil aggregates were examined using fixed effect analysis of variance (ANOVA). Post Hoc separation of means was done using Least Significant Difference (LSD) at 5%. Linear regression was used to study the relationship between MWD and SOC, aboveground biomass and soil respiration as well as that between SOC and above ground biomass (C).

## 4.3 Results

### *4.3.1 Distribution and MWD of water stable aggregates*

The values of MWD ranged from 378 µm to 525 µm (mean±SE = 423±23) (Figure 4.1). The biochar addition had no effect on MWD, but the combination of biochar with either *Tithonia*

($B_{2.5}$+$T_{2.5/5}$) or urea ($B_{2.5}$+$U_{120}$) significantly increased MWD by 34±5.2 µm (8%) and 55±5.4 µm (~13%), respectively compared to the control. The $B_{2.5}$+$T_{2.5/5}$and $B_{2.5}$+$U_{120}$ treatments were themselves not significantly different. The $T_{2.5/5}$+$U_{120}$ treatment significantly increased MWD by 17±4.1 µm (Figure 4.1) compared to the control and the rate of *Tithonia* addition had no significant effect on MWD. The MWD of the $B_{2.5}$+$U_{120}$ was comparable to that of the $B_{2.5}$+$T_{2.5}$ treatments, and was significantly lower than $B_{2.5}$+$T_{5.0}$. MWD was not significantly different from the control under the three-amendment mixture (P>0.05).

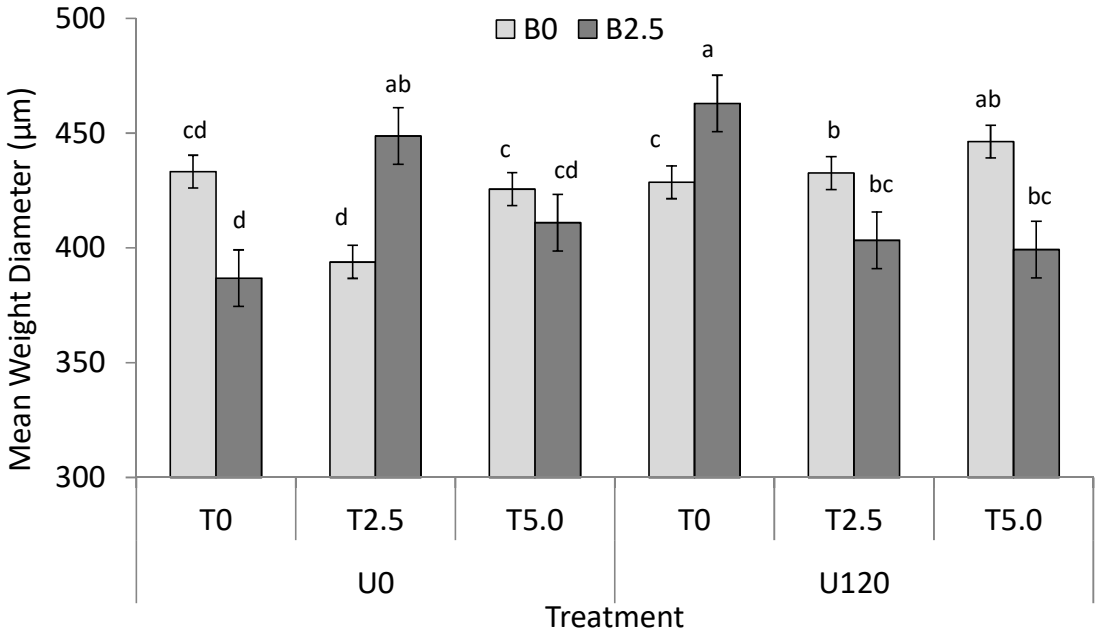



Figure 4.1: Effect of additions of biochar (B), *T. diversifolia* (T) green manure and urea (U) on mean weight diameter of soil aggregate (values indicate amendment rate in t $ha^{-1}$). Error bars are standard error, n=3, means with the same letter are not significantly different at p<0.05.

Table 4.3 shows the results of ANOVA of the main effects of each amendment as well as their interactions while Figure 4.2 shows the effect of the treatments on the distribution of the different aggregate size fraction in the bulk soil. The SM dominated the size distribution (45%) followed by M fraction (29%), then S+C (15%). The LM were the least represented fraction

(10%) (Table 3). Sole biochar treatment had no effect on size proportion. Sole *T. diversifolia* increased the S+C fraction by 8% (*F=3.8; P= 0.030*) after two years of the field trial.

Table 4.3: Variance analyses of effects of biochar, *T. diversifolia* and urea and their interactive effects on soil aggregate properties (LM large macro-aggregates; SM small macro-aggregates; M micro-aggregates; S+C silt and clay)

| Factor | LM | | SM | | M | | S+C | | Total C | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F | P | F | P | F | P | F | P | F | P |
| B | 0.06 | 0.805 | 0.0 | 0.946 | 1.0 | 0.332 | 0.0 | 0.974 | 12.31 | **0.001*** |
| T | 0.25 | 0.782 | 0.2 | 0.847 | 0.68 | 0.520 | 9.1 | **0.002*** | 1.75 | 0.176 |
| U | 0.04 | 0.853 | 10.2 | **0.005*** | 0.12 | 0.734 | 31.5 | **0.000*** | 2.84 | 0.093 |
| B x T | 1.89 | 0.178 | 0.0 | 0.960 | 0.69 | 0.513 | 1.6 | 0.225 | 0.16 | 0.856 |
| B x U | 0.00 | 0.990 | 0.2 | 0.682 | 0.88 | 0.360 | 0.0 | 0.848 | 0.17 | 0.679 |
| T x U | 0.43 | 0.659 | 1.0 | 0.404 | 0.59 | 0.568 | 9.0 | **0.002*** | 0.08 | 0.926 |
| B x T x U | 1.85 | 0.184 | 5.0 | **0.019*** | 7.1 | **0.006*** | 17.0 | **0.000*** | 0.19 | 0.827 |

**Values in bold are statistically significant*

Overall, the proportion of LM increased by 53% while the S+C fraction decreased by 46% over the two years of the field experiment (Figure 4.2). The proportion of the LM fraction reduced by 14% but the proportion of the M fraction did not change over the two years. The B+Tand B+U treatments significantly increased the proportion of the SM fractions by 15% (Table 4.3). There was no significant difference in size proportion between $T_{2.5}$ and $T_5$. Sole urea additions decreased the proportion of LM but increased the S+C fraction (Figure 2). The B+T+U mixture significantly increased the proportion of the LM by 7.0±0.8%, but significantly reduced the proportion of the S+C fraction by 5.2±0.23%, independent of the amount of *T. diversifolia* (Figure 4.2). The S+C fraction was not affected by any of the amendments, in sole or in combined application.

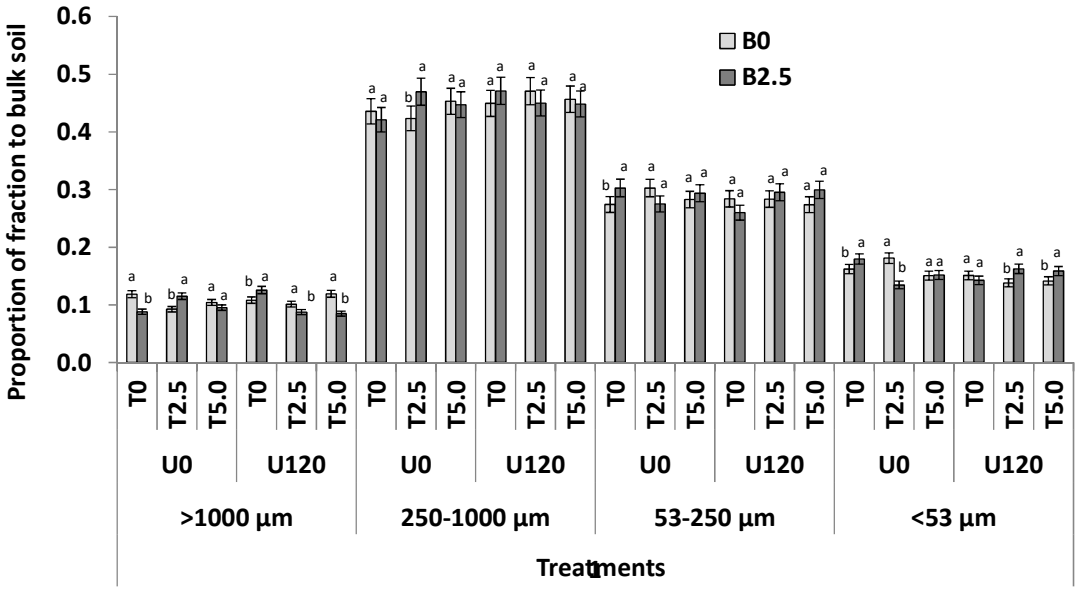



Figure 4.2: Proportion of different size aggregates in bulk soil of an Acrisol 2 years after amended with biochar (B), *T. diversifolia* (T) green manure and urea (U) (values indicate amendment rate in Mg $ha^{-1}$). Error bars are standard error, n=3, means with the same letter are not significantly different at $p<0.05$.

### *4.3.2 SOC in aggregates*

The S+C fraction contained the largest proportion of SOC (30%) followed by M (25 $gkg^{-1}$). The LM and SM had a similar mean content of SOC content (23g $kg^{-1}$). At the end of the two years, mean SOC contents in all the soil fractions had increased by a range of 0.44 to 4.69 g $kg^{-1}$ (1.92±1.06=Mean±SE). The increase in SOC was 9.6±1.0, 5.7±0.8, 6.3±1.1 and 4.2±0.9 g $kg^{-1}$ for LM, SM, M and S+C, respectively. The increase in SOC content in LM was significantly higher than for the SM, and the SM was not significantly different from M but significantly higher than the SOC content in the S+C fraction. Overall, biochar and *T. diversifolia* increased SOC. In the S+C fraction, urea and *T diversifolia* togetherincreased SOC by 2.0±0.2 g $kg^{-1}$ (~7%) without biochar but had no effect when biochar was added.

Table 4.4: Content of SOC (g $kg^{-1}$ soil) associated with different soil fractions. SE = standard Error, n=3 (LM large macro-aggregates; SM small macro-aggregates; M micro-aggregates; S+C silt and clay)

| Treatment ID | LM | | SM | | M | | S+C | | TOC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 1 ($B_0T_0U_0$) (Control) | 27.4 | (±0.24)a | 25.5 | (±0.46)ab | 26.4 | (±1.18)c | 31.6 | (±0.48)c | 29.5 | (±0.18)a |
| 2 ($B_0T_{2.5}U_0$) | 25.7 | (±0.67)b | 25.3 | (±0.43b | 28.5 | (±0.86)a | 32.4 | (±0.84)ab | 28.2 | (±0.88)b |
| 3 ($B_0T_5U_0$) | 25.7 | (±0.81)b | 25.6 | (±0.75)ab | 27.2 | (±1.06)b | 33.6 | (±1.49)a | 27.8 | (±0.65)b |
| 4 ($B_0T_0U_{120}$) | 25.9 | (±0.60)b | 23.6 | (±1.22)c | 27.9 | (±0.58)ab | 33.3 | (±0.69)a | 27.7 | (±0.06)b |
| 5 ($B_0T_{2.5}U_{120}$) | 24.9 | (±0.77)c | 26.0 | (±0.48)a | 26.4 | (±0.85)c | 31.9 | (±1.24)c | 27.3 | (±0.56)b |
| 6 ($B_0T_5U_{120}$) | 25.3 | (±1.34)a | 24.8 | (±1.56)bc | 27.7 | (±1.79)ab | 32.1 | (±1.92)c | 27.5 | (±0.66)b |
| 7 ($B_{2.5}T_0U_0$) | 24.3 | (±0.68)cd | 24.6 | (±1.44)bc | 27.0 | (±1.98)c | 32.5 | (±1.91)ab | 27.1 | (±0.90)bc |
| 8 ($B_{2.5}T_{2.5}U_0$) | 24.2 | (±1.17)cd | 23.9 | (±1.11)c | 26.8 | (±1.32)c | 31.7 | (±3.84)d | 25.9 | (±0.13)c |
| 9 ($B_{2.5}T_5U_0$) | 24.8 | (±1.17)c | 23.8 | (±1.08)c | 26.5 | (±0.38)c | 31.3 | (±1.03)d | 26.6 | (±0.65)c |
| 10 ($B_{2.5}T_0U_{120}$) | 24.2 | (±0.78)cd | 24.1 | (±1.44)c | 25.0 | (±0.75)d | 30.8 | (±1.24)cd | 26.0 | (±0.61)c |
| 11 ($B_{2.5}T_{2.5}U_{120}$) | 22.5 | (±1.41)e | 23.8 | (±0.84)c | 25.3 | (±1.13)d | 32.5 | (±0.52)b | 25.8 | (±0.01)c |
| 12 ($B_{2.5}T_5U_{120}$) | 23.9 | (±1.27)e | 24.1 | (±0.72)c | 25.4 | (±0.90)d | 32.6 | (±0.58)b | 26.3 | (±0.81)c |

*In each column, means with the same letter are not significantly different at $p<0.05$*

### *4.3.3 MWD, SOC and soil respiration*

MWD was inversely related to SOC and SOC increase explained 37% of the decrease in MWD (Figure 3A). In addition, MWD increased with increasing aboveground biomass. The amount of increase in MWD attributed to biomass production was 11% (Figure 3B). SOC and aboveground biomass were also inversely related (Figure 3C). There was no significant relationship between MWD and soil respiration ($CO_2$ emission) (Figure 3D).

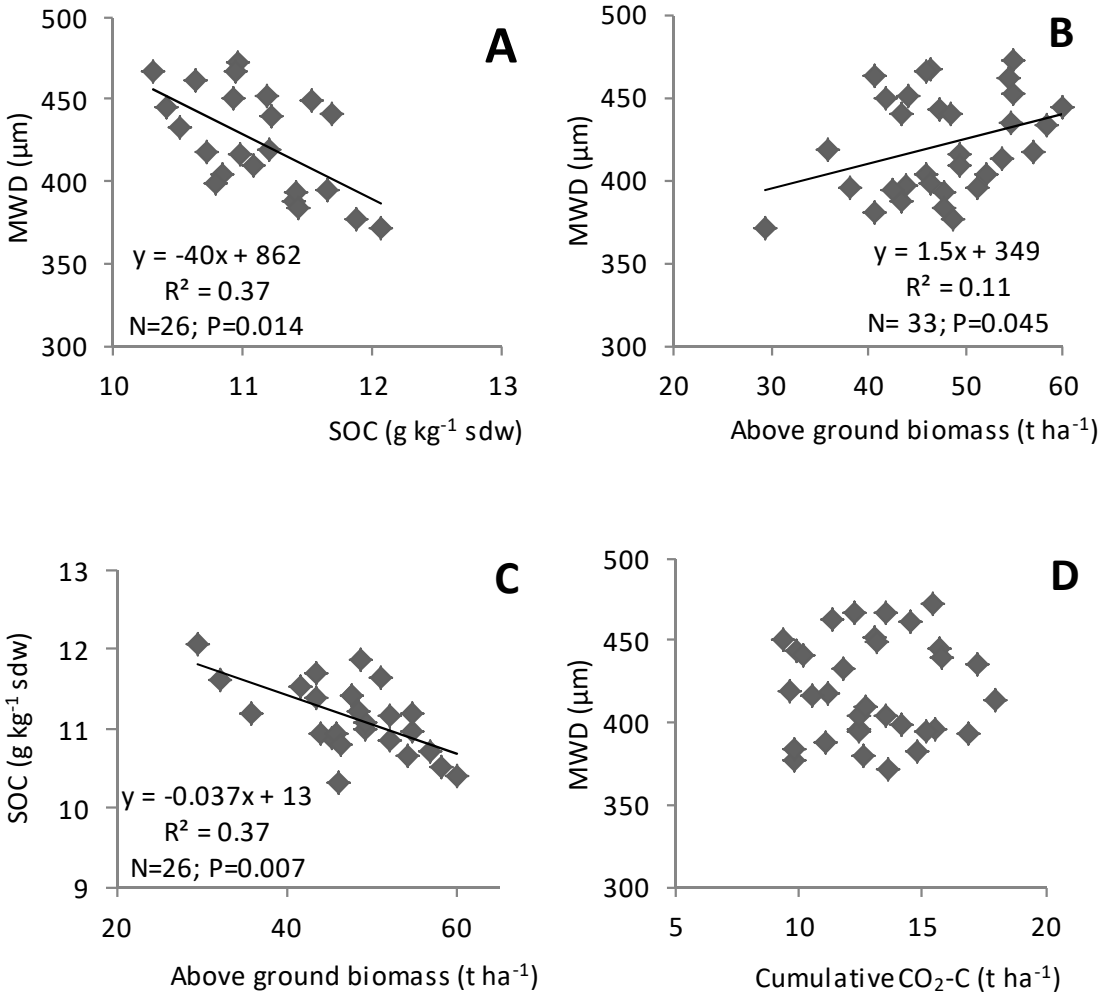



Figure 4.3: Linear relationships between MWD and SOC (A), aboveground biomass (B) and soil respiration (D) and that between SOC and above ground biomass (C)

## 4.4 Discussion

### *4.4.1 Size and distribution of soil aggregates*

Our expectation that biochar would consistently increase soil aggregate formation, was not met entirely. Biochar alone may not have increased aggregate size significantly after two years because aggregates formed in the early stages could have been broken down due to tillage at planting, and weeding. Our results agree with those of others (e.g. Herath *et al*., 2014; Peng *et al*., 2016) who found no effect of biochar on micro-aggregation. However, other studies have reported increases in aggregate size (Sun and Lu., 2013; Liu *et al*., 2014; Abdelhafez *et al*., 2014). Differences in the effect have probably occurred due to time, application rate and texture of biochar used. For example, Liu *et al*. (2014) reported increased aggregation at 40 t $ha^{-1}$, but not at 20 t $ha^{-1}$. Sun and Lu (2013) also reported increased aggregation with 90 t $ha^{-1}$ straw biochar but no difference with wood chips biochar at similar rate.

The positive relationship between MWD and biomass growth (Fig. 4.3B) could be due to increased easily mineralizable C input. Ability of biochar to improve soil structure and infiltration can also increase water viscosity, thereby increasing soil aggregation (Bandyopadhyay and Lal, 2014; Regelink *et al*., 2015). However, this relatively weak relationship suggests either short-term build-up of unstable soil aggregates, which soon break down, or time was insufficient for slow buildup of aggregates. It is possible that aggregation could have been limited by the type of microbially derived OM during the decomposition and degradation of *T. diversifolia* and biochar, respectively. According to Bronick and Lal (2005), easily decomposable inputs such as green manure have strong but transient effects on aggregate stability while more recalcitrant inputs such as decomposed manures would show weak but long-term effects. There is also evidence that mucilage types (or chemical saccharides compositions) and amounts secreted from different plant species, as well as soil moisture levels

and soil fauna also influence soil aggregate structure (Degens and Sparling, 1996; Six *et al*., 2004; Bossuyt *et al*., 2005).

The indifference in MWD with biochar additions alone could be related again to the quantity and quality of biochar applied (texture, pH, CEC). The soil used in my study was an Acrisol, which is relatively high in 1:1 clay, low in CEC, and in base cations and we would expect such a soil to respond to biochar amendment by increasing aggregation (cf Gentile *et al.,* 2010; Ouyang, *et al*., 2013). According to Gentile *et al*. (2010), aggregates of fine-textured soils are more responsive to organic matter inputs compared to the coarse-textured ones. However, Liu *et al.* (2012) found increased aggregation in two silt loam soils but not in two sandy loam soils, suggesting that higher clay content would increase likelihood of aggregation. Our soil was dominated by relatively a larger particle size fraction (250-1000 μm), which could partly explain the limited response. The carboxylic and phenolics, which are the predominant functional groups responsible for surface charge in biochar, decreased with increased pyrolysis temperature (Amonette and Joseph, 2009; Keiluweit et al., 2010). The feedstock used for making the biochar as well as the relatively high pyrolysis temperature (550°C) of my biochar could have resulted in lower surface charge as indicated by relatively low CEC (Table 4.1), hence low propensity for aggregation over the time period studied here.

The increase in the proportion of micro-aggregates with *T. diversifolia* and urea could be related to the increased biomass C from microbial C after decomposition of *T. diversifolia*, as well as plant root biomass. Indeed, Whalen and Chang (2002) showed that the increase in SOC caused by the application of manures is a direct result of the manure composition and an indirect result of the increased crop growth and crop residue in response to the nutrient supply. During SOM decomposition by microorganisms, synthesis of hydrophilic polysaccharides

promotes inter-particle cohesion through adsorption to mineral matter (Chenu, 1989; Verchot *et al*., 2011; Demisie *et al*., 2014), thus increasing soil aggregation.

### *4.4.2 SOC in soil aggregate*

We postulated that easily mineralizable C derived from *T. diversifolia* could end up in the micro-aggregates. These micro-aggregates would later be incorporated into macro-aggregates. The build-up of micro-aggregate C observed in my study (Table 4.4) is in support of the "bottom-up" process of soil aggregation proposed by Verchot *et al*. (2011) whereby micro-aggregates form through the interaction between mineral surfaces and organic matter with little protection in early stages of micro-aggregate formation (Emerson, 1959; Tisdall and Oades, 1982; Lehmann *et al*., 2007). This proposition is further supported by Kinyangi *et al*., (2006), and Verchot *et al*. (2011) that aliphatic-C, which tends to form thin films on mineral surfaces and is found throughout the microstructure of the aggregates, appears to be the responsible agent for stabilization of micro-aggregates. Verchot *et al*. (2011), using $\delta^{13}C$, also found that the portion of the new carbon from the trees in an agroforestry fallow was sequestered in the micro-aggregate.

We did not observe a significant change in macro-aggregate C content since the biochar was predominantly fine-textured (<250 μm) compared to Herath *et al*. (2014) for example, who had relatively larger-sized particle sizes of biochar. On the other hand, some studies (c.f. Herath *et al*., 2013; Zhang *et al*., 2015) found higher C in macro-aggregate fractions than in the smaller ones, indicating that biochar amendments could particularly increase C storage in these larger macro-aggregate fractions as free particulate organic matter. Although air-drying soils before fractionation can have affected on aggregation to some extent (Warkentin and Maeda, 1980), we assumed that the effect, if any, was similar for all treatments.

### *4.4.3 Relationship between MWD and SOC, soil respiration and biomass production*

The negative relationship between MWD and SOC could be explained by the fact that at this time scale, the highest values of MWD were related to the microbial activity induced by the green manure. In my case, the soil is highly weathered with free ions such as Al and Fe as well as sesquioxides, and this could significantly curtail aggregation over relatively short time scales (<10 years). Arthur*etal*. (2014) also observed no relationship between SOC and aggregation when several soil types were amended with 7.5 t $ha^{-1}$ of groundrapeshoot manure. In my case, the amount of manure added was less than that used by Arthur*etal*. (2014) and that could partly explain the absence of any significant response. The increase in $CO_2$ emission (soil respiration) is attributed to the increase in mineralizable C particularly in relatively low-C soils (Sagrilo *et al*., 2014). Improvement in soil aeration, following biochar addition has also been reported but the lack of a change in MWD and bulk density rules out this explanation from my study.

Increased plant growth was expected to increase aggregation via OM input in associated root biomass, but a reverse relationship was observed instead. Also, we did not observe a significant relationship between MWD and $CO_2$ evolution. Mizuta *et al.* (2015) found that although polysaccharides such as starch and cellulose accelerated soil aggregation, the decomposition of these amendments influenced only aggregation, not aggregate stabilization. It has also been reported that the role of organic matter (aggregating or disaggregating) depended on its chemical composition and presence of other binding agents (Goldberg *et al*., 1990; Mizuta *et al.,* 2015; Wu *et al.* 2016). The conventional hand-hoe tillage system used in this study is what is practiced by most farmers in the area and it may compromise short-term build-up of soil aggregates. Therefore, within the timeframe of this study, the aggregated distribution benefits of relatively low organic input may not be evident. Long-term trials testing various tillage

practices are required to clarify the interaction between biochar and other amendments on aggregation and stabilization of soil aggregates as a means to improve soil C sequestration.

## 4.5 Conclusions and recommendations

Application of biochar alone did not affect aggregate stability of the humid Acrisol within two years under conventional hand-hoe tillage practice. However, when applied together with easily mineralizable *T. diversifolia* at a rate of 2.5 t $ha^{-1}$, it increased aggregate proportion of medium-sized soil aggregates and resulted in increased SOM in the micro-aggregates. Mineral fertilizer reduced macro-aggregate stability at least in the short term, but SOC increased in the micro-aggregates. This may result in increased soil stability in the long term. We did not find a relationship between soil aggregation and soil respiration but biomass was positively related to the MWD as an indicator of soil aggregate stability. This indicates that OM input by plants is an important feedback mechanism for soil aggregation. The pattern observed in my data suggests that within the timeframe of the study, biochar is stored as free particulate OC in the micro-fraction. This shows a tendency to shift native SOC from the larger-size aggregates to the smaller-sized fraction in the short-term (2 years). Therefore, applying easily mineralizable organic matter such as *T. diversifolia* green manure may hasten build-up of macro-aggregates in the long term but this needs further investigation.

# CHAPTER FIVE: Emissions intensity and carbon stocks of a tropical Acrisol after amendment with Tithonia green manure, urea and biochar

**Fungo, B**., Lehmann, J., Kalbitz, K., Tenywa, M., Thionġo, M., and Neufeldt, H., 2017. Emissions intensity and carbon stocks of a tropical Acrisol after amendment with Tithonia green manure, urea and biochar. *Field Crops Research. 209, 179–188.* http://dx.doi.org/10.1016/j.fcr.2017.05.013

## 5.1 Introduction

The search for climate-smart agricultural production technologies is directing research to identify innovations that address multiple benefits such as crop productivity, carbon sequestration and mitigation of soil-atmosphere greenhouse gas (GHG) emissions. Addition of biochar (pyrogenic organic matter) to agricultural soils as a management strategy has reportedly increased crop yields in several studies but has shown variable effects on GHG fluxes (Knoblauch et al., 2011; Cayuela et al., 2014). Biochar may affect fluxes of GHGs such as $CO_2$, $CH_4$ and $N_2O$ by a variety of mechanisms, including: (i) the turnover rate of soil organic matter (SOM), which in turn determines the availability of C and N, the precursors for GHG production or consumption,(ii) soil physical properties (e.g. gas diffusivity, aggregation, water retention) (Quin et al, 2014); (iii) soil chemical properties (e.g. pH, Eh, availability of organic and mineral N and dissolved organic C, organo- mineral interactions); and (iv) soil biological properties (e.g. microbial community structure, microbial biomass and activity, macro-fauna activity, N cycling enzymes) (Van Zwieten et al., 2015).

Biochar may also change the effects of adding easily mineralizable organic matter as well as mineral N fertilizers on GHG emissions from soil. Additions of legume materials as a fertilizer

provide both N and C that typically lead to greater GHG emissions from soils (REF). Similarly, the amount of fertilizer N additions is considered proportional to the $N_2O$ emissions (REF). It is not clear, if simultaneous addition of biochar with either fertilizer N or legume mulch or a combination if the two may result in GHG emission reductions. Uncertainty also exists whether any emission reductions would persist over several cropping seasons.

It is not clear what role possible feedback through enhanced crop growth plays to the GHG budget. Greater crop growth and presumably greater C return to soil have been found where the pH is increased by biochar to neutral values (Jeffery et al., 2015) and this feedback would therefore be expected to be greatest in acid tropical soils. Whereas Spokas (2013) suggested that biochar has mainly shorter-term GHG mitigation effect (few days to several weeks) after application, Lentz et al. (2014) indicated that the effects may be long-lived. As such, questions remain concerning the long-term implications in cropping periods particularly for field-based biochar studies. Zimmerman *et al*. (2011) observed that native SOM mineralization was higher during the early incubation stage (first 90 days) and low during the later incubation stage (250-500 days). Maestrini et al. (2014) also reported pyrogenic OM (PyOM) to have promoted native OM mineralization during the first 18 days and inhibited it afterward (up to 150 days).

The objectives of this study were to determine the effect of biochar on (i) GHG fluxes ($CO_2$, $CH_4$ and $N_2O$), (ii) Emissions Intensity (EI; the net $CO_2$-equivalent for $CH_4$ and $N_2O$ per ton of grain yield), and (iii) changes in soil organic carbon (SOC) and ecosystem carbon balance of a low-fertility tropical agricultural soil when integrated with organic and mineral N inputs. The overall hypothesis is that biochar is responsible for controlling the release of labile N and C from high N mineral and organic amendments and an accompanying reduction in $CO_2$, $CH_4$ and $N_2O$ emissions. Specifically, we hypothesize that compared to un-amended soil, biochar

(i) reduces availability of N from both organic and mineral sources such as *T. diversifolia* and urea to thereby reduce $N_2O$ emission resulting from interactions of $N_2O$ with biochar; (ii) increases availability of easily mineralizable C from both soil and organic amendment to reduce the $CH_4$ soil sink; (iii) affects emissions of $CH_4$ and $N_2O$ early on, but not in later seasons as active surfaces get saturated with time; and (iv) increases plant growth as a result of biochar additions that are more important than changes in other soil processes affecting GHG emissions.

## 5.2 Materials and Methods

### *5.2.1 Study site*

The field experiment was established in September 2012 at Kapsengere on the southern Nandi hills in western Kenya (00' 09' 34''N and 34' 57' 37''E). The site receives ~2000 mm mean annual rainfall in a bimodal distribution, with two growing seasons per year, March–July and September–January. The mean annual temperature is 26°C. The soils are classified as Typic Kandiudults (USDA, 1999) developed on biotite-gneiss parent material. The experimental field was divided into three blocks. Soil properties before the experiment were determined by taking two samples from each block (six composite samples in total). The composite sample was obtained by mixing soil taken at four random locations. These were assumed to adequately represent the entire field where the experiment was established. The soil samples were analyzed using methods described in Fungo et al. (2014); in addition, particle size distribution was determined by the hydrometer method (Soil Texture Unit 1067; LaMotte Co., Chestertown, MD, USA) (soil properties in Table 1). The natural vegetation is composed of tropical rainforest of the Guineo-Congolian type. The experiment was conducted for four consecutive maize growing seasons: September-December 2012; March – August 2013; September – December 2013; March – August 2014. The seasons are henceforth referred to as Short Rains

2012 (SR2012); Long Rains 2013 (LR2013); Short Rains 2013 (SR2013) and Long Rains 2014 (LR2014), respectively.

### *5.2.2 Biochar and green manure*

Biochar was produced from eucalyptus wood by chopping and grinding to pass through a 2-mm sieve. The ground material was pyrolyzed to a maximum temperature of 550°C using a thermostat-regulated kiln with continuous agitation to provide homogeneous charring conditions and retained at this temperature for one hour before cooling to room temperature. Green manure from *T. diversifolia* was prepared by cutting leaves from the field and chopping them into 50-mm lengths, air-drying and grinding to pass through a 2-mm sieve before field application. The physical and chemical characteristics of the soil were analyzed following the same procedures as in Fungo et al. (2014) and are presented in Table 5.1.

Table 5.1: Physical-chemical properties of the soil (0-0.2 m) and the amendments used in the field trial in western Kenya (n=6 replicates for soil; triplicate measurements for amendments; means with standard errors in brackets). *nd = not determined*

| Property | Biochar | | Soil | | Green manure (*T. diversifolia*) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Property | | |
| pH | 6.3 | (0.1) | 6.0 | (0.1) | N (mg $kg^{-1}$) | 21.5 | (0.5) |
| C (g $kg^{-1}$) | 868 | (11) | 23.3 | (0.1) | P (mg $kg^{-1}$) | 2.3 | (0.1) |
| N (g $kg^{-1}$) | 27.0 | (0.9) | 21.0 | (0.9) | K (mg $kg^{-1}$) | 43.2 | (1.2) |
| P (mg $kg^{-1}$) | 135 | (3.7) | 9.30 | (0.2) | Ca (mg $kg^{-1}$) | 13.6 | (0.2) |
| K (mg $kg^{-1}$) | 1490 | (14) | 223 | (10) | Na (mg $kg^{-1}$) | 72.7 | (0.9) |
| Ca (mg $kg^{-1}$) | 1920 | (17) | 1950 | (10) | Fe (mg $kg^{-1}$) | 951 | (10) |
| Na (mg $kg^{-1}$) | 180 | (7.3) | 15.9 | (0.6) | Zn (mg $kg^{-1}$) | 89.7 | (1.6) |
| Mg (mg $kg^{-1}$) | 150 | (4.5) | 312 | (9.4) | Mg (mg $kg^{-1}$) | 2.6 | (0.0) |
| Al (mg $kg^{-1}$) | 559 | (9.8) | 939 | (16) | S (mg $kg^{-1}$) | 2.5 | (0.0) |
| S (mg $kg^{-1}$) | 36.5 | (1.4) | 14.0 | (0.8) | Mn (mg $kg^{-1}$) | 264 | (5) |
| Fe (mg $kg^{-1}$) | 164 | (5.7) | 67.2 | (1.6) | Cu (mg $kg^{-1}$) | 11.0 | (0.2) |
| Zn (mg $kg^{-1}$) | 108 | (2.4) | 13.5 | (0.4) | B (mg $kg^{-1}$) | 53.2 | (1.6) |
| Mn (mg $kg^{-1}$) | 188 | (4.9) | 782 | (14) | Mo (mg $kg^{-1}$) | 1.3 | (0.0) |
| Cu (mg $kg^{-1}$) | 0.77 | (0.1) | 1.97 | (0.1) | | | |
| B (mg $kg^{-1}$) | 1.07 | (0.0) | 0.33 | (0.0) | | | |
| C.E.C (meq 100 $g^{-1}$) | 18.2 | (0.6) | 16.2 | (0.5) | | | |
| EC (S $mm^{-1}$) | 196 | (6.5) | 88.0 | (1.2) | | | |
| Silt (%) | nd | | 17.5 | (0.3) | | | |
| Sand (%) | nd | | 10.7 | (0.4) | | | |
| Clay (%) | nd | | 71.6 | (2.0) | | | |

### *5.2.3 Experimental design*

The experiment was laid out in a randomized complete block design with three replicates. Treatments included the following: two levels of biochar (0 and 2.5 t $ha^{-1}$); three levels of *T. diversifolia* green manure (0, 2.5 and 5 t $ha^{-1}$); and two levels of urea application (0 and 120 kg N $ha^{-1}$) in a full factorial design (**Table 5.2**). Treatments were indicative of the range of conventional management practices of many small-holder farmers in integrated soil fertility management systems. Each treatment was established in 2 x 2-m plot separated by a one-meter distance within and between rows.

Table 5.2: Experimental treatments for determining the effect of biochar, *T. diversifolia* green manure and urea on fluxes of $CO_2$, $CH_4$ and $N_2O$ in a maize field in western Kenya. Biochar was applied only once at the start of the experiment while urea and tithonia were applied every season for four consecutive seasons.

| Treatment | Biochar | | *T. diversifolia* | | Mineral N (Urea) | |
|---|---|---|---|---|---|---|
| | Rate (t $ha^{-1}$)• | *Code* | *Rate* (t $ha^{-1}$)¤ | Code | Rate (kg N $ha^{-1}$)* | Code |
| 1 ($B_0T_0U_0$) (Control) | 0 | B0 | 0.0 | T0 | 0 | U0 |
| 2 ($B_0T_{2.5}U_0$) | 0 | B0 | 2.5 | T2.5 | 0 | U0 |
| 3 ($B_0T_5U_0$) | 0 | B0 | 5.0 | T5 | 0 | U0 |
| 4 ($B_0T_0U_{120}$) | 0 | B0 | 0.0 | T0 | 120 | U120 |
| 5 ($B_0T_{2.5}U_{120}$) | 0 | B0 | 2.5 | T2.5 | 120 | U120 |
| 6 ($B_0T_5U_{120}$) | 0 | B0 | 5.0 | T5 | 120 | U120 |
| 7 ($B_{2.5}T_0U_0$) | 2.5 | B2.5 | 0.0 | T0 | 0 | U0 |
| 8 ($B_{2.5}T_{2.5}U_0$) | 2.5 | B2.5 | 2.5 | T2.5 | 0 | U0 |
| 9 ($B_{2.5}T_5U_0$) | 2.5 | B2.5 | 5.0 | T5 | 0 | U0 |
| 10 ($B_{2.5}T_0U_{120}$) | 2.5 | B2.5 | 0.0 | T0 | 120 | U120 |
| 11 ($B_{2.5}T_{2.5}U_{120}$) | 2.5 | B2.5 | 2.5 | T2.5 | 120 | U120 |
| 12 ($B_{2.5}T_5U_{120}$) | 2.5 | B2.5 | 5.0 | T5 | 120 | U120 |

### *5.2.4 Management of experiment*

Precipitation and air temperature were monitored throughout the experiment with the help of a weather station on site. Application of biochar was done only once at the start of the first season

in October 2012. The same amounts of green manure (2.5 or 5.0 t $ha^{-1}$), were applied to each plot once at the start of each season (four applications in total). Mineral N (Urea; 261 kg $ha^{-1}$) was applied in two splits at a total of 120 kg N $ha^{-1}$ per season; 40% at planting and 60% at 30 days-after planting. Due to the inherently low fertility of the soil, 30 kg $ha^{-1}$ of P as Triple Super Phosphate (TSP) (55 kg $P_2O_5$ $ha^{-1}$) and 30 kg $ha^{-1}$ of K as Muriate of Potash (MoP) (45 kg $K_2O$ $ha^{-1}$) were applied to each plot at the start of each season. The amendments were applied by broadcasting on the soil surface by hand and immediately incorporating into the 0.1 m top soil. In plots where the combinations were applied, biochar was applied first, followed by Tithonia and then urea. Two seeds of a maize cultivar HB 513 were planted at the start of every season at a spacing of 0.25 m within and 0.5 m between rows (40 plants per plot). Weeding was done at 30 and 50 days after planting using a hand hoe. Thinning was done during the first weeding to retain one plant per planting hole.

### *5.2.5 Gas measurements*

Measurements of $CO_2$, $CH_4$ and $N_2O$ fluxes were conducted using a static closed chamber method (Neftel et al., 2006; Morris et al., 2013). The chamber consisted of a PVC tube (diameter = 0.3 m; height = 0.15 m) transversely divided into two parts to make a base (0.05 m) and a cover (0.1 m). The base was driven into the soil to reach ~0.02 m below the soil surface. To ensure air-tight conditions, a rubber ring was placed between the base and the cover. A photo-acoustic infrared field gas monitor (INNOVA 1402, Lumasense Technologies A/S, Ballerup, Denmark) was used to determine the gas concentrations. The accuracy and precision of standard gas concentration measurements with Photo Acoustic Spectroscopy (PAS) and Gas Chromatography (GC) have been shown to be comparable (Jung et al., 2012; Zhao et al., 2012; Iqbal et al., 2013). The gas monitor was connected to the chamber by two 0.7 m-long teflon tubes as gas inlet and outlet. Inside the cuvette, air humidity and temperature were monitored

by a digital thermo-hygrometer (PCE-313 A, Paper-Consult Engineering Group, Meschede, Germany) attached to the cover from the outside while only the sensor reached inside the chamber through a rubber screw connector. Two chambers, marked "1" and "2", were set up in each plot. All chambers marked "1" in all the treatments were sampled on day 1 and those marked "2" were sampled the following day. The values for chamber 1 and 2 for each plot were then averaged. For each gas sampling event INNOVA recorded four measurements at 2-minute intervals after closing the chamber. Flux measurements were conducted weekly except during dry periods where bi-monthly measurements were taken. Fluxes are generally constant during dry, low moisture soil conditions. A total of 53 data points was obtained and used in the analysis.

### *5.2.6 Harvesting and yield determination*

Above ground biomass was considered as the sum of stover, cobs and grain. Corn ears from each plot were removed from their shucks 120 days after planting, bagged, and dried for six days in a shed. Stover in each plot was cut ~0.02 m above the soil surface, weighed and dried in a shed for six days. All above ground residues from the plot were never returned to the plot in order to determine how much of the SOC could be credited to above ground vs. below ground inputs. After drying, ears were mechanically shelled, and cob and kernel biomass was determined for each plot. Moisture content in the samples was determined by taking biomass of five fresh randomly selected plants to the oven for 48 hours at 60°C. Soil samples were taken from a 0 - 0.2 m depth at the beginning and end of the experimental period. For the soil samples, total organic carbon (TOC) was determined by the dry combustion method (Elementar Vario EL, Hanau, Germany) and assumed that TOC = SOC since these acid soils have negligible amounts of carbonates. The available phosphate content was determined using the Lancaster method (RDA, 1988). Exchangeable cations were estimated using Inductively Coupled Plasma

Spectroscopy (OPTIMA 4300DV, Perkin Elmer, USA) after extraction with 1N ammonium acetate (pH 7.0) using a soil:water ratio of 1:20 w/v.

### *5.2.7 Data analysis*

Cumulative gas fluxes were obtained by calculating the area under the flux-time curve and summing the results while assuming linear changes in measurements between time intervals. Global Warming Potential (GWP, the sum of cumulative gas emissions of $CH_4$ and $N_2O$, multiplied by the radiative forcing factor of each gas 25 and 298, respectively, for a time horizon of 100 years) (USEPA, 2007) calculated using the following equation:

$$GWP = 25 \times (E\text{-}CH_4) + 298 \times (E\text{-}N_2O)$$

Where GWP is the emission in $CO_2$-equivalents per hectare, E-$CH_4$ and E-$N_2O$ are the emissions of $CH_4$ and $N_2O$ per hectare during a given year, respectively. Emissions Intensity (EI) was then obtained by dividing the cumulative $CO_2$-equivalent of the gas fluxes by the cumulative grain yield of each treatment over the experimental period. The cumulative emission for each treatment was derived using a linear trapezoidal rule with sampling dates as the time intervals. For seasonal comparisons, the cumulative flux was restricted to the 120 days between planting and harvesting. Changes in SOC stock were calculated as the difference between values at the beginning and end of the experiment, after subtracting the C addition from biochar and *T. diversifolia*.

### *5.2.8 Statistical analysis*

Differences in the net EI for each treatment were calculated as the difference between the treatment value and that of the control. Data for $CO_2$ was nomally distributed and did not require transformation but $CH_4$ and $N_2O$ data were natural log-transformed before ANOVA.

Treatment effects and their interaction were examined with repeated-measures ANOVA using comparisons with seasons. A fixed-effect model in Stata 12 (StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845 USA) was used with a nested design with biochar and tithonia within urea. Post hoc separation of means was done using Least Significant Difference (LSD) at a 5% level of significance using the Stata 12 statistical software.

## 5.3 Results

### *5.3.1 Dynamics in GHG fluxes*

*Daily dynamics*

The mean daily fluxes of $CO_2$, $CH_4$ and $N_2O$ were 5.4 mg $m^{-2}$ $hr^{-1}$, 39 µg $m^{-2}$ $hr^{-1}$and 1.96 µg $m^{-2}$ $hr^{-1}$, respectively (Figure 5.1). Emissions closely followed weather patterns with higher $CO_2$ and $N_2O$ emissions as well as lower $CH_4$ uptake during the wet seasons. During the dry seasons, fluxes of $CH_4$ and $N_2O$ were generally below average. Variability in daily fluxes, as expressed by the coefficient of variation (CV), was generally higher for $CH_4$ (60%) compared to $CO_2$ and $N_2O$ (21%). Biochar effects were not observable for daily $CO_2$ measurements, but its effect was observed for $CH_4$ and $N_2O$, where it generally reduced the $CH_4$ sink capacity of the soil and reduced $N_2O$ emission.

*Seasonal dynamics*

Seasonal cumulative $CO_2$ emission increased by 17% (Figure 5.2A) throughout the four seasons. $CH_4$ uptake was reduced by 17% (Figure 5.2B) in biochar-amended compared to control plots, and this reduction was observed in all the four seasons. The decrease in $CH_4$ uptake due to biochar was maintained in all the seasons, but there was no significant difference in $CH_4$ uptake among the seasons. Among all the seasons, LR2013 experienced the highest cumulative uptake of $CH_4$. Consistent 15% reduction of $N_2O$ emissions were observed due to

biochar additions, irrespective of season (Figure 2C). Similar to $CH_4$, LR2013 experienced the highest cumulative emission of $N_2O$. Emission of $N_2O$ in this season was higher (P = 0.03) than those of all the other seasons likely because the rains were highest in this season.

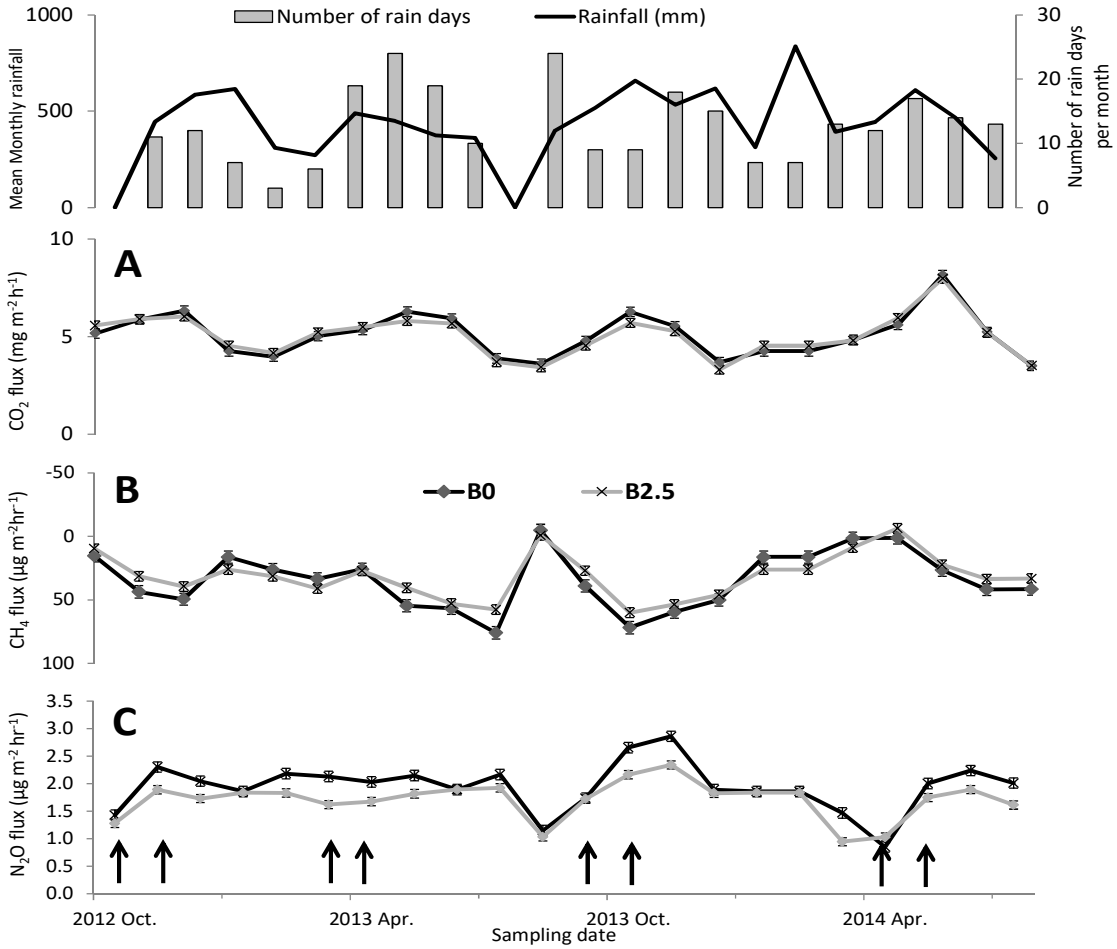



Figure 5.1: Weather patterns (top graph), $CO_2$ (A), $CH_4$ (B) and $N_2O$ (C) fluxes during four seasons of growing maize in western Kenya after amendment with mineral fertilizer (urea, 120 kg N $ha^{-1}$) and green manure (*T. diversifolia*, 2.5 and 5.0 t $ha^{-1}$) and biochar. Error bars are standard errors. Dates on the x-axis indicate the planting time. n=3. Arrows at on the x-axix show the dates when urea was applied.

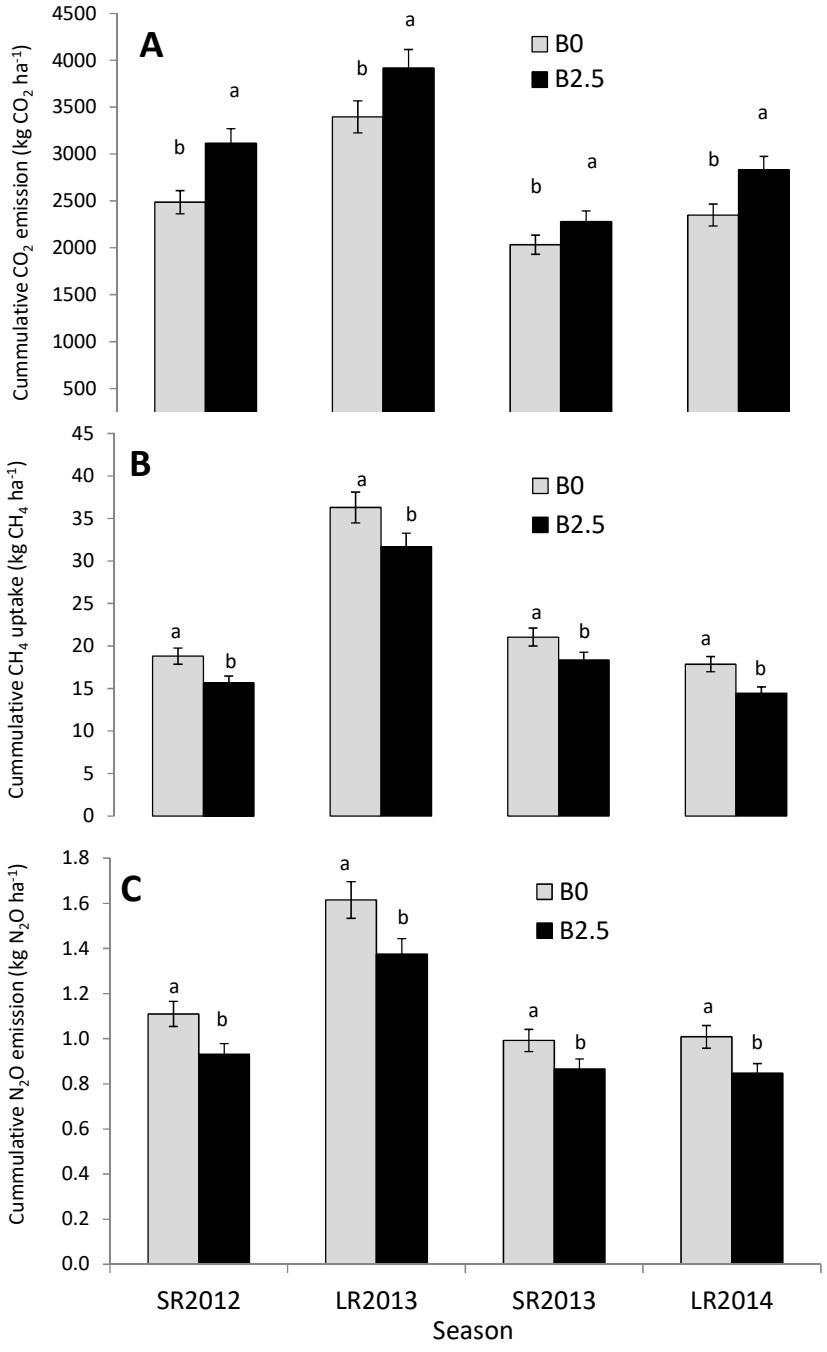



Figure 5.2: Seasonal cumulative $CO_2$, (A) emission, $CH_4$ uptake (B), and $N_2O$ emission (C) fluxes during four-season maize trial in western Kenya after amendment with Mineral fertilizer (Urea) and Green manure (*T. diversifolia*) and biochar. Within each cluster, bars with different letters are significantly different. Error bars are standard errors, n=3.

*Annual GHG emissions*

All the three amendments, except urea (which had no effect on $CH_4$), affected each of the GHGs (**Table 5.3**). Annual flux of $CO_2$ was increased by all amendments (**Table 5.3**). The increase in $CO_2$ emissions ranged from 6 to 33%. The highest increase was observed where both *T. diversifolia* and urea were present ($P = 0.02$). On average, amendments of biochar, tithonia or urea increased $CO_2$ emissions by 10% ($P = 0.034$. No interaction between either biochar and *T. diversifolia* or biochar and urea was observed ($P > 0.05$). Also, no effect of increasing *T. diversifolia* from 2.5 to 5.0 t $ha^{-1}$ in terms of $CO_2$ emissions was observed.

Table 5.3: Three-way ANOVA for the effects of biochar, *T. diversifolia*green manure and urea on fluxes of $CO_2$, $CH_4$ $N_2O$, GWP, maize grain yield and EI in maize field in western Kenya. P-values in bold show means that were significantly different p=0.05, n=3

| Source | $CO_2$ | | $CH_4$ | | $N_2O$ | | GWP | | Grain Yield | | EI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | P | F | P | F | P | F | P | F | P | F | P |
| Biochar | 6.11 | **0.021** | 6.29 | **0.026** | 5.65 | **0.011** | 12.5 | **0.007** | 10.5 | **0.031** | 10.6 | **0.010** |
| *T. diversifolia* | 12.25 | **0.000** | 3.46 | **0.047** | 5.02 | **0.015** | 10.4 | **0.025** | 1.2 | 0.96 | 12.0 | **0.017** |
| Urea | 8.62 | **0.007** | 3.01 | 0.095 | 8.43 | **0.006** | 8.0 | **0.045** | 11.4 | **0.034** | 18.0 | **0.000** |
| Biochar *xT. diversifolia* | 1.27 | 0.271 | 1.13 | 0.299 | 0.88 | 0.358 | 16.7 | **0.000** | 10.8 | **0.031** | 9.3 | **0.021** |
| Biochar *x* Urea | 1.98 | 0.160 | 1.13 | 0.339 | 4.96 | **0.037** | 11.2 | **0.016** | 10.1 | **0.038** | 15.2 | **0.000** |
| *T. diversifolia x* Urea | 1.67 | 0.208 | 1.04 | 0.370 | 1.27 | 0.098 | 0.0 | 0.095 | 0.0 | **0.095** | 11.2 | **0.018** |
| Biochar *xT. diversifoliax* Urea | 1.36 | 0.276 | 1.61 | 0.220 | 1.17 | 0.325 | 10.8 | 0.031 | 4.9 | **0.042** | 4.3 | **0.047** |

*Bold numbers show significant effect at 95% level of confidence*

Both biochar and *T. diversifolia* reduced the soil uptake of $CH_4$, while urea had no effect. Reduction in soil $CH_4$ uptake ranged from 7 to 59% (Table 5.4). There was no interaction between amendments, and the level of *T. diversifolia* additions on $CH_4$ uptake between the 2 and 4 t $ha^{-1}$ application rates. The largest reduction in $CH_4$ uptake was observed when biochar, tithonia and urea were added together at the highest quantities. $CH_4$ uptake decreased progressively as more amendments were added.

Cumulative $N_2O$ emissions progressively decreased by up to 42% (mean±SE of 19±4.1%) where biochar was amended but did not change where *T. diversifolia* or urea were added on their own compared to an un-amended control (Table 5.4). Biochar-induced decreases in $N_2O$ emission were greatest (42%) where *T. diversifolia* and urea were both present compared to when they were separately applied. $N_2O$ emissions with biochar+urea additions were significantly lower than those with urea alone (Table 5.4). The interaction between biochar and *T. diversifolia* was significant only at the higher level of *T. diversifolia.*

***Grain yield***

Maize grain yields ranged from 12.6 to 19.8 t $ha^{-1}$ $yr^{-1}$ (mean = 16.4 t $ha^{-1}$ $yr^{-1}$), corresponding to a decrease of 15% and increase of 34%, respectively, compared to the control. Decreases in maize grain yield were observed where biochar was applied either alone or in combination with low amounts of *T. diversifolia* ($B_{2.5}T_0U_0$, -15%, and $B_{2.5}T_{2.5}U_0$, -14%, respectively). High amounts of *T. diversifolia* ($T_5$) significantly increased yields by 9%. The greatest increase in maize grain yield was observed where biochar was combined with urea ($B_{2.5}T_0U_{120}$,34%). This was followed by yields where high amounts of *T. diversifolia* (5 t $ha^{-1}$) were jointly applied with urea (treatment $B_0T_5U_{120}$ at 29%) (Table 5.4). Grain yield of the combination of the three

amendments was higher than either biochar or *T. diversifolia* applied alone (Table 5.4). Grain yield comprised of 35% (SD=0.03) of total above ground biomass and this proportion was comparable for all treatments.

***Global Worming Potential (GWP)***

Biochar when applied alone caused a significantly lower GWP (8% or 6.3±0.01 t $CO_2$-eq $ha^{-1}$ $yr^{-1}$) compared to the control (Table 5.4). GWP caused by additions of *T. diversifolia* and urea compared to no additions (control) significantly increased by 32% and 12% corresponding to an increase by 7.7±0.8 and 6.5±0.6 t $CO_2$-e $ha^{-1}$ $yr^{-1}$, respectively. *T. diversifolia* or urea additions resulted in significantly higher yield compared to the control, but were not significantly different from each other. Grain yield was significantly higher with the sole biochar addition. The interactive effect between biochar and urea increased yield significantly but no difference in yield was observed where tithonia and urea were applied together or where biochar was applied with tithonia. In a combined application of biochar, urea and *T. diversifolia*, doubling the amounts of *T. diversifolia* halved the GWP. The difference between GWP as a result of biochar+*T. diversifolia* and biochar+urea additions was significantly lower than the difference in the respective sole applications of *T. diversifolia* versus urea.

***Emissions Intensity (EI)***

Biochar and tithonia applied alone caused significantly higher EI compared to the control, but EI as a result of urea additions was not significantly different from that of the control. There was no interactive effect between tithonia and urea application with respect to EI. Except for the interaction of 2.5 t biochar $ha^{-1}$ and urea ($B_{2.5}T_0U_{120}$ in Table 5.4), all the other treatments showed significantly higher EI than the control (*p=0.035*), with increases ranging from 8 to 39%. Apart from the control, the highest EI corresponded with additions of only *T. diversifolia*

green manure ($B_0T_{2.5}U_0$ and $B_0T_5U_0$) or urea additions ($B_0T_0U_{120}$). The lowest EI was observed where biochar and urea were added without or with high *T. diversifolia*. As *T. diversifolia* green manure additions increased, the EI correspondingly decreased but only in the presence of biochar. The decrease in EI with greater additions of *T. diversifolia*corresponded to increases in $CO_2$ emission by 14% and decreases in $N_2O$ emission by 16% (Table 5.4).

Table 5.4: Annual grain yield, $CH_4$ and $N_2O$ emissions, GWP (calculated only from $CH_4$ and $N_2O$) and EI of maize production over four consecutive seasons under biochar, *T. diversifolia* and urea amendment. Means (±S.E.) in the same column followed by different letters are significantly different at $p < 0.05$, $n = 3$.

| Treatment | $CO_2$ (t $ha^{-1}$ $yr^{-1}$) | $CH_4$ uptake (t $ha^{-1}$ $yr^{-1}$) | $N_2O$ emission (t $ha^{-1}$ $yr^{-1}$) | GWP (t $CO_2$-eq $ha^{-1}yr^{-1}$) | Grain yield (t $ha^{-1}$ $yr^{-1}$) | EI (t $CO_2$-eq $t^{-1}$ grain $yr^{-1}$) |
|---|---|---|---|---|---|---|
| 1 ($B_0T_0U_0$) | 3.7 (±0.6)d | 0.061 (±0.017)a | 0.003 (±0.009)a | 0.67 (±0.5)a | 14.8 (±1.0)f | 0.05 (±0.01)a |
| 2 ($B_0T_{2.5}U_0$) | 4.9 (±0.1)a | 0.057 (±0.023)b | 0.003 (±0.002)a | 0.57 (±0.34)b | 15.1 (±0.6)f | 0.04 (±0.03)b |
| 3 ($B_0T_5U_0$) | 4.8 (±0.3)a | 0.058 (±0.006)b | 0.003 (±0.006)a | 0.59 (±0.18)b | 14.9 (±0.1)f | 0.04 (±0.02)bc |
| 4 ($B_0T_0U_{120}$) | 4.1 (±0.6)ca | 0.057 (±0.007)b | 0.003 (±0.004)a | 0.60 (±0.11)b | 15.9 (±0.8)e | 0.04 (±0.02)c |
| 5 ($B_0T_{2.5}U_{120}$) | 5.0 (±0.1)a | 0.052 (±0.009)bc | 0.003 (±0.007)a | 0.47 (±0.04)c | 18.4 (±1.4)c | 0.03 (±0.03)c |
| 6 ($B_0T_5U_{120}$) | 4.9 (±0.1)c | 0.053 (±0.010)bc | 0.002 (±0.009)b | 0.59 (±0.22)b | 19.1 (±0.1)b | 0.03 (±0.05)d |
| 7 ($B_{2.5}T_0U_0$) | 4.0 (±0.6)b | 0.052 (±0.018)bc | 0.002 (±0.005)b | 0.59 (±0.32)b | 12.8 (±1.8)g | 0.05 (±0.01)a |
| 8 ($B_{2.5}T_{2.5}U_0$) | 4.4 (±0.3)a | 0.050 (±0.006)c | 0.003 (±0.005)a | 0.42 (±0.06)d | 12.6 (±1.8)g | 0.03 (±0.06)d |
| 9 ($B_{2.5}T_5U_0$) | 5.0 (±0.1)b | 0.046 (±0.029)d | 0.002 (±0.001)b | 0.47 (±0.18)d | 17.4 (±1.6)d | 0.03 (±0.01)e |
| 10 ($B_{2.5}T_0U_{120}$) | 4.6 (±0.6)b | 0.047 (±0.014)d | 0.002 (±0.007)b | 0.44 (±0.36)cd | 19.8 (±1.0)a | 0.02 (±0.03)f |
| 11 ($B_{2.5}T_{2.5}U_{120}$) | 4.6 (±0.9)b | 0.055 (±0.025)b | 0.003 (±0.001)ab | 0.58 (±0.34)b | 17.0 (±3.2)d | 0.03 (±0.01)cd |
| 12 ($B_{2.5}T_5U_{120}$) | 4.4 (±0.1)b | 0.034 (±0.020)e | 0.002 (±0.007)c | 0.26 (±0.31)e | 16.9 (±0.8)d | 0.02 (±0.03)g |

***Changes in SOC stock***

Amendments increased SOC stocks in the range of 0.7 to 7.1%, corresponding to 0.1 to 1.2 t C $ha^{-1}$ $year^{-1}$ (mean±SE of 0.8±0.09 t C $ha^{-1}$ $year^{-1}$). Except for the control (where no amendment was applied), which showed no significant difference in SOC over time, all the other treatments showed gains in SOC (Figure 5.3). The smallest increment (0.2±0.01 t C $ha^{-1}$ $yr^{-1}$) was observed without any additions while the highest increase (1.2±0.04 t C $ha^{-1}$ $yr^{-1}$) was recorded where urea was applied with *T. diversifolia* irrespective of biochar additions. The increase in SOC stocks due to biochar or *T. diversifolia*additions was comparable (0.7±0.02 t C $ha^{-1}yr^{-1}$, or 2.8%) to the control. Soil OC stocks in response to additions of 2.5 t $ha^{-1}$ of tithonia increased by 27% compared to the control, and was comparable to additions of 5 t $ha^{-1}$ of tithonia. Additions of 2.5 t $ha^{-1}$ of tithonia together with urea increased SOC by 39% compared to the control.

## 5.4 Discussion

***Dynamics of GHGs***

Stimulation of $CO_2$ emissions following biochar addition observed in this study (Fig. 5.1A and Fig. 5.2A) concurs with what has been observed in previous studies and has been attributed to the supply of easily mineralizable C and improvement in soil physical properties for microbial activity (Lehmann et al., 2011; Sagrilo et al., 2014) and increased root respiration (Major *et al.*, 2010). However, reduction in $CO_2$ emission after biochar addition has also been reported in incubations without plants, and is usually associated with limited N supply due to N immobilization by amended biochar (Laird et al., 2010; Wang et al., 2015). In my case, the amount of biochar was relatively low (0.2% compared to the average 2% w/w in most studies) and could not immobilize significant amounts of soil Nwhile crop growth significantly

increased, hence the consistent increase in $CO_2$ (that included root respiration) with all biochar plots. The lack of an interactive effect between biochar and tithonia as well as urea and tithonia with respect to $CO_2$ emission (Table 5.3) was possibly due to the fact that sufficient C was supplied by biochar or *T. diversifolia* making further inputs temporarily unnecessary for microbial use. Contrary to my findings, Rogovska et al. (2011) found reduced $CO_2$ emission after 500 days of incubation for a green manure-biochar mixture. The authors attributed this observation to the fact that biochar stabilized green manure C by influencing biochemical recalcitrance or physical protection of green manure C (Krull et al., 2003), or that green manure additions reduced the ability of biochar to enhance mineralization of soil organic matter. My results did not show interactions between biochar and green manure possibly because the mixing of the two amendments was not as uniform as it was in the incubation of Rogovska et al. (2011). Whereas biochar largely remains unchanged in space, tithonia and urea may, after incorporation into the soil, leach to lower soil layers. The subsequent reactions of these amendments may thus remain independent. Some studies on placement of amendments may elucidate this notion. It is important to note that $CO_2$ can be produced by both enhance mineralization of soil organic matter or biochar itself (Wardle et al., 2008). Our study measured only soil-atmosphere fluxes of $CO_2$, and could not attribute the increase to a particular $CO_2$ production process. Nonetheless, the fact that there was an increase in SOC with biochar-tithonia additions (Fig. 5.3) suggest an overall net positive balance towards soil C sequestration. Future studies should estimate the net effect of biochar application on soil respiration and biochar mineralization. Life cycle assessment to determine C sequestration including $CO_2$ emitted during biochar production will also be relevant.

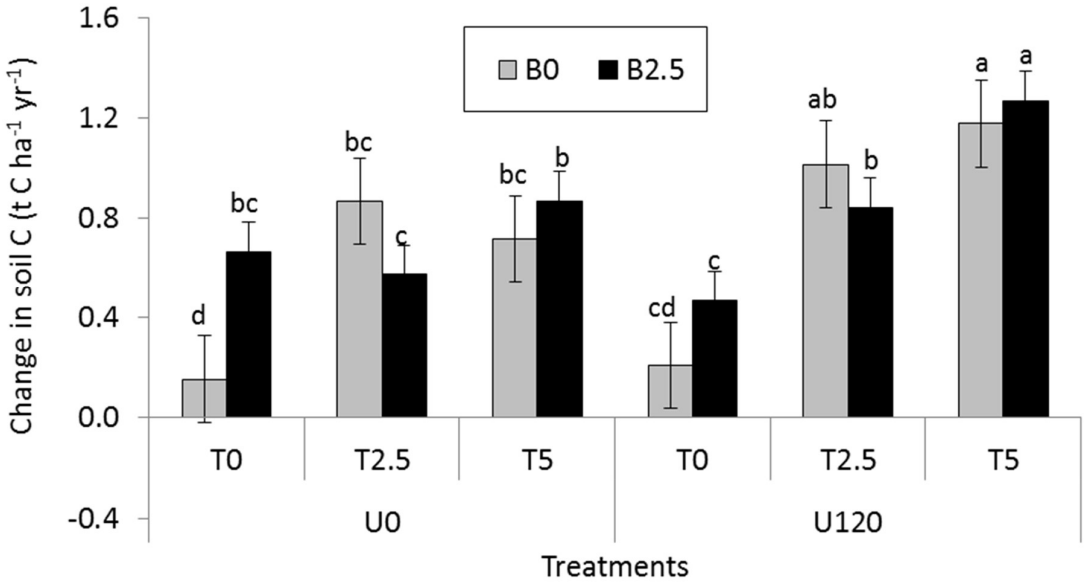



Figure 5.3: Annual changes in soil organic C stock (top 0.15 m) under biochar (B in t $ha^{-1}$), T. diversifolia (T in t $ha^{-1}$) and urea (U in kg N $ha^{-1}$) amendments compared to the control. Means (±S.E.) followed by different letters are significantly different at $p < 0.05$, n = 3.

The upland soils are generally identified as sinks for $CH_4$ (Chan and Parkin, 2001) butadditions of biochar and *T. diversifolia* in this experiment reducedthe sink capacity (Fig. 5.1B and Fig 5.2B). This can likely be attributed to the supply of easily mineralizable C that substitutes for the C oxidized by methanotrophic bacteria (Knoblauch *et al.,* 2008; Zhang et al., 2010). The greatest reduction in $CH_4$ uptake, coinciding with urea and high *T. diversifolia* input (Table 5.4), could be attributed to the availability of $NH_4^+$-N from urea, which is known to inhibit $CH_4$ oxidation (Chan and Parkin, 2001; Suwanwaree and Robertson, 2005). Whereas some studies have shown increased uptake of $CH_4$ with biochar (Liu et al., 2011; Schimmelpfennig et al., 2014), several have also shown decreases (Spokas et al., 2013; Singla et al., 2014). This suggests the complexity and variety of factors affecting $CH_4$ fluxes in biochar-amended soils. The persistence of biochar effects on fluxes over the two years of observation (Fig. 5.2B) could be related to improvements in physical properties leading to better drainage and adsorption of

dissolved organic carbon (Singh et al., 2012; Zhang et al., 2015) rather than N immobilization discussed above that is likely to be a transient phenomenon.

The consistent reduction in $N_2O$ with biochar amendments (Fig. 5.1C and Fig. 5.2C) is similar to previous studies (Rondon et al., 2005; Xiang et al., 2015). The reduction in $N_2O$ emissions in the present study is lower compared to other studies (such as Zhang et al., 2012) partly because the amounts of added biochar were relatively low (2.5 t $ha^{-1}$ compared with 20-40 t $ha^{-1}$ in all the above studies). $N_2O$ reduction could have been due to stimulation of microorganisms that can degrade more persistent SOM (Nelissen et al., 2012), resulting in more reactive surfaces. It is also plausible that adsorption of $NH_4^+$ (Taghizadeh-Toosi et al., 2012) and other potential allelochemical inhibitors of microbial metabolic pathways, such as monoterpenes and various polyphenolic compounds that are inhibitory to nitrification, could have played a role (Ball et al., 2011). That the interaction between biochar and urea (Table 5.3) resulted in reduced $N_2O$ emission could probably be due to a direct reaction between the biochar surfaces and $N_2O$. Thus, the $N_2O$ emission produced from urea is countered by the biochar. Cayuela et al. (2013) have suggested the catalytic activity of biochar as capable of enhancing the reduction of $N_2O$ to $N_2$. Direct molecular-level interaction between $N_2O$ and reducing agents has been reported (Hitoshi et al., 2002) and Fungo et al. (2014) observed that steam activation of biochar explained 56% of reduction in $N_2O$ emission from the same soil. It is plausible that such a reaction could be taking place in biochar amended soils.

The notion that a stimulatory effect of biochar on soil and/or plant respiration levels off during the first years after application (Spokas, 2013) was not seen within the two years of the experiment, as we observed consistent differences in emissions and above ground biomass production during all four cropping seasons. Several studies (e.g. Kimetu et al., 2008; Major et

al., 2010; Koide et al., 2014) have shown that biochar contents remain in soil and decreased only between 0.3 and 6% of the amount applied over a period of three years. Whereas some studies (Nelissen et al., 2014) found short-lived effects of biochar (Spokas, 2013), the two-year effects observed in my study support Lentz et al. (2014), who observed a persistent effect over a 3-year period. Lentz et al. (2014) related this multi-year effect to biochar's physical porosity and chemical binding capacity. The contrasting results in the literature indicate a need for longer-term (>5 years) studies to test this hypothesis.

Results in Fig. 5.2 show that biochar effects on $CH_4$ uptake and $N_2O$ emissions can persist over two years under field conditions. This could be attributed to the fact that non-aromatic organic materials such as sugars and fats in the pores of biochar (Mukherjee et al., 2011; Koide et al., 2014) are susceptible to mineralization over time (Kim et al., 2011). According to Lentz et al. (2014), the disappearance of these organics with soil aging likely (i) increased porosity and surface area akin to the effect that activation has on charcoal (Fungo et al., 2014); and (ii) increased negatively charged sites on biochar (Cheng et al., 2008). The charged sites can bind $NH_4$, making inorganic N only temporarily unavailable for microorganisms and leaching (Dempster et al., 2012), which can later be readily released (Wang et al., 2015). These mechanisms could have been responsible for observed persistence of biochar effect observed in this study. A change in pore size distribution in the biochar-amended soil could have influenced bacterial community composition (Lentz et al., 2014) that could conceivably also affect GHG emissions. Feng et al. (2012) reported decreased ratios of methanogenic to methanotrophic microorganisms that accounted for the decrease in $CH_4$ emission from paddy soils. Banerjee et al. (2016) found that moisture affected microbial activity, transcription, composition and ultimately, $N_2O$ emissions. The reduced $N_2O$ mitigation effect observed by

Spokas (2013) after 3 years of biochar aging could be related to the relatively large particle size (8 - 40 mm) of the biochar used.

*Changes in Soil C content*

As observed in my study, a previous study carried out in the same area (Kimetu et al., 2008) found that application of biochar increased TOC contents by 6.8 times after adding biochar compared to *T. diversifolia* green manure. In fact, the authors observed that biochar not only remained to a greater extent unchanged, but could be protected by aggregation to a greater degree in soil in the long term. The relatively fine texture of the tested soils means that the soil may allow for more SOC protection by a combination of physical occlusion and organo-mineral interactions.

Chivenge et al. (2011a) found that in clayey soils, the addition of low-quality organic resources (such as maize stover) resulted in greater stabilization of SOC and SON in the macro-aggregates, while addition of N-fertilizers enhances their decomposition and faster aggregate turnover leading to less accumulation of SOC and SON. Similar to my results, Chivenge et al. (2011b) also found no interaction between *T. diversifolia* with N fertilizers on similar soils with respect to changes in SOC. This suggests that microbial choice of the N source is independent of the source or that the utilization of freshly applied organic matter (C source) is consumed at the same rate as mineral N (source of N). Verchot et al. (2011) observed that most of the newly added SOC gains from green manures ended up in the coarser aggregates, and is therefore subject to turnover and loss in the event that OM inputs decline. However, using soils from the same experiment, Fungo et al. (2017) found that biochar is stored predominantly as free particulate OC in the silt and clay fraction and promoted a movement of native SOC from larger-size aggregates to the smaller-sized fraction. The increase in C stocks when *T.*

*diversifolia* was added but no additive effect of *T. diversifolia*+biochar may be attributed to the fact that there is a balance between increased mineralization of SOM due to biochar addition on the one hand and an increase in SOC due to greater net primary productivity of root biomass on the other.

Our results (Fig. 5.3) show that even without returning harvested above ground biomass to the soil, any of the additions were still able to demonstrate SOC gains. It follows that returning maize stover and integrated soil fertility management with biochar can achieve even greater SOC sequestration as well as GHG emission objectives. This implies that soil amendment with organic a mineral resource increases net primary productivity and hence below ground biomass. This biomass can later build up soil organic matter stocks. In terms of climate change mitigation, this is important as it demonstrates the potential for C sequestration associated with soil management.

***System C balance***

In relatively low fertility soils similar to the one used in this experiment, increases in biomass production in response to biochar application have been widely reported (Major et al., 2010; Li et al., 2015). The grain and biomass yield increase may most likely be attributed in the studied acid soils to increases in direct mineral additions from biochar, improvement in soil physical properties (e.g., bulk density), greater pH and reduced Al toxicity, improved cation exchange capacity, and possibly effects on soil biota that largely remain speculative. The proportional increase in biomass is comparable to other studies on highly weathered tropical soils (Major et al., 2010; Van Zwieten et al., 2010; Zhang et al., 2011).

The enhanced above ground biomass production that we observed (Table 5.4) could be due to improved soil physical and chemical properties resulting from biochar amendments. Olmo et al. (2014) found that grain production was correlated significantly and positively with soil moisture, EC, total N, Olsen P and available K, Cu and Zn, and negatively with soil compaction, consistent with the favorable changes in soil physico-chemical properties brought about by biochar additions. According to Lentz et al. (2014), combining biochar with green manure more effectively utilized the two soil amendments, as it eliminates potential plant growth reductions caused by N immobilization after biochar additions and maximized green manure net N mineralization potential.

The increase in net primary productivity due to soil amendments contributes to atmospheric C capture into terrestrial C (above ground and soil organic C). However, it will be necessary for future studies to investigate other types of green manures to better understand limitations over longer periods of time. Application rates used in several other trials are unrealistic for most farming systems. Despite the relatively low application rates of both biochar and *T. diversifolia* used in this study compared to those of previous studies, we demonstrate that significant ecosystem C gains are practically possible in low fertility soils even with relatively moderate amounts of biochar. The identical grain yields with additions of 2.5 t $ha^{-1}$ biochar and 5 t $ha^{-1}$ tithonia irrespective of urea additions (Table 5.4) may imply that biochar has the potential to reduce mineral N fertilizer requirements if green manure is added. In terms of GHG emissions, reduced application of mineral fertilizer is one way to reduce the impact of agriculture on climate change. This includes the avoided emissions due to fertilizer production. What remains to be determined is the trade-off between biochar production and fertilizer manufacture in terms of both cost and emissions.

Based on a yield-normalized comparison of my results (EI), biochar can potentially reduce overall GHG emission while improving crop yields and SOC. Nonetheless, the observed reduction in EI due to biochar additions needs to be further analyzed using a full Life Cycle Assessment (LCA) to account for the energy-related emissions needed to produce or transport the biochar among other emissions and emission reductions.

## 5.5 Conclusions and Recommendations

We have shown that the inclusion of biochar in integrated management of low-fertility tropical agricultural soils can reduce GHG emissions and increase ecosystem C gains. The resultant C gains and GHG emission reduction benefits of biochar can be sustained for at least two consecutive years (four seasons) from the time of application under the environmental conditions studied here. This result points to the importance of plant responses for the GHG balance in biochar systems. Our results contribute to mid-term field studies but longer trials might be necessary to better understand the C balance, including different soil types and cropping systems especially where stover biomass is returned to the plot. Longer field-based studies and field studies on soil that show different plant response to organic matter additions are needed to improve understanding of linkages between nutrient use efficiency and GHG mitigation.

# CHAPTER SIX: Ammonia and nitrous oxide emissions from a field Acrisol amended with tithonia green manure, urea, and biochar

Fungo B., Lehmann J., Kalbitz K., Thiongo M., Tenywa M., Okeyo I., and Neufeldt H., 2019. Ammonia and nitrous oxide emissions from a field Acrisol amended with Tithonia green manure, urea and biochar. *Biology and Fertility of Soils 55:135-148.* https://doi.org/10.1007/s00374-018-01338-3

## 6.1 Introduction

Ammonia ($NH_3$) and nitrous oxide ($N_2O$) are major atmospheric pollutants. $NH_3$ contributes to water eutrophication, acidification, and changes in biodiversity, through the processes of atmospheric transportation and deposition (Ciais et al. 2013). On the other hand, $N_2O$ is a long-lived greenhouse as well as an ozone layer-depleting gas (IPCC 2013). In the context of agriculture's contribution to climate change mitigation, management of agricultural soils should aimat either reducing the amount or form of these soil-derived gases in order to achieve low-emission agricultural production.

Growing evidence exists that addition of pyrogenic organic matter (biochar) can reduce NH3 and N2O emitted from agricultural soils (Cayuela et al. 2014; Sagrilo et al. 2014; Case et al. 2015). Previous studies proposed several mechanisms for both gases, which include the following: (i) retention of inorganic N through NH4+ adsorption and N immobilization (Kizito et al. 2014; Wang et al. 2015); (ii) biochar-induced changes in soil pH and other physico-chemical properties that affect the functions of ammonia oxidizers and nitrifiers at a microbial community level, directly changing the equilibrium between NH4+ and NH3; (iii) greater N

uptake by plants through enhanced plant growth (Xu et al. 2012; Abaas et al. 2012); (iv) promotion of soil aeration by reducing bulk density and increasing porosity that reduces N2O production (Ameloot et al. 2016); and (v) greater nosZ gene expression which encodes the nitrous oxide reductase and reduces N2O to N2 (Harter et al. 2013).

Hitherto, denitrification has been considered as the major pathway for $N_2O$ emissions from agricultural soils. However, there is need to draw attention to alternative pathways such as ammonia oxidation (Thomson et al. 2012) and nitrification (Kool et al. 2011; Niu et al. 2018), as may be dictated by soil moisture conditions. Ammonia ($NH_3$) volatilization is a major pathway of N loss in agricultural systems worldwide, and results in low fertilizer N use efficiency, environmental and health issues, and indirect $N_2O$ emission (Pana et al. 2016). Lower emissions of $N_2O$ in biochar-amended soils may be related to losses of $NH_4^+$ due to increased $NH_3$ volatilization. Schomberg et al. (2012) suggested that the reduced N leaching observed in their study resulted from increased $NH_3$ loss rather than adsorption by biochar surfaces. However, in many cases reduced NH3 emissions were found (Taghizadeh-Toosi et al. 2012; Mandal et al. 2016). The biochar-induced reduction in $NH_3$ volatilization may be attributed to $NH_3$ adsorption on biochar surfaces, increased nitrification, and N immobilization (Mandal et al. 2016).

$NH_3$ retention capacity depends on the properties of biochar such as its surface area, pore size, and pore structure. Very few studies have measured $NH_3$ emissions from biochar-amended soils and those that do (e.g., Taghizadeh-Toosi et al. 2012; Kizito et al. 2014; Song et al. 2014; Mandal et al. 2016) reported short-term results for $NH_3$, $N_2O$ or the processes that produce $NH_3$, leaving no clear explanation of the relationships. Clough et al. (2013) concluded that the impact of biochar on $NH_3$ varied with biochar characteristics. Soil and biochar pH influence

soil greenhouse gas (GHG) fluxes, but to a more limited extent (He et al. 2017). Zhang et al. (2017) observed that soil pH was an important factor affecting the abundance of Ammonia-Oxidizing Bacteria (AOB), which are responsible for consumption in the soil. Therefore, under field conditions, the mechanisms and duration of effect in which biochar lowers emissions of $NH_3$ remain relatively unknown.

Previous studies investigating effects of biochar aging on soil properties show changes in biochar surface chemistry, which could potentially affect C and N cycling and therefore the fluxes of $N_2O$ (Fungo et al. 2014; Li et al. 2015; Zhang et al. 2015a; Mia et al. 2017). However, experimentation under carefully controlled incubation conditions (such as Singh et al. 2012) or for short periods (Nguyen et al. 2017) does not represent well the aging processes in field soils. Griffin et al. (2017) studied soil inorganic N dynamics under biochar amendment for 4 years but did not link this to $N_2O$ emissions. Nguyen et al. (2016) linked N dynamics to $N_2O$ emissions but this was a short-term pot trial.

Whereas some studies suggest that biochar has mainly short-term effects after application, ranging from a few days to several months, others indicate that the effects may be longlived. For example, Spokas (2013) observed a reduced $N_2O$ mitigation effect by biochar after 3 years of biochar aging in the field. In contrast, Lentz et al. (2014) observed a persistent effect over a 3-year period after biochar additions, suggesting that a long-term driver is involved, possibly related to biochar's enduring porosity and surface chemistry characteristics. As such, questions remain concerning the medium- to longterm implications of biochar additions given the scant literature on field-based studies. Determining the residual effect of biochar on soil N dynamics provides opportunities to identify underlying processes and mechanisms regulating $N_2O$ fluxes

in biochar-amended soil (Nguyen et al. 2017). If biochar can provide long-term mitigation potential for N-fertilized soils, it can be a low-emission innovation for soil management.

The aim of the current study is to determine how soil mineral nitrogen (exchangeable $NH_4^+$ and $NO_3^-$) and organic matter inputs affect the long-term interaction between biochar and soil-atmosphere fluxes of $NH_3$ and $N_2O$. The specific objectives were to determine (i) the effect of continuous application of organic inputs (tithonia) and mineral N (urea) on NH3 and $N_2O$ fluxes; (ii) the residual effect of these amendments on soil mineral N; and (iii) the trend in effect size of the above amendments on $NH_3$ and $N_2O$ over a 3-year period after basal application of 2.5 t $ha^{-1}$ of biochar to an Acrisol under field conditions. We hypothesized that under low moisture, nitrification is an important $N_2O$ production pathway, and that biochar partly reduces $N_2O$ emissions via increased $NH_3$ volatilization.

## 6.2 Materials and methods

### 6.2.1 Site description

The field experiment was established in September 2012 at Kapsengere on the southern Nandi hills in western Kenya (N 00° 09′ 34″ and E 34° 57′ 37″). The sites receive ~ 2000 mm mean annual rainfall in a bimodal distribution, with two cropping seasons per year, March–July and September–January with a mean annual temperature of 26 °C. The soil is classified as Typic Kandiudult (Soil Survey Staff 1999) developed on biotite-gneiss parent material. This soil is highly weathered with the clay fraction dominated by kaolinite. The natural vegetation is composed of tropical rainforest of Guineo-Congolian region. The experiment was conducted for six consecutive maize cropping seasons from September 2012 to August 2015.

### 6.2.2 Preparation of the biochar and tithonia green manure

Biochar was produced from eucalyptus wood after chopping and grinding the organic matter to pass through a 2-mm sieve. The ground material was pyrolyzed to a maximum temperature of 550 °C and retained at that temperature for 1 h before cooling it to room temperature. In the laboratory, the resultant biochar was analyzed for pH, CEC, and elemental composition. Tithonia (Tithonia diversifolia) was prepared by cutting leaves in the field and chopping them into 50-mm lengths, air-drying, and grinding to pass through a 1-mmsieve before field application. The physical and chemical characteristics of the above materials are fully described by Fungo et al. (2017).

### 6.2.3 Experimental design

The treatments were selected to represent presence and absence of biochar as well as low and high input of tithonia green manure, with and without mineral N fertilizer. This arrangement represented a range of conventional management practices of many small-holder farmers in integrated soil fertility management systems in eastern Africa, and allowed for effects of varying organic and inorganic N to be examined. The treatments included the following: two levels of biochar (0 and 2.5 t $ha^{-1}$) applied once at the start of the experiment; three levels of green manure applied as tithonia (0, 2.5, and 5 t $ha^{-1}$) per season; and two levels of mineral N applied as urea (0 and 120 kg N $ha^{-1}$) per season (Table 6.1). Each treatment was established in 2-by-2-m plots separated by a 1-m distance within and between rows. The experimental plots were laid out in a Randomized Complete Block Design with three replicates. Due to the inherently low fertility of the soil, 30 kg $ha^{-1}$ of $P_2O_5$ as TSP and 30 kg $ha^{-1}$ of $K_2O$ as Muriate of Potash (MoP) were applied to each plot.

### 6.2.4 Management of experiment

Precipitation and air temperature were monitored throughout the experiment with the help of a weather station located near the experimental field. Application of biochar was done once at the start of the first season on October 3, 2012. Identical amounts of tithonia green manure, TSP, and MoP were applied to each plot once at the start of each season. Mineral N (urea) was applied in two splits: 40% at planting and 60% at 1st weeding (Table 6.2).

Table 6.1: Experimental treatments for determining the effect of tithonia green manure, urea and biochar on fluxes of $N_2O$ and $NH_3$ in a maize field in western Kenya

| Treatment | Biochar | | *T. diversifolia* | | | Mineral N (Urea) | | Total N |
|---|---|---|---|---|---|---|---|---|
| | (t ha$^{-1}$)[a] | *Code* | (t ha$^{-1}$)[b] | (kg N ha$^{-1}$) | Code | Rate (kg N ha$^{-1}$)[c] | Code | (kg N ha$^{-1}$) |
| 1 ($B_0T_0U_0$)(Control) | 0 | B0 | 0.0 | 0 | T0 | 0 | U0 | 0 |
| 2 ($B_0T_{2.5}U_0$) | 0 | B0 | 2.5 | 75 | T2.5 | 0 | U0 | 75 |
| 3 ($B_0T_5U_0$) | 0 | B0 | 5.0 | 150 | T5 | 0 | U0 | 150 |
| 4 ($B_0T_0U_{120}$) | 0 | B0 | 0.0 | 0 | T0 | 120 | U120 | 120 |
| 5 ($B_0T_{2.5}U_{120}$) | 0 | B0 | 2.5 | 75 | T2.5 | 120 | U120 | 195 |
| 6 ($B_0T_5U_{120}$) | 0 | B0 | 5.0 | 150 | T5 | 120 | U120 | 270 |
| 7 ($B_{2.5}T_0U_0$) | 2.5 | B2.5 | 0.0 | 0 | T0 | 0 | U0 | 0 |
| 8 ($B_{2.5}T_{2.5}U_0$) | 2.5 | B2.5 | 2.5 | 75 | T2.5 | 0 | U0 | 75 |
| 9 ($B_{2.5}T_5U_0$) | 2.5 | B2.5 | 5.0 | 150 | T5 | 0 | U0 | 150 |
| 10 ($B_{2.5}T_0U_{120}$) | 2.5 | B2.5 | 0.0 | 0 | T0 | 120 | U120 | 120 |
| 11 (B2.5T2.5U120) | 2.5 | B2.5 | 2.5 | 75 | T2.5 | 120 | U120 | 195 |
| 12 (B2.5T5U120) | 2.5 | B2.5 | 5.0 | 150 | T5 | 120 | U120 | 270 |

Biochar C = 86.8%, T. diversifolia C ~ 48%. N input from T. diversifolia application is derived from the 3% N content. The biochar contained only very low N content, and was not included in the total N applied calculations

aOne kilogram of biochar

bOne and 2 kg of T. diversifolia

cOne hundred grams, respectively, per treated 2 × 2 m plot

The biochar, manure, and mineral fertilizer were broadcast on the soil surface by hand and incorporated into the 0.1-m topsoil. Two seeds of the maize cultivar HB 513 were planted at a spacing of 0.25 m within and 0.5 m between rows (equivalent to 40 plants per plot). The 1st and 2nd weeding were done at approximately 30 and 50 days after planting, respectively, using a hand-hoe. Thinning was done during the first weeding to retain one plant per hole.

Table 6.2: Activity Calendar for the management of the field trial in maize crop western Kenya amended with biochar, tithonia and biochar.

| Activity | Season 1 | Season 2 | Season 3 | Season 4 | Season 5 | Season 6 |
|---|---|---|---|---|---|---|
| Biochar application | 3rd October 2012 | - | - | - | - | - |
| Application of manure and 40% urea; Planting | 3rd October 2012 | 5th March 2013 | 10th August 2013 | 3rd April 2014 | 7th September 2014 | 10th Aril 2015 |
| 1st Weeding and top-dressing with 60% urea | 7th November 2012 | 10th April 2013 | 10th September 2013 | 5th May 2014 | 7th October 2014 | 20th May 2015 |
| 2nd Weeding | 4th December 2012 | 12th May 2013 | 15th October 2013 | 9th June 2014 | 11th November 2014 | 17th June 2015 |
| Harvesting | 30th January 2012 | 24th July 2013 | 17th December 2013 | 7th August 2014 | 14th January 2015 | 13th August 2015 |

### 6.2.5 Gas measurements

Measurements of $N_2O$ were conducted using the static closed chamber method. The chamber consisted of a PVC tube (diameter = 0.3 m; height = 0.15 m) transversely divided into two parts to make a base (0.05 m) and a cover (0.1 m). The base was driven into the soil so that it reached ~ 0.02 m below the soil surface. To ensure air-tight conditions, a rubber ring was placed between the base and the cover. Inside the cuvette, air humidity and temperature were monitored by a digital thermohygrometer (PCE-313 A, Paper-Consult Engineering Group, Meschede, Germany) attached to the cover from the outside in a way that only the sensor

reached inside the chamber through a rubber screw connector. Two chambers were set up in each plot. The chambers were relocated every month to capture within-plot variability. Sampling was done every seven days.

Fertilizer application was done 2 days before the next sampling event. The sampling events were the same for both $NH_3$ and $N_2O$. For each $N_2O$ sampling event, four samples were taken at 0, 10, 20, and 30 min after closing the chamber, by taking 20 ml of gas from the chamber. The gas samples were collected using a 20-ml syringe and injected into pre-evacuated 10-ml gas vials. Analysis of gas samples was done on a gas chromatograph fitted with an electron capture detector (ECD) for $N_2O$ quantification (SRI GC Model 8610V). Operating conditions for the GC were as follows: injector temperature 60 °C, column temperature 400 °C, and detector temperature 320 °C. Samples were introduced using a Gilson 40-vial autosampler (Gilson Inc., Middleton, Italy). One standard $N_2O$ gas sample (CGDI, USA) was used for every four samples. Data processing was performed using the PeakSimple Chromatography software (SRI Inc., Silicon Valley, CA, USA). Coefficient of variation in mean measurements was 1.6%. Fluxes of NH3 were measured using a photo-acoustic infrared field gas monitor (INNOVA 1402, Lumasense Technologies A/S, Ballerup, Denmark). Weekly $NH_3$ gas flux measurements were conducted using chambers identical to those used for $N_2O$ and the sampling was done at the same time for both gases. A photo-acoustic infrared multi-gas monitor (INNOVA 1312–5, LumaSense Technologies A/S, Ballerup, Denmark) was used for gas analysis. To ensure accuracy of the measurements, INNOVA was calibrated every three months.

### 6.2.6 Soil sampling and analysis

Soil samples for analysis of mineral N ($NH_4^+$ and $NO_3^-$) were taken from a depth of 0–0.15mwith a soil core (0.05 l; 0.05 m d) during the third year (from October 7, 2014 to August

12, 2015). Soil from four locations within each plot were taken, composited, and approximately 200 g was packed under ice blocks in a cooler box before transfer to the laboratory for extraction and the remaining portion (~ 200 g) was used to determine water-filled pore space (WFPS). These soil samples were collected on the same days as were the N2OandNH3 gas measurements. For soil mineral N analysis, a total of 18 data points was collected during the two seasons in the third year of the experiment between October 2014 and August 2015. For bulk density, soil was taken at the beginning and end of the third experimental year. $NH_4^+$-N and $NO_3^-$-N were extracted with 2 M KCl in 100 ml of solution for 20 g of soil and the content analyzed using spectrophotometry according to International Standards Organization (ISO standard EN 14256-1). WFPS was calculated based on water content, soil bulk density, and a particle size density of 2.65 g $cm^{-3}$ according to Eq. 6.1;

$$\% \mathbf{WFPS} = \frac{\boldsymbol{soil\ water\ content\ x\ bulk\ density\ x\ 100}}{\mathbf{1} - (\boldsymbol{bulk\ sensity/2.65})} \ldots\ldots\ldots\ldots\ldots\ldots \boldsymbol{Equation\ 6.1}$$

Data management and statistical analysis The $NH_3$ and $N_2O$ cumulative flux for each treatment during the entire experimental period was derived using linear interpolation with sampling dates as the time intervals (Trapezoidal rule). Treatment-induced differences (effect size) in $NH_3$ and $N_2O$ emission for each treatment were calculated as the difference between the treatment value and that of the control. The relationship between effect size and experimental time was examined using linear regression functions. Treatment effects and their interaction on cumulative emissions were examined using three-way full factorial analysis of variance (ANOVA). Post hoc separation of means was done using least significant difference (LSD) at 5% allowable error. A power function was fitted to test dependence of $NH_3$ and $N_2O$ emission

on WFPS and mineral soil N content. For correlation between soil N and gas fluxes, only data collected on the same day was used in the analysis. Effect size of the treatments was computed as the difference between treatments with amendments and the unamended control. Positive values indicate greater emissions from the amended plots compared to the unamended control. Delta emission of N was calculated using Eq. 6.2;

$$\textbf{\textit{Prop. of biochar induced N loss}}\ (\%) = \frac{(\textbf{\textit{Cum}}\ N_{B0} - \textbf{\textit{Cum}}\ N_{B2.5})}{\textbf{\textit{Applied N}}}\ \textbf{\textit{X}}\ \mathbf{100} \ldots\ldots\ldots \textbf{\textit{Eq.}}\ \mathbf{6.2}$$

Where Cum. NB0, and Cum. NB2.5 is the cumulative N emissions for non-biochar and biochar plots, respectively, and N applied is the total amounts of applied N in the respective season (tihonia N + urea N). The resultant value was expressed as a percentage. We compare the correlation coefficients of each trend line to determine whether the differences are statistically significant. Where $p > 0.05$, it implies that the trend is not significantly different.

## 6.3 Results

### 6.3.1 Daily dynamics of $NH_3$ and $N_2O$ emissions

The mean emission rates of $NH_3$ were 1.0 µg $NH_3$-N $m^{-2}$ $h^{-1}$, with a range of 0.1 to 2.9 µg $NH_3$-N $m^{-2}$ $h^{-1}$ (Table 6.3). The mean daily emission of $N_2O$ was 1.2 ± 0.6 µg $N_2O$-N$m^{-2}$ $h^{-1}$, with a range of 0.07 to 8.2 µg $N_2O$-N $m^{-2}$ $h^{-1}$. Peaks in $N_2O$ emissions occurred 1–2 days after fertilization but those for $NH_3$ were inconsistent. Variability in daily emissions, as expressed by the coefficient of variation (cv), was higher (40%) for $NH_3$ compared to that of $N_2O$ (20%). A biochar effect was observed for both $NH_3$ and $N_2O$, where lower emissions ($p < 0.05$) were observed in biochar-amended plots. Mean emissions of $NH_3$ were 1.2 ± 0.3 and 1.4 ± 0.3 µg $NH_3$-N $m^{-2}$ $h^{-1}$ in biochar and non-biochar plots, respectively, while those for $N_2O$ were 1.4 ± 0.4 and 1.6 ± 0.3 µg $N_2O$-N $m^{-2}$ $h^{-1}$ for biochar and non-biochar plots, respectively.

### 6.3.2 Effects of amendments on emissions dynamics

***$NH_3$ emissions***

Figures 1 and 2 show comparison in the trend in effect of the treatments on emissions, Fig. 6.3 shows the direct comparisons of cumulative values for each treatment at the end of the study period. All N sources (tithonia and urea) increased emission of $NH_3$. The increase ranged from 21 to 58% for tithonia and urea, respectively, compared to the control. No significant difference in $NH_3$ emissions were observed between the tithonia application rates. Biochar amendments decreased $NH_3$ emissions with a range of 11–37% compared to the control (mean = 22 ± 2% in the first year and 10 ± 2% by the end of the third year).

Table 6.3: Mean hourly flux and effect size of $NH_3$ and $N_2O$ after soil amendment with tithonia, urea and biochar to an Acrisol under maize in western Kenya. Effect size of the treatments was computed as the difference between treatments with amendments and the unamended control.

| Treatment | Mean hourly flux ($\mu g\ N\ m^{-2}\ hr^{-1}$) | | | | *Mean effect size ($\mu g\ N\ m^{-2}\ hr^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | $NH_3$ | SE | $N_2O$ | SE | $NH_3$ | SE | $N_2O$ | SE |
| 1 ($B_0T_0U_0$) | 0.90 | 0.03 | 0.66 | 0.05 | 0.00 | - | 0.00 | - |
| 2 ($B_0T_{2.5}U_0$) | 1.09 | 0.03 | 0.73 | 0.06 | 0.64 | 0.04 | 0.52 | 0.03 |
| 3 ($B_0T_5U_0$) | 1.13 | 0.02 | 0.62 | 0.03 | 0.59 | 0.03 | 0.34 | 0.02 |
| 4 ($B_0T_0U_{120}$) | 1.23 | 0.02 | 0.49 | 0.03 | 0.85 | 0.05 | 0.59 | 0.03 |
| 5 ($B_0T_{2.5}U_{120}$) | 1.20 | 0.02 | 0.56 | 0.05 | 0.73 | 0.04 | 0.47 | 0.03 |
| 6 ($B_0T_5U_{120}$) | 1.24 | 0.02 | 0.53 | 0.05 | 0.83 | 0.05 | 0.58 | 0.03 |
| 7 ($B_{2.5}T_0U_0$) | 0.48 | 0.02 | 0.37 | 0.04 | -0.39 | 0.02 | -0.64 | 0.04 |
| 8 ($B_{2.5}T_{2.5}U_0$) | 0.37 | 0.02 | 0.44 | 0.04 | -0.34 | 0.02 | -0.59 | 0.03 |
| 9 ($B_{2.5}T_5U_0$) | 0.80 | 0.03 | 0.50 | 0.05 | 0.09 | 0.01 | -0.17 | 0.01 |
| 10 ($B_{2.5}T_0U_{120}$) | 1.05 | 0.02 | 0.49 | 0.05 | 0.38 | 0.02 | 0.13 | 0.01 |
| 11 ($B_{2.5}T_{2.5}U_{120}$) | 1.02 | 0.04 | 0.58 | 0.07 | 0.47 | 0.03 | 0.22 | 0.01 |
| 12 ($B_{2.5}T_5U_{120}$) | 0.72 | 0.02 | 0.53 | 0.04 | 0.08 | 0.00 | -0.19 | 0.01 |

Biochar lowered cumulative $NH_3$ emissions when no or low doses of N were applied but had no effect when high doses (U120 and T5 + U120) were applied. The effect size due to tithonia or urea additions did not change significantly over the 3-year period (Fig. 6.1). However, the

effect size for adding biochar with or without tithonia at low application rates (B2.5T0U0 and B2.5T2.5U0) decreased from 0.49 ± 0.1 µg NH3-N $m^{-2}$ $h^{-1}$ in the first year to 0.16 ±0.1 µg $NH^3$-N $m^{-2}$ $h^{-1}$ in the third year (Fig. 6.1).

Compared to the control, the effect size of adding biochar with a high application rate of tithonia (B2.5T5.0U0) decreased only until the 12th month after which it increased (Fig. 6.1). Adding biochar to urea (B2.5T0U120) suppressed $NH_3$ emissions compared to urea without biochar to the same level as the completely unamended control in the first year but the effect size increased progressively to 0.41 ± 0.1 µg $NH_3$-N $m^{-2}$ $h^{-1}$ by the end of the third year. High amounts of tithonia (5 t $ha^{-1}$) in combination with biochar and urea lowered the initial effect size from 0.33 ± 0.1 µg $NH_3$-N$m^{-2}$ $h^{-1}$ to zero by the $18^{th}$ month, compared to the control. However, by the $6^{th}$ season, the effect size had increased to 0.49 ± 0.1 µg $NH_3$-N $m^{-2}$ $h^{-1}$ compared to the control and not different from urea and tithonia without biochar (Fig. 6.1).

***$N_2O$ emissions***

Overall, the patterns in $N_2O$ emissions were similar to those of $NH_3$. Tithonia or urea increased $N_2O$ emissions by 13 to 47% (mean ± SE of 16 ± 3%), with the highest increase observed where urea was applied alone or in combination with high amounts of tithonia. For these treatments, changes in $N_2O$ across seasons did not vary significantly. With biochar added on its own, $N_2O$ emissions significantly lowered by 42 ± 3% in the first year but lowered by an additional 17 ± 2% by the end of the third year, representing a 59% decrease in effect size over 3 years. Biochar added with either low amounts of tithonia or urea, alone or in combination, resulted in the $N_2O$ mitigation effect lasting only until the second year compared to the control, after which the effect gradually lowered relative to the control. However, a longer $N_2O$ mitigation effect was observed when higher amounts of tithonia were applied in the

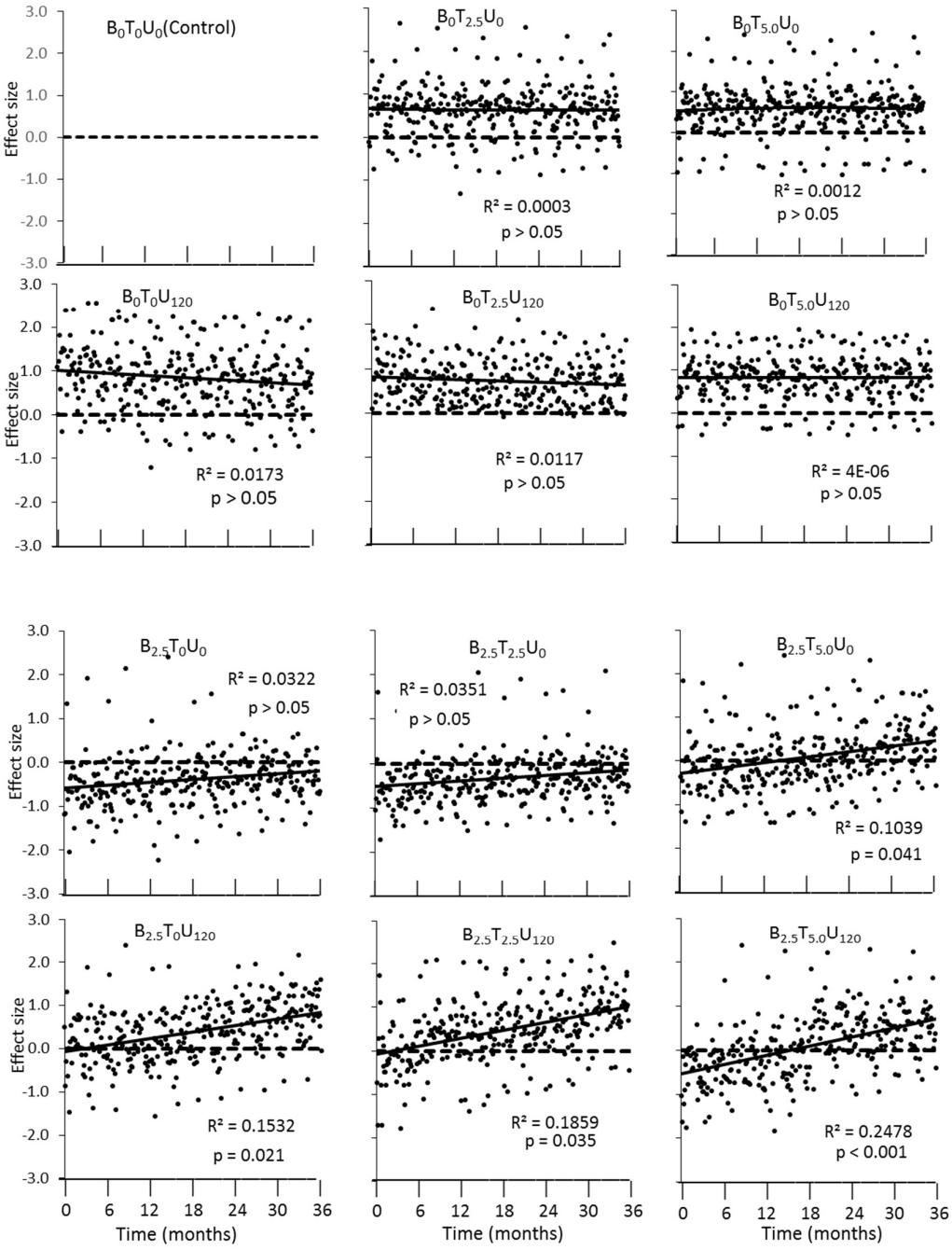



Fig. 6.1: Effect size of treatments on $NH_3$ emissions after additions of tithonia green manure, urea, and biochar in an Acrisol cropped to maize in western Kenya over three consecutive years. The dotted line is the control and the solid one is the trend of the treatment. Time after a single biochar addition. Positive values indicate greater emissions from the amended plots compared to the unamended control

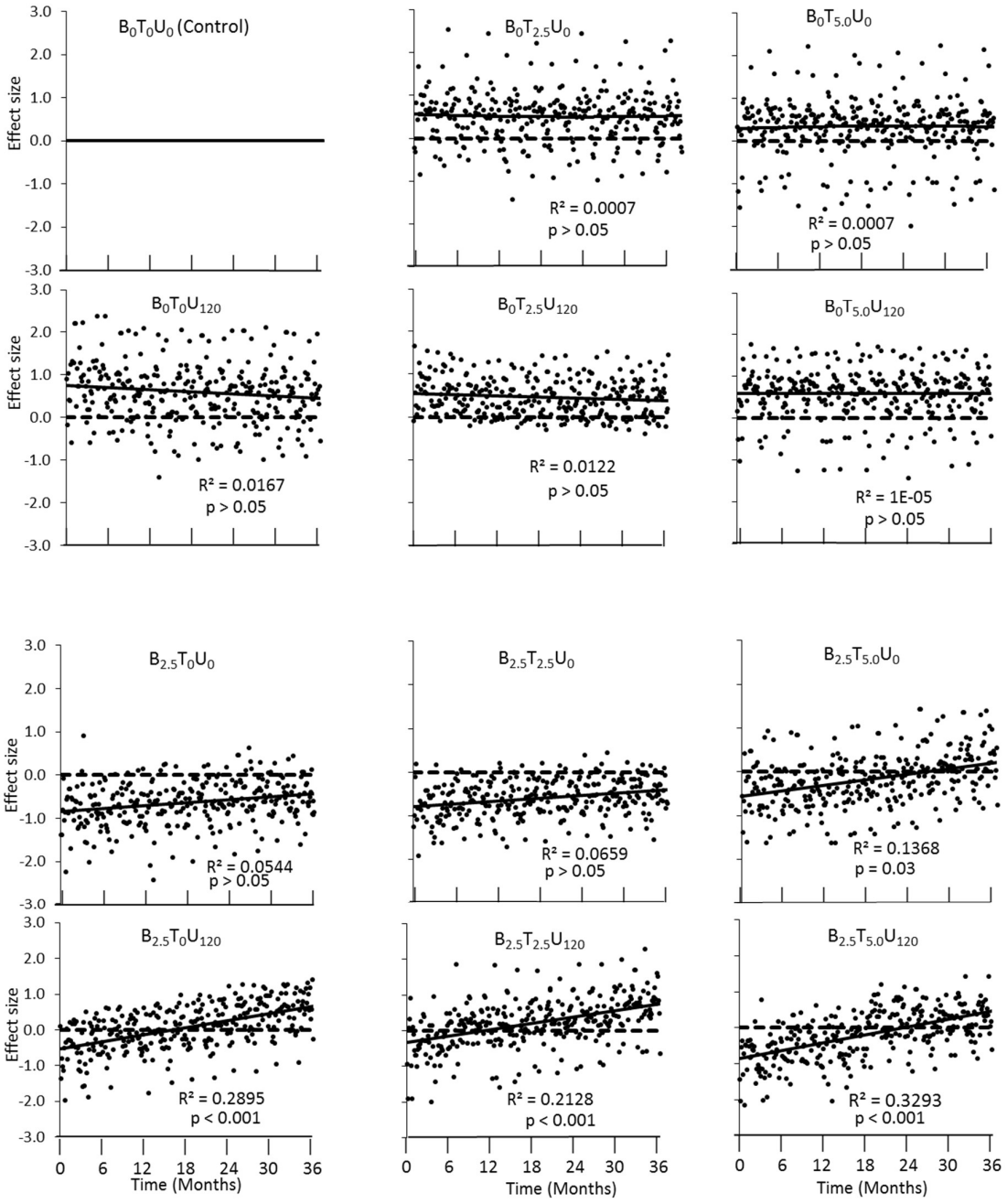



Fig. 6.2: Effect size of treatments on $N_2O$ emissions after additions of tithonia green manure, urea, and biochar to an Acrisol cropped to maize in western Kenya over three consecutive years. The dotted line is the control and the solid one is the trend of the treatment. Time after a single biochar addition. Positive values indicate greater emissions from amended plots compared to the unamended control

combination with biochar and urea (Fig. 6.2). As was the case with $NH_3$, biochar lowered cumulative $N_2O$ emissions where no or low doses of N were applied but had no effect when high doses (U120 and T5 + U120) were applied.

### 6.3.3 Cumulative emissions of $NH_3$ and $N_2O$

***$NH_3$ emissions***

Biochar applied alone significantly reduced cumulative emissions of $NH_3$ by up to 47 ± 5% without tithonia (Fig. 6.3a). The reduction was 39 and 18% when biochar was combined with 2.5 and 5 t $ha^{-1}$ of tithonia, respectively, compared to the control. For plots with only tithonia or urea, emission of $NH_3$ increased significantly (13 to 68%; average of 20%) compared to the control (Fig. 6.3a). Urea, alone or in combination with tithonia, increased $NH_3$ emissions by 50–68% compared to the control. Biochar reduced $NH_3$ emission in most treatments except where all three amendments were added (Fig. 6.3a). $N_2O$ emissions Biochar applied alone significantly decreased cumulative $N_2O$ emissions compared to the control but no difference was found where a biochar-urea combination was applied (Fig. 6.3b).

Without urea additions, $N_2O$ emissions were between 12 and 22% lower compared to the control after biochar additions, irrespective of tithonia additions. Both tithonia and urea additions significantly increased cumulative $N_2O$ emission (20±4 and 43 ± 5%, respectively) but the amount of tithonia did not significantly affect $N_2O$ emission with or without urea, compared to the control. The biochar-urea combination increased cumulative $N_2O$ by 25 ± 3% compared to the unamended control but the biochar-tithonia-urea combination (independent of the level of tithonia) increased $N_2O$ emission by 41 ± 5% compared to the unamended control.

### 6.3.4 Proportion of gaseous N emissions

The amount of $NH_3$-N lost, as a proportion of total N applied, ranged from 0.2 to 0.8% (Fig. 6.4). For all levels of applied N, the proportion of biochar-dependent $NH_3$ lowering decreased significantly over the 3 years and the decrease was highest where urea was applied without tithonia (120 kg N $ha^{-1}$) or where total N applied was highest (270 kg N $ha^{-1}$). For $N_2O$-N, the trend in proportion of N emitted was similar to that of $NH_3$ but the slope (0.4 to 1.2% $N_2O$-N) was higher compared to that of $NH_3$ (75, 195, and 270 kg N $ha^{-1}$. The continuous decline in effect size was predominantly observed where 270 kg N $ha^{-1}$ was applied without tithonia (Fig. 6.4).

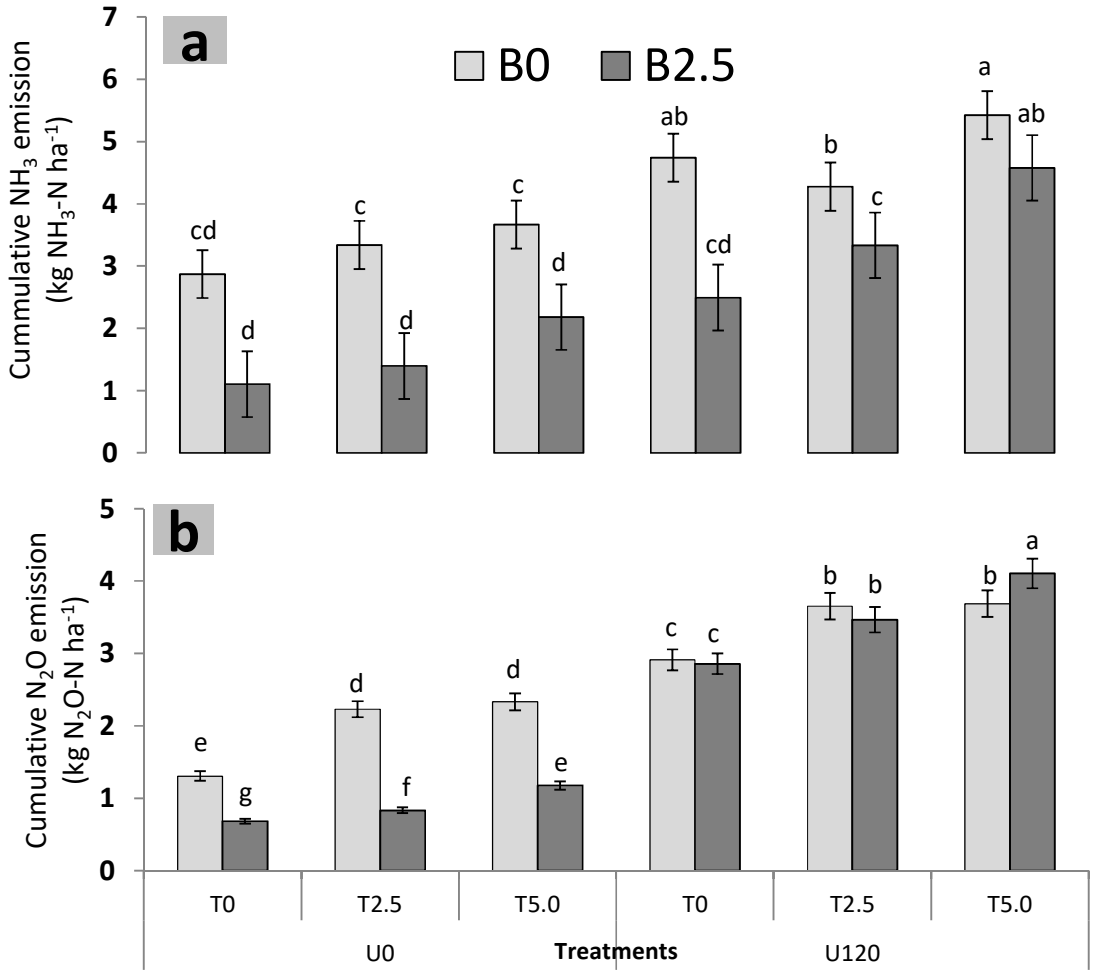



Fig. 6.3: Cumulative emissions of $NH_3$ (a) and $N_2O$ (b) during 3 years of maize cultivation on an Acrisol in western Kenya after amendment with tithonia green manure, urea, and biochar (N = 3)

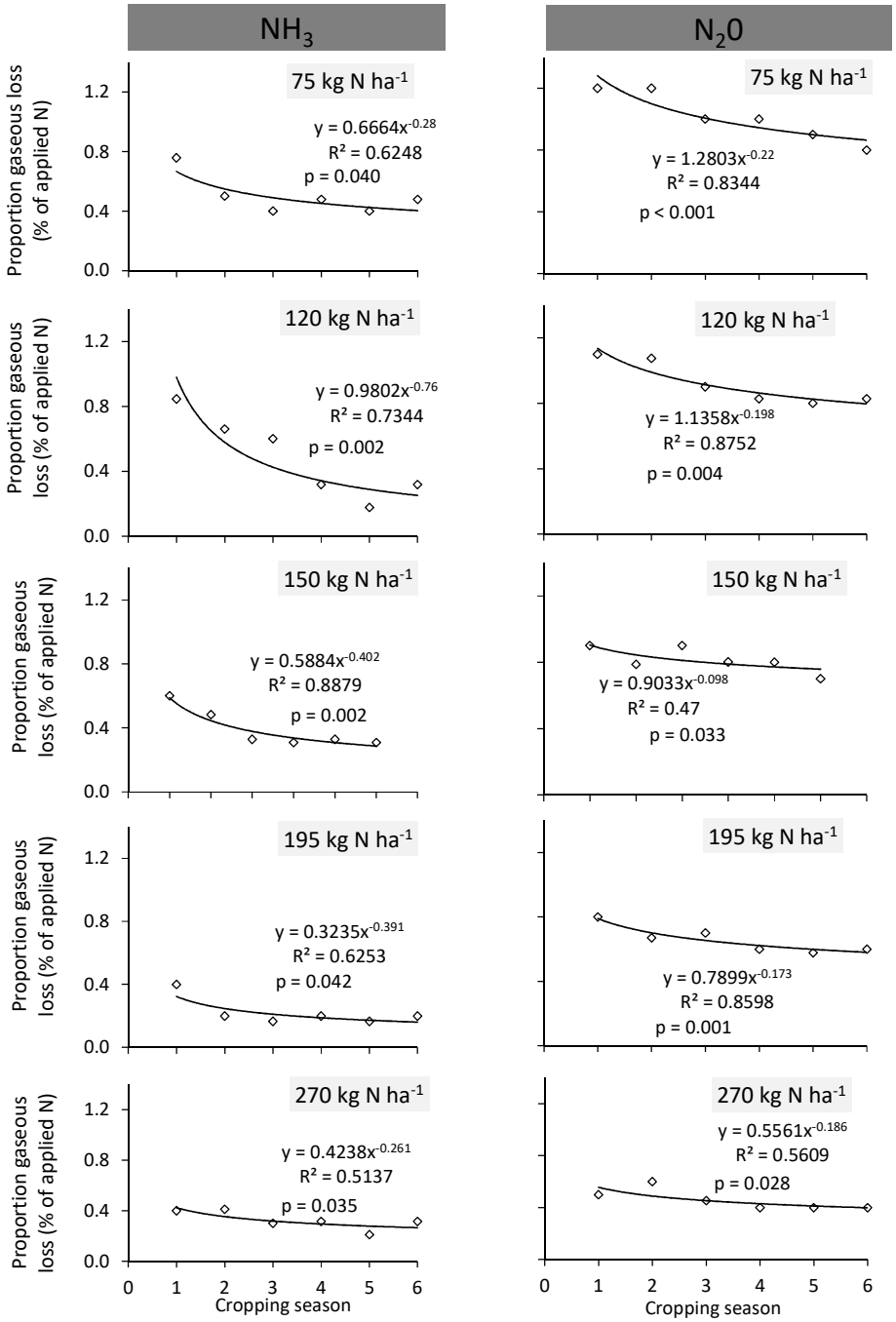



Fig. 6.4: Gaseous N losses as a proportion of the applied N for $NH_3$ (left) and $N_2O$ (right) during 3 years of maize cultivation on an Acrisol in western Kenya after amendment with tithonia green manure, urea, and biochar. Each data point represents the net proportion of loss at the completion of the crop cycle. Data was divided into 6-month periods for each season to account for the cropping period and fallow

### 6.3.5 Relationships between gas fluxes and WFPS and extractable mineral N

Emissions of $NH_3$ were neither related to soil moisture nor to $NO_3^-$-N contents (Fig. 5a, c), but were positively related to $NH_4^+$-N (Fig. 6.5b). However, WFPS significantly and positively correlated with $N_2O$ emission (Fig. 6.5d). A significant positive relationship was observed between $N_2O$ emission and NH4+-N and a positive one between $N_2O$ and $NO_3^-$-N content (Fig. 6.5f). Data were not sufficient to determine the trend in $N_2O$ emission beyond a moisture content of 60%.

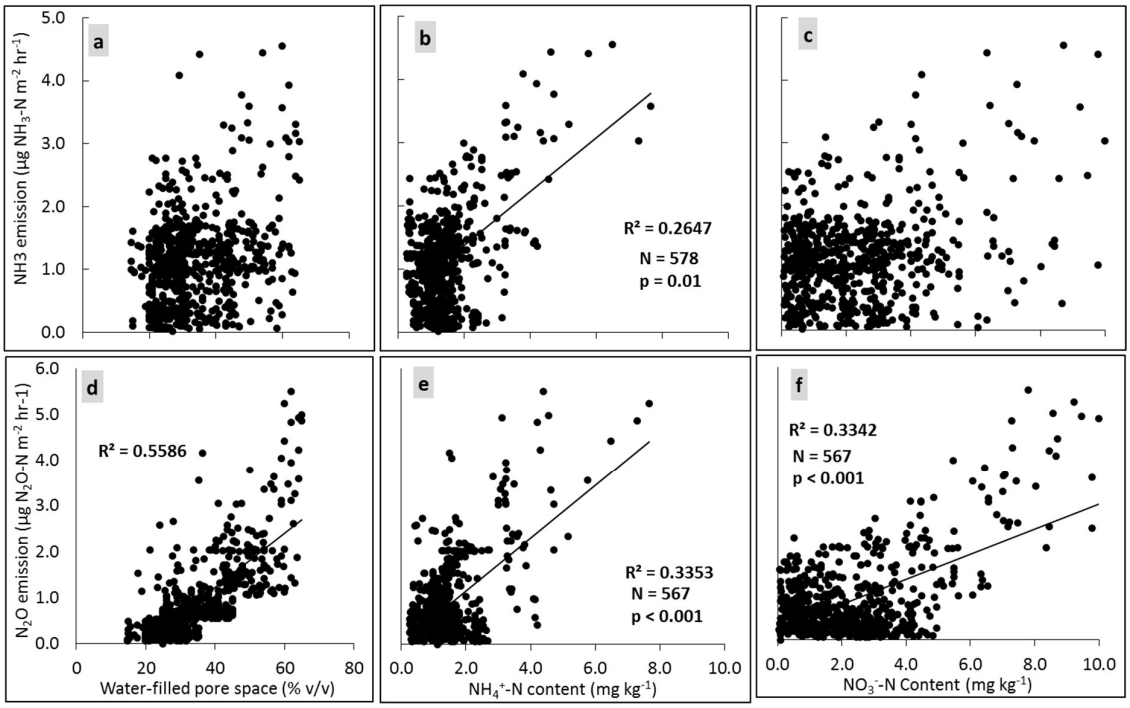



Fig. 6.5: Relationship between gas fluxes and water-filled pore space and mineral N during the third year of an Acrisol cropped to maize in western Kenya amended with tithonia, urea, and biochar

## 6.4 Discussion

### 6.4.1 Emissions of $NH_3$

Several studies have reported an effect of biochar on $NH_3$ emissions (e.g., Spokas et al. 2011; Taghizadeh-Toosi et al. 2012; Mandal et al. 2016). In all of these cases, reduction in $NH_3$ emissions due to biochar was reported, as was the case in my study (Fig. 6.2; Table 6.2). In principle, reductions in $NH_3$ emissions by biochar may be a result of (i) lower pH that would drive the equilibrium $NH_4^+ \leftrightarrows NH_3$ towards $NH_4^+$; (ii) reduced biological production of $NH_4^+$ (ammonification and urease activity); (iii) increased biological consumption of $NH_4^+$ (nitrification, immobilization, and plant uptake); (iv) increased adsorption of $NH_4^+$; or (v) increased adsorption of $NH_3$. From this study, we have provided detail of the residual effect of biochar on some of the above processes and their influence on emission of $NH_3$ and $N_2O$ under tropical field conditions. In the following, each of these mechanisms is discussed.

***pH of biochar***

The pH of biochar may differ from that of the soil and may increase or decrease and thereby also either increase of decrease $NH_3$ emissions. In my study, the pH of the biochar was slightly higher than that of the soil at the beginning of the experiment and a pH effect is therefore assumed to play no major role. López-Cano et al. (2016) observed that biochar addition could slow down the ammonification process. This is also in line with negative C priming effects as a result of biochar additions to soil (Wang et al. 2016). A positive relationship between $NH_3$ and $N_2O$ observed in my study is consistent with a similarity in the mechanisms regulating both gases such as lower $NH_4^+$ production thus reducing losses of both $NH_3$ and $N_2O$ (Steiner et al. 2010; Prendergast-Miller et al. 2011; Spokas et al. 2011; Jassal et al. 2015).

***Liming effects of biochar***

Increased biological consumption of $NH_4^+$ by biochar through increased nitrification may be a result of liming effects of biochar in acid soils or the removal of inhibiting substances such as polyphenols or tannins (DeLuca et al. 2006; Ball et al. 2010). Neither of these explanations are valid in my study. The above-mentioned positive relationship between $NH_3$ and $N_2O$ may also suggest that a biological consumption of $NH_4^+$ is unlikely to explain $NH_3$ emission reductions as it would increase $N_2O$ emissions. Increased $NH_4^+$ removal could also be the result of the improvement in soil structure that allows $NH_4^+$ to percolate from the surface to greater depths where volatilization is less likely. However, this is an unlikely explanation, because most studies report reduced $NH_4^+$ leaching for reasons including reduced water percolation, increased adsorption (Lehmann et al. 2003), increased plant uptake (Major et al. 2010), or increased microbial immobilization (Güereña et al. 2013).

***Sorption reactions***

Sorption of either $NH_4^+$, $NH_3$, or of both chemical species on biochar surfaces was proposed as a responsible mechanism (Yao et al. 2012; Taghizadeh-Toosi et al. 2012; Zhang et al. 2013). An adsorption of $NH_3$ gas is more likely to be less readily reversible as it may generate amine functional groups (Kastner et al. 2009). Oxidation of biochar surfaces, particularly in low-pH soils, over time (Li et al. 2015; Zhang et al. 2015b; Shen et al. 2016) may increase the reactivity with either $NH_4^+$ species. Thus, the observed effect size of biochar with respect to $NH_3$ emission reductions (Fig. 6.1) showed a decreasing trend over the 3 years possibly because of the oxidized surfaces on biochar. $NH_3$ emission lowering after additions of biochar was less in the presence of tithonia and urea, may be a result of the concomitant C additions. According to Anand et al. (2015), high SOC can increase soil urease activity. It follows that tithonia may have contributed to increased $NH_3$ through its additions of metabolizable C far more that its N addition. In terms of $NH_3$ emission reductions, biochar may therefore be more effective in its

effects on N than in its effects on C. Sommer et al. (1991) demonstrated that a doubling of the infiltration rate of total available N away from the soil surface could half cumulative ammonia emissions. Thus, biochar could have reduced emissions by adsorbing $NH_3$ and reducing its emission in the atmosphere.

### 6.4.2 Emissions of $N_2O$

Between the start of the first year and the end of the third year, cumulative emission of $N_2O$ had decreased by 22% due to biochar application, a value lower compared to the ~50% decrease observed from previous short-term (≤2 years) studies (cf. Zhang et al. 2015b; Fungo et al. 2017). These comparisons show a progressive decrease of the effect of biochar on $N_2O$ emission reduction over time. This trend is similar to the observation made by Zhang et al. (2015b) in a 2-year field trial where the mitigating effect of biochar on $N_2O$ decreased by 59–70% in the first year and with 54–67% slightly less in the second year. In my study, biochar additions decreased $N_2O$ emission by 32 ± 4% during the first year and by 15 ± 2% during the third year. The small mitigation of $N_2O$ emission over time reported in my study compared to Zhang et al. (2015b) could be due to the larger amount of biochar (20 to 40 t ha−1) applied by Zhang et al. (2015b). Cayuela et al. (2015) found that on average, field studies showed lower reductions in$N_2O$ emissions (28 ± 16%) compared to laboratory studies (54 ± 5%). Thus, my results fall within the range for most field studies. The decreasing effect size of biochar on $N_2O$ emission over time (Fig. 6.2) has been reported in previous studies (e.g., Singh et al. 2012). Some studies have proposed that biochar regulates denitrification and $N_2O$ emission via reducing necessary substrates such as easily mineralizable C (Joseph et al. 2010), direct retention of $N_2O$ on biochar surfaces (Cornelissen et al. 2013), and mineral N (Cayuela et al. 2013). An additional mechanism is the reaction of biochar surfaces with $N_2O$ (resulting in

greater N compounds on biochar surfaces) mediated through redox processes (Armor and Taube 1971).

Fungo et al. (2014) proposed the possible formation of complexes between $N_2O$ and transitional metals similar to that observed with Ru, favoring the observed lowering in N2O emission. Quin et al. (2015) used X-ray photoelectron spectroscopy and identified changes in surface functional groups suggesting interactions between $N_2O$ and the biochar surfaces. Quin et al. (2015) have also shown increases in -OC=N pyridine pyrrole/NH3, suggesting reactions between $N_2O$ and the C matrix upon exposure to $N_2O$. However, my data (Fig. 6.3b) suggest that in the presence of key resources (mineralizable C and N) necessary for microbial activity, improved efficiency of complete denitrification to N2 is a more likely outcome. Hence, the cumulative emission of $N_2O$ was lower when both tithonia and urea were present. However, under high N availability, the lack of difference in $N_2O$ emissions with or without biochar may be due to the excess mineral N that facilitates $N_2O$ formation.

Singh et al. (2012) found that biochar C mineralization increased between 2.5 and 4 years for 10 out of 11 biochar types and then decreased or stabilized between the 4th and $5^{th}$ year. During such mineralization, biochar surfaces may be oxidized, thereby generating quinone functional groups (Cheng et al. 2008). These surface functional groups (Chen et al. 2014; Sun et al. 2016) could be responsible for direct electron transfer, promoting $N_2$ production. Avdeev et al. (2005) hypothesized that an O atom is transferred through the 1,3-dipolar cyclo-addition of $N_2O$ to the C=C bond with the resulting intermediate decomposing to yield a Ketone and $N_2$.

Available evidence (Nguyen et al. 2008; Cheng et al. 2008) suggests that surface functional groups on biochar surfaces typically increase with longer exposure in soil. Furthermore, greater

biochar oxidation has been shown to decrease electron transfer through the biochar-C matrix (Sun et al. 2016). It should be noted though that there is a limit to this increase. Electron transfer, according to Cayuela et al. (2014), may enhance the last step of denitrification (i.e., the reduction of $N_2O$ to N2). However, we observed a reducing effect size of biochar on $N_2O$ emission (Fig. 6.2). In addition to surface functional groups, the C matrix itself can transfer electrons and do so even more rapidly (Sun et al. 2016).

As found in my study, a positive correlation between $N_2O$ emission and soil moisture (Fig. 6.5d) has been reported in previous studies (Saarnio et al. 2013; Yanga et al. 2014). The denitrification that usually occurring in the micro-pores of these relatively fine-textured soils may have contributed significantly to denitrification despite the relatively low soil moisture content. The lack of a biochar effect on $N_2O$ when urea was added but not when tithonia was added or without additions (Fig. 6.4b) may indicate that the lowering of $N_2O$ emissions by biochar may be influenced by the form of nitrogen in the soil as well as the availability of other substrates such as SOM, which support other microbial processes. The added organic matter from tithonia provides energy to the denitrification process in addition to N, and consumes $O_2$, favoring denitrification to reduce $N_2O$ to $N_2$. Furthermore, the manure could have affected the size of the soil aggregates and the microorganisms that perform denitrification. However, the response with tithonia remained unexplained because also the tithonia-N was nitrified and when only biochar was added, the nitrate continued to be produced.

The continuous decline in effect size observed where urea was applied without tithonia (Fig. 6.4) can be attributed to the more rapid decline in SOC. Mori and Hojito (2012) observed that combining tithonia manure with reduced application of mineral N fertilizer may be a possible option to mitigate $N_2O$ emissions. Therefore, application of biochar in combination with 2.5 t $ha^{-1}$ of T. diversifolia and a lower than recommended rate of mineral N (< 120 kg N $ha^{-1}$) may

be a low-emission as well as a cost-reduction strategy for resource-constrained farmers in sub-Saharan Africa. However, this optimum N application rate needs to be determined experimentally for various soil types. More importantly, the timing and method of application to optimize N use efficiently should be assessed.

## 6.5 Conclusion and recommendations

My results have demonstrated that biochar can have residual effect of lowering emissions of $NH_3$ and $N_2O$ under field conditions on low fertility tropical soils. This reduction and ancillary information were consistent with reduced ammonification and possible adsorption of $NH_3$ to biochar surfaces either or both of which could be the reason for lower $NH_3$ emissions. We also found that soil inorganic N dynamics were not affected by biochar amendment 3 years after application to the soil. However, soil nitrate content was positively correlated with $N_2O$ fluxes at relatively low soil moisture contents but it remains unclear if nitrification may have played a significant role in $N_2O$ emissions in addition to alternative mechanisms such as direct $N_2O$ adsorption on biochar surfaces, and $N_2O$ reduction to $N_2$. Tithonia and urea additions did not alter the mitigating effect of biochar on both $NH_3$ and $N_2O$ emissions. Furthermore, unlike previous studies suggesting that biochar has mainly short-term effects on $N_2O$ fluxes, we found residual effects of biochar up to the third year under field conditions although the magnitude of the effect is reduced by over 50%.

Further studies are needed to demonstrate the mechanism of $N_2O$ emission reduction by biochar and the fate of $NH_3$ captured by biochar under low moisture conditions. As the emission factors of $N_2O$ and $NH_3$ decline over time, it is worth noting that large-scale application of biochar might require reassessing $N_2O$ inventories in the context of reporting GHG emissions from

agricultural soils. Further studies using different soil properties such as SOC content are needed to improve my understanding of the regulatory mechanisms for $NH_3$ and $N_2O$.

# CHAPTER SEVEN: Synthesis and general discussion

## 7.1 Key findings

This study was set out to improve our understanding of the role played by biochar in regulating the emission of greenhouse gases in agricultural soils. The five specific objectives stated in this study were addressed as follows: Objective 1 was to find out the extent to which biochar quality (feedstock, production temperature, and biochar pre-treatment) affect fluxes of $N_2O$ and $CH_4$. The hypothesis was that surface chemistry of biochar, which is a function of its feedstock and production conditions, was responsible for its effect on soil-borne GHG emissions. It was observed (Chapter 2) that manipulating factors that affect surface chemistry of biochar resulted in changes in emission of $CH_4$ and $N_2O$. Thus, the hypothesis is supported. This implies that surface chemistry and adsorption reactions play a significant role in the mechanisms regulating emission of $CH_4$ and $N_2O$ in soils. This is one of the direct ways in which biochar affects emission of these gasses.

The second objective was to find out how biochar quantity affects nitrogen transformation pathways and hence nitrous oxide emission in contrasting tropical agricultural soils. Nitrogen dynamics (nitrification, denitrification and ammonification) were hypothesized to be slowed by the presence of biochar due to the presence of organic functional groups and molecular retentions mechanisms. The results (Chapter 3) showed that biochar affects nitrogen dynamics differently in soils with different mineralogical composition. A more suppressive effect of biochar was observed in a Ferralsol (dominated by low-activity clays (mainly kaolinite, and a high content of sesquioxides) compared to an Acrisol (with high base saturation and high-activity clays). Neither soil mineralogy nor the biochar amount had a straightforward effect on $N_2O$ emissions. The gross N transformation did not correlate significantly with $N_2O$ emission,

suggesting biochar has a decoupling effect on the relation between $N_2O$ emission and mineral nitrogen. Instead, there seems to be a significant contribution of biochar to the final step of denitrification; the conversion of $N_2O$ to $N_2$ as observed in the effect of biochar on the $N_2O$:$N_2$ ratio (Chapter 3). This observation suggests the exitance of complex mechanisms possibly related to organo-mineral interactions and/or biochemical catalysis involved in biochar-amended soils.

The third objective sought to establish if there is a linkage between biochar and soil aggregation because fluxes of GHG are known to respond to soil aggregation (Khali et al., 2005; Uchida et al., 2008; Mangalassery et al., 2013; Pramanik et al., 2014; Nie et al., 2014). I envisaged that soil aggregation affects soils aeration, which in turn affects diffusion of gasses. Compacted soils have more restricted air movement and can increase formation of $N_2O$ due to lack of $O_2$. The evidence generated by this thesis (Chapter 4) supports the assertion of the link between biochar and soil aggregation only when applied together with other easily mineralizable organic matter sources (such as *T. diversifolia* green manure).

Another important question that this study aimed to answer was whether biochar application to the soil can contribute to low-emission agriculture in tropical environments (Objective 4). It was found that biochar can reduce emission intensity of GHGs, increase SOC stocks and crop yield on a tropical soil. Therefore, the results confirm that biochar can have significant positive effect on low-emission agriculture. Greater net primary productivity in biochar-amended soils is the main reason for reduced CO2-eq.

The fifth and final objective aimed at finding out how long the residual effect of biochar remains relevant in the soil under tropical field conditions. It was found that the crop yield and

GHG mitigation benefits are maintained beyond three years, but they reduce steadily over time. This gradual decrease in the benefits of biochar are attributed to the gradual neutralization of initially active biochar surfaces charges, perhaps due to complexation and chelation in organo-mineral interactions with the soil-biochar matrix.

## 7.2 Mechanisms of biochar effect on GHG emissions

*Surface chemistry and adsorption reactions*

This thesis set out to investigate the mechanisms of biochar's influence on GHG emissions and its applicability to farm fields in tropical field conditions. The understanding of biochar mechanisms has improved after the investigations in this thesis. First, it has been found that surface chemistry generally plays a significant role as a mechanism for biochar effect on $CH_4$ and $N_2O$ from soils. $N_2O$ can break down by bonding with transitional metal cations since it can accept as well as donate electrons (Armor and Taube, 1971; Tolman, 2011). According to Sun et al. (2017), surface functional groups on biochar potentially facilitate electron transport through the carbon matrix. Surface chemistry of biochar is a function of feedstock and production conditions (Mukherjee et al., 2011; McBeath et al., 2015; Abas et al., 2018; Zhang et al, 2019).

Volatile matter content of biochar, also a consequence of production conditions, is correlated to $CO_2$ and $N_2O$ (Ameloot et al., 2013). Ameloot et al. (2013) also proposed that by providing microorganisms readily available substrate, increased emissions from biochar-amended soils may be observed. Overall, it can be inferred from these studies that to optimize biochar for reduced emissions, biochar produced at moderate temperatures (450 ºC– 600 ºC) is required.

*Nitrogen dynamics and ion exchange*

The second mechanism investigated by my study is the role of biochar on nitrogen dynamics and associated $N_2O$ emissions. In the initial stages of biochar research, it was believed that the reduced availability of inorganic-N pools [$NH_4^+$ and $NO_3^-$] (Table 1.1) is a precondition for reduced $N_2O$ emissions. These N forms are important precursors of soil nitrogen from which $N_2O$ is derived. However, the results of this thesis in the laboratory (Chapter 3) and under field conditions (Chapter 6) do not support the relationship between nitrogen dynamics as important predictor of biochar's role on GHG emission. It was also thought that $NH_4^+$ and $NH_3$ could be bound with biochar probably through ion exchange, surface complexation, hydrogen bonding, or Van der Waal's interactions (Essington, 2004). This decreases available mineral N that would otherwise be used during nitrification and denitrification (Clough and Condron, 2010; van Zwieteng et al., 2010; Kameyama et al., 2012; Yao et al., 2012; Prommer et al., 2014). To date, contradictions remain concerning biochar-induced nitrogen dynamics and GHG emissions. For example, whereas Case et al. (2015) found that nitrification, denitrification, N immobilization and $N_2O$ emission all increased with biochar amendment, the effect of biochar on N availability could not explain $N_2O$ suppression.

In contrast, Edwards et al. (2018) recently found that biochar stimulated nitrification-derived $N_2O$ emissions when soil ammonium was high. Su et al (2019) found that although pH strongly correlated with abiotic $N_2O$ production, its contribution to gross emission was minute. Results of this thesis (Chapter 3) showed reduced $N_2O$ emission that is independent of mineral N dynamics. Since, soil biochar amendment has been found to increase the diversity of nirK and typical nosZ transcripts and relative gene and transcript copy numbers of the nosZ-encoded bacterial $N_2O$ reductase, microbial species that are specialized on direct $N_2O$ reduction (Harter et al., 2014; Harter et al., 2017), I suspect a mechanistic link between biochar and $N_2O$

reductase, however the elucidation thereof remains a knowledge gap to be explored in future research.

*Soil aggregation*

The third mechanism tested by my study is that biochar can improve soil physical properties (aggregation and aeration) and hence reduce anaerobic conditions that support $N_2O$ emission (Chapter 4). I hypothesized that biochar hastens build-up of macro-aggregates, improves aeration and this could reduce denitrification in soil micropores. Under integrated biochar-manure systems, the results of Chapter 4 support earlier evidence that biochar can increase soil aggregation (Liu et al., 2014; Obia et al., 2015).

However, Bandyopadhyay and Lal, (2014) observed that emissions of $CO_2$, $N_2O$ and $CH_4$ were significantly higher from the large macro-aggregates than from other aggregate size fractions, and that the contributions of the large macro-aggregates emissions towards those of bulk soil were significantly higher than those of micro-aggregates and the mineral fraction. Nie et al. (2014) also found higher C decomposition enzyme activities in micro-aggregates, but specific enzyme activity for N decomposition was higher in macro-aggregates. Mangalassery et al. (2013) reported a higher $CH_4$ flux in both macro- and micro-fractions, and no difference for $N_2O$ fluxes.

In Chapter 4, I found that when easily mineralizable SOM sources are available, biochar increased soil aggregation, which in turn is predicted to reduce emission of GHGs. This view is contradicted by the observation of Pramanik et al. (2014), who reported that cover crop applications increased N contents in smaller aggregates (<250 μm), which proportionately increased the $N_2O$ emission potential. Therefore, I have been able to verify that biochar can

indeed increase soil aggregation. However, our understanding of the correlation between biochar -induced aggregation and GHGs needs further investigation. It is even less clear if the response is the same for different soil types, a question. that remains to be answered. My suspicion is that the proportion of particle size fraction in the whole soil is the single most important factor determining whether a soil emits more or less $N_2O$. The higher the proportion of macro-aggregates, the lower the eision of $N_2O$ ad vise versa. This hypothesis needs to be tested by further studies. Another hypothesis that needs to be tested is whether soil minerology interacts with soil texture to affect $N_2O$ dynamics in the soil.

## 7.3 Practical potential of biochar

*Emissions abatement*

Besides questions that target improving mechanistic understanding of the effect of biochar on GHG emissions, I have attempted to answer questions of a practical nature. The first is whether biochar can potentially reduce the $CO_2$-equivalent under field conditions while providing yield improvement for farmers. The results of this thesis (Chapter 4 and 5) show that through the application of biochar in combination with carefully selected combinations of other soil amendments, a significant increase in crop yields can be realized while minimizing negative environmental consequences of agricultural practices.

Although yield benefits have already been reported in previous studies (Chan et al., 2007; Major et al., 2010; Zhang et al., 2012; Qiao-Hong et al., 2014), as have the GHG effect of biochar in agricultural systems (Rogovska et al., 2010; Feng at al., 2012; Cayuela et al., 2015), this thesis provides additional information on the combined benefits in both yield and environment dimensions. Now we know that both yield benefits and GHG mitigation can be archived practically when biochar is integrated in agricultural systems in the tropics.

*Build-up of SOM and structure*

The practical benefits of biochar application to field soils as observed in arise from the increased SOC, soil aggregation (Chapter 4) and lower emission intensities coupled with increased crop yield (Chapter 5). Since soil structure is a key factor for soil maintenance and for the physical and biological processes that it involves (Celik et al., 2004; Wortmann and Shapiro, 2008), the aggregate stability brought about by biochar carbon input are partly responsible for these benefits. Lower fertilizer input required under biochar amendment, (Chapter 5) provides an avenue for avoided emissions and reduces the cost of fertilizer for resource-constrained small-holder farmers in developing economies. This is consistent with previous studies on N-use efficiency of biochar systems showing that biochar systems require less fertilixer compared to systems with mineral fertilizer only (e.g. Zhang et al., 2012; Yoo et al., 2013). Alling et al. (2014) showed that biochar provides essential mineral nutrient required for plant growth in addition to improving soil buffer capacity. These additional benefits of biochar work together with soil physical, chemical and biological properties to improve crop yields.

This study has shown that when biochar is applied to a low-fertility soil, emission intensities can be reduced and crop yields can be increased. The results of this thesis concur with those of previous ones (e.g. Wang et al., 2013; Yang et al., 2017; Rodrigues and Horan, 2018), which found that overall, system-level emissions are a lower for pyrolysis-based systems compare to combustion counterparts. They noted, however, that these results are sensitive to biochar's ability to suppress $N_2O$ emissions and increase soil organic carbon, which are subject to high uncertainty. These observations imply that biochar is a promising option for achieving triple benefits of increasing carbon sequestration, increased crop yields and low-emissions in tropical agricultural systems.

## 7.4 Further observations

This thesis found that biochar alone did not have an effect on emission intensity except where organic amendments were present (Chapter 4 and 5). Similarly, meta-analyses (Cayuela et al., 2014; Sagrilo et al., 2014; He et al., 2017) showed that whereas biochar does not affect $CH_4$, it increases $CO_2$ emissions (Mean = 25±3%) and lowers $N_2O$ emissions (Mean = 41±7%) Nonetheless, a meta-analysis of yield-scaled greenhouse gas intensity (Lui et al., 2019) showed that biochar had no effect on greenhouse gas intensity when no nitrogen fertilizer was applied. The increase in $CO_2$ and $CH_4$ in biochar-amended soils is related to the increased metabolic activity of microbes from increased supply of mineralizable C supplied by the biochar, higher SOC status and the more active soil microbial activities (Smith et al., 2010; Jones et al., 2011; Zimmerman et al., 2011; Liu et al., 2016).

The unexpected results showing that N dynamics do not affect $N_2O$ emissions may be because the main source of $N_2O$ from soils is microbial-mediated (Cayuela et al., 2013; Van Zwieten et al., 2014) and soil N is important for microbial activity. Many biological processes in soil utilize nitrogen directly or indirectly. Besides, several studies show that biochar accelerates soil N transformations, thereby increasing soil N bio-availability (van Zwieten et al., 2014). However, some studies have shown that biochar limits availability of $NO_3^-$, (Kameyama et al., 2012; Yao et al., 2012), which is a major precursor for denitrification and $N_2O$ emission. This leaves questions as to the main mechanism for biochar's role on nitrogen dynamics and $N_2O$ emission.

Another unexpected result of this thesis is the observation that biochar application rate did not affect $N_2O$ emission in the incubation study (Chapter 3). Previous studies show a consistent positive correlation between biochar rate and $N_2O$ emission (Cayuela et al., 2014; Laghari et

al., 2015; Mandal et al., 2018; Lan et al., 2019). Biochar studies have used highly variable application rates ranging from < 0.5% to >10%. The biochar rate of 2% and 4% w/w used in the incubation study (Chapter 3) was based on the average value for most studies at the time. This point to the inconsistencies still remains concerning the range within which the application rate can be responsive. For example, two separate studies (Sun et al., 2014 and Xiao et al., 2016), both using forest soils, applied widely different rates (30 t ha-1 and 5 t $ha^{-1}$) observed comparable reductions in $N_2O$ emissions (~26%). According to Jin et al. (2019), from the point of view of crop yields and economic benefits, about 11 t $ha^{-1}$ biochar is the optimal biochar application rate for the local farmers in China, while Pandit et al. (2018) has proposed 15 t $ha^{-1}$ in Nepal. Even then, this is still a high application rate for most farmers in tropical Africa.

Notwithstanding improvement in crop yields in biochar amended-soils, biochar can achieve low-emission agriculture if careful combinations of biochar and other soil amendments are applied. Noteworthy is that nitrogen is a resource that needs to be included in biochar systems because of biochar's ability to immobilize N (Zhu et al., 2019; Li et al., 2019Jin et al., 2019) to the extent that even the little nitrogen available in low-N soils such as those in the tropics. Information on the effect of biochar on GHG emission over time is largely lacking. However, previous evidence on crop yield (Glaser et al., 2002; Lehmann and Rondon, 2006; Steiner et al., 2007; Cornellison et al., 2018) supports the observed decreasing trend in a biochar effect.

The greatest challenge that remains for biochar in agricultural systems is how to access adequate materials and design appropriate production technologies to avail biochar to resource-constrained farmers for soil application (Singh, et al., 2014; Kavitha et al., 2018). Torres-Rojas et al (2011) estimated that in western Kenya, the amount of biomass available to an average household can produce an average of 0.46 t $ha^{-1}$ $y^{-1}$ of biochar if they use an improved "First-

generation pyrolytic cook stove" to reduced wood energy consumption. This will require up to five years to achieve the rate of 2.5 t $ha^{-1}$ that was used in my study.

Nevertheless, results in Chapter 5 suggest that it is possible to continue realizing yield benefits for up to three years after a single dose of biochar. As observed in Chapter 2, the production conditions for biochar significantly influence the quality, and hence the resultant benefits. Previous studies using biochar for soil amendment used application rates ranging from two to ten tons per hectare. These are large quantities that are hardly available to an average farmer. The argument is that there are several materials available for biochar production such as sugarcane bagasse from sugar factories, sawdust form carpentry workshops. At the moment, these materials are considered waste and pose challenges for disposal. moving feedstock from the gardens to where the pyrolysis takes place constitutes a large proportion of the production cost. Therefore, developing technologies for efficient conversion of such materials can improve access to biochar for soil application.

## 7.5 General conclusions and recommendations

***Conclusions***

The evidence from this thesis has demonstrated that biochar application generally increases emission of $CO_2$ and $CH_4$ but largely reduces $N_2O$ emission from agricultural soils. Similarly, it has emerged from this study, that removing volatile matter from biochar through steam activation can increase $N_2O$ emission. Activation of biochar using steam enhanced its capacity to suppress $CH_4$ and $N_2O$ emission, and the effect of steam activation is dependent on production conditions (feedstock and pyrolysis temperature) of the biochar. Based on this knowledge, pyrolysis systems should be tailor-made to produce "designer" biochar, which has specific chemical properties matched to overcome limitations of tropical soils. I also found no

direct linkage between soil nitrogen dynamics in the laboratory and in the field, indicating that ths is not a major factor in regulating $N_2O$ suppression by biochar both in the short-term (days) and long-term (years). Furthermore, biochar application rate did not affect $N_2O$ emission. As a practical benefit of biochar to farmers, this thesis has revealed that biochar can achieve low-emission agriculture by increasing soil C sequestration and crops yields if careful combinations of biochar and other soil amendments are applied. Biochar soil-amendment can contribute to reducing GHG intensity by up to 65% depending on how other soil amendments are managed. The contribution of biochar on reducing GHG emissions decreases over time but can continue for more than three years under tropical field conditions. Over the six consecutive seasons that a field trial was conducted, the effect had reduced by more than half.

***Recommendations***

While a large body of evidence supports the positive effect of biochar on reducing GHG emissions, improved yield and soil carbon sequestration, significant uncertainty remains since several studies still show no or negative results, particularly under field conditions. Multi-location field trials involving a wide range of biochar and soil properties should be undertaken to improve accuracy of the estimated effects of biochar on various agronomic and environmental benefits. Such trials should be monitored over a long time (5-10 years) to fill the knowledge gap concerning the long-term effects of biochar application under field conditions. Furthermore, field trials comparing various combinations of soil amendments should be used to determine the optimum soil management regimes that make biochar application economically feasible for low-income farmers.

Comparing the pore structure, organic by-products, and surface functional groups of biochars produced under a range of conditions is required to develop production parameters for biochar

for specific agronomic requirements. Such studies should aim at developing stoichiometric models combining a wide range of properties in order to improve the predictability of the response of biochar when applied in soils amendments.

Although numerous studies emphasize denitrification as the major source of $N_2O$ emission from soil, the role of the nitrification pathway needs to be investigated in light of the fact that biochar increases nitrification rate. This potential pathway for $N_2O$ emission in biochar amended soils should be explored further use of $^{15}N$ tracer techniques combined with nitrification inhibitors and direct $N_2$ measurements in order to distinguish pathways for $N_2O$ and $N_2$ production. Besides, other abiotic pathways for $N_2O$ production from soils such as breakdown of hydroxylamine and reduction of nitrate need to be studied under different soil types.

Further microbiological studies need to be undertaken to identify the relationships between microbial-mediated processes and the denitrification pathways. Biochemical (enzyme activity) and genetic (gene abundance) studies have been conducted and relationships with $N_2O$ emission identified but the mechanism linking them remains elusive. The tendency for $N_2O$ to act as an electron acceptor as well as donor may be the critical stage at which $N_2O$ is broken down to $N_2$ under the catalytic influence of biochar surface chemistry. There is a need to use levelled $N_2O$ in non-soil systems to investigate conditions for formation and breakdown of $N_2O$ with or without biochar. This will shade light on the role of biochar in the reduction of $N_2O$ to $N_2$.

The economics of biochar production from a wide range of feedstock such as saw dust, sugarcane bagasse and municipal biomass waste application, including its inclusion in global

carbon markets remains an important area for further investigation. It will also be useful to investigate the possibility of improving biochar behavior in Biochar-Fertilizer blending. Social studies to establish perception and acceptability of biochar use in soil fertility management among small-holder farmers in developing countries are necessary. Such studies should consider the socio-economic environment and farming systems where biochar will be applied.

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## SUMMARY IN ENGLISH

Biochar (pyrogenic organic matter) has emerged as an innovative way to improve soil fertility, increase soil carbon sequestration and reduce emission of carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) from agricultural soils. However, several questions remain as to the mechanisms responsible for the biochar effect and whether practical benefits for farmers are achievable under field conditions on low-fertility tropical soils. This study aimed at improving understanding of two broad questions: 1) By what mechanisms does biochar affect emission of GHG? 2) Can biochar contribute practical benefits for farmers on low-fertility tropical agricultural soils? The specific research questions addressed in this thesis include; (i) To what extent does biochar quality (feedstock, production temperature, and biochar pre-treatment) affect fluxes of $CO_2$, $N_2O$ and $CH_4$? (ii) How does biochar quantity affect nitrogen transformation pathways and hence nitrous oxide emission in contrasting tropical agricultural soils? (iii) Since fluxes of GHGs are known to respond to physical properties such as aeration, is there a linkage between biochar and soil physical properties such as aggregation? (iv) By how much does biochar application to soil contribute to low-emission agriculture in tropical environments? and (v) How long does the residual effect of biochar last in the soil under field conditions?

A series of experiments was used to evaluate some of the propositions about biochar's role in soil processes and associated soil-atmosphere gas fluxes. This study was conducted using Acrisol and Ferralsols both of which are generally low-fertility soils that are widespread across western Kenya, a region where sustainable soil management is extra important yet information on such management is chronically lacking.

In a greenhouse experiment, the effect of biochar quality (source material, pyrolysis temperature and biochar pre-treatment) was investigated in order to determine if surface chemistry and adsorption reactions play a role in regulating GHG emissions (**Objective 1**). maize-stover biochar lowered of $N_2O$ emission by 17% compared to that from Eucalyptus while lower-temperature biochar reduced emission by 3% compared to the higher-temperature biochar. Steam-activated biochar increased $CH_4$ emission of stover-derived biochar but decreased it for wood-derived one by 14%–70%. A 21% increase in $CH_4$ emission was observed in soils amended with activated stover-derived biochar compared to the one from Eucalyptus wood, and the 350°C recorded 10% lower values compared to the 550°C. In conclusion, surface chemistry of biochar (as affected by feedstock, pyrolysis temperature and steam activation) plays a significant role as a mechanism in GHG regulation by biochar.

To determine the role of biochar on soil nitrogen transformation and $N_2O$ emission (**Objective 2; Chapter 3**), an incubation experiment and a field trial were used. In the incubation experiment, two application rates of biochar (2% and 4% w/w) were used on two mineralogically contrasting soils (A recently converted Acrisol, an Acrisol under cultivation for >100 years and a Ferralsol under cultivation for >100 years). Gross nitrogen transformation, $N_2O$ and $N_2$ emission were monitored for 21 days using a $^{15}N$ double-labelling technique in an incubation environment. We found that biochar reduced $N_2O$ by 53 to 78 % and that the reduction was higher in the Acrisol than in the Ferralsol. The $N_2O/(N_2O+N_2)$ ratio was reduced in the high OM Acrisol but increased in the Ferralsol whereas no significant difference was observed in the low OM Acrisol. $N_2O$ emission did not follow the same pattern as mineral N, suggesting a disconnection with direct soil N dynamics. The results show evidence of the biochar application shifting the equilibrium towards more $N_2$ instead of $N_2O$ emission, but provide no indications of a relationship between mineral N dynamics and $N_2O$ production. In

a field trial, mineral nitrogen dynamics, $NH_3$ and $N_2O$ emissions were monitored for three years. The results show that biochar reduces emissions of both $NH_3$ and $N_2O$ by ~55% but the reduction was not linked to N dynamics. These results from both experiments thus imply that the observed biochar induced reduction of $N_2O$ emissions from the soil are most likely caused by a conversion of $N_2O$ to $N_2$, thus limiting $N_2O$ production.

Changing soil physical properties as a process regulating GHG emissions in biochar amended soils was also investigated (**Objective 3; Chapter 4**). A field trial was established on a long-term cultivated Acrisol in western Kenya. Biochar that was used for the experiment was produced from Eucalyptus wood and pyrolyzed to maximum temperature of 550°C. It was then applied at 2.5 t $ha^{-1}$), alone or in combination with green manure (*Tithonia diversifolia*). The green manure was applied at either 0, 2.5 or 5.0 t $ha^{-1}$. Mineral nitrogen (as urea) was introduced in as the third factor and applied at either 0 or 120 kg N t $ha^{-1}$). The experiment was a randomized complete block design with three replicates. Aggregate size distribution as affected by biochar-tithonia-urea input under field conditions was quantified over two years (Chapter 4). Results show that mean weight diameter (MWD) was not affected by biochar alone, but increased by 34±5.2 mm (8%) and 55±5.4 mm (13%), when in combination with either *T. diversifolia* (BT) or urea (BU), respectively, compared to the control. Neither soil respiration nor soil moisture correlated with MWD. SOC negatively correlated with and MWD, but positively corelated with biomass production. The conclusion is that biochar can affect the physical condition of soil, which in turn affects GHG emissions.

To quantify the practical benefits of biochar soil application in contributing to soil improvement, crop yield and low-emission agriculture (**Objective 4**) was investigated using the same field trial as above (**Chapter 5**). Soil organic carbon changes, maize yield and GHG

emissions were monitored for six consecutive seasons, on the same Acrisol as in previous trial, after amendment with manure, mineral fertilizer and biochar. The amendments increased SOC stocks by 0.1 to 1.2 t $ha^{-1}$ $year^{-1}$. When applied alone, biochar decreased maize yield by 15% but increased it by ~34% only when combined with external nitrogen sources such as urea or manure. Biochar also increased soil $CO_2$ emissions (9 to 33%; from either biochar, native soil C or root respiration), reduced soil $CH_4$ uptake and $N_2O$ emissions by 7 to 59% and 1 to 42%, respectively. No observed seasonal differences. The emission intensity, calculated by combining the contribution of SOC and GHGs, was lower than the control, ranging from 9 to 65%. Therefore, it can be concluded that biochar can offer practically benefits for farmers in the tropical farming systems since it increases SOC and crop yield, as well as contribute to low-emission agriculture.

The question how long the residual effect of biochar lasts in the soil under field conditions (**Objective 5**) was addressed through a field trial described for Question 4 above. Over the three years, the power of biochar to mitigate emission of $NH_3$ and $N_2O$ was reduced by 53% and 59%, respectively. Therefore, biochar's residual effects under field conditions persist for more than three years.

By monitoring $NH_3$ and $N_2O$ emissions for three consecutive years on an Acrisol under field conditions, it was found that nitrogen dynamics were not related to the emissions reductions observed, but the effect of biochar continued to be observed. The results of this thesis show that although previous studies report short-term mitigating effects, biochar can reduce $NH_3$ and $N_2O$ emissions for longer periods, and that N dynamics play a marginal role. Therefore, alternative mechanisms such as surface oxidation potentially explain biochar's residual effect in field soils.

From these studies, it can be seen that biochar plays several direct (surface chemistry and adsorption reactions) and indirect roles (soil aggregation and aeration) in regulating soil carbon and nitrogen turn-over and subsequent emission of $CO_2$, $CH_4$ and $N_2O$. It appears that the net effect of biochar on emission of these gasses is a non-additive contribution of the various mechanisms acting concurrently in the soil system. This study has also revealed that these GHG reduction mechanisms can translate into practical realities in the field and can benefit farmers through soil carbon build-up and crop yield improvement. Biochar can also practically contribute to low-emission agriculture because of its ability to reduce emissions intensity and increase soil carbon stocks.

It is recommended to undertake multi-location field trials involving a wide range of biochar and soil properties should be undertaken to improve accuracy of the estimated effects of biochar on various agronomic and environmental benefits. Such trials should be monitored over long time (5-10 years). The economics of biochar production from a wide range of feedstock such as sow dust, sugarcane bagasse and municipal biomass waste application, including its inclusion in global carbon markets remains an important area for further investigation.

## SAMENVATTING IN HET NEDERLANDS

Biochar (pyrogeen organisch materiaal) krijgt steeds meer aandacht als een innovatieve manier om de vruchtbaarheid van de bodem te verbeteren, de koolstofvastlegging in de bodem te verhogen en de uitstoot van kooldioxide ($CO_2$), methaan ($CH_4$) en stikstofoxide ($N_2O$) uit landbouwgronden te verminderen. De mechanismen die verantwoordelijk zijn voor het effect van biochar zijn echter nog niet geheel duidelijk. Evenmin is duidelijk of praktische voordelen van biochar voor boeren haalbaar zijn onder veldomstandigheden op tropische bodems met een lage vruchtbaarheid. Het doel van dit promotieonderzoek was daarom de volgende twee belangrijke vragen te beantwoorden: 1) Via welke mechanismen beïnvloedt biochar de uitstoot van broeikasgassen? 2) Kan biochar praktische voordelen opleveren voor boeren op tropische landbouwgronden met een lage vruchtbaarheid? De hieruit voorvloeiende specifieke onderzoeksvragen in dit proefschrift zijn; (i) In welke mate beïnvloedt de kwaliteit van biochar (bronmateriaal, productietemperatuur en voorbehandeling) de fluxen van $CO_2$, $N_2O$ en $CH_4$? (ii) Welke invloed heeft de hoeveelheid biochar op stikstofomzettingsroutes en dus op de uitstoot van stikstofoxide in contrasterende tropische landbouwgronden? (iii) Gegeven dat bekend is dat fluxen van broeikasgassen reageren op fysische eigenschappen zoals beluchting: is er een verband tussen biochar en bodemfysische eigenschappen zoals aggregatie? (iv) Met hoeveel draagt biochar-toepassing op de bodem bij aan emissiearme landbouw in tropische omgevingen? en (v) Hoe lang duurt het resteffect van biochar in de bodem onder veldomstandigheden?

Via een reeks experimenten zijn verschillende aannames over de rol van biochar in bodemprocessen en bijbehorende gasfluxen in de bodem geëvalueerd. Het onderzoek werd uitgevoerd met Acrisols en Ferralsols; beide over het algemeen weinig vruchtbare bodems die

veel voorkomen in West-Kenia, een regio waar duurzaam bodembeheer extra belangrijk is, maar waar een chronisch gebrek is aan informatie over dergelijk beheer.

In een kasexperiment werd het effect van biochar-kwaliteit (bronmateriaal, pyrolysetemperatuur en biochar-voorbehandeling) onderzocht om vast te stellen of oppervlaktechemie en adsorptiereacties een rol spelen bij het reguleren van broekasgas- (BKG) emissies (doelstelling 1). Biochar uit maïsstengels biochar verlaagde de $N_2O$-emissie met 17% vergeleken met biochar uit Eucalyptus, terwijl biochar dat bij lagere temperatuur geproduceerd werd de emissie met 3% verminderde vergeleken met biochar geproduceerd bij hogere temperatuur. Stoomgeactiveerde biochar verhoogde de $CH_4$-emissie van biochar uit stengels, maar verlaagde deze met 14% –70% voor biochar uit hout. Een stijging van de $CH_4$-emissie met 21% werd waargenomen in bodems die gemodificeerd waren met geactiveerde biochar uit stengels van Eucalyptus t.o.v. van biochar uit Eucalyptus-hout, en biochar geproduceerd bij 350 ° C leidde tot 10% lagere emissiewaarden dan biochar geproduceerd bij 550 ° C. Dit leidt tot de conclusie dat de oppervlaktechemie van biochar (zoals beïnvloed door bronmateriaal, pyrolysetemperatuur en stoomactivatie) een belangrijke rol speelt als een mechanisme volgens welke biochar de BKG-uitstoot reguleert.

Om de rol van biochar op de stikstoftransformatie in de bodem en de $N_2O$-emissie (doelstelling 2; hoofdstuk 3) te bepalen, werden een incubatie-experiment en een veldproef uitgevoerd. In het incubatie-experiment werden twee toedieningspercentages van biochar (2% en 4% op gewichtsbasis) gebruikt in twee mineralogisch contrasterende bodems (een recent tot landbouwgrond omgevormde Acrisol, een Acrisol waar reeds > 100 jaar landbouw op plaatsvindt en een Ferralsol waar reeds > 100 jaar landbouw op plaatsvindt. De bruto-stikstofomzetting, $N_2O$- en $N_2$-emissie werden gedurende 21 dagen gevolgd met behulp van

een $^{15}N$ dubbele labelingtechniek in een incubatieomgeving. De resultaten toonden dat biochar de $N_2O$ uitstoot met 53 tot 78% verminderde en dat de reductie hoger was in de Acrisol dan in de Ferralsol. De $N_2O$ / ($N_2O$ + $N_2$) -verhouding bleek na toepassing van biochar verlaagd in de Acrisol met een hoog organische stofgehalte maar nam toe in de Ferralsol. Er werd geen significant verschil waargenomen in de Acrisol met een laag organische stofgehalte. De $N_2O$-emissie volgde niet hetzelfde patroon als dat van mineraal N, wat duidt op een ontkoppeling van N-dynamiek in de bodem. De resultaten tonen aan dat de toepassing van biochar het evenwicht verschuift naar meer uitstoot van $N_2$ ten koste van de uitstoot van $N_2O$, maar geeft geen aanwijzingen voor een verband tussen minerale N-dynamiek en $N_2O$-productie. In een veldproef werden de dynamiek van minerale stikstof en de $NH_3$- en $N_2O$-emissies gedurende drie jaar gemonitord. De resultaten tonen aan dat biochar de uitstoot van zowel $NH_3$ als $N_2O$ met ca. 55% vermindert, maar de reductie was niet gekoppeld aan de dynamiek van N. De resultaten van beide experimenten impliceren daarom dat geobserveerde door biochar veroorzaakte reductie van $N_2O$-emissies uit de bodem hoogstwaarschijnlijk wordt veroorzaakt door een omzetting van $N_2O$ naar $N_2$, waardoor de productie van $N_2O$ wordt beperkt.

We onderzochten ook de rol van veranderende bodemfysische eigenschappen als proces dat de uitstoot van broeikasgassen in met biochar gemodificeerde bodems reguleert (doelstelling 3; hoofdstuk 4). Hiertoe werd een veldproef uitgevoerd op een langdurig gecultiveerde Acrisol in het westen van Kenia. De voor het experiment gebruikte biochar werd geproduceerd uit Eucalyptus-hout en gepyrolyseerd tot een maximale temperatuur van 550 ° C. Het werd vervolgens aangebracht in een hoeveelheid van 2,5 t per hectare, alleen of in combinatie met groene mest (*Tithonia diversifolia*). De groene mest werd in hoeveelheden van 0, 2,5 of 5,0 t per hectare aangebracht. Minerale stikstof (als ureum) werd geïntroduceerd als derde factor en toegepast in hoeveelheden van 0 of 120 kg N t per hectare. Het experiment volgde een

randomized complete block ontwerp met drie herhalingen. Vervolgens hebben we gedurende twee jaar de invloed van toepassing van biochar-tithonia-ureum onder veldomstandigheden op de aggregaat-grootteverdeling gekwantificeerd (hoofdstuk 4). De resultaten lieten zien dat de gemiddelde gewichtsdiameter (MWD) niet werd beïnvloed door alleen biochar, maar toenam met 34 ± 5,2 mm (8%) en 55 ± 5,4 mm (13%), wanneer biochar werd toegepast in combinatie met *T. diversifolia* (BT) of ureum (BU), respectievelijk, in vergelijking met de controle. Bodemrespiratie noch bodemvocht bleek gecorreleerd met MWD. Bodemorganische stof (SOC) toonde een negatieve correlatie met MWD, maar was positief gecorreleerd met biomassaproductie. De conclusie is dat biochar de fysieke toestand van de bodem kan beïnvloeden, wat op zijn beurt de broeikasgasemissies beïnvloedt.

Om de praktische voordelen van biochar-toepassing voor bodemverbetering te kwantificeren in de context van gewasopbrengst en emissiearme landbouw (doelstelling 4) werd gebruik gemaakt van dezelfde veldproef als hiervoor genoemd (hoofdstuk 5). Veranderingen in organische koolstof in de bodem, maïsopbrengst en broeikasgasemissies werden gedurende zes opeenvolgende seizoenen gevolgd op dezelfde Acrisol als in de vorige proef, na toepassing van dierlijke mest, kunstmest en biochar. De toevoegingen verhoogden de SOC voorraad met 0,1 tot 1,2 ton per hectare per jaar. Wanneer uitsluitend biochar werd toegepast, daalde de maïsopbrengst met 15%. Gecombineerde toepassing van biochar met externe stikstofbronnen zoals ureum of mest verhoogde de opbrengsten met ca. 34%. Biochar verhoogde ook de $CO_2$-uitstoot in de bodem (9 tot 33%; van biochar, C dat al oorspronkelijk in de bodem zat, of worteladem haling). Tegelijkertijd verminderde biocahr de opname van $CH_4$ in de bodem en de $N_2O$-uitstoot met respectievelijk 7 tot 59% en 1 tot 42%. Er werden geen verschillen tussen de seizoenen geconstateerd. De emissie-intensiteit, berekend door de bijdrage van SOC en BKG's te combineren, was lager dan de controle, en varieerde van 9 tot 65%. Daarom kan worden

geconcludeerd dat biochar praktische voordelen kan bieden voor boeren in de tropische landbouwsystemen, omdat het de SOC en gewasopbrengst verhoogt, en bijdraagt aan emissiearme landbouw.

De vraag hoe lang het effect van biochar in de bodem aanhoudt onder veldomstandigheden (doelstelling 5) werd geadresseerd via een veldproef zoals beschreven voor vraag 4 hierboven. Gedurende de drie jaar verminderde het vermogen van biochar om de uitstoot van $NH_3$ en $N_2O$ te reduceren met respectievelijk 53% en 59%. Dit leidt tot de conclusie dat de effecten van biochar onder veldomstandigheden langer dan drie jaar aanhouden.

Door $NH_3$- en $N_2O$-emissies gedurende drie opeenvolgende jaren op een Acrisol onder veldomstandigheden te volgen, bleek dat hoewel de stikstofdynamiek niet gerelateerd was aan de waargenomen emissiereducties, het effect van biochar wel degelijk werd waargenomen. De resultaten van dit proefschrift laten zien dat, hoewel eerdere studies korte-termijneffecten beschrijven, biochar in staat is $NH_3$- en $N_2O$-emissies voor langere periodes te verminderen, en dat de dynamiek van N een marginale rol speelt. Alternatieve mechanismen zoals oppervlakte-oxidatie verklaren mogelijk het residuele effect van biochar in veldbodems.

Uit ons onderzoek blijkt dat biochar verschillende directe (oppervlaktechemie en adsorptiereacties) en indirecte rollen (bodemaggregatie en beluchting) speelt bij het reguleren van de koolstofomzet en stikstofomzet en de daaraan gekoppelde uitstoot van $CO_2$, $CH_4$ en $N_2O$. Het lijkt erop dat het netto-effect van biochar op de uitstoot van deze gassen uit een niet-cumulatieve bijdrage bestaat van de verschillende mechanismen die gelijktijdig in het bodemsysteem werken. Onze studie heeft ook aangetoond dat deze BKG-reductiemechanismen zich kunnen vertalen naar praktische toepassing in het veld en boeren

kunnen helpen middels opbouw van de koolstofvoorraad in de bodem en verbetering van de opbrengst van gewassen. Biochar kan via de verlaging van de uitstootintensiteit en verhoging van de koolstofvoorraden in de bodem ook bijdragen aan emissiearme landbouw.

Op basis van het onderzoek wordt aanbevolen om in vervolgonderzoek veldproeven op meerdere locaties uit te voeren met een breed scala aan typen biochar en bodemeigenschappen. Dit is nodig om de vanuit agronomisch en milieu-oogpunt gunstige effecten van biochar nauwkeuriger te kunnen inschatten. Dergelijke onderzoeken moeten gedurende een lange periode (5-10 jaar) worden uitgevoerd. De economische aspecten van de productie van biochar uit een breed scala van uitgangsstoffen zoals zaagmeel, bagasse en huiselijk GFT afval, inclusief de opname ervan in de mondiale koolstofmarkten, vormt ook een belangrijk gebied voor verder onderzoek.

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**BIOGRAPHY**



Looking back at the first 18 years of my life, I see a young boy doing part-time work as a field assistant for scientists at Makerere University Agricultural Research Institute in Kabanyoro. This era of my life shaped the aspiration for agricultural research to this day. At 16, the choice to take Physics, Chemistry and Biology as the preferred subject combination for Uganda Advance Certificate of Education (UACE) was largely guided by the dream to be like one of those scientists I usually interacted with at Kabanyolo. This subject combination is what later enabled me to joined Makerere University for a BSc. in Forestry. Later, I went on to complete a MSc. in Soil and Water Science at University of Florida, Geinesville, Florida, USA. The three years following the MSc, I worked as National Staff for the Forum for Agricultural Research in Africa (FARA) alongside several top-notch scientists in agriculture and natural resources in Uganda, Rwanda and Congo. Discerning the need to go for further studies, I decided to makes several applications for a PhD program. Gladly, the World Agroforestry Center (ICRAF) offered me a wonderful opportunity to work as a Research Fellow to investigate the relationship between biochar-soil amendment and greenhouse gas emissions in tropical agricultural soils of western Kenya. It was from this research program at ICRAF that this PhD program at the University of Amsterdam was developed. From, ICRAF I returned to Uganda where I now work for National Agricultural Research Organization (NARO) as a Soil Scientist. My hope is that this jump into the world of philosophers will provide the necessary capabilities to contribute to building a sustainable earth. The guiding principle for my life is a quote by Albert Einstein; ***"A calm and modest life brings more happiness than the pursuit of success combined with constant restless"***







