INTERACTING EFFECTS OF BIOCHAR AND ROOT EXUDATES ON SOIL C CYCLING AND SOIL FERTILITY

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God bless you all

Festus

CONTENTS

LIST OF ABBREVIATIONS

C – Carbon

N - Nitrogen

P - Phosphorus

SOM – Soil organic matter

BC - Biochar

Re – Maize-root-exudate

OC – Organic carbon

SOC – Soil organic carbon

LMWs – Low molecular weight substances

DOC – Dissolved organic carbon

DOM – Dissolved organic matter

MBC – Microbial biomass carbon

MBN – Microbial biomass nitrogen

Pmic – Microbial biomass phosphorus

PE – Priming effect

Pspike – Phosphorus Spike

Prec – Phosphorus recovery

TOC – Total Organic Carbon

MBq - Megabecquere

WHC – Water Holding Capacity

rpm – Revolutions per minute

CFE – Chloroform fumigation extraction

PP tube – Polypropylene tube

MRT – Mean residence time

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ABSTRACT

Biochar is widely applied as a soil amendment because of its carbon storage and sequestration potential, and its ability to improve physicochemical properties related to nutrient use of the respective soils thus increasing soil fertility. The recalcitrant and slow decomposition nature of biochar compared to other sources of carbon inputs makes decomposition studies practically challenging in short-term experiments. The application of ^{14}C-labeled biochar and ^{13}C labeled root-exudates opens new opportunities for tracing multiple C sources in parallel in soils. This experiment aims to assess the impact of biochar on the stability of rhizodeposites and the possible priming of biochar decomposition by root-exudates.

In this 3-source carbon partition incubation study, we used the dual-isotope labeling techniques to trace the fate of C input and turnover in soils via ^{13}C enriched maize-root-exudates and ^{14}C enriched biochar in three soil types with clay migration from Germany, China, and Kenya. ^{14}C biochar was produced through pyrolysis of ^{14}C-labeled corncobs and maize leaves, and preincubated to remove the low molecular compounds formed by pyrolysis before addition of the root exudates. The treatments include (i) control soils as isotopic references, (ii) ^{14}C biochar, (iii) ^{13}C maize-root-exudate, (iv) combined ^{14}C biochar and ^{13}C root-exudate addition. The decomposition rates of biochar were estimated based on $^{14}CO_2$-C trapping and those of the root exudates by $^{13}CO_2$-C.

Experimental results showed strong increase in SOC mineralization after the addition of maize root exudates irrespective of the soil type and this is evident on the first day of incubation. The slow decomposition rates in biochar are consistent with studies that confirmed biochar stabilization effect on SOC through the release of stable OC fractions into the soil matrix especially in short to medium-term incubation studies. Biochar's role in SOM stabilization is more evident and beneficial in the tropical Kenyan Acrisol which have high SOM mineralization rates and thereby often resulting in rapid loss of SOC than in the temperate Luvisols where acceleration of cycling is more beneficial for nutrient availability.

CHAPTER ONE

INTRODUCTION

1.1 **Background**

Soils are central to carbon sequestration and climate change acting either as a potential C net source or sink for greenhouse gases. This makes it important to evaluate and balance the potential C inputs from various sources through rhizodeposition and soil organic matter decomposition (Kumar, et al., 2016). Rhizodeposition is the single most important process that forms a link between microbially mediated processes in soils (Pausch et al., 2013) defined as materials lost from plant roots in the form of water-soluble exudates, secretions of insoluble materials, lysates, dead fine roots, gases such as CO_2 and ethylene (Whipps & Lynch, 1985). According to Cheng & Gershenson (2007), rhizodeposits serve as sources of carbon that can be grouped into water-soluble exudates and water-insoluble materials. The water-soluble exudates mostly include sugars, amino acids, organic acids, etc. rapidly mineralized by rhizosphere microorganisms to release CO_2 through respiration by root or microbial organisms. The latter, however, are sloughed cells and root mucilage. Rhizodeposition is the main characteristic of the rhizosphere soil, and the availability of easily utilizable C substrates is a key limiting factor for microbial activity in bulk soil. As C availability is the main factor controlling SOM turnover in soil, rhizosphere priming effect can lead to relevant losses of C from soils (Fontaine, et al., 2007 and Paterson, & Sim, 2013). Thus, the introduction of C inputs may alter microbial decomposition rates leading to short-term increases or decreases in mineralization of soil organic carbon as a result of the added organic substrates, a process known as the rhizosphere priming effect (Kuzyakov, 2002).

1.2 **Biochar and C storage**

Incorporating biochar into soils has received increased attention as a measure of capturing and securing carbon storage that would, otherwise, be emitted as CO_2 into the atmosphere. It plays a significant importance in agricultural soils as it can mitigate atmospheric CO_2 and enhance soil fertility (Glaser, & Lehr, 2019 and Lehmann, 2007). Biochar is a carbon-rich compound that is produced from the thermochemical conversion of organic feedstock (rice husk, maize straw, groundnut husk, wheat, etc) under limited-oxygen conditions (Sohi, et al., 2010) at relatively low temperatures of 350 to 600^{o}C. This charred product sometimes called pyrogenic carbon or black

carbon (BC), is an important source for long-term carbon (C) sink especially if added to soils, due to its slow microbial decomposition and chemical transformations. Numerous studies present contrasting evidence of the influence of biochar on C and N transformations with unclear and little understanding of the mechanisms underlying N_2O mitigation of biochar (Wu, et al., 2018). The priming effects of biochar on soil CO_2 evolution, however, can either be positive or negative (Wu, et al., 2018) dependent on several factors such as biochar and soil type as well as several physicochemical and biogeochemical properties of the soil including pH and role of enzymes.

Interactions between biochar and soil lead to processes that (i) neutralizes soil acidity by the biochar carbonates (Wang, et al., 2020). Furthermore, the interactions between biochar and soil can also cause (ii) biochar aggregation and immobilization of nutrients required by microorganisms through their adsorption (iii) inhibition of microorganisms by some toxic compounds such as Cr, Zn, Mo, or polycyclic aromatic hydrocarbons contained in the biochar (Hale, et al., 2012) (iv) direct sorption of GHGs by biochar (Cornelissen, et al., 2013) reducing their emission from soil and (v) increased aeration of soils suppressing anaerobic processes like denitrification (Case et al., 2012). These examples demonstrate that many biogeochemical element cycles and their controlling processes can be affected by biochar application.

Studies estimating decomposition rates of biochar are usually long-term due to its slow transformation rates and long mean residence time (MRT) which were determined based on delta ^{14}C measurements of BC in soils (Schmidt, et al., 2002 and Gavin, et al., 2003). According to Lehman et al., 2015, the stability of biochar is still open for debate as estimations of biochar-C residence time show large variability ranging from decades to thousands of years. This uncertainty is a result of several factors such as differences in feedstock types, soil characteristics, and biochar production processes (Gurwick et al., 2013). More so, biochar decomposition rates and stability are influenced by the interactions between soil types, presence of fresh organic matter and duration of field and laboratory experiments.

1.3 **Root-exudates**

Plants deposit significant quantities of photosynthetically derived carbon into the rhizosphere as root-exudate (Girkin, et al., 2018), a process referred to as rhizodeposition. Root exudates refer to a collection of substances in the rhizosphere that are secreted by roots of living plants and microbially modified products of these substances (Koo, et al., 2005). According to Bolton et al., (1993) root-exudates consist (i) low-molecular-weight organic compounds (sugars, amino acids, organic acids, phenolics, etc) that are secreted and released freely and passively in association with root-cell material and (ii) high-molecular-weight compounds such as mucilage associated with roots and released by them via various secretion mechanisms. These compounds control the rhizosphere via regulating soil pH, acting as nutrient sources and chemo-attractant for soil microbial communities (Broeckling, et al., 2008). The quantity and composition of root-exudate release from living plant roots are affected by the plant species, age, and stress-associated factors (Uren, 2000). Root exudates may act as signals for microbial recognition by providing various carbon and energy sources for microbes and the different compounds that are secreted nnay influence the composition of the microbial community (Jones 1998). Thus, according to Van Overbeek & Van Elsas, (1995) and Zhalnina, et al., (2018) rhizodeposition and root exudates are the main sources of nutrient supply for the rhizosphere microbiome thereby creating a unique niche for the growth of rhizosphere microorganisms. Plant root exudate is one of the major driving force for rhizospheric interactions between microorganisms and plants (Bias et al., 2006). Several studies have linked the process of root exudation to nutrient availabilty, yet it remains unresolved (Canarini et al., 2019) whether biochar increasing sorption capacity of soils affect the fate and stability of root exudates. It is therefore important to investigate mechanisms and interactions undelining fate of root exudate stability in soils treated wit biochar amendment, which is the focus of this experiement.

1.4 **Priming Effect**

Rhizosphere priming is a necessary process that can lead not only to substantial C losses from terrestrial ecosystems (i.e., SOM) but even loss of biochar C. According to (Kuzyakov, 2002), rhizosphere priming refers to a change in SOM decomposition caused by plant root activity and is often associated with rhizodeposition. The direction and magnitude of rhizopriming are interdependent between C availability and soil nutrient status. Thus, plants and microbes require

C and nutrients within specific boundaries and at the same time affect the relative availability of C and nutrients in the rhizosphere (Dijkstra, et al., 2013). In low nutrient soils, microbes use root-exudates to release SOM-bound nutrients - a process known as microbial mining to meet their energy and nutrient requirements. Similarly, under high nutrient conditions, microbes switch from decomposing SOM or other complex compounds such as biochar, to utilizing the labile C in the root exudates for their carbon and energy requirement resulting in a negative priming effect (Blagodatskaya, et al., 2007). Possible factors explaining the wide range of observations of rhizosphere priming effects, including soil microbial community effects, quality, and stoichiometry of the root exudates, the relative availability of N and P (Dijkstra, et al, 2013).

The ability of biochar amendment to cause priming effects (PEs) which offset the effect of soil organic C sequestration has raised contentious and controversial conclusions in recent studies (Luo, et al., 2017). The inconsistent results on biochar priming effect are attributed to the differences in biochar (i.e., pyrolysis temperature, feedstock composition), soils (Awad, et al., 2017), microbial community structure, and the experimental conditions in different studies (Jones, et al., 2011). Wood and sugar bagasse-derived biochars are composed of lignin-rich feedstocks that tend to induce negative priming (Jones, et al., 2011; Zimmerman, et al., 2011), whereas grass-derived cellulose-rich biochars are more likely to cause positive priming (Luo, et al., 2011). Similarly, biochars produced at high pyrolysis temperature are likely to induce more intensive priming effects in contrast to low temperature produced biochars that induce relatively smaller priming effects (Maestrii, et al., 2015; Smith, et al., 2010; Zimmerman, et al., 2011).

1.5 **Problem statement**

There is currently limited understanding on how the stability of biochars is affected by root-exudates and how these two important components entering soil interact with each other. Furthermore, estimating BC decomposition rates directly is hardly possible as BC content changes are too small for many practical experimental periods. This approach of estimating BC decomposition rate is unsuitable because the contribution of mineralized CO_2 from BC is too small compared to soil organic matter (SOM) and plant residues (Kuzyakov et al, 2009). The application of stable isotopes has been used in trace/labeling studies not only to distinguish different SOM and C pools in relation to soil priming (Wardle et al., 2008) but also to distinguish mineralization and decomposition rates between biochar and SOC through the addition of (i) unlabeled biochar

(Wardle, et al., 2008) to a labeled soil and (ii) ^{13}C (Jones et al., 2011) or ^{14}C labeled biochar to native soils (Kuzyakov et al., 2009). To effectively evaluate the interactive effects of biochar on the stability of rhizodeposition and soil C cycling, we used a 3-source quantitative partitioning approach of CO_2-C by using dual isotopic labeling.

1.5.1 **Research questions**

A 60-day incubation was set up using ^{13}C enriched root exudates and ^{14}C labeled corn cob biochar on three soils with clay migration. This carbon trace experiment was set up to assess the:

(1) Impact of ^{14}C-labeled biochar on the stability of ^{13}C-labeled root exudation and vice versa on each of the three soil types.

(2) Potential priming of biochar and root exudates on mineralization of SOC in each soil.

(3) Short-term biochar effect on soil fertility in each soil.

1.5.2 **Hypotheses**

The hypotheses of this research are:

(1) The addition of ^{14}C biochar and ^{13}C root exudates stimulated rapid decomposition and destabilization of SOC resulting in loss of C in the short-term compared to the medium-to-long-term irrespective of the soil types.
(2) The addition of root exudates leads to activation of microbial activity and higher rhizosphere priming effects compared to combined application of root exudate and biochar in each of the soil types.
(3) Biochar and root exudate improve soil fertility via stabilizing SOC and increasing microbial biomass which serve as energy and nutrient sources and is used by microbes for microbial mineralization.

CHAPTER TWO

MATERIALS AND METHODS

2.1 **Soil sampling**

The soils used in the present study were sampled from the Ap horizon of an Acrisol from Kenya, and two Luvisols, one from China, and the other from Germany. The soils were subjected to similar soil preparation and processing techniques by air-drying and sieved through a < 2mm screen to collect the fine earth fractions and remove bigger particles and cramps. Visible, large, adventitious roots were sieved and handpicked.

2.2 **^{14}C labeled biochar production**

Biochar was produced from dried corn cobs and maize leaves (Fig. 1, a, b, c, and e) as organic feedstocks. 60 g of unlabeled corn cobs and 23 g of ^{14}C enriched maize leaves were chopped into pieces, uniformly mixed and pyrolyzed in a muffle combustion furnace with little or no oxygen for 18 hours. The temperature was gradually increased to a maximum of 400^{o}C. The biochar had a weight loss of 70 % compared to the pyrolyzed plant matter, and an activity recovery of 31.674%. The biochar produced was homogenized and thoroughly mixed by milling. The biochar activity was measured by combusting 5 mg aliquot of the homogenized biochar with 200 mg of quartz sand resulting in 120.1 Bq/mg. The labeled ^{14}C biochar was further diluted with unlabeled biochar in a 2:1 ratio and applied at 20 t ha^{-1} (equivalent to 385 mg per jar) which resulted in a total activity of 15.40 KBq per jar.

2.3 **^{13}C root exudate composition**

The preparation of maize root exudate was done according to Fan et al., (2012). The composition of the root exudates constitutes 20 compounds made up of a combination of organic acids, sugars, carbohydrates and homogenized in their correct (w/v) proportions as shown in Tab. 1 (appendix). Five compounds including malonic acid, succinic acid, glutamic acid, alanine, and glucose were labeled with ^{13}C because of their relative availability and accessibility. The total mass of maize root exudates that was produced was 209.0 mg which was applied at 1.25 ml per microcosm.

2.4 **Experimental design and layout**

The experimental design consists of 12 treatments, 4 replicates, 12 isotopic references, and 3 harvesting time points resulting in a total of 144 microcosms in a completely randomized design. The treatment combinations included (i) 3 control soils or isotopic references, (ii) ^{14}C biochar, (iii) ^{13}C root-exudate, (iv) combined ^{14}C biochar, and ^{13}C root-exudate incorporation. Each treatment combination was replicated four times in a completely randomized design and preincubated for eight days before the start of the 60-day incubation period to remove the low molecular weight (LMW) residues. The full list of the treatment combination can be found in Tab. 2 (appendix).

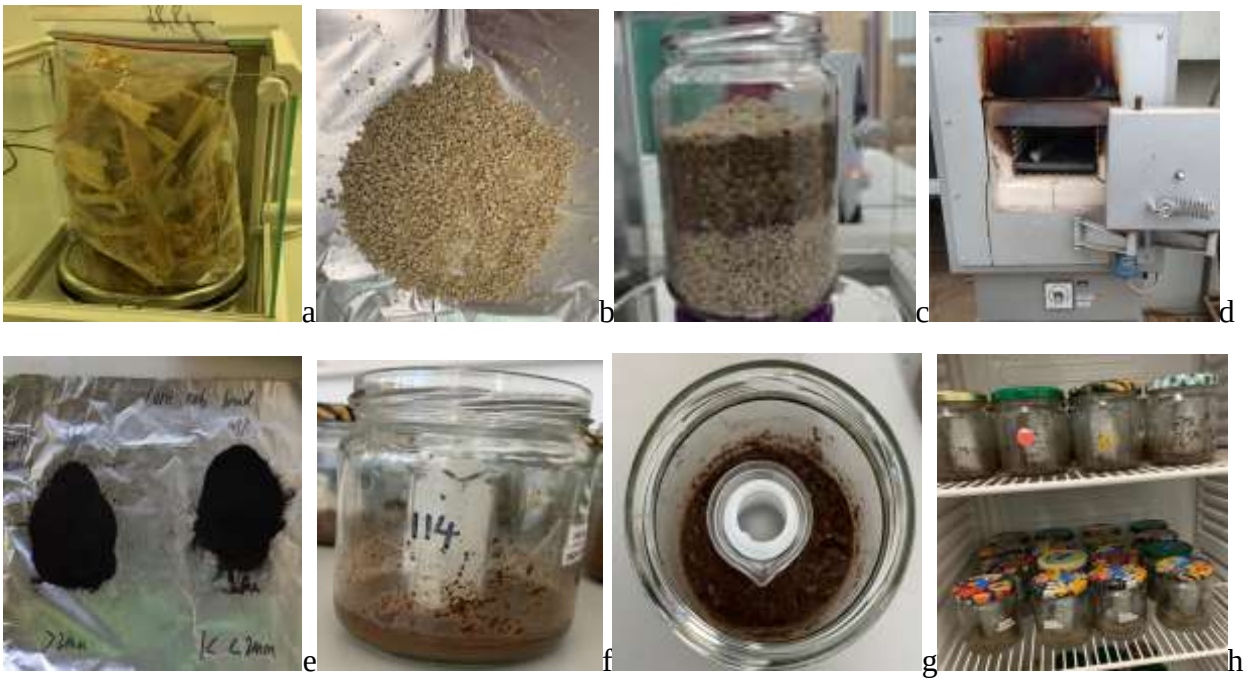

Fig. 1. a) 14C labeled maize leaves (b) Chopped corn cobs (c) Maize leaves + corn cobs (d) muffle furnace (e) 14C biochar (f) biochar + soil + biochar (g) CO_2 traps with NaOH (h) Incubation jars in incubator

2.5.1 Soil and Biochar preincubation

Fifty grams (50 g) of dried soil were balanced separately into 144 mason jars and maintained at 50% water holding capacity by adding 7.11 g, 10.87 g, and 9.12 g water to the Kenyan Acrisol, German Luvisol and Chinese Luvisol respectively. The soils were thereafter preincubated for 8 days in the dark at a constant temperature of 28^{o}C. Thereafter, 0.385 g of biochar representing a field application rate of 20 t ha^{-1} were applied to 72 of the total 144 microcosms (Tab. 2 in

appendix), mixed thoroughly, and each microcosm brought to 60% WHC. The samples were again preincubated for 3 days to remove the LMW residues formed by pyrolysis. CO_2 efflux was captured using 1M 10 ml NaOH and CO_2-C was determined in this solution (section 2.6).

2.5.2 **Incubation**

1.25 ml maize root exudates (equivalent to 1.32 mg/C per jar) was added into the predetermined treatment combinations in the microcosms after the initial soil and biochar preincubation. The incubation conditions of 28^{o}C and non-exposure to light were maintained constant throughout the 60-day incubation period. CO_2 traps were set up in each microcosm using 10 mL 10 M NaOH to trap CO_2 and $^{13/14/total}CO_2$-C respired by microorganisms at 10-time points. Subsequently, destructive sampling was done on days 3, 30, and 60, and subsamples were harvested for the determination of microbial biomass carbon and nitrogen (MBC & MBN), pH, cation exchange capacity (CEC), dissolved organic carbon (DOC), and microbial biomass P (MBP).

2.6 **Determination of CO_2-C**

CO_2 was trapped in 1M, 10 ml NaOH solutions using scintillation vials at defined days (1, 3, 5, 10, 20, 20, 30, 40, 50, and 60 days after the addition of root exudates, respectively). 0.5 ml of trapped CO_2 solution was mixed with 7.5 ml of deionized water (1:7, w/v) per sample for the determination of total inorganic carbon using the Shimadzu analyzer connected to TOC-L+ASI-L Normal Sense GC analyzer (S/W Version).

2.6.1 **Determination of ^{13}C-CO_2 in CO_2 by Sr precipitation and bulk IRMS measurement**

Five ml of trapped CO_2 were mixed with 5 mL 0.05 M $SrCl_2$ solution (1:1, w/v) to precipitate the carbonate ions (CO_3^{2-}), centrifuged at 2000 rpm for 10 minutes, and the supernatant was then discarded. The remaining $SrCO_3$ precipitate was repeatedly centrifugated and washed with distilled water until a near neutral pH was reached. Thereafter, the remaining $SrCO_3$ was oven dried for 5 days at 60^{o}C. The dried $SrCO_3$ (1.3-1.7 mg) were balanced and packed into tin capsules and their $\delta^{13}C$ analyzed on an elemental analyzer-isotope ratio mass spectrometer (DELTA V plus IRMS, Thermo Fisher Scientific, Bremen, Germany).

2.6.2 **Determination of ^{14}C-CO_2**

^{14}C activity of CO_2 trapped in NaOH solution was measured in 1 mL aliquots with 9.5 mL of the scintillation cocktail (Rotiszint® eco plus LSC-Universal cocktail), vortexed, and measured using the Hidex 300 SL analyzer-Tricarb™ B3180 TR/SL (PerkinElmer Inc., Waltham, MA, U.S.A.).

Priming effects of CO_2-C induced by the addition of maize-root exudates was calculated by

$$PE = CO2\text{-}C_{total\text{-}isotope\text{-}labeled\text{-}sample} - CO2\text{-}C_{unamended\text{-}sample} - CO2\text{-}C_{isotope\text{-}labeled\text{-}source} \quad (1)$$

2.7 **Determination of Microbial Biomass Carbon and Nitrogen**

The microbial biomass carbon and nitrogen was determined using the chloroform fumigation extraction (CFE) method.

For fumigation, 10 g soil per treatment was sampled in glass bottles and placed in a desiccator with a beaker containing about 80 ml of ethanol-free chloroform ($CHCl_3$) and anti-bumping granules to prevent retardation of boiling of the chloroform. The desiccator was closed and connected to a (vacuum) pump and a negative pressure of 200 to 300 mbar was applied to saturate the headspace of the desiccator with gaseous chloroform. The samples were afterwards incubated for 24 hours. The desiccator is evacuated at a negative pressure of 200 mbar for at least 8 times under the fume hood until no residual chloroform smell of the samples could be detected anymore.

The microbial constituents released by fumigation were directly extracted as follows to estimate the size of the soil biomass.

Fumigated and non-fumigated soil samples were extracted with 0.05 M K_2SO_4 at a ratio of 5:1 (weight of extractant to dry soil weight) for 1 hour at 25^{o}C. 10 ml of the supernatant solution was filtered through a 0.45-mm Whatman filter paper and MBC was determined by two measurements of extracted organic carbon as described below. A blank filtrate is run for each sample batch to determine the background levels of C and N in both the filter paper and the extractant.

The CFE-method for MBC is based on the equation:

$$C_{mic} = (\text{Fumigated } C_{mic} - \text{Non fumigated } C_{mic}) / K_{ec} \quad (2)$$

where,

K_{ec} = Proportion of microbial C that is extracted from the soil. As it is not possible to determine the extraction factor sample specific, K_{ec} is given by an empirical correction factor of 0.45 (Wu et al., 1990).

Similarly, MBN is determined by the

CFE-method using the equation:

N_{mic} = (Fumigated N- Non fumigated N)/K_{en} (3)

where,

K_{en}= Efficiency of the extraction of organic microbial N and inorganic N from the soil. Theoretically, K_{en} is given the value of 0.54

2.8 **Microbial Biomass Phosphorus (MBP)**

To estimate the amount of P in the microbial biomass, the fumigation-extraction method with anion-exchange membranes as detailed by Kouno et al., (1995) was used.

2 g of the harvested moist samples were weighed into 50 mL PP tubes and shaken horizontally for 16 hours along with one 6*2cm anion exchange resin strips in each PP tube. Three subsamples were prepared for each soil sample namely, (1) unfumigated with only distilled water (2) fumigated with 1-ml of 1-hexanol (3) P-33 spike (1 mL of a P solution containing 50 µg P/mL) added to the soil. Blanks with H_2O only (no soil) or H_2O + P-spike plus resin strips were prepared and included in the determination. The adhering soil particles were removed on the resin strips using distilled water and then the cleansed strips were given into new 50 mL PP tubes containing 30 ml 0.1 M NaCl+HCl. The samples were allowed to stand in the fume chamber for about 30 minutes to facilitate CO_2 bubbles escape and then shaken for 2 hours to allow desorption of P from the resin strips and thereafter, the strips were removed. 0.5 ml of the NaCl/HCl eluate was dissolved in 2 ml H_2O and 0.4 ml reagent 1(14.2 $mmolL^{-1}$, ammonium molybdate) followed by 0.4 ml Reagent 2 (0.35g L^{-1}, malachite green solution).

The Pmic concentration in µg/g is calculated as follows.

Pmic = (P fum – P resin) / Prec (4)

Where P fum, P resin are concentrations of fumigated and unfumigated samples in µg g^{-1} and P rec is P spike recovered from the resin membrane and is calculated by

Prec = (Pspike – Presin/ Pspike (5)

Where P spike is the concentration P added with spike in µg P g^{-1}.

3.0 **Statistical Analysis**

Statistical analyses were done using R version 3.6.3 software and graphs constructed using SigmaPlot v14.5. The means and standard errors were calculated for all the measured parameters and subjected to a normality test using the Shapiro Test at $P < 0.05$. Repeated two-way analysis of variance (ANOVA) was used to evaluate the differences in CO_2-C between the biochar and root exudate treatments in each of the three soil types (Kenyan Acrisol, German and Chinese Luvisols). Means of the treatments were compared for significant differences using least significant differences (Tukey HSD) at the 5% level. A one-way ANOVA was performed for MBC, MBN, MBP DOC parameters, and the stoichiometric ratios. Pearson correlation test was performed between all the parameters separately for the soils (Figs.14-16 in appendix).

CHAPTER THREE

RESULTS

4.1 **Total and Cumulative CO_2-C-efflux from soil**

The general pattern of total CO_2 evolution shows rapid CO_2-C decomposition rates at day 1 especially after addition of root exudates, followed by a steadily slow decomposition phase from day 5 until the end of the 60-day incubation period (Fig. 2). The results also highlight that regardless of the soil type, sole applications of root exudate (Re) and its combined application with biochar (Re+BC) recorded the highest total CO_2 efflux on day 1 but then decreased to a comparable efflux than soils without Re addition as the incubation progressed further. At the end of the incubation, the total CO_2-C efflux was about 4-fold higher in the Luvisols than the Kenyan Acrisol.

In the Kenyan Acrisol, total CO_2 efflux was enhanced by the addition of root-exudates and was significantly different ($P < 0.05$) from soils with sole biochar addition and control soils at the first incubation day. The addition of root exudates increased the total rate of CO_2 emission and subsequently induce a high priming effect and thus a loss of soil organic carbon. Sole biochar treatments tend to inhibit and decrease the decomposition of CO_2-C but have no significant effect when combined with the root exudate amendment at each sampling point except for day 1. At the end of incubation, the highest cumulative CO_2 efflux was recorded in the root exudation treatment with an increase of 25.36 % relative to the control soil, whereas the lowest CO_2 efflux was recorded in the control soil (100.4050 ± 8.69 µg C g^{-1}).

Similar results are depicted in the German and Chinese Luvisol. Root exudation enhanced CO_2 efflux significantly $P < 0.05$ compared to biochar amended and control soils (Fig. 2). However, in the German Luvisol, sole root exudate (Re) and combined root-exudate and biochar (BC+Re) application both reported a cumulative negative priming effect (-38.96 and -39.87). Cumulative CO_2-C was highest in the control of the German Luvisol reporting a value of 413.294 ± 32.73 µg C g^{-1} and the least CO_2-C evolved in the sole root-exudate application (377.563 ± 11.07 µg C g^{-1})

The decomposition rate of CO_2-C in the Chinese Luvisol showed that biochar incorporated in soils enhanced CO_2 evolution but the cumulative biochar, root exudate and SOC mineralization rates were not significantly different irrespective of the soil type.

The cumulative CO_2 emissions from biochar-amended and root-exudate soil showed similar trends as in the two Luvisols (Fig. 2). Compared to majority of studies showing an exponential increase in the first 8-10 days of incubation, our results show a linear cumulative CO_2-C efflux. The differences in the cumulative CO_2 fluxes between biochar and root-exudate treated soils are however statistically insignificant. Thus, at the end of the 60-day incubation period, the Kenyan Acrisol recorded the lowest amount of totally respired cumulative CO_2-C means representing 125.867 ± 12.32 µg C g^{-1} in the root exudate amended soil (Re) compared to 413.294 ± 32.73 µg C g^{-1} in the control soil of the German Luvisol and 454.153 ± 55.83 µg C g^{-1} combined root exudation and biochar treatment (C+Re+BC) in the Chinese Luvisol.

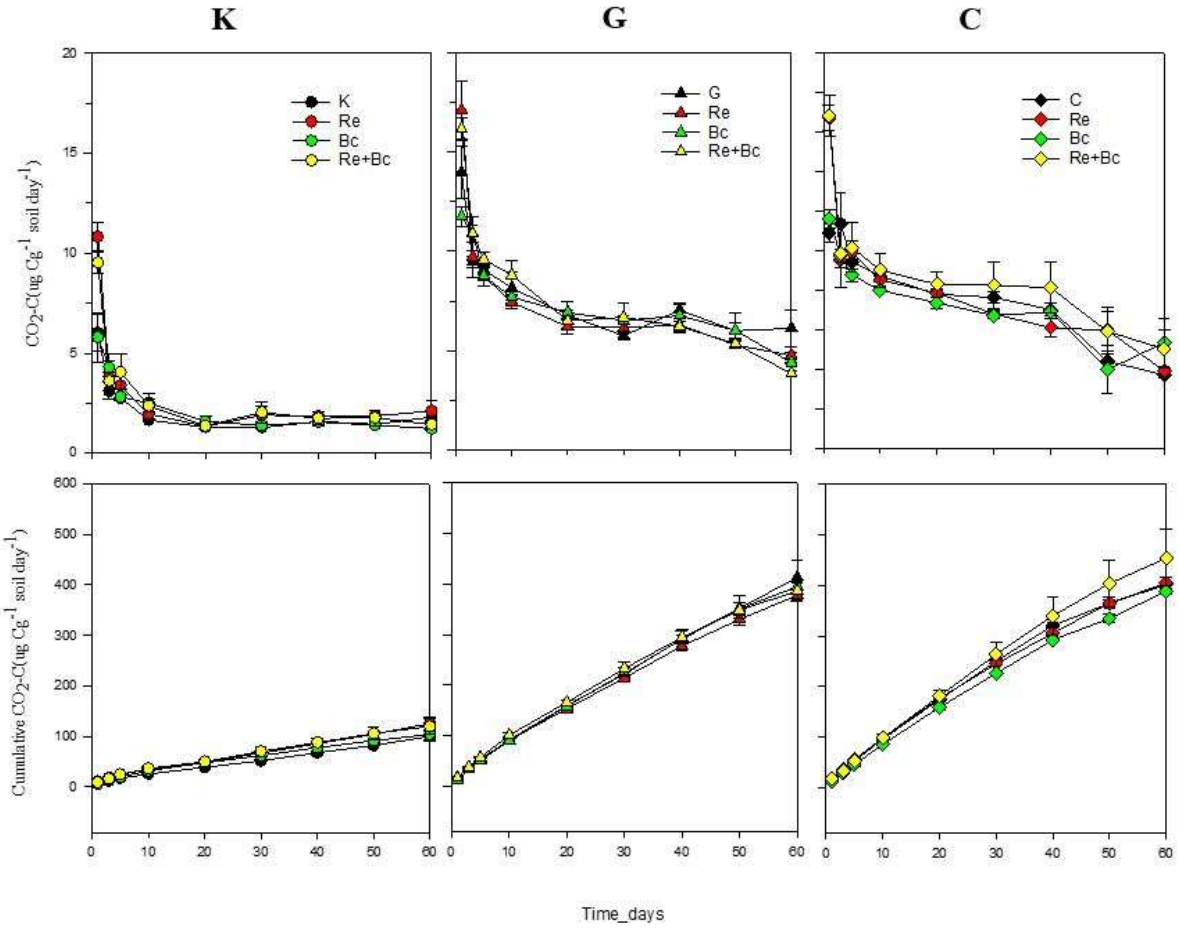

Fig. 2. CO_2 efflux rates (µg C g^{-1} soil day^{-1}) and cumulative CO_2 efflux (µg C g^{-1} soil day^{-1}) in three soils amended with sole applications of maize-root exudates, biochar, or a combination of biochar and root exudates on three soil types (K) Kenyan_Ascrisol (G) German_Luvisol and (C) Chinese_Luvisol. Re, BC, and Re+BC are maize root exudates, biochar, and co-applied biochar and root-exudate.

4.2 **Partitioning soil CO_2**

The addition of ^{13}C labeled maize-root-exudates was associated with higher $^{13}CO_2$-C mineralization of the root exudates compared to the co-applied biochar and root exudate treatment (Re+BC) on the first day of incubation and decreased rapidly to a steadily or flattened line as the incubation day progresses in the Kenyan Acrisol and Chinese Luvisol. However, in the German Luvisol, the co-application of biochar and root exudate (G+Re+BC), has higher biochar and root exudate mineralization rates than the sole Re exudation treated soil suggesting a retardation or stabilization effect of biochar on root exudate mineralization (Fig. 3).

Because changes in CO_2 efflux in short-term incubation studies are too small for any deductive conclusions on biochar mineralization, estimated decomposition rates of biochar are mostly based on the $^{14}CO_2$ efflux from soil. In Fig. 4, biochar-derived carbon incorporated in the soil shows evidence of two phases of mineralization in the Luvisols, an initial intensive phase starting from day 1 to day 10 and a second less intensive mineralization phase from day 20-60. Biochar and root exudate mineralization in the Luvisols began at initial higher $^{13}CO_2$-C (µg C g^{-1}soil $^{-1}$) quantities, about 2.5-fold higher than the same treatments in the Acrisol. Thus, the first and second intensive root exudate mineralization phases began at a slightly higher means above 0.4 and 0.2 µg C g^{-1} soil $^{-1}$ in the German and Chinese Luvisols respectively, representing a 2.5-fold relative CO_2-C increase compared to the Kenyan Acrisol. Thus, at the end of the 60-day incubation, the decomposition rates of biochar and root exudate were below 0.2 µg C g^{-1} soil $^{-1}$ in the three studied soils.

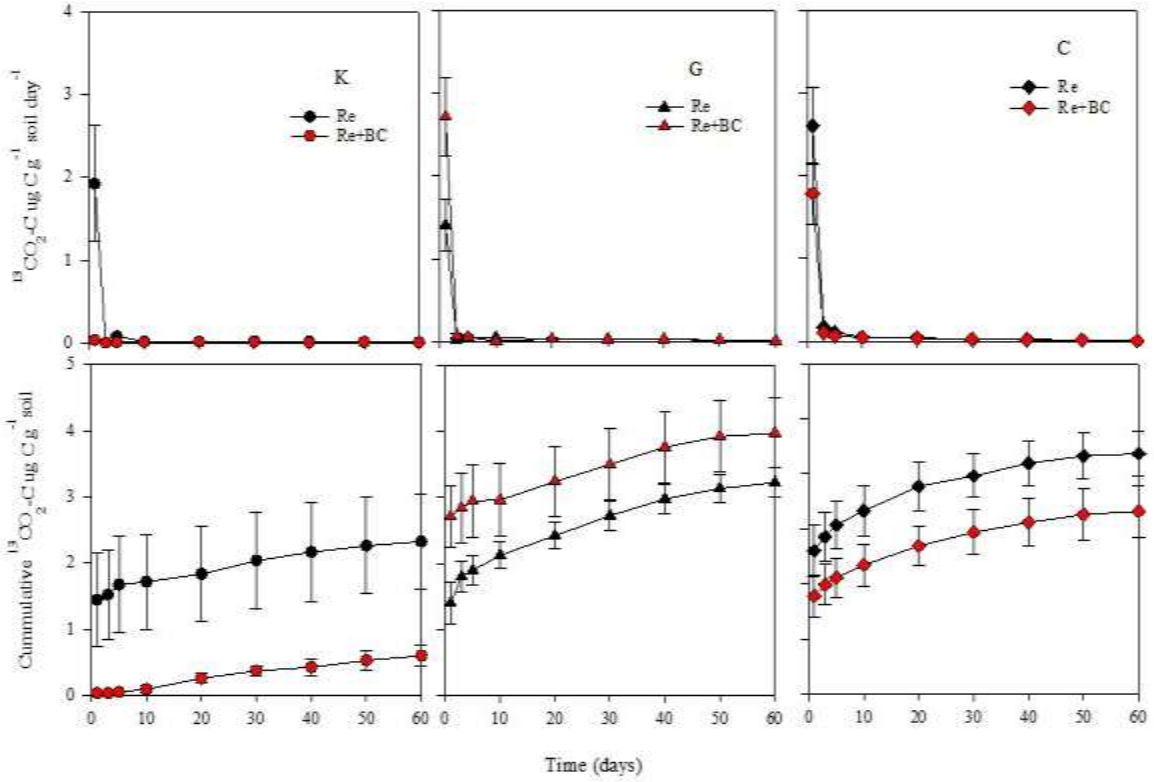

Fig. 3. $^{13}CO_2$ efflux rates (µg C g^{-1} soil day^{-1}) and cumulative $^{13}CO_2$ efflux (µg C g^{-1} soil day^{-1}) from three soil types (K) Kenyan_Ascrisol (G) German_Luvisol and (C) Chinese_Luvisol amended with ^{13}C labeled maize root exudate. Re and Re+BC are maize root exudates, and combined biochar and root-exudate.

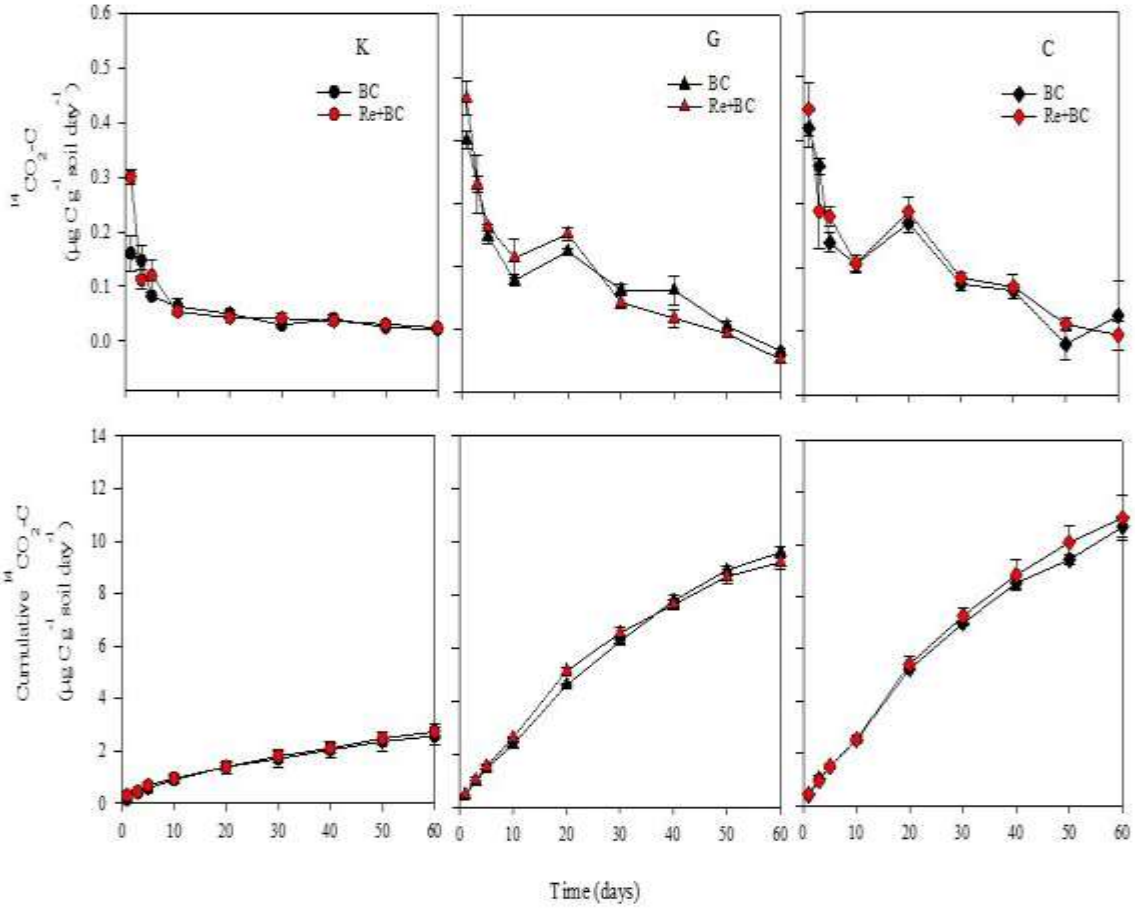

Fig. 4. $^{14}CO_2$ efflux rates (µg C g^{-1} soil day^{-1}) and cumulative $^{14}CO_2$ efflux (µg C g^{-1} soil day^{-1}) from three soil types (K) Kenyan_Ascrisol (G) German_Luvisol (C) Chinese_Luvisol amended with ^{14}C-labeled corn cob biochar. BC and Re+BC are biochar, and combined biochar and root-exudate.

4.3 **Priming effect**

Application of biochar and root exudate induced a response in microbial activities which may in turn accelerate both the decomposition of biochar and rhizodeposits. The high root exudate decomposition rates of CO_2-C were significantly higher compared to all other treatments irrespective of the soil types at day 1 of incubation. A single root-exudate application induced positive priming effects of +3.36, +1.74, and +3.23 µg CO_2-C g^{-1} soil, in the Kenyan Acrisol, German and Chinese Luvisol, respectively. Similarly, co-applied Re and biochar amendment resulted in higher positive priming effect compared to the sole Re applications except in the German Luvisol where the co-applied Re+BC was negative (Fig. 5).

At the end of the incubation, we observed a higher rhizopriming and lower biochar priming in the Acrisol, negative rhizosphere and biochar priming effects in the German Luvisol and a higher positive biochar priming relative to the rhizopriming in the Chinese Luvisol (Fig. 6). In the Kenyan Acrisol, Re+BC priming decreased by a factor of 0.39 from the Re soil whereas in the Chinese soil, the PEs in Re+BC increased by a factor of 25.48 also from the sole Re application. In the German soil, however, where we recorded negative priming effects, the Re+BC further decreased by a factor of 0.023 from the Re soil.

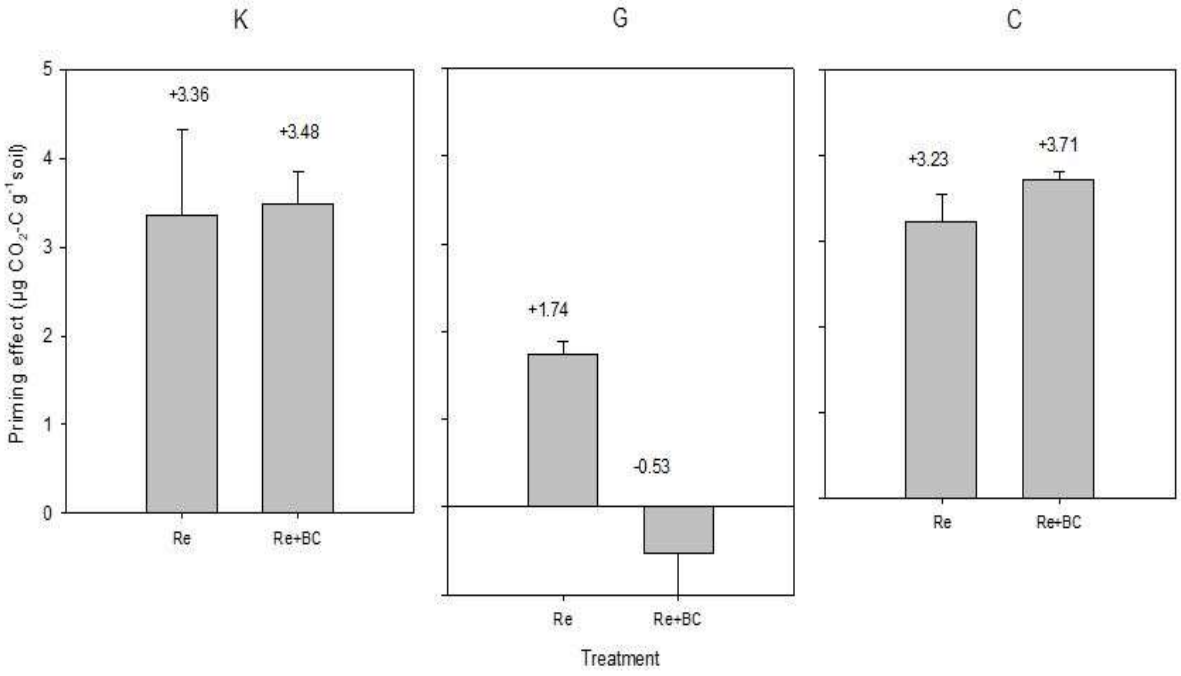

Fig. 5. Effect of maize root exudate addition on PE (µg CO^2-C g^{-1} soil) on three soil types (K) Kenyan_Ascrisol (G) German_Luvisol (C) Chinese_Luvisol at the day 1 during a 60-day incubation. Re, and Re+BC are maize-root-exudate and co-applied biochar and root-exudate.

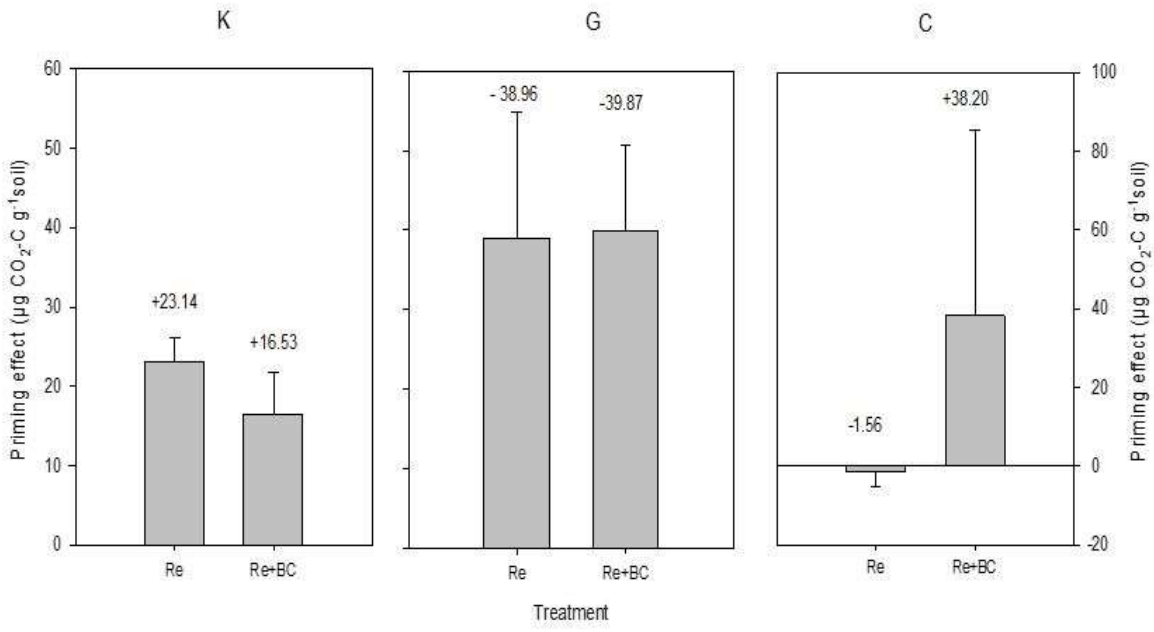

Fig. 6. Effect of maize root exudate addition on PE (µg CO^2-C g^{-1} soil) on three soil types (K) Kenyan_Ascrisol (G) German_Luvisol (C) Chinese_Luvisol during a 60-day incubation. Re, and Re+BC are maize-root-exudate and co-applied biochar and root-exudate.

4.4 **Biochar Effect on Microbial Biomass Carbon, Nitrogen and Phosphorus**

The quantity of MBC measured by the fumigation extraction methods decreased gradually with time irrespective of the soil types. The differences in the pools of microbial biomass carbon were significantly different between treatments and sampling time in the Acrisol. Furthermore, the combined biochar and root exudate treated soil have higher MBC pools that was significantly higher compared to the control soil but not significantly different from the sole biochar and root exudate application (Fig. 7). In comparing the different treatments of the Luvisols, the controls have insignificantly higher microbial carbon pools than the sole or co-application of biochar and root exudates. Microbial biomass carbon decreased with the addition of biochar to the German Luvisol. In contrast, in the Chinese Luvisol, the addition of biochar carbon stimulated microbial biomass growth compared to the control and root exudate treatment.

In the Acrisol, the microbial N pools decreased with both root exudate and biochar addition as well as with increasing days of incubation. However, in the German Luvisol, microbial N increased with the time of sampling, most strongly towards day 60. Except for the control, microbial N was

significantly higher in the co-applied compared to the sole treatments. As depicted in Fig. 7, microbial N pools decreased with increasing sampling days, biochar, and root exudate addition in the Chinese Luvisol resulting in the control having highest N pools.

Available phosphorus was measured at the end of incubation (60 days), and the results show higher available phosphorus in the biochar-treated soils. Co-applied root exudation and biochar recorded a 20.38 % increase available P compared to the sole biochar application to about 145.99 ± 5.95 mg P kg^{-1} in the C+Re+BC treatment, which was significantly different from non-biochar amended treatments in the Chinese Luvisol.

Similarly, in Fig. 8, the Luvisols have much higher pools of microbial P than the Acrisol. The maximum of microbial P (1.934 ± 0.89 mg kg^{-1}) was found in the sole biochar-treated Acrisol. The addition of biochar increased microbial P in the Kenyan Acriosl and Chinese Luvisol but decreased in the German Luvisol (Fig. 8).

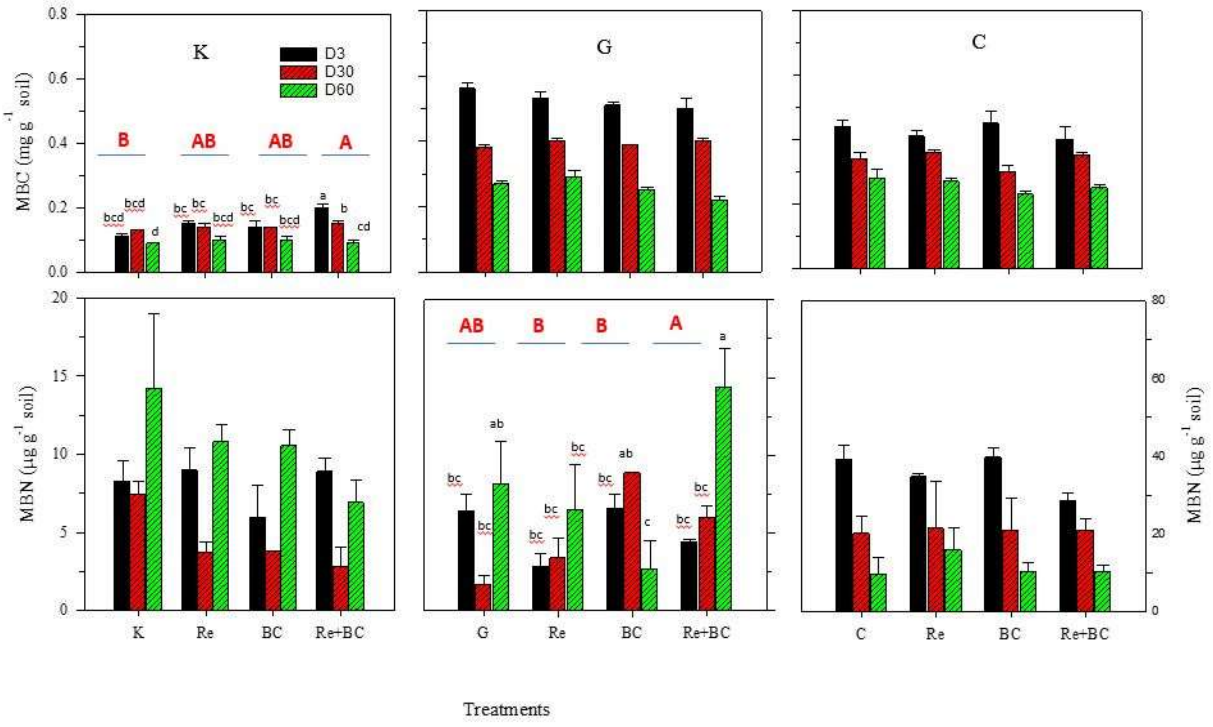

Fig. 7. Effect of biochar and maize root exudate on MBC (mg g^{-1} soil) and MBN (ug g^{-1}) at day 3, 30 and 60 of three soil types (K) Kenyan_Acrisol, (G) German_Luvisol and (C) Chinese_Luvisol. Re, BC and Re+BC are maize root exudates, biochar, and combined biochar and root-exudate. Block letters represent statistical differences between the treatments and small letters indicate statistical differences between the three sampling days. All statistical analyses were done at $p < 0.05$ and PostHoc analysis using Tukey LSD.

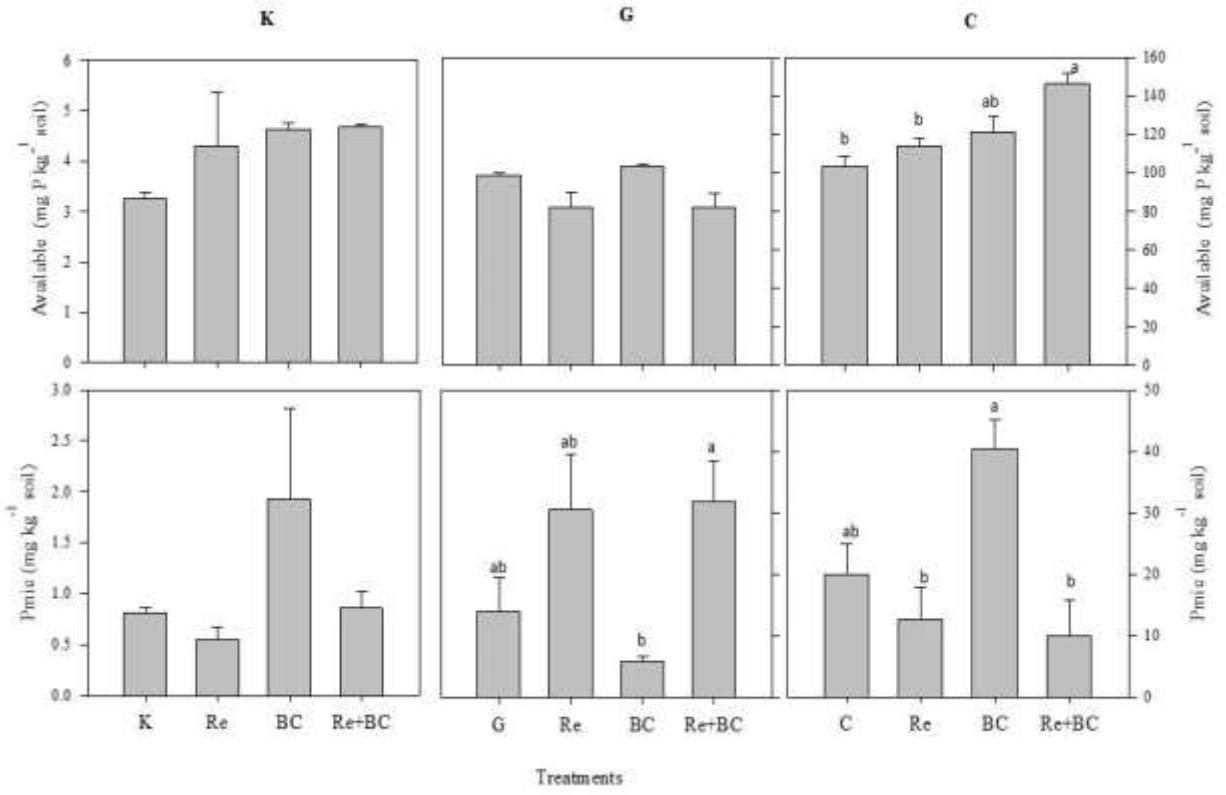

Fig. 8. Effect of biochar and maize root exudate on microbial P (mg P kg^{-1} soil) and available P (mg P kg^{-1}) sampled at the end of the 60-day incubation period from the three soil types (K) Kenyan_Acrisol, (G) German_Luvisol and (C) Chinese_Luvisol. Re, BC, and Re+BC are maize root exudates, biochar, and combined biochar and root-exudate. Small letters highlight statistical differences between treatment replicates at $p < 0.05$.

4.5 **Effect of Biochar on DOC, Available N and pH**

Biochar and root exudation addition have no significant effect on DOC and available nitrogen. The DOC and available nitrogen pools decreased as the incubation day progressed, with the Luvisols having higher overall pools (DOC and available nitrogen) than the Acrisol. The available nitrogen pool in the Luvisols was about 5-fold higher than that in the Acrisol, as shown in Fig. 9.

Generally, Acrisols are acidic soils, and the incorporation of root exudate further increased their acidity from an average of 5.7 to 5.3. However, the sole addition of biochar or co-applied biochar and root exudate significantly increased the pH to about 5.9 compared to the control. The German

Luvisols showed a similar result with a significant increase in pH in the biochar treated soils compared to control soils. As shown in Fig.10, the Chinese Luvisol generally has higher pH values (near neutral, 6.8) but similar trends to the other two soils could be observed.

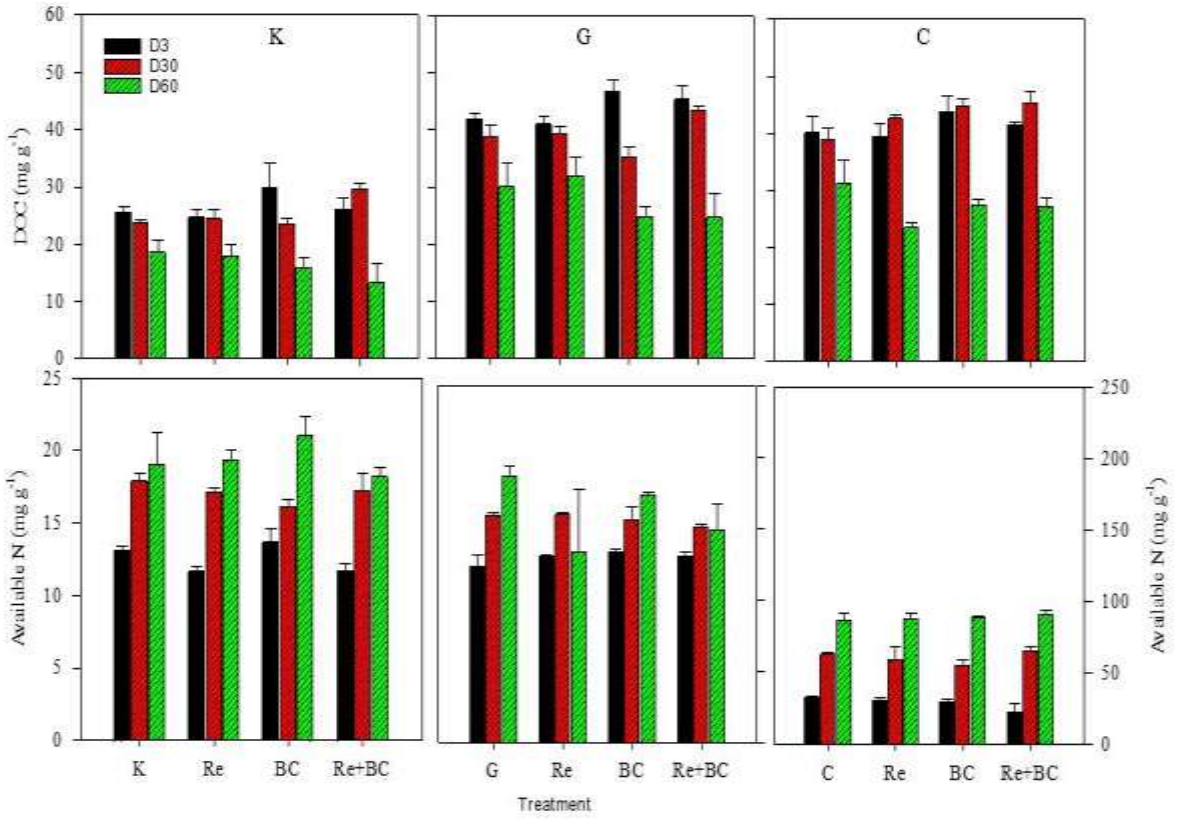

Fig. 9. Effect of biochar and maize root exudate on pools of DOC (ug g^{-1} soil) and Available N (g g^{-1}) sampled at the days 3, 30, and 60 days of three soil types (K) Kenyan_Acrisol, (G) German_Luvisol, and (C) Chinese_Luvisol. Re, BC, and Re+BC are maize root exudates, biochar, and combined biochar and root-exudate.

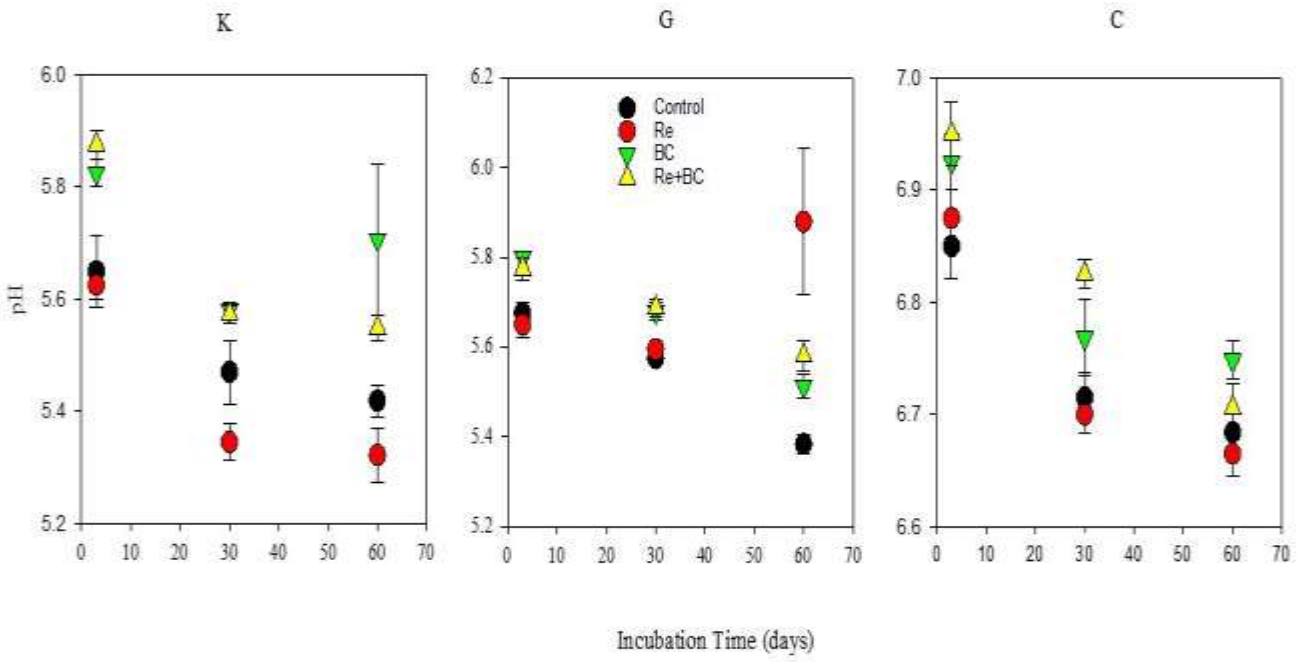

Fig. 10. Effect of biochar and maize root exudate on pH sampled at the days 3, 30, and 60 of the incubation periods from the three soil types (K) Kenyan_Acrisol, (G) German_Luvisol, and (C) Chinese_Luvisol. Re, BC, and Re+BC are maize root exudates, biochar, and combined biochar and root-exudate.

CHAPTER FOUR

DISCUSSION

5.1 **Decomposition of CO_2**

In this experiment, we evaluated the impact of ^{14}C-labeled biochar on the stability of ^{13}C-labeled maize root exudates and vice versa. The application of C sources including biochar to soils may facilitate short to long-term stabilization of SOC to support nutrient cycling for crop consumption in temperate soils (Awad, et al., 2013). In tropical soils, there is rapid decomposition of SOM hence the need for increased fertilizer and nutrient application. Tropical soils such as the Acrisol used in this study is a strongly weathered acidic soil with low levels of plant nutrients (e.g., CEC, carbon, and nitrogen limitation) but are not limited in their mineralization rates. The accumulation of SOM has the potential to increase CEC by providing negatively charged sites on the SOM surfaces that can absorb and hold positively charged ions (cations), thus, soils with generally large quantities of negative charge are resumably more fertile because of their ability to retain more cations (McKenzie et al., 2004). As such, SOM stabilization is more beneficial in tropical soils than in temperate soils where acceleration of cycling is more beneficial for nutrient availability.

The maximum total CO_2 emissions were found at the start of the incubation after the addition of root exudates and thereafter, they decreased steadily with ongoing incubation. Root exudates contain easily degradable substances such as sugars and organic acids that are rapidly decomposed. This contrasts with the slow biochar decomposition rates due to presence of the more recalcitrant structural compounds such as lignin and or microbial-inhibiting phenolic compounds contained in biochar (Chen, et al., 2009 and Dilly & Munch, 2004). Peak CO_2 efflux at day 1 could be attributed to the presence and availability of readily accessible labile C in added root exudates that stimulate microbial activity and hence increased C mineralization. More so, the presence of soluble components of maize root exudates provides energy in the form of carbon and nutrients for microbiota which may increase soil respiration and hence soil-derived CO_2-C named priming effect. According to Ameloot, et al., 2013 and Qayyum, et al., 2012, biochar may contain small fractions of bioavailable C in the form of aliphatic and volatile organic carbons, and these were easily respired by microorganisms and lost during the biochar preincubation. Generally, compared with short-term studies, medium to long-term experiments result in lower biochar decomposition rates (Wang et al., 2016). Biochar consists of both labile and recalcitrant fractions, and hence the

decomposition rates estimate during short-term trials may be due to degradation of the most labile biochar-C and not reflect the persistency of the polyaromatic backbone (Kuzyakov et al., 2014; Wang et al., 2016). The incorporation of biochar altered the mineralization of root exudate primarily at the initial phase resulting in a direct destabilization of the root exudates. In the Kenyan Acrisol and Chinese Luvisol, root-exudates are sorbed to biochar and therefore become less available for microbial decomposition in the Re+BC treatment as shown in Fig. 2. This is confirmed by studies suggesting that decreases in labile carbon pools may result from sorption from other carbon sources (in this experiment- root-exudate carbon) either within the biochar pores or at the external biochar surface with its functional groups. The addition of biochar resulted in an increase in the DOC pools in all the soil types and this was confirmed by Yin et al., 2014 who reported that the increase in DOC was contributed by the stable OC of biochar, because the labile OC in the soil was gradually and completely decomposed during the incubation. Furthermore, the subsequent addition of root-exudate increased microbial mineralization in the combined Re+BC treatment to a higher level than that in sole Re treatment (Fig. 2).

The differences in cumulative CO_2 emissions between biochar, maize root exudate, and control were insignificantly higher and followed similar trends in the three soil types. The differences between the cumulative mineralization in the Acrisol and Luvisol could be attributed to differences in soil texture, pH, microbial community among others. The higher CO_2-C evolved from biochar amended soils in the Luvisols is consistent with Sigua et al. (2014), that cumulative CO_2-C emission was two to three-fold higher in loamy than sandy soils. Soil pH may alter the composition of the microbial community, which influences microbial activity and consequently limit the decomposition of applied organic amendments (Motavalli, et al., 1995 and Huang & Chen, 2009).

Thus, at the end of incubation, the quality and quantity of composition of Re (comprising soluble and easily degradable sugars and organic acids) contributed to higher Re mineralization rates whereas slow biochar mineralization was attributed to the release of stable OC fractions from biochar to soils and mixture with SOC pool in all the soil types.

5.2 **Priming Effect**

The magnitude and direction of PE in relation to microbial C is a crucial factor dependent on the quality and quantity of the composition of the added of substrates (Blagodatskaya and Kuzyakov, 2008). Root exudates stimulate microbial activity (Yin et al., 2013) and in effect positive rhizosphere PE (RPE) in the Kenyan Acrisol. This is supported by theoretical (Cheng, et al., 2014 and Wutzler & Reichstein, 2013) and experimental studies (Drake, et al., 2013 and Phillips, et al., 2011) that show that root exudates may accelerate rhizosphere priming for SOM decomposition, thus increasing the flux of nutrients to forms available for plants uptake. In our study, microbes induced a high RPE after addition of root-exudates which was accompanied by activation of previously dormant microorganisms that responded to the extra C inputs resulting in a loss of soil organic carbon (Fig. 6). The Acrisols are characteristics of low fertile conditions, under which the observed positive priming effects may be a necessary consequence based on the need of the microbial community to mine for nutrients via the production of extracellular enzymes that release nutrients otherwise locked in SOM (Blagodatskaya et al., 2007). During biochar preincubation, the labile C pools of the biochar were removed, therefore, in the co-applied Re+BC treatments of the Acrisol and Chinese Luvisol, we assume a preferential microbial substrate utilization i.e., switch of substrate conditions by microbes from the stable OC factions in biochar to the easily accessible added alternative C sources of the root-exudate for their carbon and energy requirements. In addition, Dudley & Churchill, 1995 noted that biochar's negative PE or at least a reduced of the other compounds positive PE was caused not only by adsorption and protection of DOC on biochar surface but also by changes in microbial diversity and activity as a consequence of the biochar addition. Zimmerman et al., 2011 further showed using labeled biochar the inhibition effects of biochar on SOC mineralization. Biochar repressed SOM turnover by releasing humic substances, which binds to and inhibits extracellular enzymes involved in the breakdown of SOM and sorption of extracellular enzymes on biochar surfaces, causing inactivation and disconnection with potential substrates (Virchenko, et al., 1986). That is, induced RPE are based on accessibility of microbes to extra sources of C of the root exudates for their nutrient or energy requirements.

5.3 **Incorporation of biochar into microbial biomass**

Changes in microbial biomass reflect the process of microbial growth, death, and necromass accumulation in organic matter (Zhang, et al., 2014). Biochar and root-exudate addition increased microbial biomass compared to the control, suggesting a stimulation and acceleration of microbial activity due to the availability of new C sources especially in the Kenyan Acrisol. This may probably be due to the presence of favorable conditions such as soil pH and texture that provided habitat and energy for microbial activation resulting in higher TOC mineralization causing rapid SOC loss in tropical compared to temperate soils. Domene et al., 2015 reported that the addition of biochar to soil has a positive correlation between the quantity of microbial biomass and biochar application rate. The large surface area and porous structure created by the introduction of biochar are noted to provide favorable conditions and habitats for microbial colonization (Luo, et al., 2013). Similarly, Kolb et al. (2009) found that charcoal addition improves the soil surface area, promotes soil microbial growth, and thus significantly increased soil microbial biomass and activity.

Biochar's effects on microbial biomass are highly soil specific, with reports from (Dempster et al., 2012 and Luo et al., 2013) often indicating either a positive, negative, or no effect. These variations may depend on soil and biochar type: biochar pyrolyzed at a temperature > 600^{o}C was found to promote microbial biomass in fine but not in coarse-textured soil, where biochar had negative effects on microbial biomass (Gul et al., 2015). In the German Luvisol, the labile root exudate accelerated internal microbial metabolism within a few hours to days resulting in a shift from dormant to active microorganisms. As more microorganisms are activated, biochar-C are gradually reduced and utilized by microbes causing a reduction in MBC and microbial metabolic quotient (Fig. 12 in appendix). The difference in observations between the two Luvisols can be explained by the changes in the soil texture and C:N ratios, which are factors influencing mineralization of SOM (Tab. 3 in appendix). Furthermore, changes in microbial community and activity, could be a factor causing the differences in MBC of the Luvisols considering that the incubation conditions i.e., biochar type, and soil properties were nearly constant during the period. However, the study of the microbial community structure was beyond the scope of this experiment.

5.4 **Effect of biochar and root exudation on microbial P and DOC**

The application of biochar may lead to direct or indirect alterations of soil P dynamics via several processes. Zhai et al., 2015 reported that biochar contains various P species, through which soluble P may be released into the soil after its application causing a significant increase in the available P pool (Fig. 8). Biochar contains large P quantities and a slow-discharge P resource capable of continuously and reliably replenishing labile P inputs as H_2O-soluble P in the short term (Zheng et al., 2012 and Qian et al., 2013) compared to the non-biochar-amended soils. According to Glaser & Lehr (2019), maize biochar produced under low-mid temperatures (450 - 600^oC) comparable to what was used in this study significantly improved P availability in soil. Thus, biochar produced at a pyrolysis temperature of 400^oC released more phosphate into the soil matrix compared to biochar produced at 700^oC (Jian et al., 2019). Despite biochar's ability to increase phosphate ability, it has also been proved to be a robust, and efficient phosphate absorbent due to its high surface area and internal porosity, which aids in its P adsorption, and this is highly soil specific (Liard et al., 2010). As such, the reduced soil P after biochar addition in the German Luvisol could be attributed to the interaction between the soil and biochar thereby increasing the soil's surface area and capacity for phosphate sorption (Matin et al., 2020).

CHAPTER FIVE

CONCLUSION

In this study, we demonstrated the possibility of adopting a three-source partitioning approach in estimating the decomposition and stability of carbon by combining ^{14}C labeled biochar and ^{13}C labeled maize-root-exudates of three soils in a 60-day incubation experiment. Using this approach, we were able to separate CO_2 emissions from biochar, SOC and root exudates. Our results showed that SOC mineralization responds differently to the addition C sources from root exudate amended and biochar amended soils. The addition of root exudates serves as an alternative labile C source and energy substrate used by microorganisms that strongly influenced biochar-carbon decomposition and stabilization via stimulation of microbial activity in the biochar-amended treatments. The mineralization of SOC after the addition of root exudate by microbes induced a positive rhizosphere PE in the Kenyan Acrisol and Chinese Luvisol compared to the negative rhizosphere PE in the German Luvisol. Biochar decomposition rates decreased over the course of incubation – showing that biochar amendment can buffer against rhizosphere priming avoiding C loss by the input of easily available C irrespective of the soil type.

Throughout the study, the Acrisol has higher limitations of microbial biomass quantities (MBC, MBN, MBP and DOC) and lower SOC, root exudate and biochar decomposition rates compared to the two Luvisols. It was also evident that root exudates stimulated microbial activity resulting in higher SOC mineralization causing a rapid loss of SOC which was higher in the Luvisols compared to the Acrisol.

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APPENDIX

Tab. 1. Complete list of the composition of labeled and unlabeled root exudate

Name of compound		ratio	Target weight mg/each compound
Lactic acid		16.41	23.5
Malic acid		15.86	33.8
Malonic acid	^{13}C-labeled	11.94	20.3
Succinic acid	^{13}C-labeled	11.69	22.7
Glucose	^{13}C-labeled	6.43	19
t-Aconitic acid		5.88	16.3
Melibiose		5.76	31.3
Alanine	^{13}C-labeled	3.06	4.5
Histidine			
Glycine		3	3.6
Aspartic acid		2.63	5.6
Leucine		2.57	5.4
Glutamic acid	^{13}C-labeled	2.14	5.2
Tyrosine		1.65	4.8
Phenylalanine		1.53	4
Glutamine		1.53	3.6
Valine		1.29	2.4
Threonine		0.92	1.7
Serine		0.86	1.4
sum			209

Tab. 2. Experimental set-up; treatment combinations and code

	Code	Isotope reference	Treatment code		
Kenyan_Acrisol	K	K	K	G	C
German_Luvisol	G	G	K+Re	G+Re	C+Re
Chinese_Luvisol	C	C	K+BC	G+BC	C+BC
Maize Root exudate	Re		K+Re+BC	G+Re+BC	C+Re+BC
Biochar	BC				

Table. 3. Properties of the soil types

	Kenyan Acrisol	German Luvisol	Chinese Luvisol
sand	59.71	11.41	4.46
silt	8.57	72.41	89.43
clay	31.71	16.19	6.11
C:N ratio	8.20	9.22	9.55

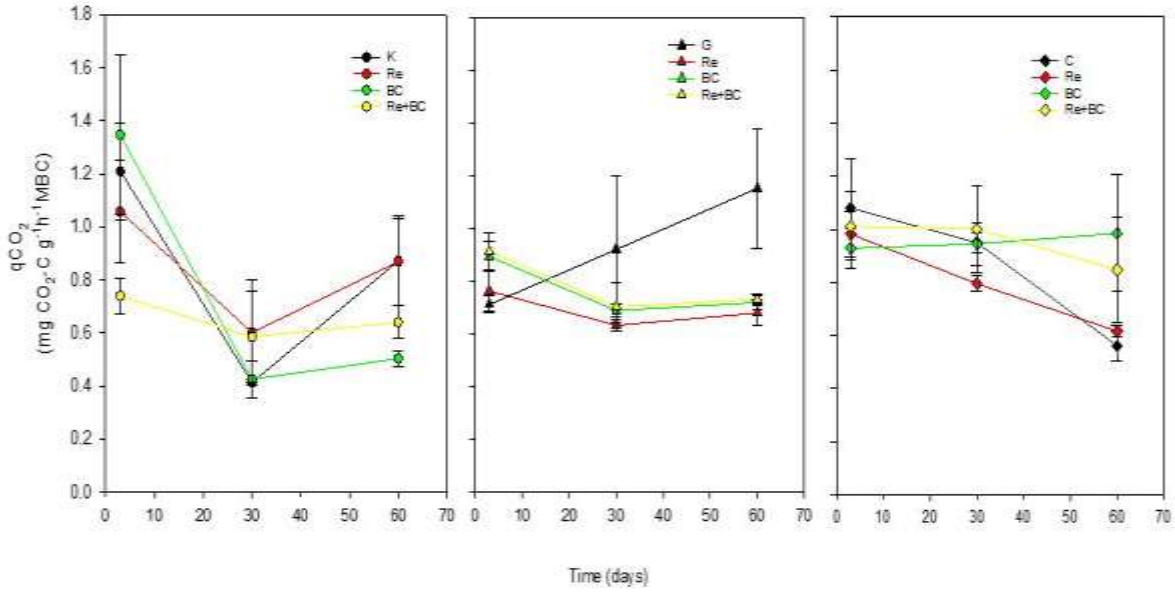

Fig. 11. Effect biochar and maize root exudation on metabolic microbial quotient (qCO_2 mg CO_2-C $g^{-1}h^{-1}$ MBC) during a 60-day incubation on three soils (K) Kenyan_Ascrisol (G) German_Luvisol (C) Chinese_Luvisol.

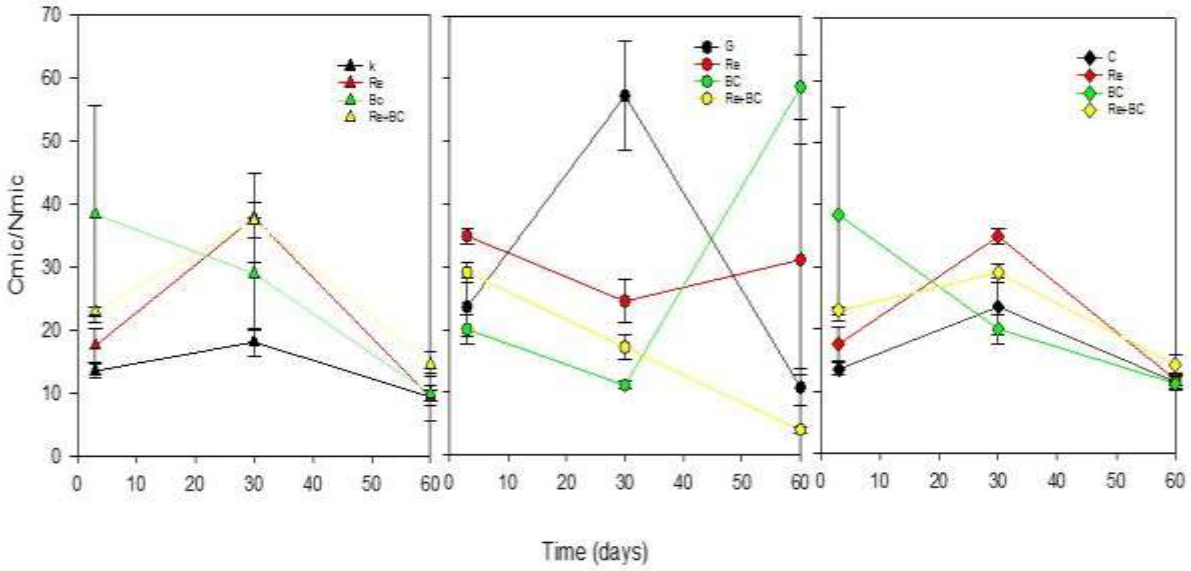

Fig. 12. Effect biochar and maize root exudation on C/N ratio at three sampling times during a 60-day incubation on three soils (K) Kenyan_Ascrisol (G) German_Luvisol (C) Chinese_Luvisol.

	CO2_ rate	Cum_co2	DOC	MBC	available N	MBN	pH	qCO2	MBC/MBN
CO2_ rate	1	**-0.46***	**0.39***	**0.38***	**-0.71***	0.04	0.28	**0.76***	0.22
Cum_co2		1	**-0.62***	**-0.53***	**0.73***	0.13	**-0.58***	-0.18	**-0.38***
DOC			1	**0.31***	**-0.32***	**-0.50***	**0.31***	0.13	**0.61***
MBC				1	**-0.56***	-0.1	**0.34***	-0.24	**0.61***
available N					1	-0.03	**-0.37***	**-0.47***	**-0.30***
MBN						1	0.05	0.20	**-0.83***
pH							1	0.20	0.20
qCO2								1	-0.26
MBC/MBN									1

Fig. 13. Pearson correlation matrix between the parameters of the Kenyan_Acrisol

	CO2_ rate	Cum_co2	DOC	MBC	available N	MBN	pH	qCO2	MBC/MBN
CO2_ rate	1	**-0.46***	**0.39***	**0.38***	**-0.71***	0.04	0.28	**0.76***	0.22
Cum_co2		1	**-0.62***	**-0.53***	**0.73***	0.13	**-0.58***	-0.18	**-0.38***
DOC			1	**0.31***	**-0.32***	**-0.50***	**0.31***	0.13	**0.61***
MBC				1	**-0.56***	-0.1	**0.34***	-0.24	**0.61***
available N					1	-0.03	**-0.37***	**-0.47***	**-0.30***
MBN						1	0.05	0.20	**-0.83***
pH							1	0.20	0.20
qCO2								1	-0.26
MBC/MBN									1

Fig. 14. Pearson correlation matrix between the parameters of the German_Luvisol

	CO2_ rate	Cum_co2	DOC	MBC	available N	MBN	pH	qCO2	MBC/MBN
CO2_ rate	1	**-0.46***	**0.39***	**0.38***	**-0.71***	0.04	0.28	**0.76***	0.22
Cum_co2		1	**-0.62***	**-0.53***	**0.73***	0.13	**-0.58***	-0.18	**-0.38***
DOC			1	**0.31***	**-0.32***	**-0.50***	**0.31***	0.13	**0.61***
MBC				1	**-0.56***	-0.1	**0.34***	-0.24	**0.61***
available N					1	-0.03	**-0.37***	**-0.47***	**-0.30***
MBN						1	0.05	0.20	**-0.83***
pH							1	0.20	0.20
qCO2								1	-0.26
MBC/MBN									1

Fig. 15. Pearson correlation matrix between the parameters of the Chinese_Luvisol

AFFIRMATION

I hereby affirm that this master thesis titled "Interacting effects of biochar and root exudates on soil C cycling and soil fertility" is done in partial fulfillment of the requirement for the award of the Joint degree of International Master of Science in Soils and Global Change. I declare that this work does not include or incorporate, without proper citation and acknowledgment of any material previously submitted for a degree in any university or institution. To the best of my knowledge, this dissertation contains no material published or written by another person except where due reference is made in the thesis itself and is solely produced from the effort of the student under the proper supervision of my supervisors and the guidelines outlined by the University of Göttingen and the partner universities.

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