MINERALIZABLE NITROGEN POOLS AND ASSOCIATED ENZYMATIC ACTIVITIES IN SOILS AMENDED WITH BIOCHAR MANURE CO-COMPOST

BY

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DECLARATION

I hereby declare that, except for references to other works which have duly been cited, this work is a result of my research, and it has not been presented either in whole or in part for the award of another degree.

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DEDICATION

I dedicate this work to my husband (Mr. Daniel Kekeli Tsatsu), my four children (Ezra, David, Kharis and Cedar Tsatsu) and my mother (Mrs Elizabeth Ivy Agbenyega) for their love and immeasurable support towards my education.

Also, to my late father (Mr. William Agbenyega), who supported me throughout my education. May your soul rest in perfect peace.

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ABSTRACT

Biochar-manure co-compost (BMC) produced from the addition of biochar to nitrogen (N) rich materials has increasingly been promoted as an organic amendment to improve fertility constraints in soils. However, studies on the composition of organic N (ON) pools, N releasing capacity and associated microbial and enzyme activities of BMC-amended soils are lacking. In this study, the effects of co-composted market wastes (MW), cattle (CM) and poultry (PM) manures, alone (manure compost-MC), or in combinations with rice husk biochar (RB), sawdust biochar (SB) and coconut husk biochar (CB) on the dynamics of N mineralization, soil microbial biomass (SMB) carbon (C) (C_{mic}) and N (N_{mic}), amidohydrolase activities (AA) (L-Asparaginase, L-Glutaminase and Amidase) distribution and changes of ON fractions and their relationships were investigated in three soils, namely Adentan, Denteso and Keta series. The soils were amended with 200 mg N kg^{-1} of the BMCs and MCs, incubated at 30 ºC and periodically leached for 26 weeks. Using the first-order kinetic model, the potentially mineralizable N (N_o) and the mineralization rate constant (k) were obtained. The AA, C_{mic}, N_{mic} and selected chemical properties such as pH, total carbon (TC), organic N (ON), permanganate oxidizable carbon (POXC) of the BMC and MC-amended soils were also determined. Similarly, the distribution and changes in ON fractions namely: Total Hydrolysable N (THN), Hydrolysable Ammonia N (HAN), Hydrolysable Amino Sugar N (HASN), Hydrolysable Amino Acid N (HAAN), Hydrolysable Unknown N (HUN) and Non-Hydrolysable N (NHN) in the amended soils were also determined.

The results showed that N_o values ranged from 120 to 205, 53 to 170 and 71 to 174 mg

kg^{-1} in Adentan, Denteso and Keta series, respectively, with SB and CB-based compost recording the lowest N_o values, probably due to immobilization and sorption of liable N. The k values were lowest in BMCs compared to MC due to C and N stabilization. Except for TN and ON, all the chemical and biochemical properties (lignin, cellulose, C/N ratio, NH_4^+-N) correlated significantly with N_o. Except for ON, BMC had a higher influence on the chemical and microbial properties of the soils compared to MCs due to the initial ON of the MCs. The RB, CB and CM-based BMCs had the highest influence on SMB and AA, probably due to the increased labile C and N in the amended soils. Also, the AA and SMB were positively and significantly correlated with each other and with N_o ($r \geq 0.229$; $p \leq 0.05$) as well as with chemical properties (TC, ON, pH, POXC) of amended soils. Soils amended with BMC and MC had higher ON fractions compared to soils alone which was attributed to an increase in the extractable organic N by the amendments. A negative relation between the THN, HAN, HAAN and N_o indicates that these fractions contributed to N_o of the amended soils. A stepwise regression analysis showed that the reduction in HAN, HAAN and THN explained about 60% of the variations in N_o. A positive correlation between HAAN, HAN, THN and AA in BMC-amended soils suggests that the enzymes were hydrolyzing these organic N fractions to release inorganic N. It was concluded that BMC moderated the N releasing capacity of the soils in addition to enhancing the microbial properties and organic composition of the amended soils.

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CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Due to the relatively low level of agro-processing in Ghana, a high proportion of agricultural products are marketed in their natural state, generating large amounts of vegetative waste, with about 12,710 tons of municipal and market waste (MW) generated daily nationwide (Lissah *et al*., 2021). Furthermore, animal husbandries produced about 2.4 million tons of waste in the form of manure in 2020 (Seglah *et al*., 2022). Incineration is the major form of disposal of municipal and agricultural related wastes (Zhang *et al.,* 2001), emitting large amounts of CO_2 which would contribute to global warming. Another waste disposal method in Ghana is the dumping in landfills or inappropriate disposal in the open, serving as breeding grounds for disease causing agents (Addo *et al.,* 2015; Boadi and Kuitunen, 2005).

Conceivably, an alternative and environmentally sound and economically feasible waste treatment system can be used to produce nutrient enrich organic amendment, to supplement chemical fertilizers (Li *et al*., 2011). Such methods could involve composting, which can transform manures and MWs into usable forms in agriculture by concentrating nutrients that can alleviate the declining nutrients in tropical soils and prevent negative impacts such as immobilization of plant nutrients and phytotoxicity when wastes are applied directly to the soil (Dias *et al*., 2010; Bernal *et al*., 2009; Lim *et al*., 2018). Although compost materials and direct application of waste can enhance the physical, chemical and biological characteristics of soils, the high tropical temperatures result in rapid decomposition of the carbon (C) and nitrogen (N) in MWs and manures, necessitating yearly re-application

(Bernal *et al*., 2009; Lim *et al*., 2018; Sanchez-Monedero *et al*., 2019; Dodor *et al*., 2024). Further, the rapid release of easily leached nutrients such as dissolved nitrate produced from the compost materials constitutes not only an economic loss but also has an adverse environmental impact (Yadav *et al*., 2013; Burgos *et al*., 2006). Consequently, there is a need to stabilize the N and C in MWs and manure-derived composts to improve their recalcitrance in soils and moderate the release of nutrients for sustainable maintenance of the fertility of tropical soils.

1.2 Problem statement and hypothesis

Recently, biochar, a carbonaceous material generated through the pyrolysis of organic material under limited or no oxygen conditions, has been explored as a bulking agent in composting (Barthod *et al*., 2018; Antonangelo *et al*., 2021, Mirsha *et al*., 2022; Dodor *et al*., 2024). The addition of biochar to compost is reported to improve the composting mixture in terms of stabilizing nutrients from manure and MWs, modifying the surface functional groups of biochar and promoting the formation of humic substances (Sanchez-Monedero *et al*., 2018; Dias *et al*., 2010; Xiao *et al* 2017; Wang *et al*., 2014). This process tends to regulate the rate and extent of organic C (OC) and N (ON) decomposition thereby enhancing stability, reducing emission and N loss in the environment (Agyarko-Mintah *et al*., 2017a; Thiele-Brhm and Ngigi, 2021; Mirsha *et al*.,2022; Jindo *et al*., 2012). Such material, referred to as biochar manure co-compost (BMC) contains stable organo-mineral complexes that can regulate the rate of N release in addition to stimulating microbial proliferation and its associated enzymatic activities, immobilization of toxic pollutant, increasing of organic matter (OM) and nutrient load, resulting in enhanced crop yields (Mackie *et al*., 2015; Schulz *et al*., 2013; Kammann *et al*., 2016; Guo *et al*., 2020).

Due to the stability of N in BMCs, it has been suggested that incorporating BMCs into soils would impact the potentially mineralizable N (N_o), thereby increasing crop productivity (Schulz *et al*., 2013; Kammann *et al*., 2015). The N_o can be described as the active ON fraction that can easily be converted to inorganic N over time (Curtin and Campbell 2008). The N_o, which is an estimate of N mineralization in soils (Curtin and Campbell 2008), is controlled by edaphic factors (soil moisture, soil temperature), the composition of organic material (C, N concentration, lignin content etc.) and decomposing organisms (Cabrera *et al* 2005).

Though a relatively large body of studies on the N_o of different organic materials such as composts and manures are available in the literature, much of these works are focused on temperate regions. Some examples of studies of the dynamics of N_o of manures and composts with regard to soil fertility improvement include Chae and Tabatabai (1986), Li and Li (2014), Lazicki *et al*. (2020) and Cassidy-Duffery *et al*., (2020). Observations from these studies have shown that the chemical properties of manure or compost such as C/N ratio, initial ammonium, C, lignin, polyphenol and total N were related closely to N_o of amended soils and could be used to predict N releasing capacity of these organic amendments (Gale *et al*., 2006; Lazicki *et al*., 2020, Azeez and Van Averbeke, 2010; Burger and Venterea, 2008; Calderon *et al*., 2005; Palm and Sanchez, 1990; Vahdat *et al*., 2011; Palm and Sanchez., 1991). The understanding of the N release rates from manures and composts and in general agricultural wastes is useful in the overall soil fertility management to prevent excessive application of fertilizers where compost and manures have been previously applied as well as to minimize environmental health risks associated with excessive nutrient leaching.

However, whereas BMCs are gaining usage in the tropics as soil amendments, for the reasons previously advanced, knowledge of their composition, the soil and environmental controlling factors have not been adequately studied to synchronize N release with plant demand in varied soil types. In particular, BMCs containing different ON pools may differ in the kinetic parameters of N mineralization such as N_o and k compared to single N pool BMCs. It is plausible that the incorporation of biochar into manure, though increases the overall C content, can also introduce C of different stabilities. The same argument may be valid for N, as the C/N ratio of biochar is different from that of manure.

Indeed, studies have shown that biochar type, rate of biochar application and the composting mixture have a significant influence on the release and availability of N as well as the rate of mineralization (Antonangelo *et al.*, 2021, Thiele-Bruhm and Ngigi, 2021, Tsai and Chang, 2020). For instance, Tsai and Chang (2020) reported a 19% decrease in N mineralization rate when 2% woody biochar and compost were incorporated into the soil. Further, a 12% biochar rate decreased N content of the final compost, resulting in N immobilzation (Awasthi *et al*., 2017). Agyarko-Mintah *et al.* (2017a) also reported that plant-based biochar with relatively larger surface area tends to absorb labile N and increases recalcitrant ON, reducing N mineralization compared to manure-based biochar. Yuan *et al*. (2017) and Singh *et al*. (2010) reported a decrease in N mineralization when co-composted rice hull and woody biochar and manure were applied to the soil owing to stable OC and ON. These characteristics of biochar types and rates influence the rate and extent of N availability, preventing N loses and yearly re-application of BMCs, and hence improve crop productivity (Kammann *et al*., 2015; Agegnehu *et al*., 2016, Schulz *et al*., 2013; Tsai and Chang, 2020). However, the effect of BMCs produced from different biochar types, biochar rates and composting mixtures on N availability in tropical soils has scarcely been explored.

Application of BMC to soils has also been reported to improve microbial activities through increases in microbial biomasses and their associated enzymatic activities such as amidohydrolase activities (AA) (Manirakiza *et al*., 2019, Guo *et al* 2020, Antonangelo *et al*., 2021; Yuan *et al*., 2017; Lu *et al*., 2015; Sekaran *et al*., 2020). Amidohydrolases are enzymes responsible for hydrolyzing the C-N bond in amino acids and other amides releasing ammonia as a by-product (Tabatabai, 1994; Dodor, 2002; Dodor and Tabatabai, 2003). Their function in soils is critical in the release of inorganic N for microbial assimilation and plant uptake. Soil microbial biomass C (C_{mic}) and N (N_{mic}) are, however, labile components of organic matter in the soil, which play a role in the C and N cycling (Dalal, 1998). As a result, BMC incorporation into soil enhances these microbial parameters indirectly enhancing nutrient cycling, OM decomposition and nutrient availability hence enhancing soil fertility and crop productivity (Azeem *et al*., 2020; Schulz *et al*., 2013).

The incorporation of BMC into soils not only increases the availability of labile C and N, but also the porous structure of the biochar improves aeration, maintains the soil's water holding capacity and creates microhabitats that enhances SMB and associated amidohydrolase activities. Due to their sensitive biological nature and ability to react quickly to changes in management practices, SMB and AA are often employed to evaluate the health and quality of soil (Dodor, 2002; Dodor and Tabatabai, 2003; Tabatabai *et al*., 2010). Whereas many studies have shown the positive influence of BMC on SMB and associated enzyme activites, few studies have also indicated the converse. For example, Dempster *et al*. (2012) found a decrease in C_{mic} when composted pig manure and Eucalyptus biochar were applied to the soil. While the authors attributed their findings to biochar type, another study by Elzobair *et al*. (2016) and Feng *et al.* (2019) which indicated similar effects on extracellular enzyme activities, attributed this occurrence to the rate of biochar and the composting feedstock. Therefore, not only the biochar type, but also the rate of biochar and composting feedstock in the co-composting mixture, are important in determining the overall biochemical and microbiological properties of the BMC. The manner in which biochar types and composting feedstock affect the microbiological and biochemical properties, N_o and N release dynamics in tropical soils, however, is yet to be unraveled.

Furthermore, it is known that the magnitude and activities of biological indicators of OM often depend on one another. For example, studies by Tabatabai *et al*. (2010) found that the activity of four amidohydrolases in limed agricultural soils showed significant correlation with the amount of N mineralized in a 20-weeks incubation study. In a 24-week incubation at 30°C, Dodor and Tabatabai (2003) similarly discovered a positive and significant relationship among the activities of amidohydrolases, C_{mic}, N_{mic}, and the quantity of N mineralized. Indeed, microbes are the main originators of enzymes, as a result SMB could correlate positively with amidohydrolase activities. In the light of these findings, it could also be hypothesized that BMCs can also have direct influence on C_{mic}, N_{mic} and amidohydrolase activities, and hence influence N_o and N release in soils amended with BMCs.

Soil ON can be grouped into hydrolysable ammonia N (HAN), hydrolysable amino acid N (HAAN), hydrolysable amino sugar N (HASN), hydrolysable unknown N (HUN), total hydrolysable N (THN) and non-hydrolysable N (NHN) pools or fractions based on biochemical composition (Bremner *et al*., 1965). Mineralization of these ON pools involves the breakdown of various ON bonds brought about by the activity of the amidohydrolases. Observations by Sekhon *et al*. (2011) indicated that manure application increased the proportion of HAAN, HASN and HAN in soils. However, the manner in which the application and ON composition of BMC affect the enzymes and their ability to hydrolyze N bonds to N release remains a lingering research question.

1.3 Objectives

The overall objective of this study was to investigate the influence of compost produced from agricultural and municipal solid waste, with or without biochar, on the N releasing capacity, microbial and enzymatic activities of three tropical soils. Specifically, the study was designed to:

1. determine the parameters of N mineralization dynamics: N_o and mineralization rate constant (k) for the various BMCs produced from three biochar types (rice husk, sawdust, and coconut husk), market wastes and two manure types (poultry and cattle manures), using the long-term (26-week) earobic incubation procedure proposed by Stanford and Smith (1972),
2. asses the impact of the BMCs when amended to three different soils (Adentan, Denteso and Keta) on selected soil properties (pH, TC, ON, POXC contents), activity of amidohydrolases (L-glutaminase, L-asparaginase and amidase) and soil microbial biomass (C_{mic} and N_{mic}).
3. assess the ON composition of BMCs and their relation to N mineralization potential and amidohydrolase activities involved in hydrolyzing ON bonds in soils.

1.4 Organization of the thesis

The thesis contains six chapters with Chapters 1 and 2 being the general introduction and literature review, respectively. Chapters 3, 4 and 5 are prepared manuscripts to be submitted for publication in refereed peer-reviewed journals. Chapter 3 describes the potentially mineralizable N (N_o) and the rate constant of N mineralization (k) of different BMCs in three tropical soils (Adentan, Denteso and Keta series). This chapter also assessed the relationship between N_o and properties of the BMCs as well as the differences in N_o among the three soils. Chapter 4 assessed the effect of BMCs on the microbial biomass C (C_{mic}) and N (N_{mic}) as well as amidohydrolase activities (AA) (L-asparaginase, L-glutaminase and Amidase). In addition, Chapter 4 investigated the relationship among C_{mic}, N_{mic}, AA, N_o and properties of the BMC-amended soils. The various ON fractions and their distributions in the BMC-amended soils and their relationships with N_o and AA are discussed in Chapter 5. Chapter 6 summarizes the results of the studies as the General Conclusion and outlined some recommendations.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Soil: Natural fertility and management

2.1.1. Natural soil fertility

Soil is a product of weathering of rocks. The fertility of soils is derived in part from the types of rocks that formed the parent material (Brady and Weil, 2008). For example, parent materials consisting of minerals such as feldspar and biotite will weather to form soils with high calcium (Ca), Magnesium (Mg) and potassium (K), whilst those from hornblende will generate magnesium (Mg), calcium (Ca) or sodium (Na) (Brady and Weil, 2008). Quartz or koalinitic soils have low fertility due to fewer nutrients released during weathering (Sanchez, 2019). However, since the inherent fertility of the soil from parent material can be limited, particularly in areas where the parent rock is low in nutrients or the soil has been subjected to weathering over an extended length of time, practices such as fertilization, crop rotation and cover cropping are necessary to maintain its productivity. By far, however, soil fertility in tropical lands is derived from organic matter addition. These are added to the soils as plant and animal residues that decompose to release nutrients. Data by Dawoe *et al.* (2010) indicate that about 8 to 10.4 tons/ha of forest litter are added to the soils in the humid zones of Ghana, whereas additions of residue in tropical savannahs range from 172.4 to 874 metric t/ha (Fisher *et al.,* 1994).

2.1.2 Soil nitrogen

The soil is a repository of many elements. However, for the purposes of supporting agriculture, the elements of importance can be grouped into two main classes: (i) the macro-nutrients such as nitrogen, phosphorus and potassium, and (ii) the micro-ntrients, e.g., Zinc, Boron, Manganese, Iron, Copper etc (Sanchez, 2019). Of the macro-nutrients, nitrogen, which is the focus of this study, is most important and is required in large quantities as a vital constituent of proteins, enzymes and other cellular constituents such as nucleotides and chlorophyll (Leghari *et al*., 2016). Air as the major reservoir of nitrogen constitutes about 78% of nitrogen gas (N_2). The N_2, however, is not readily usable because of the strong triple bonds between the N atoms in the N_2 molecules making it relatively inert (Sanchez, 2019). However, the N_2 can be made available to plants through a series of natural activities such as lightning and microbial transformation (Lim *et al*., 2018). The microbial transformation of nitrogen is through the symbiotic association between bacteria (rhizobia) and leguminous crops as well as the non-symbiotic organisms (Kormondy, 1996). Another means of making N_2 available is the addition of synthetic nitrogen fertilizers produced by the Haber Borch process (Smil, 2004). The decomposition of plant residues and Soil Organic Matter (SOM), such as composts, manure, sludge etc. also comprise major ways in which nitrogen is added to the soil for plant uptake and use. The decomposition process is by various microorganisms in the soil (González-Ubierna *et al*., 2012). Nitrogen is absorbed by plants from the soil as nitrate or ammonium ions, but these are converted into amino acids, proteins and DNA in plant tissue (Masclaux-Daubresse, 2010). Animals consume plants while both plants and animals are consumed by humans, where these nitrogen compounds are an essential part of human nutrition. Other forms of nitrogen can be found in anaerobic conditions in the reduced forms e.g., NO_3^- are reduced to nitrous oxide (N_2O), nitric oxide (NO) and nitrogen dioxide (NO_2) (Hofman and Cleemput, 2004).

2.1.3 Management of soil fertility in temperate regions

The pre-historic management of soil fertility, which partly continues today is the application of animal manures and composts (Loss *et al.,* 2019). The practice, according to Sanchez (2019) dates from the dawn of agriculture and till now continues globally even in mechanized agricultural systems. Manure is rich in a number of elements such a nitrogen, potassium and phosphorus and their content in the manure is related to the type of feed taken by the animals (Azeez and Van Averbeke, 2010). The nutrient content in manure is necessary for optimum crop growth in addition to promoting soil microbial activity, building soil structure, and improving the soil's capacity to retain water (Ilahi *et al.,* 2020; Loss *et al.,* 2019). The organic matter that manure adds to the soil improves its ability to hold onto nutrients (i.e Cation exchange capacity of soil) for plant use (Loss *et al.,* 2019). These improved characteristics of soil by manure help enhance the productivity and hence promote crop yields (Antoneli *et al.,* 2019; de Moura Zanine and de Jesus Ferreira, 2015). Furthermore, animal manure can be an environmentally sustainable way to dispose off waste products, to reduce greenhouse gas emission and soil erosion (Diacono and Montemurro, 2011; Zhang and Schroder, 2014). Also, the use of manure helps reduce the use of inorganic fertilizers serving as a cost-effective soil amendment with several environmental advantages while maintaining crop yields (Kumar *et al.,* 2019). Because of these benefits, manure can help crop production systems become more profitable and environmentally sustainable. Despite the fact that animal manure has been applied to improve soil and serve as crop fertilizer, it has several disadvatges which include difficulties in storage and handling of manure due to its bulkiness and volume, the need for labour in transporting and spreading on farms, can be time-consuming to properly apply and manage manure on farm, the need to reapply yearly may result in large quantities needed and high concentration of nitrogen and phosphorus in manure can contribute to nutrient pollution and algal blooms in nearby waterways (Jensen, 2013; Khoshnevisan *et al.,* 2021; Dubrovsky and Hamilton, 2010). Additionally, excessive manure use may result in unpleasant scent, carriers of disease-causing pathogens and visual pollution during handling (Spiehs and Goyal, 2007; Kusiluka *et al.,* 2012).

A more effective way of applying such nutrients to soil is by composting. This method has been the most popular for processing organic waste because of its cheap operational costs and significant social and environmental benefits (Lim *et al*., 2018). Composting may be described as a biological breakdown of organic materials into a stable product through mineralization and partial humification of organic matter by action of diverse microorganisms which transforms the organic matter into CO_2, H_2O, NH_3, organic acid and other compounds, improving nutritional quality and recycling (Bernal *et al*., 2009) This product generated has soil quality enhancing effects and prevent negative impacts such as immobilization of plant nutrients and phytotoxicity when wastes are applied directly to the soil (Dias *et al*., 2010). In animal manure fertilization, composting has been suggested as a sustainable method of dealing with fecal waste since it can reduce the volume and bulkiness of manure in addition to eliminating harmful pathogens and weed seeds (Chen *et al*., 2010; Larney *et al*., 2006; Bernal *et al*., 2009).

Also, composting is known to produce a more stable form of organic fertilizer which controls nutrient release especially N and provides a safer method of fertilization to the environment and crop production (Chen *et al*., 2010; Bernal *et al*., 2009). It is important to note that applying compost to the soil may limit nitrogen losses such as nitrate through leaching and ammonium through volatilization.

Also, incorporation of compost to agricultural lands is a practice that has gained much importance since its inception many years ago and has been discovered to provide positive effects in terms of soil organic carbon enhancement, soil water holding capacity, nutrient replenishment, microbial population enhancement and other related soil properties (Lim *et al*., 2018; Yuan *et al*., 2017; Sharifi *et al.,* 2014; Helgason *et al*, 2005; Ofosu-Budu *et al.,* 2008). Due to these effects, soil fertility and productivity enhancement has been identified and documented by many authors (Boakye *et al.,* 2023; Yuan *et al*., 2017; Helgason *et al*., 2005; Abdel-Rahman, 2009; Ofosu-Budu *et al.,* 2008).

Working on a Ferric Acrisol, Boakye *et al.* (2023) discovered a maize grain yield of 4.4 t/ha and 3.6 t/ha in the major and minor cropping seasons respectively when composted municipal solid waste, pig manure and goat manure were applied. These results according to the authors were due to the enhancement of the soil's fertility and improvement in nutrient availability. Working with composted phosphate rock, cocoa pod husk, poultry manure and sawdust at a rate of 875 kg ha^{-1}, Ofosu-Budu *et al.* (2008) found an increase in nodules numbers, number of leaves, plant biomass, number of pods per plant and grain yield of cowpea (*Vigna unguiculata*) compared to control (no amendment). According to the authors grain yield of cowpea was 25.8% greater than the control with the application of compost, via the enhancement of the fertility of soil. Additionally, Abdel-Rahman (2009) reported an improved sorgum grain production by 45% by applying 5 t ha^{-1} of compost compared to no compost amended plots (control). The modern form of soil fertility management relies on fertilizer application which improves and maintains soil fertility as well as sustain crop productivity due to the rapid nutrient release (Pahalvi *et al.,* 2021). In chemical fertilizers, nitrogen (N), phosphorus (P), and potassium (K) are the three main nutrients, which are used to supplement the natural nutrients present in soil (Pahalvi *et al.,* 201; Kumar *et al.,* 2019). The usage of fertilizer application on crops and pastures in developing countries can be high (Ahmed *et al.,*2017; Pahalvi *et al.,*2021). According to estimates, 10.8 million metric tons of chemical fertilizers were utilized in the year 1960, which increased to 82 million metric tons in 2000 and expected to rise to 249 million metric tons in 2050 (Ahmed *et al.,* 2017). Data has shown that the rate of N applications often reaches 360 kg N/ha while P rates also reach 150 Kg P/ha and K reaches 120 Kg K/ha (Duan *et al.,* 2019). The yields of crops that receive fertilizer applications are also high. Maize yields as high as 12 tons/ha have been harvested in most farms (Chen *et al.,* 2022). Even though applying chemical fertilizers to crops improves crop productivity, its inappropriate use has a negative influence on the soil and environmental health which is a cause for worry (Ahmed *et al.,* 2017; Kumar *et al.,* 2019; Dar *et al.,* 2016; Dervesh *et al.,* 2020). During application to the soil, excessive fertilizer may result in slow growth of plants, delayed maturity and low quality of leaf which eventually lead to low productivity. Also, soil acidity, decrease organic matter, humus and loss of beneficial soil organisms may result from excessive chemical fertilization on soil (Kumar *et al*., 2019). It has also been documented that soil acidity may reduce phosphate intake by crops increasing its toxicity in soil and inhibiting plant crop growth (Kumar *et al.,* 2019). Pollution in the environment result when excessive chemical fertilizers are utilized resulting in algae bloom (eutrophication) in aquatic bodies and limiting oxygen supply to aquatic life (Liu *et al.,* 2014), contamination of groundwater due to nitrate leaching (resulting in methemoglobinemia in infants, birth defects and gastric cancer which is linked with nitrosoamines) (Kumar *et al.,* 2019) emission of greenhouse gases (NH_3, NO, CO_2, N_2O, CH_4) which destroy the ozone and exposes humans to harmful ultraviolet radiation (Savci, 2012). Furthermore, chemical fertilizer applied excessively contaminates the soil with heavy metals including uranium, cadmium, and arsenic which builds up in cereals, fruits, and vegetables (Sonmez *et al.,* 2007). For example, trace elements such as arsenic and cadmium found in fertilizers like triple superphosphate accumulate in plants and can have an impact on human health through the food chain. In conclusion, it can be stated that overuse of chemical fertilizers has detrimental effects on the ecosystem and soil, that are substantial and long-lasting (Kumar *et al*., 2019; Pahalvi *et al.,* 2021; Ahmed *et al.*, 2017)

2.1.3 Management of soil fertility in tropical regions

Tropical soils are formed in the tropics under high temperature and rainfall conditions. It is often found that the mean temperature at a depth of 50 centimeters in the soil is greater than 5 °C (Sanchez and Boul, 1975). The high ambient temperatures influence the speeds of chemical reactions and hence the pace of parent rock weathering (Aguiar, 2022). Also, high rainfall leads to the leaching of basic cations out of the soil profile, thereby concentrating oxides and hydrooxides of iron, and aluminum sesquioxides (Aguiar, 2022). These processes finally lead to lateritic soils, which differ from soils created in temperate climates (Aguiar, 2022). Also, the processes of surface erosion and deposition, which actively weather the parent material have resulted in the formation of many more soils in the tropics (Sanchez and Boul, 1975).

One important characteristic of tropical soils is the levels of organic matter in these soils. Although Sanchez and Boul, (1975) documented that variations in the levels of organic carbon between tropical and temperate soils may be statistically or agronomically insignificant, the high temperatures often lead to high biological activity, leading to a rapid decomposition of organic matter. Consequently, the soil fertility of cropped fields declines rapidly and with external inputs, the fertility remains low (Sanchez, 2019).

The traditional technique that has evolved in the tropics to maintain soil fertility is linked to cropping or farming systems. The most common is the shifting cultivation system (SCS) which is probably the oldest farming system in the tropics practiced for thousands of years (Bellwood, 2005). Formally SCS was practiced throughout the world but over the past three centuries it has been practiced by small-scale and rural communities in the tropical forest due to population increase in the temperate regions (Sanchez, 2019; Altieri, 1999). This system involves the clearing of forest or woodland of its vegetation and the land is burnt for agricultural purposes for a period of 1 to 3 years followed by secondary fallow system for 15 to 25 years (Filho *et al.,* 2013; Sanchez, 2019). Though the burning process decrease in organic matter, loses of nutrients (N, P and S) through volatilization, decrease in forest biodiversity and soil structure occurs but soil fertility is increased through increase in soil pH, basic cations and cation exchange capacity of the soil (Mg, Ca, Na and K) by the wood ash produced (Filho *et al.,* 2013; Brady, 1996; Gafur *et al.,* 2000). Data by Gafur *et al.* (2000) showed that about 234 kg Ca ha^{-1}, 55 kg Mg ha^{-1} and 20 kg K ha^{-1} was returned to the soil during a forest clearing and burning in the Chittagong Hill Tracts of Bangladesh. Pests and diseases are also controlled when the vegetation is burnt (Sanchez, 2019). Furthermore, removal of forest cover increases the soil temperature thereby accelerating the SOM decomposition and nutrient mineralization which provide nutrients during the cultivation stage (Filho *et al.,* 2013). However, the removal of nutrients during the cultivation stage, runoff, leaching or erosion during the clearing and burning stages causes a decline in nutrients (Filho *et al.,* 2013). The two most important disadvantages of this system are the emission of CO_2 into the atmosphere and deforestation. According to Fearnside (2005) and Brown and Lugo, (1990) areas where shifting cultivation are mostly practiced may be a major contributor to global warming. and the soil as an emitter of CO_2 to the atmosphere. Housgton *et al.* (1991) and Serrao *et al.* (1996) documented that deforestation due to SCS was 10% in Latin America and 30-35% on the Amazon Forest. Reports by Achard *et al.* (2010) also showed 300 million ha of loss of forest areas from the year 1990 to 2012 to SCS. The authors also estimated a contribution of 12 Pg C yr^{-1} (12%) of the world's greenhouse emission from tropical deforestion by SCS in the year 1997 to 2006. On the other hand, the fallow stage which is abandoning the cultivated land to the regrowth of secondary forest restores the forest to its original state before agricultural management (Kleinman *et al.,* 1995; Mertz *et al.,* 2009). This stage reduces the negative impact of SCS on the soil and the environment such as rehabilitating the soil physical properties, accumulation plant biomass and nutrients through the secondary fallows as well as reducing deforestation and CO_2 emission. Although SCS helps to reestablish the soil, increasing population pressures affect the length of the fallow period making it less likely for the soil to regain high productivity levels (Gafur *et al.,* 2000; Sanchez, 2019). The short fallows are mostly less than 10 years and commonly 2-4 years which is inadequate for the soil to accumulate enough biomass and regain its fertility (Sanchez, 2019).

The challenges facing the SCS have apparently compelled many tropical countries to adopt other soil fertility maintenance methods. The application of manure is common where animal production is a major landuse system in northern and coastal savanah zones. Intensive vegetable cultivation is often supported by manures application (Awadzi *et al.,* 2008). More recently composting of manure and organic waste (e.g., market waste) are becoming a common practice as this concentrates the nutients and improves crop productivity (Bernal *et al.,* 2009). It is estimated that each year, a total of 760,000 tons of market waste is generated in Accra alone (Anomanyo, 2004). The estimates for the whole of Ghana lie in 4.5 million tons (Obirih-Opareh, 2002; Anomanyo, 2004). The disposal of these wastes is now a major challenge but can be converted to compost. Data by Boakye *et al.* (2023) showed that 4.4 tons/ha of maize could be attained when the crops received MSW compost application compared with soil without compost application.

By far, the application of soluble chemical fertilizers has become the major method of soil fertility management. A major pillar of the Government of Ghana's agricultural policy such as "Planting for Food and Jobs (PFJ)" is the promotion of fertilizer use for crop production due to the ferililzer subsidy program implemented (Pauw, 2022; MoFA, 2017). It is estimated that an average of 429,261 metric tons of chemical fertilizer was imported into Ghana in the year 2016 to 2020 (IFDC, 2021). According to the Food and Agriculture Organization of the United Nations (FAO), Ghana's fertilizer consumption has been increasing steadily over the years from 165,119 metric tons in 2006 to 476,872 metric tons in 2019, indicating an increase of 189% due to the PFJ program. Also, on the average it is estimated that N fertilizer use in Ghana by farmers has increased from about 12 kg N/ha to 60 kg N/ha giving an average yield of 1500 kg/ha of maize (Ragasa *et al.,* 2014, Tetteh *et al.,* 2017) and contributing only about 20% of what is produced in the advanced countries.

However, despite the increase in fertilizer use, due to the PFJ program, there are still challenges such as inadequate access to credit facilities and poor extension services, which affect the adoption and proper usage of fertilizers (Pauw *et al.*, 2022). Additionally, the quality of fertilizers sold on the local market is a concern as some imported fertilizers are of low quality, which affects crop yields (Pauw *et al.*, 2022).

2.2 Soil Nutrients

2.2.1 Nitrogen in tropical soils

Nitrogen is a vital nutrient for plant growth and development in tropical soils (Leghari *et al.,* 2016), and its availability varies widely depending on the soil type, climate and management practices (Sanchez, 2019). In tropical regions, nitrogen is often limiting in soils due to rapid weathering and organic matter decomposition, nitrate leaching, and microbial immobilization. According to Sanchez and Boul, (1975) variations in the levels of organic carbon between tropical and temperate soils may be statistically or agronomically insignificant, however a 10ºC rise in temperature in the tropics can cause biological activity to double. This phenomenon causes an increase in organic matter decomposition and nutrient mineralization, especially nitrogen in tropical soil. With heavy rainfall, nutrient loss, particularly nitrate leaching resulting when crops do not utilize the nutrient quickly (Aguiar, 2022). The inherent high weathering nature of the soils is also attributed to the low soil fertility (Brady and Weil, 2008). As a result, many tropical soils are described as N-limiting which have low nitrogen availability and limits plant growth and crop productivity (Sanchez, 2019; Brady and Weil, 2008).

To increase nitrogen content in tropical soils, different approaches have been used, such as the application of organic (plant residue, animal manure, compost etc) and inorganic fertilizers (chemical fertilizers) or through biological fixation where leguminous crops are used (Sanchez, 2019). Despite its benefits of increasing crop production, continuous application of inorganic (chemical) fertilizers often reduces soil organic matter and acidifies the soil leading to adverse effect on microbial and N dynamics (Kumar *et* al., 2019; Pahalvi *et al.,* 2021; Ahmed *et al.*, 2017). In addition, Motasim *et al.,* (2021) also reported that more than 50% of inorganic fertilizer are lost into the environment through ammonia volatilization, denitrification, leaching, and runoff resulting in human and environmental health issues such as eutrophication of water, loss of biodiversity, and stratospheric ozone depletion as well as reducing N use efficiency of crops in tropical soils. Burgos *et al.,* (2006) also documented similar problems with the application of organic N amendment in tropical soils. Therefore, careful management is needed to prevent losses and its associated negative impact in the environment.

2.2.2. Animal manure as N source in tropical soils

Large quantities of organic waste are often produced as a result of production of livestock and poultry products for human consumption which is driven by urbanization, population growth and changes in consumer behavior (Hoornweg *et al.*, 2013, Delgado, 2005). These wastes consist of a mixture of faeces, urine, used bedding and waste fed with varying water content (Szogi *et al.*, 2015). According to Hoornweg *et al.* (2013), waste from the production of livestock and poultry is projected to exceed 11 million tons on a daily basis at the end of the twenty-first century. In Ghana, poultry and livestock production has generated about 2.4 million tons of wastes in the form of manure in the year 2020 which is projected to increase in the subsequent years (Seglah *et al.*, 2022). Therefore, proper disposal of these wastes is essential for the efficient management of organic solid waste.

To curb this problem, animal waste has been converted to manure which are incorporated into the soil in addition to its nutritional benefits. According to Issaka *et al*., (2012), poultry, goat, sheep, pig and cow manures are beneficial to the soil in terms of their nutritional benefits. Poultry manure is high in magnesium and calcium due to the addition of calcium enriched dietary ingredients to chicken feeds especially layers (Issaka *et al*., 2012).

All essential nutrients especially N, P and K are abundant in animal manure (de Moura Zanine and de Jesus Ferreira, 2015). Among all the nutrients in manure, nitrogen is described as the most important because it is limiting in the soil. High levels of nitrogen are found in sheep, goat and cow manure. These animals typically mix their manure with their urine which contributes to the relatively high nitrogen content of their manure (Issaka *et al*., 2012, de Moura Zanine and de Jesus Ferreira, 2015). Other authors have also observed high amounts of N in poultry manure (Azeez and Van Averbeke, 2010; Calderon *et al*., 2005, Li and Li, 2014) compared to livestock manure, which is attributed to animal feeding and management practices. According to Azeez and Van Averbeke (2010), chickens mostly feed on grains and cakes with high levels of proteins and fats compared to forages fed to livestock of low N content.

Manures have a long-term effect on the N content in soil due to their slow release thereby improving soil and crop productivity. As a result, tropical soils, especially those used for intensive vegetable cultivation, are supplied with manure to alleviate the nutrient deficiencies in the soil, especially N. In northern Ghana, where animal rearing is a major land use, large quantities of cowdung are applied to the otherwise low N soils to enhance soil fertility (Ayamba *et al.,* 2021; Issaka *et al*., 2012). In the southeast part of Ghana, where the soils are sandy, farmers use poultry and cattle manure to sustain crop productivity. According to reports, the application of the manure which is purchased from kraals and poultry farms from the environs of Keta lagoons enhances the soil fertility, structure and water holding capacity of the soil (Awadzi *et al*., 2008). The manure application has also been found to increase the microbial consortia and enhance the release of N (Awadzi *et al*., 2008; Timsina, 2018). The enhanced physical, chemical and biological characteisics of the soil by manure application have been translated into increased crop growth and yield. Studies by Boateng *et al.* (2006) in the semi-deciduous rain forest zone of Ghana on a Ferric Acrisol demonstrated a 2.07 t/ha increase in maize grain yield when a 4 t/ha poultry manure was applied compared with the soil alone. In addition, the authors registered a 53% increase in soil N as well as an increase in exchangeable cations which contributed to the enhanced yield of maize. Mpanga *et al.* (2021) also reported an enhanced garden egg growth (dry matter by 73%) and yield by 66% in a slightly acidic sandy-loam in Ghana when poultry manure was applied to the soil compared to the unamended soil of which the authors attributed to the soil mineral enhancement.

Despite the generally beneficial impact of manure application to soils and plant growth, some health hazards have also been associated to excessive N loss by leaching (Szogi *et al*., 2015). According to Bai *et al*. (2016) approximately 78% of the manure nitrogen can be lost to the environment. The environmental problems can be categorized into three which include issues with the soil (nutrient buildup), the water (eutrophication) and the air (global warming, odors) (Kumar *et al.,* 2013). An excessive amount of manure N applied to the soil might lead to $N0_3$ and NH_3 buildup as a result of microbial mineralization of organic N (Kumar *et al*., 2013). Although these types of reactive nitrogen are necessary for plant growth, their losses to the environment can have an adverse effect on the quality of air and water (Kumar *et al*., 2013). Elevated NO_3 level resulting from NH_4 oxidation leads to eutrophication of freshwater and groundwater sources through leaching and runoff, which in turn promotes excessive algal growth, which occasionally causes a significant loss of fish as well as reducing the overall quality of water (Dubrovsky and Hamilton, 2010). In general, less biodiversity may result from environmental enrichment of N (Stadler, 2012; Isbell *et al.,* 2013). Further losses of N into the environment include the gaseous emissions such as NH_3 and nitrogen oxides (NOx) due to collection, storage, and land disposal of manure. These gases contribute to N-containing fine particulate matter in the air, ground level ozone, rain acidification, which in turn affects adversely water and air quality as well as human and animal health (Sutton and Bleeker, 2013). Also, the unpleasant odour associated with livestock husbandry as a result of the production of CH_4 gases are of concern. Nitrous oxide and CH_4 emissions indirectly contribute to global warming (Suddick *et al.,* 2013). According to Bhatia *et al*. (2013), CH_4 and N_2O emissions contribute 15 to 20% and 6%, respectively to global warming. Other environmental issues with excessive use of manure also include dust, noise, visual pollution, animals and their manure as carriers of pathogens (Sobsey *et al.,* 2006; Liu *et al.,*2022).

As indicated in section 2.1.3 above, composting provides a means of concentrating nutients in organic manures and hence improves its use efficiently. Nevertheless, ammonia volatilization results in a number of gas losses and the composting and application processes release greenhouse gases such carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). (Lim *et al*., 2018; Steiner *et al*., 2015). Among all the gases emitted, ammonia (NH_3) is a major gas that generates nuisance odours to humans and it is toxic to plants. According to Martin and Dewes (1992), N loss through NH_3 gas accounted for 46.8–77.4%

of the initial nitrogen in straw-manure compost. Kithome *et al*. (1999) showed a 62% loss of initial nitrogen in a composted manure as NH_3 gas. Ogunwande *et al*., (2008) also showed that 50-80% of total N in poultry manure was lost as NH_3 during composting. In addition, the agronomic value of composted manure is often reduced due to NH_3 volatilization (Ermolaev *et al*., 2015). Another problem with composting of organic materials is nitrate (NO_3) leaching which has been documented by several authors (Dias *et al* 2010; Sanchez-Monedero *et al*., 2018). As a result, several researchers have investigated the use of chemical additives, microbial amendment, modification of aeration and bulking agents to aid in composting as well as to curb the issue of N losses (Liu *et al*., 2015; Yuan *et al*., 2017; DeLaune *et al*., 2004; Zhang *et al*., 2016). The use of biochar as a compost addictive or bulking agent has received a lot of attention recently. (Guo *et al*., 2020; Xiao *et al*., 2017; Barthod *et al*., 2018).

2.3 Effect of biochar on compost quality

2.3.1 Biochar

Biochar has been identified as an effective material in composting and production of high-quality compost (Xiao *et al*., 2017, Antonangelo *et al*., 2021; Mirsha *et al*., 2022; Chen *et al*., 2017a). Described as a carbon-rich solid substance created when biomass and other carbonaceous materials undergo thermochemical breakdown in the absence or low availability of oxygen (pyrolysis), its origin is traced backed to 2,000 years ago in the pre-columbian amazon where biochar produced by igniting and smoldering biomass in pits or trenches enhanced the productivity of soils (Verheijen *et al.,* 2010; Lehmann and Joseph, 2009; Lehmann, 2007). In the South American Amazon, intentional mixing of the burned biomass with the soil produced layers of enriched soils known as terra preta, which sparked increased interest and investigation into biochar (Lehmann, 2009).

The pyrolysis of biomass into biochar involves the breakdown of polymeric building blocks of biomass such as cellulose, hemicellulose and lignin through several processes such as cross-linking, depolymerisation and fragmentation resulting in the production of char, oils and gases (Armah *et al.,* 2022). The yield of biochar is dependent on the feedstock type, pyrolysis temperature, residence time, heating rate and reactor type (Al-Rumaihi *et al.,* 2022). Different pyrolysis temperatures mostly employed range from 300 to 1000 °C with feedstock used originating from agricultural, municipal, animal or industrial source (Duku *et al.,* 2011; Al-Rumaihi *et al.,* 2022). As a result, biochar is mostly composed of carbon with other elements such as calcium, magnesium, potassium and nitrogen which is dependent on the feedstock type and pyrolysis temperature (Tomczyk *et al.,* 2020). Water and volatile organics in the biomass may evaporate during pyrolysis, raising the aromatic content and stability of the resulting biochar (Das *et al.,* 2021). As pyrolysis temperature increases above 500ºC volatile matter loss increases enhancing the formation of aromatic C component. Woody biochars can produce aromatic carbon up to 90% by weight in biochars (Tomczyk *et al.,* 2020; Verheijen *et al.,* 2010). In addition, the pyrolysis temperature and feedstock are a determinant of the physicochemical properties of the biochar produced (Verheijen *et al.,* 2010, Ippolito *et al.,* 2020; Al-Rumaihi *et al.,* 2022). For example, high temperature pyrolysing increases the alkalinity of biochar because it removes functional groups that form unpaired negative charges like carboxyl (COO−) and hydroxyl groups (OH−). These charges can then attract positive charges, increasing the cation exchange capacity (CEC) of the material (Weber and Quicker, 2018; Singh *et al.,* 2021). Also, high pyrolysis temperature leads to formation of pores through the release of volatile organics enhancing the porosity, pore size and surface area of biochar (Chowdhury *et al.,* 2016; Tomczyk *et al.,* 2020). According to Spokas *et al.,* (2009) biochar produced from woody biomass has high C content, low nutrient status, relatively large surface area, and high pH.

Because of its many advantages, biochar has recieved more attention. (Zhang *et al.,* 2021; Duku *et al.,* 2011, Verheijen *et al.,* 2010). Over the years it has been used to enhance soil fertility (Laghari *et al.,* 2015; Schulz and Glaser, 2012; Yeboah *et al.,* 2009; Güereña *et al.,* 2013) immobilize and sorb contaminants (Zheng *et al.,* 2012; Zhang *et al* 2021), sequester carbon (Sohi, 2012; Verheijen *et al.,* 2010), reduce greenhouse gases (Spokas *et al.,* 2009; Sri Shalini *et al.,* 2021) as well as increase structure stabilization and infiltration of water (Mukherjee and Lal, 2013; Blanco-Canqui, 2017; Dugan *et al.,* 2010) in soils of which many reviews and studies have documented

As a soil fertility and structure stabilization enhancer, biochar has been reported to improve the physical, chemical and biological characteristics of the soil. Due to the porous nature of biochar, a range of the physical properties such as soil moisture content, water holding capacity (WHC), infiltration, total porosity, bulk density, structure stability and pore size distribution are improved (Sohi *et al.,* 2010; Aslam *et al.,* 2014; Blanco-Canqui, 2017; Mukherjee and Lal, 2013) Studies by Sohi *et al.* (2010) found that biochar contributed to new pores in the soil which were mostly less than 0.002 µm in diameter although other studies have shown degree of macroporosity which is in the range of 1 to 10 µm. These pores contributions from biochar have been found by Mukherjee and Lal (2013) to increase water storage as well as pore diameter in amended soils hence increasing the porosity, WHC and lowering the bulk density of amended soils. Similar work in Ghana by Dugan *et al.* (2010) showed an increased in WHC in three Ghanaian soils especially in the coarser textured soil when corn stover and sawdust biochar was applied at 5 t/ha compared to untreated soils. The authors attributed this occurrence to the smaller pores size and water retention capacity of biochar.

In addition, biochar is known to increase organic matter content in soil and supply nutrients (e.g., P, N, K, Ca, Mg) previously existing in the feedstock thus improving soil fertility and productivity. This can be achieved by the influence of biochar on the physical, chemical and biological characteristics of soil through the alteration in soil pH, improving nutrient retention through cation adsorption and enhancing pore size and surface area of soil (Zhang *et al.,* 2021). An experiment conducted by Laghari *et al.* (2015) with the application of pine sawdust biochar on sandy desert soil revealed an enhancement in the water holding capacity, carbon content and total phosphorus of the soil by 32%, 11%, 70% respectively thereby improving in sorghum dry weight yield by 22% compared to the control. Also, workdone by Schulz and Glaser (2012) by the application of biochar on an infertile sandy soil revealed a significant increase in pH, total N, available P and organic C hence increased the growth of oa. Yeboah *et al.* (2009) showed a 4 and 5% N recovery in two contrasting soils in Ghana when biochar was added through the enhancement of organic C and exchangeable Ca of the soil which translated into improved shoot and root yield of maize. Furthermore, biochar has been identified to reconstitute microbial environment by increasing microbial activities, enzymatic activities and improving microbial community structure which can translate into increase crop productivity through nutrient cycling and OM decomposition. Many researches have documented such positive effects by biochar

(Muhammad *et al.*, 2014; Oladele, 2019, Gul *et al.,* 2015). Working with some sandy clay loam soils under rice cultivation, Oladele (2019) found that biochar increased soil invertase, urease and phosphatase activities especially when higher rates were applied. In addition, Chen *et al.,* (2017a) reported a higher increase in microbial abundance and activity, higher total PLFA concentration, and altered community structure when biochar was applied in a soil under bamboo plantation. Güereña *et al.* (2013) reported that these changes in community structure could alter microbial mediated nitrogen dynamics such as nitrification. These positive influences by biochar are as a result of changes in the physicochemical properties of soil which causes a change in microbial habitat and metabolism and induce responses in microbial species (Gul *et al.,* 2015; Zhu *et al.,* 2017). Although the majority of literature points out the beneficial aspect of biochar as soil amendments, there are some limitations. Firstly, biochar is unable to provide nitrogen in N limiting soils due to its high C: N ratio, a situation that can be counter-productive to plant growth (Lehmann *et al.,* 2003). Furthermore, biochar can absorb and react with soil nutrients (eg. N, P, Fe) to act as a competitor instead of producing nutrients (Kim *et al.,* 2015). Research conducted by Joseph *et al.* (2018) and Xu *et al.* (2016) highlighted these limitations of biochar. Therefore, it has been proposed that biochar should either be co-composted or co-applied with other organic materials for maximum utilization in soils (Lentz and Ippolito, 2012)

2.3.2 Biochar manure co-composting

Adding biochar to manure or other compost feedstock mixture (also known as co-composting) has the aim of improving the physical, chemical and biological characteisics of the final compost (Sanchez-Garcia *et al*., 2015; López-Cano *et al*., 2016, Steiner *et al*., 2010; Jindo *et al*., 2012; Dias *et al*., 2010). As a bulking agent, biochar acts as a suitable physical structure which lowers the bulk density of the composting pile and promotes aeration by increasing the air voids of the pile (Dias *et al* 2010; Sanchez-Monedero *et al*., 2018). Again, the inclusion of biochar enhances the particle size distribution of the composting mixtures by reducing the development of huge clumps greater than 70 mm and increasing the proportion of small particle fractions that is between 0.25 and 2.0 mm (Sanchez-Garcia *et al*., 2015, Zhang and Sun, 2014). Biochar has been reported to improve the water holding capacity and control moisture variations in composting piles (Prost *et al*., 2013; López-Cano *et al*., 2016). This accelerates the composting process by stimulating microbial activity, increasing temperature (thermophilic stage) and shortening the overall time for composting (Barthod *et al.,* 2018; Steiner *et al.,* 2010; Liu *et al*., 2017; Jindo *et al*., 2012).

Another important function of biochar in composting is related to the chemical composition of compost. The C and energy sources provided by biochar enhance microbial proliferation (Sanchez-Monedero *et al*., 2018). Enhanced microbial activity results in the accelerated degradation of organic matter and the breakdown of complex biopolymers to more simple organic compounds (carbohydrates, phenolic compounds, and amino acids) leading to reduced dissolved organic carbon (DOC) (Sanchez-Monedero *et al*., 2018; Wei *et al*., 2014; Akdeniz, 2019; Zhang *et al*., 2016). In general, due to the recalcitrance nature of the aromatic structure of biochar, it does not decompose throughout the composting process (Prost *et al*., 2013; Khan *et al*., 2016). However, based on the feedstock and pyrolysis temperature of biochar, organic C compounds may be released as C and energy source for microorganisms to undergo partial degradation (Dias *et al* 2010). Additionally, NH_3, NH_4^+, and H_2S that would have had an inhibitory influence on microbial activity during composting are easily adsorbed on biochar surface.

Reduced effluent output from the compost pile is another benefit of biochar's chemical absorption of DOC (Zhang *et al*., 2016). Biochar addition to composting enhances humification and regulates the degree of polymerization of the humic-like substances during composting (Jindo *et al*., 2012; Dias *et al*., 2010; Wang *et al*., 2014). Furthermore, the promotion of humic acids and humic like substances (such as fulvic acid, humic acid and humin) during composting results in the modification of the surface functional properties of the biochar improving the sorption capacity of the biochar (Jindo *et al* 2012; Dias *et al*., 2010; Awasthi *et al*., 2017). The high C: N ratio of the mixture as a result of the stable C of the biochar, reduces N loses during composting and enhances the quality of the final composts (Mirsha *et al*.,2022; Thiele-Bruhn and Ngigi, 2021). Other mechanisms for reducing N loses and other gases in compost by biochar include the large surface area, cation exchange sites, surface acid groups especially carboxylic groups and micropores formed on biochar due to modification such as oxidation during composting can adsorb and prevent loses (Agyarko-Mintah *et al*., 2017b; Steiner *et al*., 2010; Wang *et al*., 2014; Sanchez-Monedero *et al*., 2018). Several authors have shown the ability of the biochar in minimizing N losses and other gases in composting of manure and improving on the overall N content of final product. According to Steiner *et. al*. (2010) a reduction of NH_3 volatilization by 64% and total N lose by 54% was recorded when 20% of biochar was applied during composting poultry manure. Work done by Agyarko-Mintah *et al*. (2017b) revealed a reduction in ammonium emission by 60% and 55% when greenwaste and poultry manure biochar were applied in poultry manure composting. On the contrary, Akumah *et al.* (2021) showed an increase in ammonia volatilization when 15% ricehusk biochar was added to market waste composting thereby reducing the N content of final compost. The authors attributed this occurnces to the high pH of the biochar. Sonoki *et al.* (2013) also observed a decline in methane emission when biochar was applied during composting of poultry manure. However, during the process the authors reported no reduction in N_2O emission, giving an idea that biochar inclusion in composting mix may not always cause a reduction in greenhouse gas (GHG) emissions. In addition, the high CEC of biochar retains nutrients and prevents NO_3^- leaching in composting.

Composting with biochar has a significant positive effect on the macro-nutrients of compost. Mostly, biochar addition to compost reduces the NH_3^+ production and increases NO_3^- concentration due to enhanced nitrification which is brought about by increased aeration in the compost pile (Chen *et al*., 2010; Malińska *et al*., 2014). Also, the inorganic phases containing oxides of Fe, Al, and Si, carbonates of Ca and Mg as well as phosphates of Ca, Fe, and Al create a positive surface charge on biochar which allows for NO_3^- absorption (Archanjo *et al*., 2017). Furthermore, Hua *et al*. (2011) discovered through FTIR spectroscopy that biochar may develop surface acid groups after composting which may protonate and react with NH_4^+ to form stable complexes. As a result, biochar-amended compost often exhibits greater total nitrogen (N) levels than compost without biochar (Antonangelo *et al*., 2021). In addition to nitrogen, phosphorus (P) content in compost also increases with the addition of biochar which is as a result of P reserves in the added biochar (Vandecasteele *et al*., 2016; Sulemana *et al*., 2021). Other nutrients such as potassium (K^+), calcium (Ca^{2+}) and Magnesium (Mg^{2+}) also show improve retention by biochar in biochar-amended compost due to the negatively charged biochar surface (Zhang *et al*., 2016).

Biochar has the tendency of increasing the organic matter and total carbon content of the compost indirectly increasing its C: N ratio. (Wang *et al*., 2013; Wei *et al* 2014; Jindo *et al*., 2012; Dias *et al*., 2010). However, other studies have found no significant difference in C: N ratio of biochar-amended compost and compost alone which was attributed to the C: N ratio of the raw materials (Khan *et al*., 2014; Malińska *et al*., 2014). Other benefits derived from biochar in composting include the reduction of organic pollutants and heavy metals (Stefaniuk and Oleszczuk, 2016; Wu *et al.,* 2017; Zhang *et al.,* 2013), increase or decrease in compost pH (Li *et al*., 2015; Wu *et al.,* 2017; Chen *et al.,* 2017b; Zhang *et al*., 2016), achieve phytotoxicity-free levels (Mirsha *et al*., 2022; Wu *et al* 2017) and increase in population of microorganisms and enzyme activities (Jindo *et al*., 2012; Du *et al*., 2019; Wu *et al* 2017). The latter is as a result of the provision of water-soluble carbon, source of energy, microporous space, ability to retain water and nutrient and protective habitat by biochar for proliferation of microorganisms (Sanchez-Monedero *et al*., 2018; Wei *et al*., 2014; Thies and Rillig, 2009).

2.3.3 Effect of biochar type, rate, and feedstocks on co-composting

The effectiveness of biochar amendment in composting may be influenced by a number of factors, including biochar application rates, time of biochar application and biochar types. With a maximum rate of 50% (w/w), the rates of biochar addition commonly vary from 2% to 20% (w/w) (Xiao *et al*., 2017). The effects of applying biochar at rates ranging from 3% to 50% (by weight) have been studied in the past (Awasthi *et al*., 2017; Dias *et al*., 2010; Khan *et al*., 2014; Steiner *et al*., 2010). A rate of more than 20% (on a fresh weight basis) (40% w/w) has been found to be unsuitable and probably can adversely affect the composting process (Antonangelo *et al*., 2021; Tsapekos *et al*., 2018). This is because biochar addition at a high dosage may impair the biodegradation of manure, decrease the availability of readily degradable compounds, cause severe water loss and heat dissipation (Camps and Tomlinson, 2015; Liu *et al*., 2017; Antonangelo *et al*., 2021; Tsapekos *et al*., 2018).

According to Antonangelo *et al*. (2021) the optimal biochar rate is typically considered to be between 10% and 15%, however higher rates of 20% (Steiner *et al*., 2010) and 27% (Chowdhury *et al*., 2014) have also been recommended. Different biochar rates have been applied in manure composting to achieve desired compost quality and composting process which have been documented by several authors. Jain *et al*. (2018) demonstrated the highest temperature, fastest rate of degradation and highest N transformation in composting was achieved with 5% biochar. According to Czekala *et al*. (2016) compost material with 10% biochar attained the maximum temperature (72ºC on day 2) which was due to the faster decomposition of organic matter due to biochar addition. Also, Awasthi *et al*. (2017) observed a much humified compost within 35 days of composting, increased water soluble nutrients including DOC, dissolved organic nitrogen (DON), NO_3^-, total Kjeldahl nitrogen (TKN), K^+ and Na^+ and lower NH_4^+ when higher biochar rates (8%, 12% and 18%) was applied to compost material compared with the lower rates (2%, 4%, and 6%) and control. The authors also found a minimum NH_3 and high CO_2 emission with higher biochar rates. Jia *et al*. (2016) also reported that composting with 20% biochar reduced N_2O and CH_4 emissions by up to 59.8% and 54.9%, respectively in chicken manure composting. However, according to Sánchez-Garcia *et al*. (2015), the relatively low biochar application rate (3%, dry weight) did not significantly reduce the cumulative total CH_4 emissions in the composting of poultry manure. This was likely because the lower biochar rate was insufficient to raise the required level of aeration conditions.

It has been demonstrated that production factors like feedstock, residence time and pyrolysis temperature etc. affect the properties of biochar which in turn influence the composting process and compost quality (Antonangelo *et al.*, 2021). Among these, feedstock properties are the primary determinants of biochar bulk and surface characteristics (Bird *et al.*, 2011; Enders *et al.*, 2012). Woody biomass and agricultural wastes are commonly pyrolyzed at temperatures between 400 and 700 °C in order to create biochar that is appropriate for composting. (Zhang *et al.*, 2016). This is due to the highest levels of biochar's characteristics, including porosity, functional groups, CEC, and water-holding capacity (Antonangelo *et al.*, 2021). Chen *et al.* (2017b) reported a decrease in NH_3 and CH_4 volatilization in cornstalk biochar amended chicken compost compared to woody, layer, coir and bamboo biochar amended compost. The authors attributed this occurrance to higher nitrification and lower pH, as well as larger surface area, pore volumes, total acidic functional groups and CEC of cornstalk biochar and its increased ability to sorb gases and their precursors, like ammonium nitrogen from composting mixes (Tsutomu *et al.*, 2004; Asada *et al.*, 2006). In addition, treatment with cornstalk and woody biochar had lower NH_4^+-N contents and higher NO_3^--N contents compared to the control. This demonstrated that the biochar formed an ideal habitat for nitrifying bacteria, which converted ammonia into nitrate and helped the compost retain N. Also, Lui *et al.* (2017) observed that 10% bamboo biochar added ensured aerobic conditions and decreased CH_4 generation. Czekala *et al.* (2016) showed an increase in CO_2 emissions (6.9%–7.4%) in response to the addition of woodchip biochar, which was attributed to the increased aerobic breakdown of the organic material. It is, however, essential to conduct more studies using various biochar types in composting since limited studies that are now available show that impacts depend on surface area, porosity, and chemical surface characteristics, including the biochar's potential for sorption (He *et al*., 2019; Chen *et al*., 2017b).

Composting feedstock type also has an influence on the overall composting and compost quality. In manure biochar co-compost, it has been determined that the observed difference in final compost properties is due to the type of manure used which is dependent on the animal dietary (Chen *et al.,* 2019). It is well known that omnivores (e.g poultry and pigs) are fed mostly with commercial feed (which are protein and phosphorus based) while herbivores (e.g., cattle and goats) consume more roughage-based diets (Chen *et al.,* 2019; Sharpley and Moyer, 2000; Wan *et al.,* 2021). As a result, manure from omnivores may contain more N and P compound and less C compared to herbivores. These differences in diet influence the physicochemical properties of their manure which is easily exhibited in the composts produced (Chen *et al.,* 2019, Wan *et al.,* 2021) According to Wan *et al.,* (2021) composts prepared from sheep and cattle manure had higher carbon and C: N ratio compared to pig and poultry manure which had higher total N and total P. The observed difference in properties of the compost was however attributed to the difference in the animals' diet. Also, the use of market waste as a composting feedstock has an influence on composting and final compost. According to Akumah *et al.* (2021) the use of low N feedstocks e.g plantain peduncle and increasing the biochar rate resulted in compost of low N. The authors also emphasized that feedstocks of high-water content e.g fruit wastes influence negatively on decomposition process and delays compost maturity which was attributed to less aeration.

2.3.4 Impact of BMC on soil properties and crop productivity

The application of biochar-manure co-compost (BMC) to soils has been shown to enhance the physical, chemical, and microbiological properties and hence the growth and productivity of crops. With respect to the physical properties of soil, Głąb *et al*. (2018) reported that the application of BMC to sandy soils improved water retention compared to applying compost without biochar. Chen *et al*. (2020) also documented that BMC is stable, increases the aeration, water holding capacity of soil, stabilizes soil aggregates and reduces the bulk density of soil. Overall, BMC has been observed to increase soil C, N, and P levels and promote the growth of microorganisms (Mackie *et al*., 2015; Novak *et al*., 2019; Vandecasteele *et al*., 2016; Yuan *et al*., 2017; Schulz *et al*., 2013; Ye *et al*., 2019). The research by Chen *et al*. (2020) demonstrated that the application of BMC enhanced the electrical conductivity (EC) and pH of contaminated soil in addition to increased organic matter and nutrients. Similar observation was made by Maru *et al*., (2023) when biochar and chicken litter or cowdung or leuceana were composted and applied to a tropical soil thereby increasing the growth and productivity of rice. Applying BMC also increases the CEC of the soil which helps in the binding or holding capacity for plant nutrient cations and are kept and made more readily available for plant uptake (Kammamm *et al.,* 2015; Qayyum *et al*., 2017; Cao *et al*., 2018). According to Kammann *et al*. (2015), biochar amended compost was nutrient-rich, especially in nitrate and phosphate anion. The high CEC in addition to the micro- and nano-pores of biochar captured the $N0_3$ and protected it from leaching. According to the authors this process resulted in an increase in the biomass yield of *Chenopodium quinoa* of up to 305% when 2% (w/w) of co-composted biochar was applied to a poor sandy soil.

Furthermore, due to increase in organic C, carbon sequestration is enhanced in BMC-amended soil for at least in the short term (Khan *et al.,* 2016; Busch and Glaser, 2015). Khan *et al.* (2016) demonstrated that making and adding co-compost to the soil improves the sequestration of recalcitrant biochar carbon. The authors, therefore, urged composters and farmers to increase the soil organic carbon stock (SOC) for a longer time by applying BMC to the soil. The demonstration by Steiner *et al*. (2010) revealed that biochar was mainly unaffected by microbial decomposition during composting meaning that adding biochar did not alter the effective C: N ratio. As a result, the rates of labile C mineralization stayed low and the BMC in the soil increased the longevity of carbon when used, having a substantial impact on C sequestration. The SOC content of BMC is also known to provide plant mineralizable nutrients in soil which was documented by Schulz *et al.* (2013) leading to an increase in plant growth in sandy and loamy soil. Chen *et al*. (2020) also found a significant increment of 25.1% to 249.3%, 17.0% to 125.4% and 34.1% to 212.5% of N, P and K respectively in contaminated soil in addition to an increase in organic matter when biochar-amended compost was applied. Other researchers have reported the impact of BMC on chemical properties of soil which have translated to improve growth and productivity of crops. Work done by Pandit *et al*. (2019) observed an increase in biomass yield by 243% in maize productivity with the application of co-composted bokashi-biochar and attributed this improvement to the enhanced available N, P and exchangeable base cations. A similar report was also documented by Sanchez-Monedero *et al*. (2019) on tomato and grapes cultivation with BMC application. Compared to the control and non-enriched biochar, Kizito *et al*. (2019) revealed substantially higher biomass yield in maize production when the soil was amended with non-digestate enriched biochar. Studies by

Sulemana *et al.* (2021) in concretionary ferric lixisols of Northern Ghana showed an increase in soil C, pH, total and available P when biochar compost was incorporated in the soil translating into 2.71 to 3.71 and 1.51 to 2.33-fold increase in maize P uptake and dry matter respectively compared with mineral fertilizer. Co-composting feacal sludge, oil palm empty fruit bunch, cocoa pod husk and ricehusk biochar, Nartey *et al.* (2017) found an increase in total and available P, Nitrate-N and total C which increased the growth parameters of tomatoes in the greenhouse. In addition, the microbiology of the soil is enhanced when amended with BMC which is detailed in section 2.7.

2.4 Mineralization of BMC in soil

2.4.1 Nitrogen mineralization from BMC.

Nitrogen mineralization from BMC is an important process for plant nutrition and soil sustenance (Bass *et al*., 2016; Kammann *et al*., 2015). This is often assessed in terms of the Potentially Mineralizable N (PMN) which is described as the fraction of N that can be mineralized under favorable conditions (Curtin and Campbell, 2008; USDA-NRCS, 2014). Mostly generated from soil organic matter, PMN is an indirect measure of N availability that estimates N mineralization in the soil (Curtin and Campbell, 2008). The addition of BMC is known to improve the PMN of soil (Schulz *et al*., 2013) hence increase in crop growth. Schulz *et al*. (2013) reported an increase in organic matter with the addition of BMC which was the main source of mineralizable N generating an increase in plant growth. Sanchez-Monedero *et al*. (2019) reported a 44% increase in extractable N after the application of BMC to the soils. Yuan *et al*. (2017) also reported an increase in extractable organic nitrogen in biochar chicken manure co-compost compared to control. Kammann *et al*. (2015) documented an increase in biomass yield of *Chenopodium quinoa* when 2% co-

composted biochar was applied to a sandy poor soil compared to the untreated biochar. The authors attributed this occurrence to the capture and slow release of available N by biochar.

However, according to Thiele-Bruhn and Ngigi (2021), the recalcitrant nature of biochar to degradation in co-composted biochar causes less N availability or PMN compared to manure or compost. The biochar also stabilizes the labile C from the N rich feedstock such as manure during composting through the formation of organo-mineral complexes (Plaza *et al*., 2016). The N is then retained as constituent of the complexed organic matter (Šimanský *et al*., 2018). Ippolito *et al*. (2016) and Kocatürk-Schumacher *et al*. (2019) then generalized that co-composted biochar can be termed as a slow-release N fertilizer which results in lower levels of available and easily extractable N in soil but enhances the storage of plant N due to fertilization effect which is significant for increase plant growth.

These differences in the N availability or PMN of BMC may be attributed to the biochar type and its rate of application in the compost mix. Generally during composting the oxidation of biochar surface is enhanced due to elevated temperatures or microbial activity and sorption of organic compounds (Dias *et al*., 2010; Prost *et al*., 2013). The process enhances the cation exchange capacity (CEC) and functional groups of biochar thus affecting the N adsorption and slow release of N (Wang *et al* 2018; Weidner *et al*., 2015). Furthermore, the porous nature, functional groups and large surface area of biochar also aids in N adsorption in BMC-amended soil (Weidner *et al*., 2015; Agyarko-Mintah *et al*., 2017a; Khan *et al*., 2016). In addition, the recalcitrant carbon content of biochar which increases the C: N ratio of BMC promotes N immobilization (Thiele-Bruhn and Ngigi, 2021; Manirakiza *et al*. 2019; Singh *et al*., 2010). According to Manirakiza *et al*. (2019) woody and lignocellulose biochar pyrolyzed at high temperature used in manure composting produces BMC that contain biochar having higher CEC, porosity and surface area compared with manure and crop-based biochar pyrolyzed at lower temperature. Tsai and Chang (2020) also reported a much decrease in N mineralization when 2% biochar was applied in combination with poultry manure compost to soils in a 371-day incubation study compared to when 0.5% and 1% were applied. These mechanisms of slow N release of BMC profit the growth and yields of crops and prevent losses as reported by Kammann *et al* (2015). According to Kammann *et al.* (2015) experiment conducted using pure biochar and co-composted biochar revealed less nitrate leaching with the application of the BMC to the soil compared to the pure biochar and control.

2.4.2 Factors influencing N mineralization of BMC.

Several factors affect the rate and extent of nitrogen mineralization of BMC in soils. These include the composition of organic material, soil properties and environmental factors. To better predict N release from BMC, there should be a deeper comprehension of these three controlling variables.

2.4.2.1 Composition of BMC

The composition of BMC is a major factor that controls the rates, patterns and amount of N mineralized in soil. Studies have demonstrated that the most widely used parameters for determining N mineralization or availability in organic materials include N and C concentration, C: N ratio, cellulose, hemicellulose, lignin and polyphenol content and their interactions (Palm and Sanchez, 1991; Van Kessel and Reeves, 2002; Nakhone and Tabatabai, 2008; Vahdat *et al*., 2011). Most research on BMC have documented that due to the carbonaceous and recalcitrant C of biochar, BMC-amended soils have high C stability, decomposing less rapidly and mineralizing N slowly (Manirakiza *et al*., 2019;

Schofield *et al*., 2019; Jien, 2019; Thiele-Bruhn and Ngigi, 2021, Qayyum *et al.,* 2017). Therefore, Thiele-Bruhn and Ngigi (2021) reported that composted biochar causes less N availability or PMN compared to manure or compost. As a result, Kammann *et al*. (2015) documented that the release of organic N from co-composed biochars and the release of dissolved organic carbon had a significant correlation. By forming organo-mineral complexes, the biochar addition stabilizes the labile carbon from N-rich feedstocks like manure during composting, where the N remains in the complexed organic matter (Plaza *et al*., 2016; Qayyum *et al.,* 2017). In addition, N can be sorbed to surface charges of oxidized biochar making it unavailable for mineralization (Wang *et al* 2017; Weidner *et al*., 2015). Although the BMC has high amount of N in the final compost (Antonangelo *et al*., 2021), N is slowly released (Kammann *et al*., 2015, Ippolito *et al*. 2016). The use of C: N ratio as a predictor of N mineralization of BMCs is based on a threshold value of 30:1. When C: N ratio is < 30:1, the BMC-amended soil has a high potential to mineralize N. On the contrary, when the C: N ratio >30:1, N immobilization by microorganisms is favoured (Hodge *et al*., 2000). Schofield *et al*. (2019) observed a 44% reduction of total dissolved N concentration when 10% biochar was applied to a manufactured soil composed of bark, sand, light clay and composted greenwaste. According to the authors the reduction was as a result of nitrogen immobilization by microbes due to high C: N ratio of the amendment. A similar report was made by Tsai and Chang (2020) and Manirakiza *et al*. (2019) in biochar and compost-amended soil as well as biochar and biosolid-amended soil respectively. Bonanomi *et al*. (2017) also documented a decrease in lettuce growth due to the immobilization of N by microbes because of high C: N ratio of biochar and leaf litter amended soil. Therefore, C: N ratio is inversely proportional to N availability and plant growth (Palm *et al*., 2001). The lignin and polyphenol contents are better predictors of N mineralization of most organic material amended soils (Palm and Sanchez, 1991, Vahdat *et al*., 2011). In general, lignin and polyphenol contents are inversely related to N availability, whilse cellulose content is directly proportional to N availability (Vahdat *et al.,* 2011). According to Antonangelo *et al*. (2021) biochar feedstocks with high lignin content may generate biochar with high yield and lignin content. Lignin which is known to degrade into phenolics compounds and form complex structures with N containing groups and amino acid groups may make BMC resistant to decomposition and thereby limit N release and N availability (Fox *et al*., 1990).

2.4.2.2 Soil moisture

Soil moisture plays an important role in organic matter decomposition and mineralization of N by influencing the activities of microbes involved in the processes and indirectly influencing soil aeration (Luce *et al*., 2011). Microorganisms involved in mineralization are aerobes with nitrification proceeding slowly or ceasing in submerged soils. In submerged soils, less diffusion of oxygen occurs resulting in less proliferation of aerobic microbes (Paul *et al*., 2003). Furthermore, reduced oxygen and substrate diffusion, combined with decreased microbial mobility and growth lowers microbial activity and N mineralization (Luce *et al*., 2011). According to Whalen and Sampedro, (2010) soil moisture content is regarded as optimum for mineralization when the soil is at 50 to 80% Field Capacity (FC), providing enough moisture and O_2 for microbial activities. De Neve and Hofman (2002) also reported 60% FC as optimal for N mineralization. The effect of soil moisture on N mineralization in BMC-amended soil has been the subject of many investigations. For example, Uddin *et al*. (2021) in an incubation experiment studied the N release patterns of compost, poultry manure, rice husk biochar, poultry manure biochar and cowdung (CD) combined with chemical fertilizer in two contrasting soils and two water regimes (field capacity-FC and continuous standing water-CSW) for 120 days). Their results showed that NH_4^+-N was dominant at CSW water regime while NO_3^--N was prevalent at FC water regime. In addition, drying and wetting cycles have been observed to stimulate the mineralization of labile organic substrates which are broken down by rapidly growing bacteria (Broken and Matzner, 2009).

2.4.2.3 Soil temperature

Temperature is a major environmental factor that affects the decomposition of organic matter, BMC and N mineralization (Ellert and Bettany, 1992; De Neve *et al.,* 1996; Guntiñas *et al.,* 2012). According to Whalen and Sampedro, (2010) the maximum decomposition and N mineralization rate often occurs between 24 and 30 ºC whilse mineralization essentially ceases near freezing point. This is because low temperatures injure the soil microflora and thus impedes soil N transformations (Schimel *et al*., 2004). Above 30 ºC, ammonification continues but nitrification ceases above 45 ºC (Stanford *et al*., 1975; Schimel *et al*., 2004). From 24 to 30 ºC, complete conversion of ammonium to nitrite occurs under optimum soil moisture conditions in aerated soils (Zaman amd Chang 2004). In general, the accumulation of mineralized N increased with increasing temperature.

2.4.2.4 Soil texture

N mineralization of BMC is mostly influenced by soil texture (Tsai and Chang *et al*., 2020; Manirakiza *et al*., 2019). Soils with high clay content and high exchange capacity reduce N mineralized compared to soils with high sand contents (Sørensen and Jensen, 1995;

Mubarak *et al*., 2010). This could be due to physical protection of organic matter by the clay content due to their small pore spaces and the prevention of microbial attack on SOM (Thomsen and Olesen, 2000; Hassink *et al.,* 1993). As a result, BMC-amended soils are noted to release less N, especially in clayey soils. Studies by Manirakiza *et al*. (2019) revealed that N release from biochar and paper mill biosolid was reduced in two agricultural soils (Kamouraska clay soil and St-Antoine sandy-loam) and the reduction was dominant in the clayey soil. A reverse occurrence is often observed in soils with high sand content and large pore spaces (Hassink *et al*., 1993; Motavalli *et al*., 1995; Cote *et al*., 2000). Studies conducted by Hassink *et al*. (1993) to determine N mineralization rates on grassland soils of different textures revealed that the percentage of N mineralized from organic N was higher in sandy soils than in clays.

However other studies have shown a contrasting effect and attributed it to the drying and sieving of clayey soil. Franzluebbers (1999) showed that N mineralization following soil disturbance (i.e drying and sieving) and varying in texture led to an increase in net N mineralization in a 14-day incubation, when soils were sieved through < 0.018mm sieve compared to < 2mm sieve. This occurrence was attributed to the extent of breakdown of the soil macroaggregate exposing the organic matter content for decomposition. The author also emphasized that the type of clay influences the protection of organic matter. Koalinitic soils with less surface activity may not offer the same degree of protection to organic matter compared to montmorillonitic soil. When BMC is applied on the soil, a similar observation was made by Tsai and Chang (2020), especially when a clayey soil was amended with biochar and 5% compost. Other authors have found no relationship between soil textural composition and N mineralization. According to researchers such as Jia *et al*., (2019), Fu *et al*. (1987), Bai *et al*. (2012), and González-Prieto *et al*. (1996), soil factors such as pH, heavy metal content, presence of other nutrients; salinity etc. are important in N mineralization.

2.5 Methods of estimating N availability by BMC

Estimating N availability is very important for N management during crop growth, as well as due to environmental concerns. A lot of research has been conducted to develop methods for this purpose which have been outlined in reviews by Sharifi *et al*. (2008), Serna and Pomares, (1991), Schomberg *et al*.(2009) and among others. The N mineralization indices have been grouped into two classes, namely: the biological and chemical.

2.5.1 Biological indices on N availability

The most widely used biological method to estimate N availability is to incubate soil and/or N amendment in a constant environmental condition and quantify inorganic N released over time (Stanford and Smith, 1972). To find the N mineralization potential (PMN or N_o), this approach typically entails incubation for 30 weeks at an optimum moisture content (80 kPa tension) and temperature (35 ^oC). The mineralized N is extracted by periodic leaching. The mineralization dynamics is described in terms of a rate constant , k, (week^{-1}) (Serna and Pomares, 1991). Stanford and Smith (1972) assumed that the net N production rate follows first-order kinetics and was used to determine 39 soils. The potentially mineralizable N (N_o) and a rate constant of 0.054 week^{-1} was determined. However, Wang *et al.* (2003) reported varying figures for the rate constant (k) for different soils, indicating that there is no universal k for all soils. Aside from this shortcoming, the time-consuming factor, laborious and expensive nature of this method makes it only partially adopted by scientists (Mariano *et al*., 2013). Therefore, short term or rapid incubation methods have been explored which includes 7 to14 days aerobic and anearobic incubation (Keeney and Bremner, 1967). The potentially mineralizable N (N_o) obtained by these methods have been correlated with the long term incubation and a strong positive relation was found between them (Schomberg *et al*., 2009). In addition, plant cultivation procedures, which estimate N uptake by crop is also used as a proxy for N availability (Serna and Pomares, 1991).

2.5.2 Chemical indices on N availability

The fastest procedures of N mineralization assessment are by chemical methods, leading to chemical indices. Some of the methods which require only 1-hour include the 2 M KCl extractable mineral N (Bremner and Keeney, 1966), oxidizable N by acidified $KMnO_4$ (Stanford, 1978), sulfuric acid extractable NH_4^+, acid permanganate-extractable NH_4^+-N (Stanford, 1978), etc. According to Serna and Pomares (1991), chemical procedures, though are rapid and inexpensive, need to be correlated with biological procedures to be use as predictors of N availability in soils. Work done by Safarzadeh *et al*. (2010) found out that N extracted by 0.05 M acidified $KMnO_4$ correlated with results of anaerobic method of determining N mineralized in a manure-amended soil and could be used as an index of N availability. Also, Serna and Pomares (1991) found out that N extracted by autoclaving 6 M HCl, 0.5 N $KMnO_4$ and pepsin correlated with results of N uptake by maize. A simple alkaline hydrolysis method for estimating the N availability index of soil has been described by Dodor and Tabatabai (2020). According to the authors using 1 M KOH and NaOH showed a significant correlation among potentialy hydrolysable N (N_o) and the amount of N mineralized in an aerobic and anaerobic incubation in 2 weeks at 30

°C as well as 1 M KCl extraction at 80 °C for 2 hrs and initial NH_4^+-N. Comparing the N availability from the alkaline hydrolysis method and the long term aerobic incubation in four Ghanaian soils, Dodor *et al., (*2022) found a strong association between the potentially hydrolyzable N (N_{max}) and potentially mineralizable N (N_o) as the maximum N mineralized fom the long term incubation method. The authors therefore concluded that this chemical method of estimating N availability in soils can be a good predictor of the labile pool of mineralizable soil organic N and can also be used in laboratories to quantify the N releasing capacities of soils. A work by Zoglie (2021) also compared the N availability from the alkaline hydrolysis method with the the long-term incubation method in a biochar manure co-compost amended soil. According to the author, N_o and $\mathrm{N}_{\mathrm{max}}$ differed significantly among the various BMC amended soils which was attributed to the chemical composition of amendments. Also, there was a strong positive and significant correlation between N_o and $\mathrm{N}_{\mathrm{max}}$ $(p<0.01)$ as well as $\mathrm{N}_{\mathrm{max}}$ and N uptake by maize $(p < 0.05)$.

2.6 Modelling N mineralization in soil amended with BMC.

Predicting the amounts of N mineralized by BMC in soil over time is important to evaluate its N supply capacity and thus minimizing adverse N impacts on the environment (Benbi and Richter, 2002; Wang *et al*., 2004). To study the N transformations in soil, 2 factors are of concern, namely the potentially mineralizable N, (PMN), defined as the maximum amount of inorganic N that can be mineralized, and the rate of mineralization (Benbi and Richter, 2002). The latter depends on the type of organic matter and the former on the soil properties, edaphic and composition of organic matter (Wang *et al*., 2006). These factors are incorporated into models for N dynamics description (Gil *et al*., 2011). The models are mathematical equations that are fitted to experimental data. However, modelling of N release in BMC amended soils may be non-trivial due to the multiple interactions between the BMC and soil properties, turnover processes of the different N pools, among others (Sistani *et al*., 2008; Gil *et al*., 2011).

According to Azeez and Van Averbeke (2010), the properties of organic material in general influence the amount of N mineralized. For example, BMC with higher amount of biochar applied during composting may mineralize a lower amount of N compared to that with lower biochar applied (Thiele-Bruhn and Ngigi, 2021, Antonangelo *et al*., 2021). At the same time BMC without biochar may mineralize N at a shorter time (Tsai and Chang, 2020). Most often, the estimation of the N mineralized in soil amended with organic materials is using short- and long-term biological methods through incubation of samples in the laboratory. Furthermore, laboratory incubation method also gives a better understanding of the mineralization kinetics of BMC amended soils as it mimicks N mineralization under field conditions.

Numerous equations have been used to model N mineralization. However, the most used are the first-order and second-order (Kaur *et al*., 2018, Masunga *et al*., 2016, Chae and Tabatabi, 1986; Lazicki *et al*., 2020; Cassidy-Duffery *et al*., 2020). The first order kinetic for evaluating N transformation is exponential since microbial activity in soil is determined by enzymatic dynamics, which is often exponential. Stanford and Smith (1972) proposed a first-order exponential model of the form:

$$\frac{dN}{dt} = -kN \tag{2.1}$$

where N is the amount of mineralizable substrate and t is time

Integrating this equation gives:

$$N_t = N_0 \, exp(-kt) \tag{2.2}$$

where N_0 is the potentially mineralizable N, N_t is the amount of substrate at time *t*, *k* is the rate constant. The equation may be modified by substituting $N_t = (N_o - N_m)$ producing equation 2.3 known as first order N kinetics

$$N_m = N_0(1 - \exp[-kt]) \tag{2.3}$$

where N_{m} is the N mineralized per time t.

Campbell *et al*., (1981), working in Australia observed wide range of the *k* value indicating variations in the active organic N and microbial turnover of soils (Wang *et al*., 2004). Amending the soil with manure, Li and Li (2014) also found that the first-order kinetics fitted their data well for soils amended with cattle, pig and poultry manure of different sources. Furthermore, Cassidy-Duffery *et al.* (2020), investigating the potentially mineralizable N and the rate of mineralization from 22 commercial organic fertilizers, 15 poultry manures and 11 compost discovered that the N mineralization data form the amendments fitted well with first order kinetics. Gil *et al*. (2011) also found the best fit for a 1-year experimental data from compost amended soils was the first -order model. Studies by Dodor *et al.* (2022) showed that the model fitted well with N mineralization data of some soils from Ghana, with N_o and k values ranging from 127.2 to 215.7 mg kg^{-1} and 0.076 to 0.107 week^{-1}, respectively indicating variations among the soils studied.

Although the first order kinetic model has been extensively used to predict the N supplying capacity of organic material amended soils, a series of shortfalls have also been associated to this model (Molina *et al*., 1980; Deans *et al*., 1986; Matus and Rodriguez, 1994). Lindemann and Cardenas (1984) identified that the first order N kinetic model was not able to describe the N mineralization kinetic in sewage sludge amended soils. Also, Seyfried and Rao, (1988) observed an underestimation of N mineralization at the end and beginning of the incubation and overestimation at the intermediate times. As a result, the linearized form of the model was employed to obtain values of N_o as the best fit, using a regression of log(N_o-N_m) on t (Stanford and Smith 1972; Campbell *et al* 1981). Moreover, the nonlinear least square gives more accurate estimation compared to the linear least square (Smith *et al*., 1980; Benedetti and Sebastaini, 1996).

Furthermore, a major underlying assumption of the first-order model is the homogeneity of the N pool in the orgnic material. In most cases, however, there are often more than one N pool in organic matrials, with each pool having different kinetic parameters. Therefore, authors such as Molina *et al*., (1980) and Deans *et al*., (1986) proposed models beyond the first-order to second order models. The second order models contain more than one component used to analyze experimental cumulative mineralized N as a function of time. Each component is assumed to mineralize according to the first-order; however, the first component represents the active (easily mineralizable) organic fraction and the other recalcitrant which mineralizes independently and simultaneously at different rate constants.

$$N_m = N_{0l}(1 - \exp(-k_l t) - N_{0s}(1 - \exp(-k_s t) \tag{2.4}$$

where N_{ol} is the potentially mineralizable N of labile fraction, N_{os} is the potentially mineralizable N of the recalcitrant fraction, k_{l} is the rate constant of labile fraction, k_{s} is the rate constant of recalcitrant fraction and N_{m} is the N mineralized per time (t). Other modifications were made to the double first kinetic model by replacing the recalcitrant pool with a constant, suggesting a mixture of the first and zero order exponential model. This was known as the special model (Bonde and Rosswall, 1987). Hyperbolic and parabolic models have also been suggested to estimate the N supplying capacity of organic material amended soils (Juma *et al*., 1984; Broadbent, 1986).

2.7 Effect of BMC on soil biological properties

2.7.1 Microbial biomass

Soil microbial biomass (SMB) is described as that part of soil organic matter (SOM) that consist of living microorganisms smaller than 5×10^3 µm (Chowdhury *et al*., 2008; Sparling, 1985). Soil microbial biomass is the most physiologically active labile C and N pools making up 1 to 3% of the total C and 5% of the total N for both microbial carbon (MBC) and microbial biomass nitrogen (MBN), respectively. Functions of SMB include organic matter decomposition, nutrient cycling, degradation of organic contaminants, suppression of soil pathogens and diseases, secretion of plant growth promoters and soil structure formation (Dalal, 1998). Measurement of SMB is using chloroform fumigation incubation (CFI), substrate-induced respiration (SIR), chloroform fumigation extraction (CFE) and adenosine triphosphate (ATP) analysis (Jenkinson *et al*., 1979; Webster *et al*., 1984; Anderson and Domsch, 1978; Vance *et al*., 1987). Among the four, the most used technique for determining MBC and MBN concentrations in agricultural soils is the chloroform fumigation extraction method by Vance *et al*. (1987), which was further detailed by Voroney *et al*. (2008). SMB has therefore been described as an important and sensitive parameter for measurement of soil quality and health (Chowdhury *et al*., 2008; Dalal, 1998).

Soil microbial biomass is sensitive to rapid decline in soil nutrients and to the application of organic inputs to replenish the nutrient status of the soil. Therfore, the SMB is one instrument used to detect the response of the soil to these amendments. Application of BMC has resulted in similar SMB response, generating a higher microbial proliferation, and hence abundance of soil biota, compared to soils alone (Manirakiza *et al*., 2019, Guo *et al.,* 2020, Antonangelo *et al*., 2021; Yuan *et al*., 2017). Investigating the influence of biochar-chicken manure co-compost on biological properties of the soil, Yuan *et al*. (2017) observed an increase in the SMB-C and SMB-N with the addition of biochar chicken manure co-compost, compared the soil alone. Manirakiza *et al*. (2019) also reported similar observation in soils amended with biochar paper mill biosolid. The mechanisms for which biochar compost amendment increase SMB include provision of nutrients for microbes, retention and provision of labile carbon for metabolism, creation of porous and maintenance of high-water holding capacity for aeration and suitable moisture, thus creating suitable microhabitat (Lehmann *et al.,* 2011; Antonangelo *et al*., 2021; Guo *et al*., 2020; Sanchez Monedero *et al.,* 2018).

According to Birk *et al*. (2009), the availability of liable C in BMC stimulates microbial activity resulting in N mineralization, which reflects an increase in MBC and N measured in amended soils. However, the extent of effect may be dependent on feedstock type, biochar type and their ratios used in preparation of the BMC (Antonangelo *et al*., 2021). Studies by Dempster *et al*. (2012) showed a decrease in SMB when composted pig manure and eucalyptus biochar were applied on a coarse textured soil. The authors attributed this occurrence to the type of feedstock used in preparation of the biochar which resulted in reduced organic matter decomposition. Elzobair *et al*. (2016) also observed similar findings to that of Dempster *et al*. (2012) and attributed this occurrence to the rate of biochar application. According to Quyang *et al*. (2014) manure biochars produce more volatile matter and available nutrients and hence increase microbial activities compared with woody biochars.

Relating MBC and MBN to the PMN (N_o) of soils have been undertaken by numerous researchers (Deng *et al*., 2000; Sharifi *et al.,* 2007; Carter and Macleod, 1987; Yuan *et al*., 2017; Manirakiza *et al*., 2019). This process enables prediction of N availability using the MBC and MBN measured, since PMN mostly takes longer time to assay especially in the long-term incubation experiment. In soils from Prince Edward Island, Carter and Macleod (1987) observed that MBN was highly correlated with N_o ($R^2 = 0.94$) while Sharifi *et al*. (2007) showed that MBC was poorly correlated with N_o ($R^2 = 0.11$) in soils from humid and semi-arid climatic zones of USA and Canada. Burket and Dick (1998) also found that MBN and MBC were highly related with N_o under different management systems. However, there are few studies that link MBC or MBN to N availability in biochar and manure or BMC amended soils. According to Yuan *et al*. (2017), MBC and BMN were related positively with N availability in biochar chicken manure co-compost. Manirakiza *et al*. (2019) reported a negative correlation between MBC and N availability in biochar and biosolid amended soil. According to the authors, as MBC increased, NH_4^+-N and NO_3^--N decreased which was attributed to less N and recalcitrant carbon of biochar although biochar created a suitable habitat for microbial proliferation and abundance. This may therefore imply that the recalcitrant nature of carbon in biochar may not be a good source of substrate metabolism by soil biota. However, Zimmerman *et al*., (2011) documented that biochar produces liable carbon immediately after application to stimulate microbial proliferation and absorbs it on the surfaces and pores of biochar making them unavailable for microbes as a nutrient source. This occurrence may have a negative impact on microbial biomass in the long term. Furthermore, the decrease in SMB in biochar and manure amended soil according to Deenik *et al*. (2010) has been attributed to presence of toxic organic compounds.

2.7.2 N cycling enzyme

Enzymes are protein molecules whose main role in the soil is to catalyze biochemical activities such as the decomposition of organic matter and the mineralization of organic inputs, which make vital nutrients for agricultural productivity available (Tabatabai, 1994). Similarly, N cycling enzymes are involved in catalyzing N transformation making N available or lost in the soil without undergoing alteration themselves and are specific to the type of transformation they participate in the N cycle (Tabatabai, 1994; Kandeler e*t al.,* 2011). For example, amidohydrolases (urease, L-asparaginase, L-glutaminase and amidase) are involved in the hydrolysis of amino acids to release ammonium whereas peptidases, protease, chitinase and N -acetylglucosaminase are involved in the hydrolysis of proteins and chitin to release amino acids and amino sugars respectively (Kandeler *et al.,* 2011). Other enzymes include nitrite reductase and ammonia monooxidase that are involved in denitrification and nitrification reactions respectively as well as arylamidases which hydrolyzes N-terminal amino acid from arylamides (Kandeler *et al.,* 2011; Acosta-Martinez and Tabatabai, 2000).

Researchers have investigated and recorded the impact of BMC on the activity of N cycling enzymes as well as other enzymes. For example, Lu *et al.* (2015) discovered that applying BMC and pyroligneous acid to a saline soil increased the activities of urease and phosphatase enzymes, which alleviated the salinity stress on plants. Yuan *et al.* (2017) also found that the incorporation of biochar manure chicken compost increased the activities of cellulose enzymes but lowered that of glucosaminidase activity compared to compost alone. Further the authors found an increase in peroxidase activity in the biochar manure compost treated soil and attributed this occurrence to the stable organic C. Further work by Sekaran *et al*. (2020) also showed 80% increase of urease activity in an eroded soil amended with manure and biochar compared with the control. The authors attributed this increase in urease activity to the availability in substrate as well as increase in microbial activity which could increase the turnover rate of N, therefore enhancing the fertility of the degraded soil. On the contrary, Elzobair *et al*. (2016) reported no increase in Leucine aminopeptidase and N-acetyl-b-glucosaminidase when biochar and manure were applied to the soil compared to no amendment.

The addtiton of BMC to the soil has a great influence on the N cycling enzymes that cause N transformations in soil. Mostly, the additions of BMC and other organic residues change the physico-chemical properties of the soil, stimulating enzyme activities by enhancing microbial proliferation or enzyme induction in response to the amendment (Lu *et al*., 2015; Antonangelo *et al.,* 2021; Deng *et al*., 2006). On the contrary, inorganic N materials (N fertilization) tend to decrease enzymatic activities through their impact on soil pH although the type of fertilizer is a determining factor (Tabatabai, 1994). Nitrogen cycling enzymes, therefore serve as an early indicator to changes induced by management because of their rapid change to management practices and ease and accuracy of their assay (Dick, 1992; Tabatabai *et al*., 2010). A study by Dodor and Tabatabai (2003) to identify the influence of crop rotation and N fertilization on four amidohydrolases and arylamidase activity showed that multicroping increased the activity of the enzymes whereas monocropping decreased their activity. The authors attributed this occurrence to the type of organic matter generated under the 2 cropping systems as well as enhanced rhizosphere because of secretion of root exudates by legumes under the multicroping system.

Through their catalytic processes N cycling enzymes make N available for plant uptake. This was because the acid hydrolysis (6 M) of 15-25% of total organic N released as NH_4^+ was identified by Sowden (1958) to have a portion released by the hydrolysis of amides by the action of L-glutaminase and L-asparaginase. Also, Stevenson (1996) showed that 30-45% and 5-10% of total organic N was identified through acid hydrolysis to be amino acid and amino sugar, which could also be hydrolyzed by N cycling enzyme as sources of inorganic N in soils. As a key element and limiting nutrient for plant growth, the prediction of soil N mineralization has been a long-standing objective. Most research on N mineralization has held N cycling enzymes as N mineralization indicators (Dodor and Tabatabai *et al.,* 2003; Tabatabai *et al.,* 2010; Burket and Dick, 1998; Acosta-Martinez and Tabatabai, 2000). Research conducted in 2003 by Dodor and Tabatabai shown a significant correlation between the activity of arylamidases and the quantities of N mineralized using chemical and biological methods in soils sampled from six agro ecological zones in North Central region of the United States. According to Burket and Dick (1998), the activities of amidase, asparaginase, urease and dipeptidase correlated positively (r = 0.65 to 0.85) with the amount of N mineralization by the plant uptake method in the greenhouse. Similarly, Tabatabai *et al*. (2010) observed among all the eight N cycling enzymes studied that N-acetylglucosaminase was most significantly correlated with cumulative amount of N mineralized at 20 °C (r = 0.87) and 30 °C (r = 0.95). It is well known that microbes are the dominant originators of N cycling enzymes and hence microbial biomass significantly correlates with N mineralization making them a predictor of N mineralization (Sharifi *et* *al*., 2007; Carter and Macleod, 1987; Tabatabai *et al*., 2010). However, Deng *et al*. (2000) suggested that biochemical processes such as N mineralization are predominantly controlled by enzymes and best used as indices of soil fertility other than overall microbial activity. Relating the N mineralized or potentially mineralizable N to the activities of N cycling enzymes in BMC amended tropical soils has not been evaluated yet. Amidohydrolase activity and SMB determination needs to be conducted especially in tropical soils to assess the relation between PMN in BMC-amended soils and N cycling enzymes to establish nutrient turnover and N fertility by these enzymes.

2.8 N fractions in soils

Nitrogen mostly occurs in the organic forms in surface soils, which is estimated to be about 95-98% of total N (Stevenson, 1982). Mainly from proteins, organic N (ON) can easily be transformed by proteolytic enzymes into available N forms (Nitrate and ammonium) for plant uptake (Li *et al*., 2014). The available N from mineralization of ON is a concern in the environment due to its participation in reactions that yields other N components (Li *et al.,* 2014). This shows the ability of organic nitrogen in influencing the soil's N supplying capacity. So further research has been conducted on the composition and properties of organic N compounds in soils to further adjust the N transformations, formulate proper fertilizers and improve the nitrogen supplying capacity of soils (Stevenson, 1982). The ON is often classified into different types based on its availability and chemical composition. Labile N and stable N are the two main types of ON according to availability (Polgalse *et al*., 1992). Labile N comprises particulate organic N, dissolved organic N, light fraction N and microbial biomass N (Yan *et al.,* 2007). The particulate organic N is a short-term N pool between the fresh plant residue and the humified organic matter, which forms a major source of C and energy for heterotrophic microorganisms (Haynes, 2005). However, the dissolved organic N is ON that can dissolve in HCl or water and is readily taken by plants. (Zhou *et al*., 2005). The light fraction organic N mainly originates from animal debris and plant residues while microbial biomass N is mainly from the biomasses of microbes which are mostly affected by soil management and plays important roles in C and N transformation (Bremer *et al.,* 1994; Yan *et al.,* 2007). Studies by Yan *et al.* (2016) showed an increase in labile N with a long-term combined application of inorganic fertilizers and rice straw or manure in a black soil compared to no and single fertilized soil, although dissolved organic N increased with single fertilization. Gong *et al.* (2008) also observed an increase in particulate organic N by a combined application of NPK and organic fertilizers while Xu *et al.* (2003) evidenced the increase in labile N pool by the application of manure alone.

For classification of ON based on the chemical component, Bremner (1965) proposed that ON can be grouped into amino acid N, ammonia sugar N, ammonia N, acid-hydrolysable unknown N and non-hydrolysable N. The author therefore carried out a procedure by heating a soil containing an appreciable ON with 6 mol L^{-1} HCl for 12-24 h, after which the N separated was grouped into acid-insoluble (non-hydrolysable N) and acid soluble N (hydrolysable N). The latter comprised of the hydrolysable known and unknown N. The hydrolysable known N was recovered from hydrolysate by steam distillation with MgO to obtain NH_3-N (Stevenson, 1994). And that obtain with oxidation by ninhydrin was amino acid N and steam distillation with phosphate borate buffer at pH 11.2 and correction for NH_3-N generated ammonia sugar or hexosamines (Stevenson, 1994). The hydrolysable unknown fraction was determined as the N not accounted for and occurs mostly as non-$\alpha-$amino N in arginine, tryptophan, lysine, and proline (Li *et al*., 2014).

Acid insoluble N (non-hydrolysable N) remained in the soil was obtained by the difference of total N minus the acid soluble N (hydrolysable N). Using the method by Bremner (1965) to determine ON in soil has yielded a variety of N compounds, however modification have been proposed, such as the diffusion method instead of the distillation method. Using this method accounts for only half of the ON in the soil while others remain unknown. However new information has been generated into the instrumentation and analytical techniques to specialize ON in bulk or fractionated soil samples within which combination of the various techniques is appropriate to gain insights into the quality of ON (Leinweber *et al*., 2013).

2.9 Effect of BMC on N fractions in soil

Continuous cultivation of soil, especially tropical soils, has resulted in an appreciable decline in N because of mineralization processes. According to Sammi Reddy *et al*. (2003) and Subba Rao and Ghosh (1981), hydrolysable components of organic N such as amino acid, hydrolyzable ammonium, amino sugar, and hydrolysable unknown forms often referred to as the liable forms of N, are more susceptible to mineralization and subsequent losses under cultivation than the non-hydrolyzable fractions. However other studies have shown depletion of non-hydrolysable fraction under arable cropping (Kaur and Singh, 2014; Sammi Reddy *et al*., 2003, Sekhon *et al.,* 2011). Application of organic fertilizers has a great impact on organic N fractions in the soil by changing the composition of soil N and indirectly affecting crop growth (Sekhon *et al.,* 2011; Durani *et al.,* 2016). Its combination with either inorganic fertilizer or alone not only lead to an improvement in organic N fractions in the soil profile, but also contributes to N mineralization (Sammi

Reddy *et al*., 2003). Sammi Reddy *et al*. (2003) reported an increase in total hydrolysable N with the incorporation of farmyard manure and 100% NPK compared to control. A similar report was made by Kaur and Singh (2014) in a rice-wheat system. Xu *et al*. (2003) found the highest amino sugar and amino acid-N contents with pig and NPK fertilizers and the lowest with NPK fertilizer treatment. However, inorganic N alone are sometimes unable to translate into active fractions of N especially over a longer period. Studies by Singh *et al*. (2001) revealed a decline in total hydrolysable N with the application of inorganic fertilizers. Also, Campbell *et al*. (1986) observed an increase in the proportion of amino acid-N and reduction in the proportion of hydrolysable unknown N under long term organic fertilization compared to inorganic fertilization, which did not change the composition of N fractions. According to Durani *et al*. (2016) in a long-term study of the effect of fertilizer on N fractions, results revealed that organic N fertilization alone increased hydrolysable N compared to inorganic fertilizers. Similarly, Sekhon *et al*. (2011) observed an increase in total N and hydrolysable N with the incorporation of farmyard manure, green manure and press mud which translated into increase in nitrogen uptake and grain yield of rice and wheat compared to mineral fertilizer treatment. As a result of these findings Singh *et al*. (2001) suggested that inorganic fertilizer alone may not sustain soil productivity over a longer period and therefore its combination with organic fertilizer is appropriate.

The addition of fresh C sources such as biochar to soils has the ability of influencing the lability or recalcitrance of soil organic N fractions (Schomberg *et al.,* 2012). According to Schomberg *et al*. (2012), the addition of poultry manure, pecan shell and peanut hull biochar pyrolyzed at high temperature to a sandy soil showed a higher amount of recalcitrant N (non-hydrolysable N) and less effect on labile N compared to the control. A similar report was made by Wang *et al*. (2012) during an investigation on the influence of different types of feedstocks, and pyrolysis temperature on N fractions and lability in biochar. Using cow manure and biosolid biochar in their experiment, the authors observed that these biochars contain hydrolysable organic N forms such as amino acids, which could enhance N uptake by plant. However, a decrease in hydrolysable N fractions was recorded under high temperature, which was due to an increase in formation of aromatic and heterocyclic structures or the degradation of labile N forms. Therefore, to improve the availability of labile N fractions, biochar can be applied with other sources of N.

Recently, N turnover and availability in BMC-amended soils have been extensively studied. According to Awasthi *et al.* (2017), application of BMC increases the ON, which translates to N availability in soil. Sanchez-Monedero *et al*. (2019) showed an increase of 44% in extractable N after application of biochar compost to the soils. Similarly, Yuan *et al*. (2017) reported a significant increase in KCl-extractable ON contents of soils following application of biochar-chicken-manure compost. These studies show how BMC contributes to the ON component of soils which could easily translate into their N availability. However, the contribution of various ON classes, based on their chemical composition to N availability in BMC-amended soils has not been fully investigated. It is therefore essential to fill this knowledge gap. This is one of the major focuses on this thesis.

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CHAPTER 3

3.0 NITROGEN MINERALIZATION POTENTIAL OF TROPICAL SOILS AMENDED WITH BIOCHAR MANURE CO-COMPOST

3.1 Abstract

The use of biochar in composting of nitrogen (N)-rich materials has been proposed as a strategy to produce compost containing stable organic N (ON) to prevent N losses and frequent re-application to the soils. In the present study, the effect of co-composted market waste (MW), cattle (CM) and poultry (PM) manures alone (manure compost- MC) or with rice husk (RB), sawdust (SB) and coconut husk (CB) biochars (biochar manure compost-BMC) at a rate of 20% v/v dry weight on the dynamics of N mineralization was investigated. Three soils series, i.e., Adentan, Denteso and Keta were amended with the BMC and MC at a rate of 200 mg N kg^{-1} soil and incubated for 26 weeks at 30ºC. The amount of NH_4^+-N and NO_3^--N mineralized was leached periodically using 0.01 M $CaCl_2$ and analyzed. The kinetic parameters, namely, the potentially mineralizable N (N_o) and the rate constant (k) were calculated by fitting the cumulative N mineralized to the first order kinetic model. The findings demonstrated significant variations in N_o across the soils amended with BMC amd MC, indicating that the chemical nature of ON in the amendments differed. Estimated N_o values ranged from 120 to 205, 53 to 170 and 71 to 174 mg kg^{-1} for Adentan, Denteso and Keta series, respectively. Sawdust (SB)- and coconut husk (CB) biochar-based compost recorded the lowest N_o, probably due to immobilization and sorption of liable N. Estimated k values ranged from 0.064 to 0.083, 0.058 to 0.089, and 0.059 to 0.075 week^{-1} in Adentan, Denteso, and Keta series, respectively and was lowest in the BMCs compared to MCs. Except for total N and organic N (ON), all the chemical

and biochemical properties of the composts (lignin, cellulose, C/N ratio, NH_4^+-N) were significantly correlated with N_o. Multiple regression analysis showed that C: N ratio, lignin, NH_4^+-N and cellulose contents could predict 66% of the variation in N_o. The results suggest that BMC can stabilize ON in soil and prevent N losses.

3.2 Introduction

The supply of food to urban market centers and the intensive livestock production systems in most developing countries, including Ghana generate substantial amounts of organic waste such as market waste (MW) and manure (M), which are disposed at landfill sites (Chen *et al*., 2017). Also, these wastes are often left in the open where they become a breeding ground for disease-causing animals or are burnt off producing large amounts of CO_2 which contribute to global warming (Ayilara *et al*., 2020). To forestall the continuous threat these waste pose to the environment, there is a need for economically feasible and environmentally sound treatment that can generate a nutrient-rich organic fertilizer to serve as an alternative to chemical fertilizers (Vandecasteele *et al*., 2016; Li *et al*., 2011).

Composting has been identified as one of the preferred methods for recycling MWs and manures. Composting concentrates nutrients in wastes materials through biological decomposition and converts them into organic amendments that can be applied to agricultural lands to alleviate the rapid nutrient decline, especially nitrogen (N) in tropical soils (Bernal *et al*., 2009; Lim *et al*., 2018). Composting also helps in preventing immobilization of plant nutrients and phytotoxicity when these wastes are applied directly to soils (Dias *et al*., 2010). In addition, composting manure and MWs termed as manure compost (MC) enhances the organic matter (OM) content as well as the physical and biological properties of soils that are important for sustainable plant growth (Bass *et al*., 2016; Yadav *et al*., 2013; Lim *et al*., 2018). However, the high contents of readily decomposable carbon (C) and N in MC predisposes them to rapid decomposition, thereby necessitating yearly re-application (Yadav *et al*., 2013). Moreover, the high temperatures in the tropics favor rapid mineralization of N in these composts, which may result in nitrate leaching and groundwater pollution as well as eutrophication (Yadav *et al*., 2013; Burgos *et al*., 2006). Thus, to enhance the recalcitrance of these composts as well as to sustain soil fertility, there is the need for stabilization of C and N in them to increase their mean residence time in soils.

Recently, the use of biochar, a stable carbonaceous material produced by pyrolysis of biomass under limited oxygen condition, has been shown to act as a bulking agent to stabilize nutrients from MW and manure-based composts (Yuan *et al*., 2017, Oledele *et al*., 2022; Thiele-Bruhn and Ngigi, 2021; Zoglie, 2021). Biochar interacts with the varied organic N (ON) forms in MW and manure to form N-enriched compost that are more C and N stable during co-composting (Antonangelo *et al.,* 2021, Thiele-Bruhn and Ngigi, 2021; Mirsha *et al*., 2022). The combined composting of various organic wastes and biochar, termed "co-composting", modifies the surface functional groups of biochar, promotes the formation of humic substances, intensifies humification and regulates the rate and extent of degradation of labile organic C and N, resulting in reduced emissions and enhanced compost stability (Jindo *et al*., 2012; Casini *et al*., 2021; Dias *et al*., 2010; Agyarko -Mintah *et al.,* 2017a). Such material referred to as biochar-manure co-compost (BMC) will contain stable organo-mineral complexes with varying degrees of susceptibility to decomposition and N mineralization. Biochar-manure compost influences crop productivity through stimulation of microbial diversity, increasing OM and nutrient load as well as immobilization of potentially toxic organic pollutants (Wu *et al*., 2017; Guo *et al*., 2020; Kammann *et al*., 2016; Cooper *et al*., 2020; Agegnehu *et al*., 2017; Hagemann *et al*., 2017).

The influence of biochar on N mineralization is determined by the biochar type, feedstock type, pyrolysis temperature, residence time and loading rate (Antonangelo *et al*., 2021). For instance, plant-based biochar with relatively larger surface area tends to absorb more labile C and N and produces more recalcitrant ON, reducing N mineralization and loss relative to manure-based biochar (Agyarko-Mintah *et al*. 2017a). Yuan *et al*. (2017) and Singh *et al*. (2010) reported that co-composed rice hull and woody biochar with manure produced a highly stable OC which slowed N mineralization owing to the high C: N ratio of the final compost. Also, Awasthi *et al*. (2017) showed that a biochar loading rate of 12% increased the C content of the final compost which influenced N immobilization when applied to the soil. The above references indicate that BMC derived from woody and plant biochar and applied at a higher loading rate is likely to sorb more labile OM, increase the C: N ratio and release N slowly thereby facilitating crop productivity and reducing frequent application (Kammann *et al*., 2015, Agegnehu *et al*., 2016, Schulz *et al*., 2013; Tsai and Chang, 2020).

For mineral N to be made available for crop uptake, the ON in BMCs needs to be mineralized. Therefore, it is imperative to estimate the N releasing capacity or the potentially mineralizable N (N_o) of BMCs for making recommendations on the rate and timing of application to meet crop N needs and to avoid environmental pollution associated with excessive MC application (Sharifi *et al*., 2014, Cabrera *et al*., 2005; Li and Li, 2014). The N_o, considered as the active fraction of ON that can be converted to available N by the action of microorganisms during the growing season of plants (Curtin and Campbell, 2008; USDA-NRCS, 2014), and associated mineralization rate constant, k is controlled by edaphic factors (moisture, temperature, soil properties), composition of organic material (C, N concentration, lignin content etc.) and decomposing organism (Cabrera *et al.,* 2005; USDA-NRCS, 2014).

The composition of BMCs and the properties of the soils are important factors that can be used to estimate N_o in BMC-amended soils. According to D'Hose *et al.* (2020), biochar blended compost and compost applied in a field trial released mineral N in proportion to the C content and rate of application. However, the authors did not assess the influence of soil properties, especially texture on N availability and N_o of the amendments. Hassink *et al.* (1993) reported higher N mineralization in sandy compared to clayey soils and attributed it to the physical protection of OM and smaller pore spaces in clays, which reduced microbial decomposition and N release. Currently, only few studies evaluated the influence of soil properties on N mineralization from BMCs (Tsai and Chang, 2020; Manirakiza *et al*., 2019; Schulz *et al*., 2013; Manolikaki and Diamadopoulos, 2019), even though the C: N ratio, the initial ammonium, total C, lignin, polyphenol and total N of compost and manure have been shown to relate closely to N_o of amended soils (Gale *et al*., 2006; Lazicki *et al*., 2020, Azeez and Van Averbeke, 2010; Burger and Venterea, 2008; Calderon *et al*., 2005; Vahdat *et al*., 2011, Hartz *et al*., 2000; Palm and Sanchez., 1990).

Application of BMCs containing different ON pools can affect the kinetics parameter of mineralization such as N_o and k. Various mathematical equations have been used to model and describe N mineralization kinetics in compost amended soils, of which the first order exponential equation developed by Stanford and Smith (1972) is the most frequently used

(Cassidy- Duffery *et al*., 2020; Lazicki *et al*., 2020; Hartz *et al*., 2000; Gale *et al*., 2006; Zoglie, 2021; Dodor *et al*., 2022; 2024). Some researchers have employed this equation to investigate the N mineralization of other organic materials under controlled conditions in the laboratory (Chae and Tabatabai, 1986; Cassity-Duffey *et al*., 2020; Kaur *et al*., 2018; Zoglie, 2021; Dodor *et al*., 2022; 2024).

It is hypothesized in the present study that biochar and manure types of varying ON pools will moderate the rate and extent of N mineralization from BMCs. This study aims at (i) determining the N_o and k of BMC and MC (control) derived from a range of combinations of poultry and cattle manures, and biochar types (ii) evaluating the relationship between N_o and properties of compost (iii) determining the effect of soil types on the N mineralization kinetics.

3.3 Materials and Methods

3.3.1 Soil sampling and characterization

The soils used in the present study belong to the Adentan, Denteso and Keta series. The soils were selected from locations to cover a wide range of agroecological zones in Ghana. The Adentan series was sampled from the University of Ghana farms, Legon, Ghana (5° 39’3516” N, 0°11’37.026” W) located in the coastal savannah agro-ecological zone of Ghana. The area experiences total annual rainfall of about 800 mm which is bimodally distributed, and a mean temperature of 28°C (Dowuona *et al.,* 2012). The soil is classified as Ferric Acrisol according to the IUSS-WRB criteria (IUSS-WRB, 2022) and Typic Kandiustalf by Eze (2015) according to the USDA classification system. Denteso series was sampled from Donkorkrom in the Sekyere Afram plains district of the Eastern Region of Ghana (7° 1’42.66” N, 0°5’43.56” W) located in the forest-savannah transitional agro-ecological zone of Ghana. The area experiences an annual temperature of 26 ºC and a mean annual rainfall of 1332 mm which is bimodally distributed (Adu and Mensah-Ansah, 1995). The soil is classified as Dystric Fluvisols according to the IUSS-WRB classification system (IUSS-WRB, 2022). Keta series was taken from Anloga, a town located in the Keta municipality in the Volta Region of Ghana (5º79'74.28" N 0º92'17.51 E) in the semi-arid coastal savanna zone of Ghana. It is classified as Gleyic Arenosols according to the IUSS-WRB classification system (IUSS-WRB, 2022) and Quartzipsamments according to the soil Taxanomy criteria (Soil survey staff, 1998). The soil is a narrow sand spit consisting of marine sands and forming narrow elongated sandbars with narrow depressions (Awadzi *et al*., 2008). The climate at the area is dry equatorial with a mean annual temperature of about 28-30ºC and a mean annual rainfall below 900 mm which is unevenly distributed over the year (Dickson and Benneh, 1988; Dodor *et al*., 2018; 2019).

The three soils were sampled from farmers' fields. This selection was because farmers' fields are mostly nutrient-deficient due to continuous cultivation and to assess the influence of the amendment on the soil's nutrient status, this choice of soil was relevant for the study. On Keta series, crops such as carrots, okro, lettuce and maize had been cultivated whilst maize was cultivated on Denteso series. However, on Adentan series, the soil had been left to follow for about 5 years after the cultivation of okro. After about four weeks of harvesting of crops on Keta and Denteso series, surface soil samples (0-20 cm) on each field including Adentan serie were randomly collected in an area of 5 m x 5 m, passed through a sieve of 2 mm mesh and transported to the laboratory in polyethene bags. Afterwards samples were divided into two portions with one portion kept in the refrigerator for about 3 days at a temperature of 4 ºC for the incubation studies and the other portion air-dried at room temperature for the measurement of selected physical and chemical characteristics.

The soil pH was determined in a soil to water ratio of 1:1 (Watson and Brown, 1998), bulk density was measured by the core sampler method (for Adentan series) and excavation method (for Denteso and Keta seies) (Blake and Hartge, 1986) while particle size distribution was determined by the Bouyoucos hydrometer method (Gee and Bauder, 1986). The total C (TC) and total N (TN) were determined on < 180 µm air-dried samples by dry combustion using LECO CNS analyzer (LECO Corp., St. Joseph Michigan). The mineral N (NH_4^+-N and NO_3^--N) was determined using the steam distillation method described by Mulvaney (1996). The differences between mineral N and TN were used to determine the Organic N (ON) of the soils. All laboratory analyses were done in triplicate and presented in Table 3.2.

3.3.2 Preparation of biochar manure co-compost (BMC) amd manure compost (MC)

The biochar materials used in the present study was produced from rice husk, sawdust and coconut husk which were collected from the Ghana Irrigation Development Authority Project site located at Ashiaman, in the Gretaer Accra Region of Ghana, local wood mill and coconut vendors, respectively. The different biochars were produced at pyrolysis temperature of 450 °C using a Kuntan kiln, cooled, crushed, and passed through a 2 mm sieve to ensure uniformity. Market waste (MW), mainly vegetable and fruit wastes, were collected from the Madina market located at the La Nkwantanang Municipal Assembly in Accra, Ghana at two consecutive days. The cattle (CM) and poultry (PM) manures were collected from the Livestock and Poultry Research Center (LIPREC, University of Ghana).

Co-composting was done on a platform at the University of Ghana, where eight (8) compost types (i.e., 6 BMC and 2 MC) were prepared in triplicate, i.e., 24 experimental units (2 manure types × 4 biochar types × 3 replicates). Biochar was applied at 20% (v/v). The piles were prepared and labelled as percentages of various feedstocks used such as $B_0CM_{40}MW_{40}$, $B_0PM_{40}MW_{40}$, $RB_{20}CM_{40}MW_{40}$, $CB_{20}CM_{40}MW_{40}$, $SB_{20}CM_{40}MW_{40}$, $RB_{20}PM_{40}MW_{40}$, $CB_{20}PM_{40}MW_{40}$ and $SB_{20}PM_{40}MW_{40}$. The various proportions of feedstock used in the compost preparation are presented in Table 3.1. The raw materials were moistened with water (60% water holding capacity), mixed thoroughly, and transferred into 100 L plastic containers with perforations for gas and heat exchange with the environment. A vertical pipe with a hanging thermometer was inserted through one of the holes for temperature readings. The mixture was stirred weekly, and the composting process was completed after 90 days. After air-drying, each compost was sieved using a 10-mm mesh. Random sampling was performed on the mature compost for chemical and biochemical analyses in triplicate. Mineral N content was determined by the procedure described by Mulvany (1996), lignin (L) and cellulose (CE) by the van Soest and Wine (1968) method, pH and germination index by Watson and Brown (1998) and Zucconi *et al*. (1981) procedures, respectively. The TC and total TN contents were determined by the CNS analyzer. The properties of the compost feedstocks and biochars as well as the final compost are presented in Tables 3.2 and 3.3, respectively.

Table 3.1 Biochar manure compost (BMC) and manure compost (MC) with their composition.

BMC	Manure type	Biochar type	Market waste
$B_0CM_{50}MW_{50}$	CM	0B	MW
$CB_{20}CM_{40}MW_{40}$	CM	CB	MW
$RB_{20}CM_{40}MW_{40}$	CM	RB	MW
$SB_{20}CM_{40}MW_{40}$	CM	SB	MW
$B_0PM_{50}MW_{50}$	PM	0B	MW
$CB_{20}PM_{40}MW_{40}$	PM	CB	MW
$RB_{20}PM_{40}MW_{20}$	PM	RB	MW
$SB_{20}PM_{40}MW_{40}$	PM	SB	MW

0B: No biochar; PM: Poultry manure; CM: Cattle manure; MW: Market waste; RB: Ricehusk biochar; CB: Coconut husk biochar; SD: Sawdust biochar

3.3.3 Nitrogen mineralization experiment

The procedure outlined by Stanford and Smith (1972) was used in the long-term N mineralization study. Briefly, 100 g field-moist Adentan and Denteso soils were mixed with 100 g acid-washed sand (for percolation of leacheate) and amended with appropriate amount of the BMC and MC (control) prepared to provide 200 mg N kg^{-1} soil. For the Keta series, 200 g of soil (without acid-washed sand) was used since the soil is made up of about 80% sand (refer to Table 3.2). To prevent soil loss, the compost-soil mixtures were put in leaching tubes with non-absorbent wool lining the bottom. Soil alone was also included in the experiment. The tubes were covered with perforated cling film to allow for the exchange of gases and incubated at 30 ° C for 26 weeks. By weighing and adding water as needed, the moisture level was maintained at 60% water holding capacity (WHC) during the incubation period. The tubes were leached with 75 mL of 0.01 M $CaCl_2$ and 25 mL of N free nutrient solution (0.002 M $CaSO_4.2H_2O$, 0.002 $MgSO_4$, 0.005 M $Ca(H_2PO_4)_2.H_2O$, and 0.0025 M KCl) on the 2nd, 4th, 6th, 12th, 16th, 22nd and 26th week of incubation (Chae and Tabatabai, 1986). Leachates were analyzed for nitrate (NO_3^--N) and ammonium N (NH_4^+-N) using steam distillation (Mulvany, 1996).

During the 26-week incubation period, the cumulative N mineralized was fitted to the first-order kinetic model developed by Stanford and Smith (1972):

$$N_{min} = N_o[1 - \exp(-k_m t)] \qquad (3.1)$$

where N_{min} is the cumulative N mineralized (mg kg^{-1}) at time *t* (week), N_o is potentially mineralizable N (mg kg^{-1}), and *k* is the first-order mineralization rate constant ($week^{-1}$)

The percentage of total N mineralized (% TN) from the applied ON in each treatment at the end of the incubation period was calculated as:

$$\% TN = \frac{Total\ N\ mineralized\ (treatment)}{Total\ organic\ N\ Applied} \times 100 \qquad (3.2)$$

3.3.4 Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate differences in the chemical properties of the BMC and MC and their effect on N_o and *k*. For mean comparisons, the Tukey Posthoc test was used, and a $p < 0.05$ was shown to be significant statistically. The relationship between N_o and biochemical properties of the BMC and MC were evaluated using regression and correlation analysis. All statistical analyses were done using GenStat statistical software (12th edition, 2009). GraphPad Prism 8 for Windows (version 8.0.) was used to determine the first-order kinetic parameters of N mineralization and for graphical representation of N mineralization data.

3.4 Results

3.4.1 Soil properties

Selected physical and chemical properties of the soils are presented in Table 3.2. The texture of the soils is sandy clay loam, sandy loam and sandy for Adentan, Denteso and Keta series, respectively. The pH of the soils was 5.7, 6.1 and 6.7 for Adentan, Denteso and Keta series, respectively. The TC content varied considerably among the soils, with values ranging from 2.3 to 7.5 g kg^{-1}. The TN and ON ranged from 0.18 g kg^{-1} to 0.51 g kg^{-1} and 0.12 g kg^{-1} to 0.44 g kg^{-1}, respectively in all soils. The NH_4^+-N content was 46.5 mg kg^{-1} in Adentan series, 47 mg kg^{-1} in Denteso series and 48 mg kg^{-1} in Keta series. The NO_3^--N content in the three soils ranged from 9.5 mg kg^{-1} to 21.3 mg kg^{-1}.

Table 3.2 Physical and chemical properties of the soils used.

Soil series	Textural class	Sand	Silt	Clay	pH	TC	TN	ON	NH_4^+-N	NO_3^--N
		-----------------%------------				---------------------g kg^{-1}-------------			--------mg kg^{-1}-----	
Adentan	Sandy clay loam	66	10	24	5.7	7.5	0.51	0.44	46.5	19.0
Denteso	Sandy loam	65	24	11	6.1	2.3	0.18	0.12	47.0	9.5
Keta	Sandy	87	11	2	6.7	3.3	0.24	0.17	48.7	21.3

TC: Total carbon; TN: Total nitrogen; ON: organic N

3.4.2 Chemical and biochemical properties of the BMCs and MCs.

The chemical and biochemical properties of the BMCs and MCs were significantly *($p <$ 0.05)* affected by biochar and manure types (Table 3.4). The highest and lowest pH values were recorded in $CB_{20}PM_{40}MW_{40}$ and $B_0CM_{50}MW_{50}$, respectively. The TN contents of the BMC and MC ranged from 9.7g kg^{-1} in $CB_{20}PM_{40}MW_{40}$ to 14.5g kg^{-1} in $B_0P_{50}MW_5$ while the ON ranged from 9.1 g kg^{-1} in $CB_{20}CM_{40}MW_{40}$ to 13.6 g kg^{-1} in the $B_0PM_{50}MW_{50.}$ The highest TC was observed in $SB_{20}PM_{40}MW_{40}$ followed by $CB_{20}CM_{40}WM_{40}$ and $SB_{20}CM_{40}MW_{40}$ and the least was in $B_0CM_{50}MW_{50}$. Cellulose and lignin contents of the BMC ranged from 27 g kg^{-1} in $RB_{20}CM_{40}MW_{40}$ to 93 g kg^{-1} in $B_0CM_{50}MW_{50}$ and 203 g kg^{-1} in $B_0CM_{50}MW_{50}$ to 496 g kg^{-1} in $CB_{20}PM_{40}MW_{40}$, respectively. In general, biochar addition to compost decreased cellulose content compared to compost without biochar while lignin content increased with biochar addition. The NH_4^+-N and NO_3^--N contents ranged from 53.67 mg kg^{-1} to 114.33 mg kg^{-1} and 189.00 mg kg^{-1} to 1103.70 mg kg^{-1}, respectively. The C: N ratio also ranged from 7.4 in $B_0CM_{50}MW_{50}$ to 17.8 in $SB_{20}CM_{40}MW_{40}$. After the co-composting procedure, the germination index (GI) for each compost was more than 80%.

Table 3.3 Selected chemical and biochemical properties of the feedstocks used in preparation of the BMCs and MCs.

Feedstocks	pH	Total carbon	Total nitrogen	Organic nitrogen	NH_4^+-N	NO_3^--N	C: N ratio	Cellulose	Lignin
		-----------------g kg^{-1}	--------------------	--------------------	--------mg kg^{-1}	---------		------------g kg^{-1}	-------------
Market wastes	5.12	349$_c$	21.6$_f$	21.4$_f$	131.8$_b$	7.6$_b$	16.2$_a$	142$_f$	230$_a$
Cattle manure	7.61	172$_a$	12.1$_d$	11.9$_d$	166.8$_c$	10.1$_c$	14.3$_a$	93$_d$	303$_b$
Poultry manure	8.15	200$_b$	16.3$_e$	16.1$_e$	229.8$_d$	16.1$_d$	12.3$_a$	100$_e$	334$_c$
Coconut husk biochar	10.81	466$_e$	4.8$_b$	4.7$_b$	25.7$_a$	6.8$_b$	98.4$_b$	72$_c$	436$_d$
Rice husk biochar	7.43	391$_d$	3.3$_a$	3.2$_a$	16.0$_a$	0.3$_a$	119.2$_c$	27$_a$	340$_c$
Sawdust biochar	8.90	670$_f$	6.8$_c$	6.8$_c$	24.3$_a$	0.1$_a$	98.4$_b$	51$_b$	531$_e$

Values followed by the same letter(s) within each column do not differ significantly at $p < 0.05$ level.

Table 3.4 Selected properties of the BMCs and MCs used.

BMC	pH	TC	TN	ON	NH_4^+-N	NO_3^--N	C: N ratio	CE	L	GI
		-----------------g kg^{-1}--------------------			--------mg kg^{-1}---------			-------g kg^{-1}-------		%
$B_0CM_{50}MW_{50}$	7.96	76_{a}	11.2_{bc}	10.0_{ab}	114.3_{c}	1103.7_{e}	7.4_{a}	93_{d}	203_{a}	92
$CB_{20}CM_{40}MW_{40}$	8.39	125_{b}	9.7_{a}	9.1_{a}	84.0_{b}	445.7_{b}	13.0_{bc}	28_{a}	266_{b}	103
$RB_{20}CM_{40}MW_{40}$	8.09	147_{c}	10.0_{ab}	9.2_{a}	70.0_{ab}	669.7_{c}	14.8_{cd}	27_{a}	240_{b}	97
$SB_{20}CM_{40}MW_{40}$	8.12	206_{d}	11.6_{cd}	10.8_{bc}	77.0_{b}	800.3_{d}	17.8_{f}	42_{b}	452_{d}	95
$B_0PM_{50}MW_{50}$	8.62	126_{b}	14.5_{e}	13.6_{f}	81.7_{b}	863.3_{d}	8.7_{a}	60_{c}	393_{c}	84
$CB_{20}PM_{40}MW_{40}$	9.43	208_{d}	12.5_{d}	12.2_{de}	56.0_{a}	189.0_{a}	15.0_{de}	34_{ab}	496_{e}	88
$RB_{20}PM_{40}MW_{20}$	8.70	140_{bc}	12.2_{cd}	11.7_{cd}	53.7_{a}	476.0_{b}	11.5_{b}	40_{b}	475_{de}	88
$SB_{20}PM_{40}MW_{40}$	8.76	232_{e}	13.8_{e}	13.3_{de}	56.3_{a}	485.3_{b}	16.8_{ef}	32_{ab}	461_{d}	86
Significance of F values										
Manure type (M)	-	***	***	***	***	***	**	***	***	-
Biochar type (B)	-	***	***	***	***	***	***	***	***	-
M × B	-	***	***	***	***	***	***	***	***	-

Ns = not significant, *** =p<0.001, ** =p<0.01, * =p<0.05; GI: Germination Index; CE: Cellulose; L: Lignin; TC: Total Carbon; ON: Organic Nitrogen; TN: Total Nitrogen

Values followed by the same letter(s) within each column do not differ significantly at *$p < 0.05$* level.

3.4.3 Pattern and amount of N mineralized from the BMCs and MCs.

The pattern and cumulative amount of N mineralized in five of the BMC and MC-soil mixtures are illustrated in Figures 3.1A, 3.2C and 3.3E, with the other four falling within those shown. Generally, there was an initial rapid mineralization of N up to the 16th week of incubation in the BMC and MC-amended and unamended soils (soil alone) (Figures 3.1A, 3.2C and 3.3E), followed by a slow N release during the later stages of incubation, resulting in a curvilinear pattern of N mineralization at the end of the incubation period. The total amount of N mineralized ranged from 100 mg kg^{-1} in the soil alone to 176 mg kg^{-1} in Adentan series amended with $B_0CM_{50}MW_{50}$, 48 mg kg^{-1} in soil alone to 129 mg kg^{-1} in Denteso series amended with $B_0CM_{50}MW_{50}$ and 61 mg kg^{-1} in soil alone to 137 mg kg^{-1} in $B_0CM_{50}MW_{50}$ in Keta series (Figures 3.1B, 3.2D and 3.3F). Expressed as percentage of applied N from the amendment, the amount of N mineralized ranged between 14% to 25% in Adentan series, 13% to 34% in Denteso series and 14% to 31% in Keta series. According to an analysis of variance, the total amount of N mineralized differed significantly ($p < 0.05$) among the BMCs and MCs, depending on the type of manure and biochar.

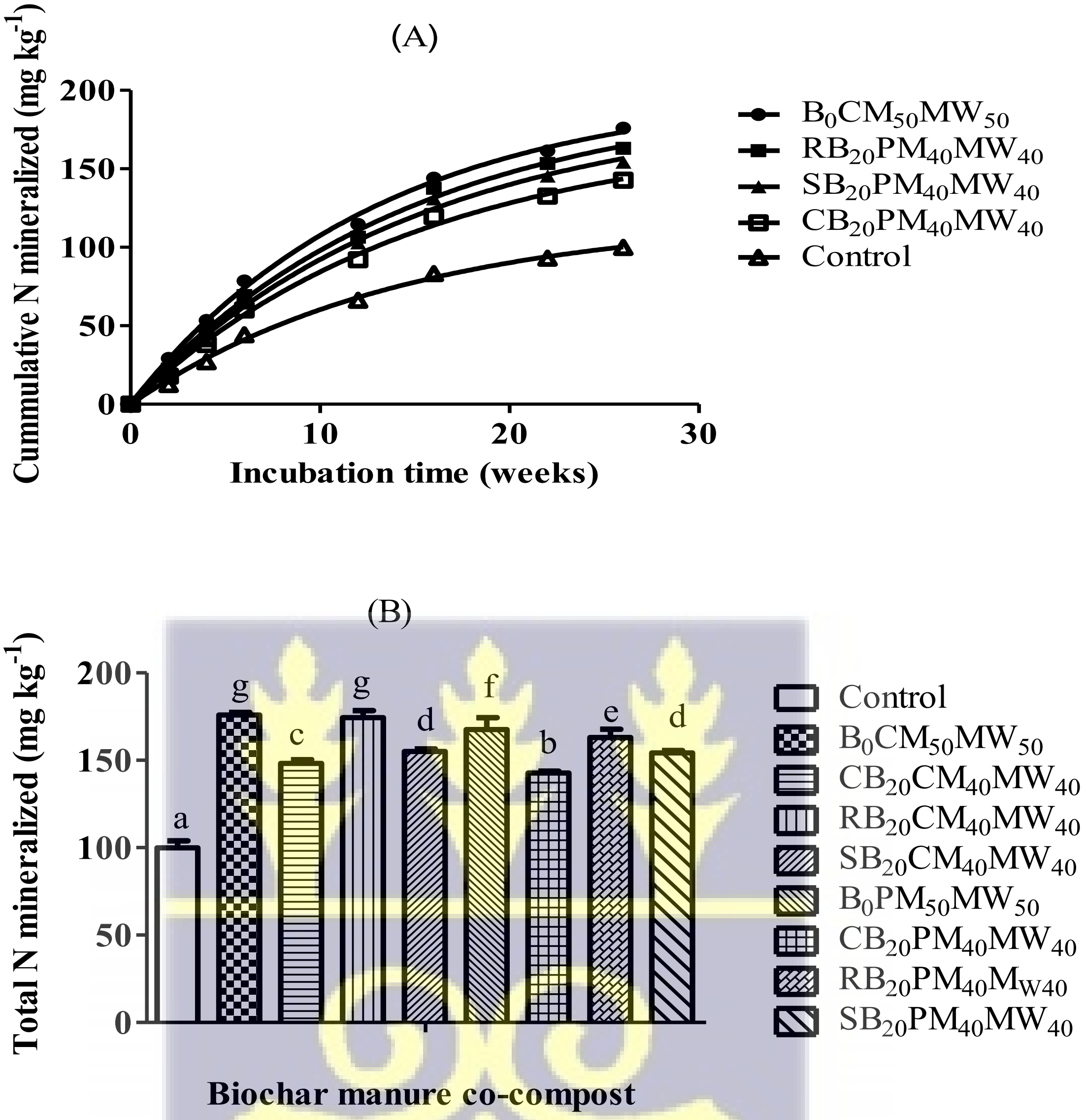

Figure 3.1: Cumulative N mineralized (A) and total amounts of N mineralized (B) from BMC and MC in Adentan series after 26 weeks of incubation. At the $p < 0.05$ level, bars with the same letters do not differ significantly.

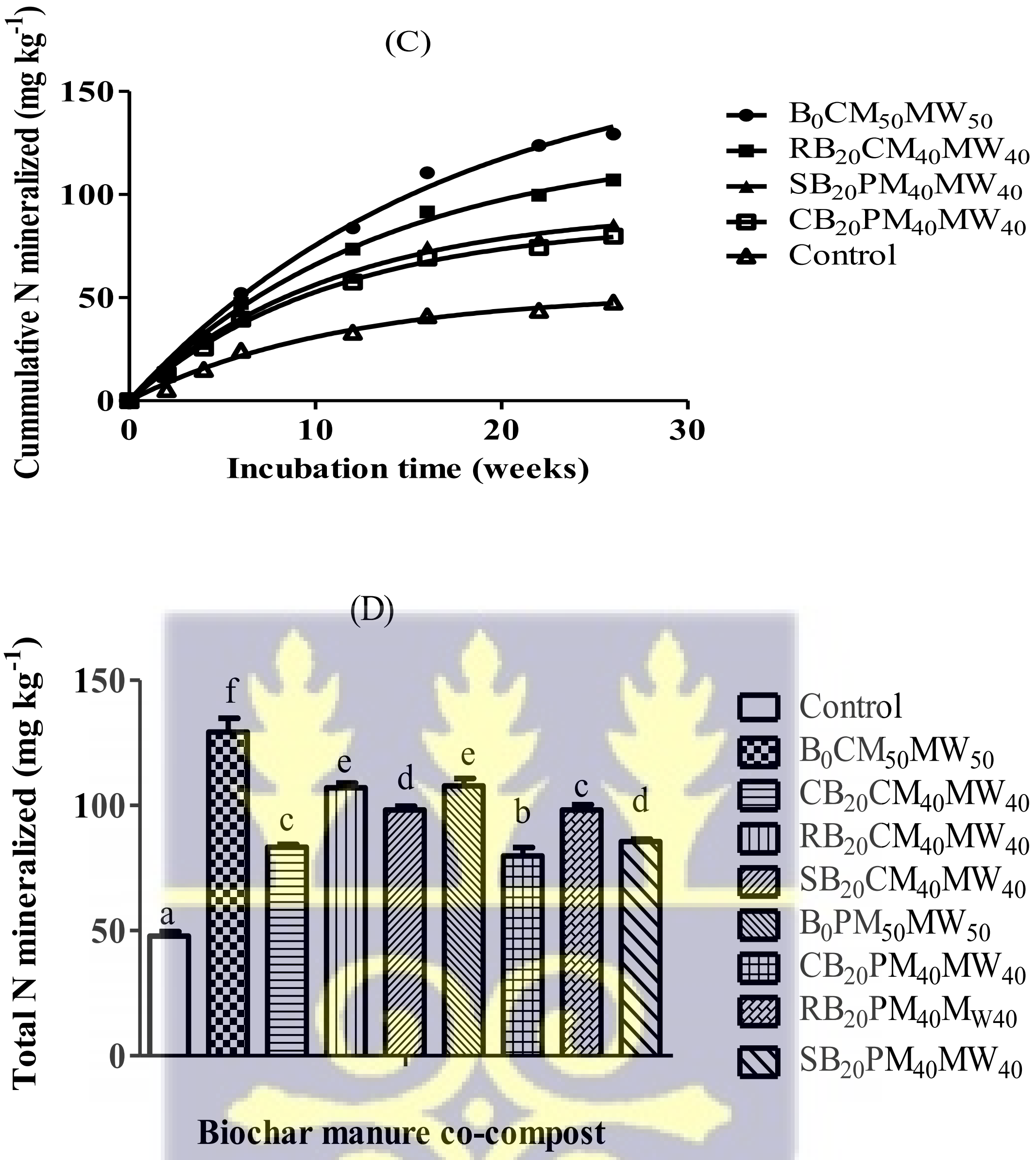

Figure 3.2: Cumulative N mineralized (C) and total amounts of N mineralized (D) from BMC and MC in Denteso series after 26 weeks of incubation. At the $p < 0.05$ level, bars with the same letters do not differ significantly.

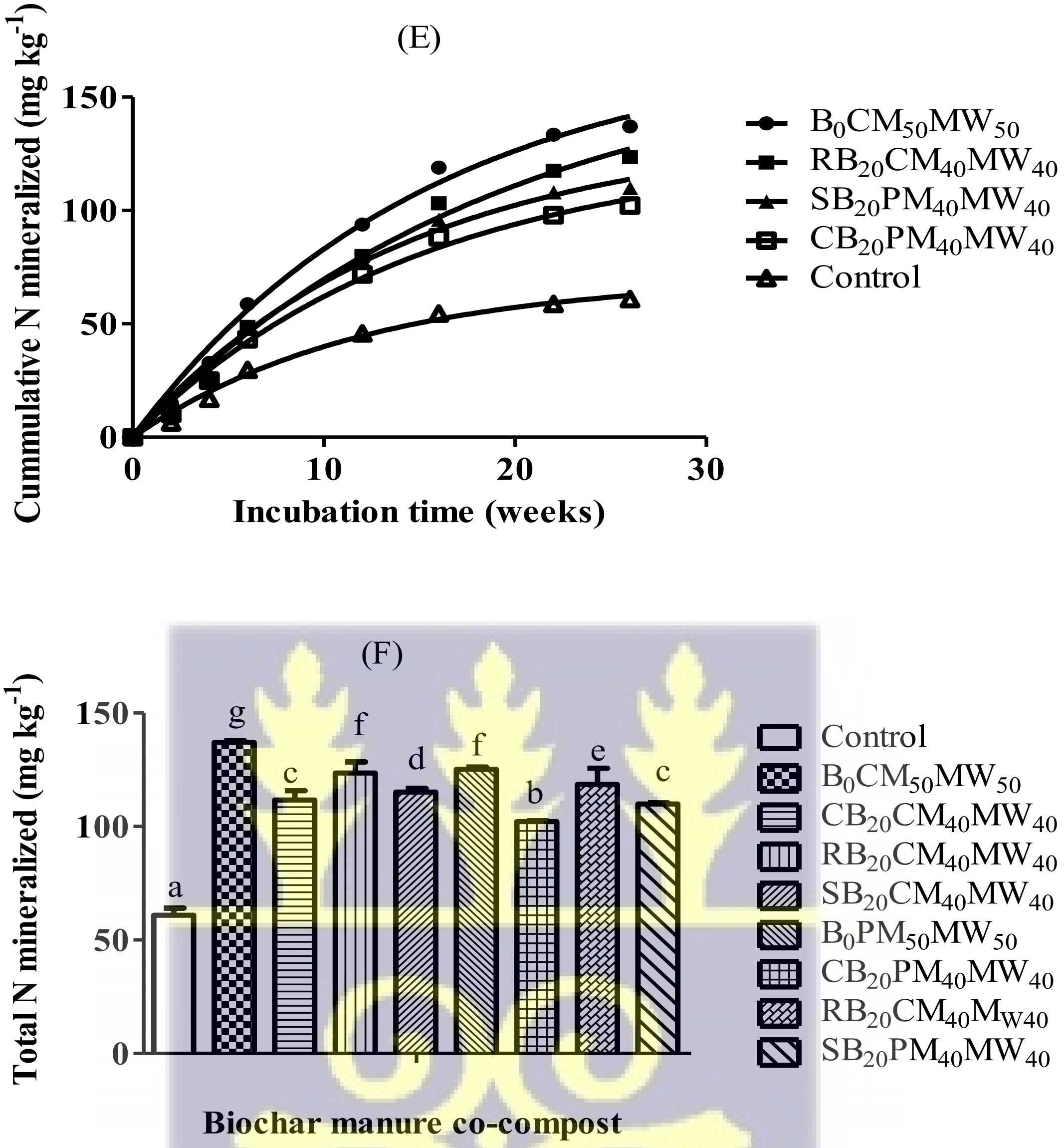

Figure 3.3: Cumulative N mineralized (E) and total amounts of N mineralized (F) from BMC and MC in Keta series after 26 weeks of incubation. At the *$p < 0.05$* level, bars with the same letters do not differ significantly.

3.4.4 Kinetic parameters of N mineralization from the BMCs and MCs

The application of the non-linear first order regression equation to estimate the kinetic parameters (N_o and k) of N mineralization from the BMC and MCs-amended soils indicated that the data conformed very well to the model, with R^2 values ≥ 0.993 across the three soils. The estimated N_o varied among the BMC and MC- amended soils and soils alone, with values ranging from 120 mg kg^{-1} to 205 mg kg^{-1} in Adentan series, 53 mg kg^{-1} to 170 mg kg^{-1} in Denteso series and 71 mg kg^{-1} to 174 in Keta series (Table 3.5). Expressed as a percentage of TN applied, %N mineralized ranged from 17% to 29% in Adentan series, 14% to 45% in Denteso series and 16% to 40% in Keta series (Table 3.5). Regardless of the treatment type, the unamended soils had lower N_o values compared to the BMC and MC amended soils. Among the compost types, $B_0CM_{50}MW_{50}$ had the highest N_o followed by $RB_{20}CM_{40}MW_{40}$ and $B_0PM_{50}MW_{50}$, with N_o values being 0.71, 0.64 and 0.63 folds higher in Adentan series, 2.21, 1.38 and 1.32 folds higher in Denteso series and 1.45, 1.42 and 1.20 folds higher in Keta series compared to the soil alone (Table 3.5). The N_o values among the BMC and MCs-amended soils differed significantly $(p < 0.05)$, according to analysis of variance. The highest N_o values were recorded in MC followed by BMC (RB-based compost). The least N_o values were observed in CB and SB-based compost. Further, CM-based compost had the highest N_o compared with PM-based counterparts. Overall, the N_o values of the soils followed the order Adentan< Denteso < Keta series.

The k values varied among the compost in the amended soils, with values ranging from 0.063 to 0.083 $week^{-1}$ in Adentan series, 0.058 to 0.089 $week^{-1}$ in Denteso series and 0.059 to 0.075 $week^{-1}$ in the Keta series (Table 3.5). Generally, the estimated k values of BMCs were slower than the MCs.

Table 3.5 Kinetic parameters of N mineralization from the BMCs and MCs in the soils.

Soil Series	Adentan series			Denteso series			Keta series		
BMC	N_o (mg kg^{-1})	k (week^{-1})	%TN	N_o (mg kg^{-1})	k (week^{-1})	%TN	N_o (mg kg^{-1})	k (week^{-1})	%TN
Control	120_{a}	0.070_{c}	17	53_{a}	0.073_{b}	14	71_{a}	0.064_{bc}	16
$B_0CM_{50}MW_{50}$	205_{e}	0.083_{d}	29	170_{h}	0.089_{e}	45	174_{f}	0.075_{f}	40
$CB_{20}CM_{40}MW_{40}$	183_{bc}	0.064_{a}	26	98_{d}	0.072_{b}	26	140_{d}	0.063_{b}	32
$RB_{20}CM_{40}MW_{40}$	197_{de}	0.077_{c}	28	126_{g}	0.081_{d}	33	172_{f}	0.072_{e}	39
$SB_{20}CM_{40}MW_{40}$	180_{b}	0.077_{c}	25	106_{e}	0.074_{b}	28	146_{d}	0.06bc	33
$B_0PM_{50}MW_{50}$	196_{de}	0.078_{c}	27	123_{g}	0.081_{d}	32	156_{e}	0.068_{d}	35
$CB_{20}PM_{40}MW_{40}$	176_{b}	0.063_{ab}	25	87_{b}	0.058_{a}	23	128_{b}	0.059_{a}	29
$RB_{20}PM_{40}MW_{20}$	192_{cd}	0.068_{ab}	27	114_{f}	0.078_{c}	30	154_{e}	0.066_{cd}	35
$SB_{20}PM_{40}MW_{40}$	191_{cd}	0.065_{ab}	27	93_{c}	0.074_{b}	24	136_{c}	0.062_{b}	31
Significance of F value									
Manure type (M)	***	***	-	***	***	-	***	***	-
Biochar type (B)	***	***	-	***	***	-	***	***	-
M × B	***	***	-	***	***	-	***	***	-

No: potentially mineralizable N; *k*: rate of N mineralization; %TN: Percentage total N mineralized

3.4.5 Relationship between N_o values and BMC properties.

The relationship between selected properties of the compost and the estimated N_o values is shown in Table 3.6. In general, estimated N_o values were negatively correlated with C: N ratio, lignin, and TC, with *r*-values of -0.574 ($p < 0.001$), -0.554 ($p < 0.001$) and -0.634 ($p < 0.001$), respectively (Table 3.6). Cellulose and NH_4^+-N content of the composts were positively and significantly correlated with N_o values, with *r*-values of 0.683 ($p < 0.001$) and 0.513 ($p < 0.01$), respectively. The N_o values were negatively correlated with TN and ON, however, the correlation was not significant (Table 3.6).

To assess the importance of BMC properties in predicting N_o, a stepwise multiple regression analysis was employed. The results indicated that the C: N ratio explained about 32% of the variation in N_o (Table 3.7). When cellulose was added to the equation, the model explained 45% of the variations in N_o. The variation in N_o explained by the model increased to 53% when lignin, cellulose and C: N ratio were used in the equation. Finally, combination of C: N ratio, cellulose, lignin and NH_4^+-N content of the amendments explained about 66% of the variation in N_o (Table 3.7).

Table 3.6 Pearson correlation coefficient (*r*) between selected properties of the BMCs, MC and N_o in three soils.

BMC and MC properties	N_o (Correlation coefficient, *r*)
C/N ratio	-0.574^{***}
Cellulose	0.683^{***}
Lignin	-0.554^{***}
NH_4^+-N	0.513^{***}
TC	-0.634^{***}
TN	-0.228^{ns}
ON	-0.237^{ns}

Ns = not significant, $^{***} = p < 0.001$, $^{**} = p < 0.01$, $^{*} = p < 0.05$

Table 3.7 Multiple regression analysis between properties of the BMCs, MC and N_o.

Regression model ($Y=b_1X_1 \ldots b_nX_n+b_o$)	R^2	*P*
N_o = -4.12C: N +187	0.32	<0.001
N_o = -3.29C: N +0.22CE +166	0.45	<0.001
N_o = -1.58C: N +0.30CE -0.09L +175	0.53	<0.001
N_o = -1.63C: N +0.35CE -0.1 +0.156 NH_4^+-N +190	0.66	<0.001

CE: Cellulose; L: Lignin

3.5 Discussion

The pH values of the BMCs and MC fall within the range reported by na Mona (2003) for a mature compost. The observed differences in the pH values of the BMCs and MC may be due to the initial pH of the feedstock and those of the biochar produced (see Table 3.3).

The significant reduction in the TN and ON contents of the BMC compared to the MC may be attributed to the dilution of N content of the final product because of the low N in the biochar, especially CB and RB (Table 3.4). This agrees with the findings of Steiner *et al.* (2010), who found a reduction in N content of BMC when poultry manure was co-composted with 20% pine chip biochar compared to the 5% and 0% biochar addition. The reason could also be due to ammonia volatilization because of the high pH of biochar. This result is in line with reports by Akumah *et al.* (2021) who reported a reduction in TN of compost when 15% rice husk biochar was co-composted with market waste. The higher N content recorded in the PM-based compost may be due to the higher N content of the PM feedstock. According to Azeez and Van Averbeke (2010), PM has higher N content compared to CM due to differences in the animals' diets. Mostly chickens are fed grains and cakes with higher protein and fat contents compared with cattle forages and straws (Azeez and Van Averbeke, 2010). The higher C content of the BMCs, especially SB-based compost compared to the MCs, can be attributed to the recalcitrant C content in the biochar. This finding agrees with those of Khan *et al*. (2016) who identified the recalcitrance of hardwood shavings during and after composting with chicken manure as a contributor to the higher C content of the final product.

Cellulose and lignin contents of the BMCs and MCs are similar to those reported by Vahdat *et al*. (2011) and Marshall *et al*. (2016) for organic residues and commercial composts made for high tunnel tomato production, respectively. The low cellulose content in the BMC compared to the MC could be attributed to accelareted decomposition of organic matter due to the incorporation of biochar which was reported by Sanchez-Monedero *et al.* (2018) and Dias *et al.* (2010). For a compost to be considered matured generally, NH_4^+-N concentration must be less than 400 mg kg^{-1}, NO_3^--N concentration must be mostly more than 400 mg kg^{-1} while the C: N ratio must be lower than 20:1 (Brinton, 2000; Bernal *et al.,* 2009; Guo *et al.,* 2012). According to the results obtained for BMCs and MCs prepared, the composts were matured. The observed > 80% GI for the BMCs and MCs also indicated that phytotoxicity has been eliminated and the composts are matured (Zucconi *et al*., 1981).

The very high R^2 values achieved when the cumulative N mineralized from the BMCs and MCs were fitted to the first-order kinetic model showed that the model proposed by Stanford and Smith (1972) is a useful approximation of N mineralization in BMC and MC-amended soils, as has been reported previously by other researchers (Gale *et al*., 2006, Lazicki *et al*., 2020; Zoglie, 2021; Dodor *et al*., 2024). The curvilinear pattern of N mineralization in the BMCs and MC-amended and unamended soils could be attributed to the progressively declining levels of the readily mineralizable N pool. This result corroborates the findings of other authors who monitored the N release of composted manure in soils and reported a decreasing N mineralization as incubation progressed (Lazicki *et al*., 2020; Zoglie, 2021; Dodor *et al*., 2024). The results, however, contradict reports by other authors that mineralization of N in manured soils increased linearly with time which was attributed to the type of manure and the type of soil amended (Chae and Tabatabai, 1986; Gale *et al*., 2006)

In contrast to the soil alone, the BMC and MC-amended soils had higher N_o values which is in line with the results of other researchers who reported that composting N rich material with biochar increased the ON and TN contents of the soils compared to the unamended control (Agegnehu *et al*., 2016; Awasthi *et al*., 2017; Zoglie, 2021). Also, Sanchez-Monedero *et al*. (2019) reported a 44% increase in extractable N after applying biochar compost to the soil. Luo *et al*. (2017) also found higher soil organic carbon (SOC) and potentially available N when degraded coastal soil was amended with co-composted biochar and other additives such as commercial humate, seafood shell powder, inorganic nutrients and peanut shell biochar compared to soil alone. The current study's N_o values are more than the 5.3 mg kg^{-1} to 73 mg kg^{-1} reported by Li and Li (2014) for poultry and cattle manured soils. Chae and Tabatabai (1986) reported N_o values ranging between 456 mg kg^{-1} and 844 mg kg^{-1} for soils amended with animal manure. In addition, Zoglie (2021) reported N_o values between 127.8 and 180.3 mg kg^{-1} for some coastal savanna soils amended with BMCs of varied C: N ratios. The observed higher N_o values when the soils were amended with $B_0CM_{50}MW_{50}$ and $B_0PM_{50}MW_{50}$ (MC) could be attributed to the higher content of easily mineralizable organic N and C, with the latter stimulating the activities of heterotrophic microorganisms and enhancing mineralization of the organic N (Dodor *et. al*., 2018; 2019). This finding is consistent with the results by Yuan *et al*. (2017) who found a higher N availability in CM compost compared to biochar-CM co-compost. The result is also consistent with that of Tsai and Chang (2020) who reported an increase in available N when 2% compost without biochar was applied to soils and attributed it to less N immobilization due to readily available N.

The observed decrease in N_o values following amendment with BMCs can be attributed to the formation of stabilized humic substance when biochar was co-composted with manure, leading to N immobilization (Thiele-Bruhn and Ngigi 2021, Mirsha *et al*., 2022, Ippolito *et al*., 2016; Dodor *et al*., 2024). The results are consistent with those of Ippolito *et al*. (2016) who reported a lower N mineralization following co-application of hardwood biochar and manure to soils. Dodor *et al*. (2024) also reported lower N_o values in soils amended with BMCs compared to MCs. Similar lower N mineralization was observed by Manirakiza *et al*. (2019) when they amended the soils with paper biosolid and pine biochar. The authors attributed this occurrence to the increased sorption capacity of biochar due to higher cation exchange capacity (CEC), porosity and surface area as well as lower N content of the amendment.

According to Antonangelo *et al*. (2021) and Sanchez-Monedero (2018), biochar properties can have a significant effect on the composting process, as well as properties of the final product and biochar. The biochar loading rate and biochar feedstock are mostly used in determining the influence of BMC on N mineralization capacity of soils (Awasthi *et al*., 2017). Since the biochar loading rate used in this study is one, the observed differences in the N_o values could be attributed to the biochar feedstock types. The observed lower N_o values in CB and SB-based BMCs are due to the woody and lignocellulose nature of the feedstock and its resultant biochar. According to Singh *et al*., (2010) lignocellulose feedstocks produce biochar of low N content, high C: N ratio and high aromatic C content that influence N immobilization by microorganisms compared to manure or crop biochar. The results corroborate those of other authors (Manirakiza *et al.,* 2019; Singh *et al.,* 2010, Ippolito *et al*., 2016; Tsai and Chang, 2020) who reported lower N mineralization from biochar produced from lignocellulose or woody feedstocks. Further, during composting of woody and lignocellulose biochar with manure, higher CEC, porosity, and surface area is achieved due to aging and OM adsorption which promote low mineralization (Weidner *et al*., 2015; Agyarko-Mintah *et al*., 2017b; Khan *et al*., 2016). Based on these results by other authors it could be deduced that the low N_o values from the CB and SB-based composts can be attributed to prevalence of the above properties, which increased the biochar adsorptive capacities. The high adsorptive capacity may have decreased the available N concentration in CB and SB-based compost amended soils. This result agrees with those of Manirakiza *et al.* (2019) who reported a lower available N in biochar and paper biosolid-amended soils and attributed it to higher CEC, surface area, and porosity of the biochar. Hagemann *et al*. (2017) also observed a capture and slow release of inorganic N by BMC and attributed this occurrence to the biochar component.

The observed higher N_o values for CM-based compared to PM-based composts may be due to the stable ON in PM-based compost after composting and the high C content in the manure as a result of the bedding used (refer to Table .3.3). This finding contradicts reports by Azeez and Van Averbeke (2010) and Li and Li (2014) who reported a high N mineralization from poultry manure compared to the cattle manure and attributed this result to the high N in poultry manure as a result of the diet fed to the animals. The higher N_o for CM-based compared to PM-based composts could probably be due to the high labile C in the CM-based composts providing substrate for microbial decomposition and N mineralization (refer to Table 4.2).

The estimated higher N_o in Adentan series compared to the other soil series could be attributed to the relatively higher C and ON due to the management practice on the field prior to sampling (see Table 3.2). Microbial decomposition is enhanced by the availability of labile C and N which promote biosynthesis of proteins, nucleic acids, and enzyme activities (Jien *et al*., 2017; Farrell *et al*., 2014), as have been reported in the literature (Busby *et al*., 2007; Duong *et al*., 2012). For example, Busby *et al*. (2007) observed higher net N immobilization in soils containing less N and C when amended with composted municipal waste. In contrast, Mubarak *et al*. (2010) found increased N mineralization in coarse-textured (sandy soil) manured soils and attributed this occurrence to the soil texture.

The slower mineralization rate constant (*k)* in the BMCs compared with MCs suggests differences in the nature of the mineralizable ON content of the two amendments. This result is consistent with the reduced N mineralization from BMCs, probably due to the stabilization of ON during composting. Several studies have reported the impact of biochar on the rate of N mineralization in BMC-amended soils (Yuan *et al*., 2017; Schofield *et al*., 2019; Tsai and Chang 2020). Tsai and Chang (2020) reported 6%, 9% and 19% decrease in N mineralization rate when 0.5, 1% and 2% biochar were applied in combination with poultry manure compost to soils in a 371-day incubation study. Organic N stabilization by biochar and its resistance to mineralization categorizes BMCs into a slow N release fertilizer, profiting the growth and yields of crops as well as preventing excessive leaching of nitrates in light textured soils (Kammann *et al*., 2015).

The negative correlation between N_o and C: N ratio of the BMCs indicated that as the C: N ratio of the BMCs increases the N_o decreases, which is consistent with previous studies (Lazicki *et al*., 2020; Cassity-Duffey *et al*., 2020; Qian and Schoenau, 2002). According to Qian and Schoenau (2002), a negative correlation was observed between C: N ratio of CM and N mineralization at a value greater than 15, which decreased the N availability in the short term. According to the authors, C: N ratio was the best predictor of N mineralization in the short term. Likewise, van Kessel and Reeves (2002) reported a significant negative correlation between N mineralization and C: N ratio of manure. On the contrary, Li and Li (2014) observed non-significant correlation between C: N ratio and N mineralization in manured soils. According to the authors, other factors such as ON fractions other than C: N ratio could have accounted for N mineralized.

The positive relationship between NH_4^+-N and N_o is consistent with the results of Marshall *et al*. (2016) showing that the initial inorganic N in compost positively influenced the amount of N mineralized in a high tunnel tomato production. The negative relationship between lignin content and N mineralized has been attributed to the aromatic-based polymer content of biochar which forms complexes with ON compounds in compost (Toumela *et al*., 2000), thereby reducing N mineralization (Vigil and Kissel, 1995; Flavel and Murphy, 2006). The positive correlation between N_o and cellulose content is in line with results by Marshall *et al.* (2016) and Flavel and Murphy (2006). The insignificant relationship between TN, ON and N_o could be due to the complexity of the availability of N in the BMC as a result of the biochar component (such as its adsorption and sorption of N) (Manirakiza *et al.,* 2019; Hageman *et al*., 2017; Kammann *et al.,* 2015)

The stepwise regression analysis using all the compositional parameters indicated that the properties of the composts such as cellulose, C: N, lignin and NH_4^+-N have a predictive value for N_o. This shows that standard BMC compositional factors and N mineralization exist for this data set and can be used to predict variability in N mineralization from BMC.

3.6 Conclusions

The study indicated that application of BMC to the soils resulted in lower N_o values compared with MC. In all, CB- and SB-based composts had the lowest N_o values when amended with the three tropical soils. Also, the BMC-amended soils had low k values compared with the MC-amended soils. Among the soils used, Adentan series had the highest *N_o*. The results also showed a negative correlation between C: N ratio, lignin content, TC and *N_o,* while positive correlation was observed between NH_4^+-N, cellulose content and *N_o* values. In all, BMC properties such as lignin, cellulose, C: N and NH_4^+-N were identified as good predictors of *N_o*.

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CHAPTER 4

4.0 AMIDOHYDROLASES ACTIVITY AND SOIL MICROBIAL BIOMASS AS AFFECTED BY BIOCHAR MANURE CO-COMPOST AND ITS RELATION TO NITROGEN MINERALIZATION IN THREE TROPICAL SOILS

4.1 Abstract

The addition of organic amendments such as manure compost (MC) to soils has been reported to enhance microbial biomass and associated activity of amidohydrolase enzymes (AA, L-glutaminase, L-asparaginase and amidase) which are involved in cycling of nitrogen (N) in soils. However, the influence of biochar manure co-compost (BMC) on these parameters has not been examined in tropical soils. In this study, an incubation study was conducted to investigate the influence of BMC and MC (control) amendment on soil microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) and the activities of amidohydrolases in relation to N mineralization in three tropical soils (i.e. Adentan, Denteso and Keta series). The soils were amended with compost prepared from poultry (PM) and cattle (CM) manures alone (MC) or in combination with three biochar types made from ricehusk (RB), sawdust (SB) and coconut husk (CB) at a rate of 20% v/v (BMC) and the amount of N mineralized during 26-week of incubated was determined. The cumulative N mineralized was fitted to first-order exponential model to determine the potentially mineralizable N (N_o) and the mineralization rate constant, k. The soils were also analyzed for pH, total C (TC), organic N (ON), permanganate oxidizable carbon (POXC), C_{mic}, N_{mic} and AA at the end of the incubation period. The results demostrated that applying BMCs and MCs significantly increased the contents of TC, pH, ON, POXC, C_{mic}, N_{mic} and AA compared to the soil alone. However, except for ON, BMCs had a higher influence on chemical and

microbial properties of the soils compared to MCs due to the initial ON content of the MCs. The RB, CB and CM-based BMCs had a higher influence on SMB and AA, probably due to the increased labile C and N in the amended soils. Also, AA and SMB were positively and significantly correlated with each other and with N_o ($r \geq 0.229$; $p \leq 0.05$) as well as chemical properties (TC, ON, pH, POXC) ($r \geq 0.239$; $p \leq 0.05$) of the amended soils. It was concluded that application of BMC modified the micro-biochemical properties of the soils, resulting in increased SMB and AA, which can be used to predict N_o of BMC-amended soils.

4.2 Introduction

The Environmental quality and health implications associated with improper disposal of agricultural and municipal solid wastes require the development of appropriate technologies for creating value-added products from these waste streams for alternate uses (Lim *et al*., 2018; Li *et al*., 2011; Dodor *et al*., 2024). Recently, conversion of mixtures of biochar and agro-municipal organic waste materials, such as market waste and manure, to produce high-value nutrient-enriched biochar manure co-composts (BMCs) has received increasing attention (Akumah *et al.,* 2021; Zoglie, 2021; Dodor *et al.,* 2024; Antonangelo *et al.,* 2021). This amendment helps to improve soil fertility in tropical agricultural soils and has been received as an environmentally friendly and sustainable approach in managing the ever-increasing generation of agro-municipal wastes across the world (Antonangelo *et al*., 2021; Yuan *et al*., 2017, Oledele *et al*., 2022; Thiele-Bruhn and Ngigi, 2021; Zoglie, 2021; Dodor *et al.,* 2024).

Biochar, a carbon (C) rich material produced from pyrolysis of agricultural biomass under limited or no-oxygen condition (Lehmann, 2007; Clough *et al.,* 2013), improves the performance of composting by increasing humification, immobilization of toxic metals and pollutants associated with compost as well as increasing microbial and enzymatic activities (Dias *et al.,* 2010; Jindo *et al*., 2012; Sanchez Monedero *et al*., 2018; Antonangelo *et al.,* 2021; Wang *et al*., 2019). Biochar promotes microbial and associated enzyme activity of the composting process by enhancing the physicochemical characteristics of the composting matrix through the retention of dissolved organic C and N compounds and minerals which serve as nutrients for microbes. Furthermore, biochar enhances an aerobic environment and maintains suitable moisture content during the composting process (Lehmann *et al.,* 2011; Antonangelo *et al*., 2021; Sanchez Monedero *et al*., 2018). As a result, these positive effects promote the composting process, yielding a high-quality compost (Guo *et al*., 2020). Biochar manure co-compost exhibits a similar mechanism by enhancing the microbiology of the soil to facilitate nutrient cycling, organic matter decomposition and nutrient availability, resulting in increased crop productivity compared to manure only compost (Azeem *et al*., 2020; Schulz *et al*., 2013).

However, other studies have shown a negative or neutral impact of BMC on soil microbes and associated enzymatic activities after amendment. For example, Dempster *et al*. (2012) reported that application of composted pig manure and eucalyptus biochar decreased the microbial biomass C (C_{mic}) content of the soil and attributed it to the biochar feedstock. Studies by Elzobair *et al*. (2016) and Feng *et al.* (2019) attributed the observed negative impact of BMC application on the rate of biochar and composting feestocks used.

The mineralization of organic amendments added to soils is mediated by the activities of amidohydrolases associated with microbial biomass. Amidohydrolases (amidase (E.C. 3.5.1.4), L-asparaginase (E.C. 3.5.1.1) L-glutaminase (E.C.3.5.1.2) are the enzymes that catalyze the hydrolysis of C-N bonds other than the peptide bonds in linear amides (Tabatabai, 1994; Dodor and Tabatabai, 2003), releasing nitrogen (N) for microbial assimulation and plant uptake. The importance of the amidohydrolases in soils is based on evidence that a significant amount (15 – 25%) of NH_4^+-N produced from acid hydrolysis of organic N (ON) is derived from linear amides such as L-asparagine and L-glutamine in organic matter (OM) residues (Sowden, 1958). The amidohydrolases in soil are believed to originate mostly from microbes even though some may originate from plants and animals (Tabatabai, 1994). Soil microbial biomass C (C_{mic}) and N (N_{mic}) are labile and a small component of the soil organic matter (SOM) that influence the rate of C and N cycling in soils (Dalal, 1998; Dodor, 2002). Because it responds quickly to management practices, soil microbial biomass (SMB) is a significant and sensitive measure of the quality and health of the soil (Dodor, 2002; Dodor and Tabatabai, 2003; Tabatabai *et al*., 2010). Therefore, due to a linkage between enzymatic activities and microbial biomass, estimating C_{mic} and N_{mic} can help to measure the response of microorganisms to management practices (Dodor, 2002; Balota *et al*., 2003; Dodor and Tabatabai, 2003). Management practices, such as application of BMCs, will affect properties of the soil (e.g., pH, organic N (ON), total C (TC) and labile carbon) which have a direct influence on microbial and enzyme activities (Dodor and Tabatabai, 2003; Sandhu *et al* 2019). Therefore, the activities of soil enzymes have been suggested as an early indicator of changes in management practices (Dodor, 2002). Additionally, SMB has been proposed as a soil fertility index and a predictor of N mineralization in soils (Burket and Dick 1998). Extensive research has been conducted in temperate regions to examine the effects of management strategies on amidohydrolase activity and microbial biomass in connection to

N mineralization in soils. (Deng *et al*., 2006, Dodor and Tabatabai, 2003). For instance, research by Dodor and Tabatabai (2003) showed that amidohydrolase activities, C_{mic} and N_{mic}, were increased in multi-cropping systems which was found to be strongly related to the quantity of N mineralized over a 24-week incubation period at 30 ºC. In a 20-week incubation investigation of limed agricultural soils, Tabatabai *et al.* (2010) also found a strong correlation between the activity of four amidohydrolases and the amount of N mineralized. Indeed, because BMC production involves the combination of feedstocks, which differ in decomposition rates, factors such as feedstock type, biochar rate and composting feedstock may also influence the microbial and enzymatic activities of amended soils (Antonangelo *et al.,* 2021; Sanchez-Monedero *et al.,* 2018; Feng *et al.,* 2019; Dempster *et al.,* 2012). Therefore, it is imperative to examine the impact of biochar type and composting feedstock properties as well as their combinations favorable for microbial and enzymatic activities. Although it is generally known that the activity of amidohydrolases controls the transformation of N in soils, the impact of BMC-amendment induced changes on the activity of N cycling enzymes and the associated microbial biomass have not been evaluated in tropical soils.

It is hypothesized that AA, C_{mic} and N_{mic} will be affected by the application of BMC prepared from different biochar and manure types through changes in the pH, ON, TC and labile C contents of soils. The study aims to (i) investigate the effect of BMC from different biochar and manure types, manure compost (MC- control) from different manure types as well as soil types on the activity of amidohydrolases (L-glutaminase, L-asparaginase and amidase), C_{mic}, N_{mic} and soil chemical properties (pH, permanganate oxidizable carbon (POXC) (a measure of labile carbon), TC (both labile and non labile carbon) and ON), (ii) evaluate the effect of C_{mic} and N_{mic} on the activities of AA (iii) to assess the relationship between AA, C_{mic}, N_{mic} and potentially mineralizable N (N_o) of BMC-amended tropical soils, and (iv) assess the relationship between AA, C_{mic}, N_{mic} and BMC-amended soil chemical properties.

4.3 Materials and Methods

4.3.1 Soils and Biochar Manure Co-Composts (BMCs)

The soils utilized in this current investigation were sampled from three contrasting agro-ecological zones in Ghana. A Ferric Acrisol (IUSS-WRB, 2022), corresponding to Typic Kandiustalf (USDA), was sampled from University of Ghana farms located in the coastal savannah agro-ecological zone of Ghana (also known as Adentan series). The area experiences total annual rainfall of about 800 mm which is bimodally distributed, and a mean temperature of 28 °C (Dowuona *et al.,* 2012). The second soil, a Dystric Fluvisols (IUSS-WRB, 2022), which is locally called Denteso series was collected from Donkorkrom located in the transitional agro-ecological zone of Ghana. The area experiences an annual temperature of 26 °C and a mean annual rainfall of 1331.7 mm which is bimodally distributed (Adu and Mensah-Ansah, 1995). The third soil, Keta series (Gleyic Arenosols (IUSS-WRB, 2022), corresponding to Quartzipsamments (USDA), was sampled from Anloga, in the semi-arid coastal savanna zone of Ghana. The region has an equatorial dry climate with an average yearly temperature of 28 to 30 °C and erratic annual rainfall of less than 900 mm (Dickson and Benneh, 1988). Surface soil (0 – 20 cm depth) samples were collected from cultivated sites and transported to the laboratory. Samples were divided into two; one-half was kept in the refrigerator for about 3 days for incubation studies, and the other half was allowed to air dry, sieved through a 2-mm mesh sieve and thoroughly homogenised. Sub-samples of the air-dry soils were used for the laboratory analysis for the initial chemical properties, which is presented in Table 3.2 (see Chapter 3 section 3.4.1).

The biochars used for the BMCs were produced from rice husk, sawdust, and coconut husk at pyrolysis temperature of 450 °C using a Kuntan kiln. Market wastes (MW) were collected from the Madina market (La Nkwantanang Municipal Assembly, Ghana) and cattle manure (CM) and poultry manure (PM) were collected from the Livestock and Poultry Research Center (LIPREC, University of Ghana). In all, eight (8) composts were prepared in triplicate using varied proportions of MW, CM and PM alone (MC) or in combinations with rice husk biochar (RB), coconut husk biochar (CB), sawdust biochar (SB)(BMC). The piles were labelled using subscripts of proportions of feedstocks which are $B_0CM_{40}MW_{40}$, $B_0PM_{40}MW_{40}$, $RB_{20}CM_{40}MW_{40}$, $CB_{20}CM_{40}MW_{40}$, $SB_{20}CM_{40}MW_{40}$, $RB_{20}PM_{40}MW_{40}$, $CB_{20}PM_{40}MW_{40}$, (Table 3.1, Chapter 3 section 3.3.2) and composting lasted for 90 days. Selected chemical properties of BMCs and MCs are presented in Table 3.4 (see Chapter 3 section 3.4.2).

4.3.2 Incubation studies (Experimental procedure)

A static incubation technique was used to determine the effect of the different BMCs and MCs on C_{mic}, N_{mic}, activities of L-asparaginase, L-glutaminase and Amidase, pH, ON, TC and labile carbon (POXC). The BMCs and MCs (control) were uniformly mixed with 500 g field-moist soils at a rate equivalent to 200 mg N kg^{-1} soil and placed in 1-L plastic jars with 0.5 cm diameter hole in the lid to provide aeration. Other treatments without composts amendment (soil alone) were also included. In all 81 experimental units were used (i.e., 9 composts (including without amendment) × 3 soils ×3 replicates). The soil-compost mixtures were thoroughly mixed and moistened to 60% water holding capacity (WHC), arranged in completely randomized design, and incubated at 30°C. Throughout the incubation period, the moisture level was kept at 60% WHC by periodically weighing the jars and adding distilled water as needed. At the end of the incubation period (26 weeks) the soils were analyzed for pH, POXC, TC, ON, C_{mic}, N_{mic}, and the activities of L-asparaginase, L-glutaminase and Amidase, as describe below.

4.3.3 Laboratory analysis of BMCand MC-amended soils

The pH of BMC and MC-amended soils was measured in soil to water ratio of 1:1. The total N (TN) and TC were measured on < 180 μm air-dried samples by dry combustion using the LECO CNS analyzer (LECO Corp., St. Joseph Michigan). Mineral N was determined by the steam distillation method described by Mulvany (1996). The ON was determined by subtracting the mineral N from TN (sum of NO_3^--N and NH_4^+-N).

4.3.3.1 Potassium permanganate extractable organic carbon

The potassium permanganate extractable organic carbon (POXC) was determined using the method described by Weil *et al*. (2003). Briefly, 2.5 g of compost-amended soils were weighed into centrifuge tubes, 18 ml of water and 2 mL of 0.2 M $KMnO_4$ were added and shaken for 2 min on an oscillating shaker. After taking out the tubes and letting them stand for ten minutes, 0.5 mL of the supernatant was placed into additional 50 mL centrifuge tubes, and distilled water was added to reach the 50 mL mark. The absorbance of the samples and four standard solutions (0.00005, 0.0001, 0.00015, and 0.0002 mol L^{-1} $KMnO_4$) were read at 550 nm using spectrophotometer. The absorbance and concentration values of the standards were used to derive a standard curve from which the concentration of POXC content of the samples were determined using the equation below:

$$POXC\left(\frac{mg}{kg}\right) = [0.02M - (a + b\,x\,c)]x\,\frac{(9000\,mg\,C\,/mol)x\,(0.02\,L\,solution)}{mass\,of\,soil\,(kg)} \qquad (4.1)$$

where, 0.02 M = concentration of the initial $KMnO_4$ solution, *a* = intercept (standard curve), *b* = slope (standard curve), *c* = absorbance of the unknown soil sample, 9000 mg is the amount of C oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{4+}, 0.02 L is the volume of $KMnO_4$ solution. All laboratory analyses were done in triplicates.

4.3.4 Microbial biomass C and N

Soil microbial biomass C (C_{mic}) and N (N_{mic}) were measured using the chloroform fumigation extraction procedure described by Vance *et al*. (1987). Briefly, 10 g of the BMC and MC-soil mixtures were fumigated using ethanol-free chloroform followed by extraction with 0.5 M K_2SO_4. The concentration of organic carbon (OC) in the extract was determined calorimetrically by measuring Cr_3^+ produced from the reduction of Cr_6^+ at 578 nm after acidification with H_2SO_4. The C_{mic} was calculated as the difference in the C content of the fumigated and non-fumigated samples (Joergensen and Mueller, 1996):

$$Cmic\ (\mathrm{mg}/kg) = \frac{(C_{fumigated} - (C_{unfumigated})}{KEC} \qquad (4.2)$$

where *KEC* = 0.38 (efficiency constant).

Microbial biomass N (N_{mic}) was determined on the extract using the Kjeldahl digestion and distillation technique described by Brookes *et al*. (1985). The difference in the labile N between fumigated and unfumigated samples was used to calculate N_{mic} as follows:

$$Nmic\ (\mathrm{mg}/kg) = \frac{(N_{fumigated} - (N_{unfumigated})}{KEN} \qquad (4.3)$$

Where K_{EN} (correction factor = 0.54)

4.3.5 Amidohydrolase activity

The activity of the amidohydrolases was assayed using the method described by Tabatabai (1994). The methods are based on the determination of NH_4^+-N released when 1 g BMC and MC-soil mixtures were incubated with 0.2 mL toluene, 0.1 M tris(hydroxymethyl) aminomethane (THAM) buffer, and 0.5 M L-asparagine, L- glutamine or formamide solution (for L-asparaginase, L-glutaminase and amidase activity, respectively) at a temperature of 37 °C for 2 hrs. The incubated samples were treated with 2.5 M KCl containing Ag_2SO_4 to stop the activities of L-glutaminase and L-asparaginase or 2.5 M KCl containing uranyl acetate to deactivate amidase. An aliquot of the resulting solution was steam-distilled for 4 min with 0.2 g MgO (Mulvaney, 1996). Controls were performed for each enzyme by adding substrates after deactivation of the enzymes. The activity of the enzymes was measured on moist samples at their optimum pH values and expressed on a moisture-free basis. Amidohydrolase activities were expressed in concentration of NH_4^+-N released per duration of assay (mg NH_4^+-N kg^{-1} $2hrs^{-1}$).

Table 4.1 Summary of the procedures used for the assay of Amidohydrolase activities.

Enzyme	Substrate (Concentration)	Reaction involved	Opt. pH and temperature	References
L-asparaginase	L-asparagine (0.5 M)	L-asparagine + $H_2O \rightarrow$ L-asparatic acid + NH_3	10 (37 ºC)	Tabatabai (1994)
L-glutaminase	L-glutamine (0.5 M)	L-glutamine + $H_2O \rightarrow$ L-glutamic acid + NH_3	10 (37 ºC)	Tabatabai (1994)
Amidase	Formamide (0.5 M)	Amides + $H_2O \rightarrow$ carboxylic acid + NH_3	8.5 (37 ºC)	Tabatabai (1994)

The figures in parenthesis are the concentration of substrate and optimum (opt.) temperature of enzyme reaction respectively.

4.3.6 N mineralization

The amounts N mineralized during 26 weeks of incubation of the soil-BMC and MC mixtures were determined using the method by Stanford and Smith (1972). The cumulative amounts of N mineralized was fitted to the first-order kinetics equation described by Stanford and Smith (1972):

$$N_{min} = N_o[1 - \exp(-k_m t)] \tag{4.4}$$

where, N_{min} (mg N kg^{-1}) is the cumulative amounts of N mineralized at time, t (week), N_o (mg N kg^{-1}) is the potentially mineralizable N and k_m (week^{-1}) is the first-order mineralization rate constant.

4.3.7 Statistical analysis

The normality of data for the experiment was tested using Shapiro-Wilk test. Data were then statistically analyzed using Genstat version 12 and means separated using Tukey

Posthoc test. The relationships among enzymatic activities, microbial biomass, N_o and properties of the compost-amended soils were evaluated using Pearson correlation analysis.

4.4 Results

4.4.1 Effect of BMCs and MCs on selected chemical properties of the soils

The effect of the BMCs and MCs on pH, POXC, TC and ON contents of the soils are represented in Tables 4.2 and 4.3. At the end of the incubation, BMC and MC amendment increased pH by 0.81 to 1.85, 2.21 to 3.44, and 0.37 to 0.75 pH units in Adentan, Denteso and Keta series, respectively compared to their unamended soils. Across the three soils, amendment with $CB_{20}PM_{40}MW_{40}$ resulted in the highest increase in pH compared to the other composts. Analysis of variance indicated that manure and biochar types had significant effect on pH values (Table 4.4). The Keta series had the highest pH among the soil types (Table 4.4).

Amending the soils with the BMCs and MCs significantly ($p < 0.05$) increased the POXC content from 626 to 870 mg kg^{-1}, 16 to 294 mg kg^{-1}, and 18 to 498 mg kg^{-1} in Adentan, Denteso and Keta series, respectively (Table 4.2). The highest increase in POXC content was observed when the soils were amended with $CB_{20}CM_{40}MW_{40}$ and $RB_{20}CM_{40}MW_{40}$, while the least was recorded in $B_0PM_{50}MW_{50}$ amended soils. Analysis of variance showed that biochar and manure types had a significant ($p<0.05$) influence on the POXC (Table 4.4). The soil type also had a significant effect on POXC, with Adentan series having the highest increase in POXC compared with Keta and Denteso series.

Application of the BMCs and MCs significantly ($p < 0.05$) increased the ON content of the soils compared to the soil alone (Table 4.3). Across the three soils, the highest increase in ON content was observed in soils amended with $B_0PM_{50}MW_{50}$, which was statistically similar to $SB_{20}PM_{40}MW_{40}$ and $B_0CM_{50}MW_{50}$, and the lowest was in the soil alone. Generally, manure and biochar had significant ($p < 0.05$) effect on ON content. (Table 4.4). Adentan series had the highest ON content with the least recorded in Denteso series.

Averagely, the TC content of BMC and MC-amended soils significantly increased from 3.57 to 14.22 g kg^{-1}, 0.75 to 7.96 g kg^{-1}, and 2.02 to 8.22 g kg^{-1} for Adentan, Denteso and Keta series, respectively, with the highest increase in TC content occurring in soils amended with $SB_{20}PM_{40}MW_{40}$ and the least in those amended with $B_0CM_{50}MW_{50}$ and $B_0PM_{50}MW_{50}$ (Table 4.3). The soil type had a significant effect on TC with Adentan series having the highest TC among the three soils studied.

Table 4.2 The pH and Potassium permanganate extractable organic carbon as influenced by BMCs and MCs in three soils.

Treatment	pH			POXC (mg kg^{-1})		
	Adentan	Denteso	Keta	Adentan	Denteso	Keta
Control	5.38$_a$	5.41$_a$	6.76$_a$	626$_a$	16$_a$	18$_a$
B$_0$CM$_{50}$MW$_{50}$	6.22$_b$	7.61$_b$	7.13$_b$	830$_c$	223$_d$	421$_f$
CB$_{20}$CM$_{40}$MW$_{40}$	6.73$_e$	7.86$_f$	7.40$_f$	870$_d$	294$_h$	485$_g$
RB$_{20}$CM$_{40}$MW$_{40}$	6.30$_c$	7.73$_{cd}$	7.24$_c$	853$_{cd}$	247$_{fg}$	498$_g$
SB$_{20}$CM$_{40}$MW$_{40}$	6.52$_d$	7.78$_{de}$	7.27$_{de}$	840$_c$	186$_c$	434$_f$
B$_0$PM$_{50}$MW$_{50}$	7.03$_f$	7.66$_{bc}$	7.24$_{cd}$	774$_b$	154$_b$	258$_b$
CB$_{20}$PM$_{40}$MW$_{40}$	7.23$_h$	7.89$_f$	7.51$_g$	780$_b$	227$_{de}$	296$_d$
RB$_{20}$PM$_{40}$MW$_{40}$	7.10$_g$	7.72$_{cd}$	7.33$_e$	802$_b$	248$_g$	322$_e$
SB$_{20}$PM$_{40}$MW$_{40}$	6.80$_e$	7.76$_{de}$	7.41$_f$	792$_b$	236$_{ef}$	277$_c$

Table 4.3 The organic N and total carbon as influenced by BMCs and MCs in three soils.

Treatment	Organic N (g kg^{-1})			Total C (g kg^{-1})		
	Adentan	Denteso	Keta	Adentan	Denteso	Keta
Control	0.60$_{a}$	0.34$_{a}$	0.53$_{a}$	3.57$_{a}$	0.75$_{a}$	1.71$_{a}$
B$_{0}$CM$_{50}$MW$_{50}$	1.91$_{de}$	1.64$_{e}$	1.83$_{de}$	6.10$_{b}$	3.67$_{b}$	3.93$_{a}$
CB$_{20}$CM$_{40}$MW$_{40}$	1.65$_{b}$	1.40$_{b}$	1.55$_{b}$	13.59$_{d}$	6.66$_{cd}$	6.35$_{bc}$
RB$_{20}$CM$_{40}$MW$_{40}$	1.72$_{bc}$	1.43$_{bc}$	1.62$_{b}$	9.61$_{c}$	5.73$_{c}$	6.06$_{b}$
SB$_{20}$CM$_{40}$MW$_{40}$	1.80$_{cd}$	1.52$_{d}$	1.75$_{cd}$	12.75$_{d}$	6.01$_{c}$	8.11$_{c}$
B$_{0}$PM$_{50}$MW$_{50}$	1.98$_{e}$	1.76$_{f}$	1.94$_{f}$	7.08$_{b}$	3.25$_{b}$	3.76$_{a}$
CB$_{20}$PM$_{40}$MW$_{40}$	1.71$_{bc}$	1.51$_{cd}$	1.65$_{bc}$	14.22$_{d}$	7.96$_{d}$	7.53$_{bc}$
RB$_{20}$PM$_{40}$MW$_{40}$	1.81$_{cd}$	1.55$_{d}$	1.75$_{cd}$	12.31$_{d}$	5.52$_{c}$	6.79$_{bc}$
SB$_{20}$PM$_{40}$MW$_{40}$	1.91$_{e}$	1.66$_{e}$	1.86$_{ef}$	13.69$_{d}$	7.85$_{d}$	8.22$_{c}$

Table 4.4 Analysis of variance of selected chemical properties in three tropical soils as affected by manure, biochar types, their interaction (final compost) and soil types.

Soil series	Source of variation	df	pH	POXC	ON	TC
Adentan	Manure (M)	2	***	***	***	***
	Biochar (B)	3	***	*	***	***
	M*B	6	***	*	***	***
Denteso	Manure (M)	2	***	***	***	***
	Biochar (B)	3	***	***	***	***
	M*B	6	***	***	***	***
Keta	Manure (M)	2	***	***	***	***
	Biochar (B)	3	***	***	***	***
	M*B	6	***	***	***	***
All soils	Soil	2	***	***	***	***

Ns= not significant, *** = $p < 0.001$, **= $p < 0.01$, $p < 0.05$,

4.4.2 Effect of BMCs and MCs on amidohydrolase activities

Generally, the activity of amidohydrolases increased significantly ($p < 0.05$) following amendment with the BMCs and MCs in the three soils compared to the soil alone, with the highest relative increase in activity observed in Adentan series. The L-glutaminase activity was the highest among the amidohydrolases studied, while amidase and L-asparaginase had the least activity values (Table 4.5, 4.6 and 4.7). Amendment with $RB_{20}CM_{40}MW_{40}$ and $CB_{20}CM_{40}MW_{40}$ resulted in the highest increase in enzymatic activity in most of the soils studied. According to analysis of variance, the activity of amidohydrolases in the BMC and MC-amended soils were significantly ($p < 0.05$) affected by manure and biochar types (Table 4.8), with CM-based compost giving a higher activity compared to PM-based composts. Among the biochar types, amendment with RB and CB-based composts resulted in a higher promotion of the enzymatic activities, with the least observed in SB and no biochar-based composts (MC).

4.4.3 Effect of BMCs on C_{mic} and N_{mic}

Application of BMCs and MCs resulted in significant increase in C_{mic} of the soils compared to the soil alone, with the highest and the lowest increase occurring in soils amended with $RB_{20}CM_{40}MW_{40}$ and $B_0PM_{50}MW_{50}$, respectively (Table 4.5, 4.6 and 4.7). However, the response of N_{mic} to compost application varied within each soil. The highest N_{mic} value was observed with the application of $RB_{20}CM_{40}MW_{40}$, $CB_{20}CM_{40}MW_{40}$, $CB_{20}PM_{40}MW_{40}$, and $RB_{20}PM_{40}MW_{40}$ in Adentan series, $RB_{20}PM_{40}MW_{40}$ $CB_{20}CM_{40}MW_{40}$ and $RB_{20}PM_{40}MW$ in Denteso series and $RB_{20}CM_{40}MW_{40}$ and $CB_{20}CM_{40}MW_{40}$ in Keta series (Table 4.5, 4.6 and 4.7). Analysis of variance indicated that manure and biochar had significant effect on C_{mic} and N_{mic} content of the soils, with the effect of manure type on C_{mic} following the order PM < CM in all the amended soils (Table 4.8). Among the biochar types, RB had the highest effect on C_{mic} followed by CB and the least observed in SB and no biochar (MC). The trend in biochar effect on N_{mic} was also similar to C_{mic}. (Table 4.8).

Table 4.5 Effect of BMC and MC on microbial biomass and amidohydrolase activities in Adentan series

Treatment	C_{mic}	N_{mic}	L-Glutaminase	L-Asparaginase	Amidase
	($\mu g\ g^{-1}$)		($mg\ NH_4^+$-$N\ kg^{-1}\ 2h^{-1}$)		
Control	101_a	14_a	77_a	12_a	3_a
$B_0CM_{50}MW_{50}$	162_b	31_{bc}	157_c	39_{bcd}	8_b
$CB_{20}CM_{40}MW_{40}$	237_e	63_e	195_{de}	59_e	19_f
$RB_{20}CM_{40}MW_{40}$	597_f	54_{de}	217_e	52_{de}	22_h
$SB_{20}CM_{40}MW_{40}$	220_{de}	45_{cd}	161_c	32_{bc}	13_d
$B_0PM_{50}MW_{50}$	144_b	26_{ab}	129_b	29_b	11_c
$CB_{20}PM_{40}MW_{40}$	198_{cd}	54_{de}	178_{cd}	45_{de}	21_g
$RB_{20}PM_{40}MW_{40}$	329_e	49_{de}	216_e	43_{bcd}	17_e
$SB_{20}PM_{40}MW_{40}$	189_c	45_{cd}	166_c	30_b	15_e

Table 4.6 Effect of BMC and MC on microbial biomass and amidohydrolase activities in Denteso series

Treatment	C_{mic}	N_{mic}	L-Glutaminase	L-Asparaginase	Amidase
	(µg g^{-1})		(mg NH_4^+-N kg^{-1} $2h^{-1}$)		
Control	178_a	9_a	23_a	2_a	4_a
$B_0CM_{50}MW_{50}$	366_c	27_b	70_{cd}	8_{bc}	12_b
$CB_{20}CM_{40}MW_{40}$	422_d	54_{de}	95_e	11_{bcd}	22_d
$RB_{20}CM_{40}MW_{40}$	639_g	63_{de}	117_f	16_e	21_d
$SB_{20}CM_{40}MW_{40}$	405_d	27_b	61_{bcd}	10_{bc}	17_{bcd}
$B_0PM_{50}MW_{50}$	323_b	27_b	49_b	7_b	12_b
$CB_{20}PM_{40}MW_{40}$	475_e	50_{cd}	73_{cd}	16_e	20_{cd}
$RB_{20}PM_{40}MW_{40}$	566_f	67_e	77_{de}	14_d	21_d
$SB_{20}PM_{40}MW_{40}$	313_b	36_{bc}	54_{bc}	10_{bcd}	14_{bc}

Table 4.7 Effect of BMC and MC on microbial biomass and amidohydrolase activities in Keta series

Treatment	C_{mic}	N_{mic}	L-Glutaminase	L-Asparaginase	Amidase
	($\mu g\ g^{-1}$)		($mg\ NH_4^{+}$-$N\ kg^{-1}\ 2h^{-1}$)		
Control	105_{a}	18_{a}	26_{a}	4_{a}	4_{a}
$B_0CM_{50}MW_{50}$	326_{e}	45_{bc}	63_{bc}	13_{bc}	12_{b}
$CB_{20}CM_{40}MW_{40}$	236_{d}	98_{f}	115_{f}	26_{de}	20_{cd}
$RB_{20}CM_{40}MW_{40}$	548_{g}	85_{ef}	91_{de}	29_{e}	24_{d}
$SB_{20}CM_{40}MW_{40}$	257_{d}	63_{cde}	50_{b}	19_{bcd}	14_{bc}
$B_0PM_{50}MW_{50}$	142_{b}	36_{ab}	46_{b}	12_{b}	14_{bc}
$CB_{20}PM_{40}MW_{40}$	191_{c}	72_{de}	73_{cd}	24_{de}	20_{cd}
$RB_{20}PM_{40}MW_{40}$	393_{f}	54_{bcd}	101_{ef}	21_{cd}	21_{cd}
$SB_{20}PM_{40}MW_{40}$	169_{c}	45_{bc}	57_{bc}	14_{bc}	14_{bc}

Table 4.8 Analysis of variance of the effect of manure, biochar type and their interaction (final compost) on microbial biomass and amidohydrolases activities in the soils

Soil series	Source of variation	Df	C_{mic}	N_{mic}	L-Glutaminase	L-Asparaginase	Amidase
Adentan	Manure (M)	2	***	***	***	***	***
	Biochar (B)	3	***	***	***	**	***
	M*B	6	***	**	***	*	***
Denteso	Manure (M)	2	***	***	***	***	***
	Biochar (B)	3	***	***	***	***	***
	M*B	6	***	***	***	*	**
Keta	Manure (M)	2	***	***	***	***	***
	Biochar (B)	3	***	***	***	***	***
	M*B	6	***	*	***	**	**
All soils	Soil	2	***	***	***	***	*

Ns =not significant, $^{***} = p < 0.001$, $^{**} = p < 0.01$, $^{*} = p < 0.05$

4.4.4 Relationship among amidohydrolases activities, C_{mic}, N_{mic} and N_o

The estimated N_o values were positively and significantly correlated with the activity of the amidohydrolases in the compost-amended soils with r-values ≥ 0.409 ($p \leq 0.01$) (Table 4.9). The N_o was positively and significantly correlated with C_{mic} and N_{mic} with r-values ≥ 0.426 ($p \leq 0.01$) (Table 4.9). The activity of the amidohydrolases were positively correlated with C_{mic} and N_{mic} content of the amended soils, with *r*-values ≥ 0.229 ($p \leq 0.05$) (Table 4.10)

Table 4.9 Pearson correlation coefficient (*r*) among amidohydrolase activities, soil microbial biomass and N_o in BMC and MC-amended soils.

AA/SMB	N_o (Correlation coefficient, *r*)
Amidase	0.409^{**}
L-asparaginase	0.728^{***}
L-glutaminase	0.775^{***}
C_{mic}	0.426^{**}
N_{mic}	0.445^{**}

Ns =not significant, $^{***} = p < 0.001$, $^{**} = p < 0.01$, $^{*} = p < 0.05$; AA: Amidohydrolase Activity; SMB: Soil Microbial Biomass; $C_{mic:}$ Microbial biomass C; $N_{mic:}$ Microbial biomass N.

4.4.5 Amidohydrolases activities and SMB in relation to selected soil chemical properties

The activity of the amidohydrolases were positively and significantly correlated with ON content of the soils, with *r*-values ≥ 0.279 ($p < 0.05$) (Tables 4.10). The results also indicated a strong positive correlation between amidohydrolase activities and POXC (*r*-values ≥ 0.513 ($p < 0.001$). Also, the activity of amidohydrolases was positive and significantly ($p < 0.05$) correlated with TC content of the amended soils with *r*-values ranging from 0.394 ($p < 0.01$) to 0.628 ($p< 0.001$). The correlation between pH of amended soils and amidohydrolase activities was positive, with *r*-values ≥ 0.445 ($p < 0.001$).

According to the pearson correlation analysis, there was a significant and positive correlation between SMB and ON, with *r*-values ≥ 0.239 ($p < 0.05$). The SMB also correlated positively with POXC, TC and pH with r-values ≥ 0.243 ($p < 0\ 05$), ≥ 0.357 ($p < 0\ 01$) and ≥ 0.312 ($p < 0.05$).

Table 4.10 Pearson correlation coefficient between enzymatic activities, microbial biomass and selected chemical properties.

	Amidase	L-asparaginase	L- glutaminase	C_{mic}	N_{mic}	ON	POXC	TC	pH
Amidase	1								
L-asparaginase	0.523***	1							
L-glutaminase	0407**	0.901***	1						
C_{mic}	0.677***	0.374**	0.329*	1					
N_{mic}	0.805***	0.282*	0.229*	0.489***	1				
ON	0.535***	0.289*	0.279*	0.239*	0.330*	1			
POXC	0.513***	0.759***	0.856***	0.569***	0.243*	0.125^{ns}	1		
TC	0.628***	0.394**	0.398**	0.357**	0.722***	0.392**	-0.687***	1	
pH	0.445***	0.423***	0.400**	0.312*	0.509***	0.625***	-0.665***	0.826***	1

Ns= not significant, *** $=p < 0.001$, ** $=p < 0.01$, * $=p < 0.05$; TC: Total Carbon; ON: Organic N; POXC: Permanganate oxidizable carbon

4.5 Discussion

4.5.1. Effect of BMCs on selected properties of soils

The increase in pH of the soils amended with BMCs compared to MCs and soil alone can be attributed to the initial pH of the amendments. This observation is consistent with the results of Chen *et al*. (2020) who reported an increase in pH of heavy metal contaminated soil following the application of sewage sludge wheat straw biochar compost. The increased POXC content of the BMC-amended soils compared to MC-amended soils is due to the higher amounts of labile C following application of the BMCs (Zimmerman *et al*., 2011; Thiele-Brhm and Ngigi, 2021; Antonangelo *et al*., 2021). The higher ON content observed in soils amended with $B_0PM_{40}MW_{40}$, $SB_{20}PM_{40}MW_{40}$ and $B_0CM_{40}MW_{40}$ can be attributed to the initial N in these amendments. The relatively higher TC in the BMC-amended soils compared to MC-amended soils could be due to the addition of biochar during co-composting which increased the C content of the finished product. This is consistent with the findings of Steiner *et al.* (2010) who reported that soil amended with biochar poultry manure co-compost had a greater C content compared to soils amended with compost alone.

The higher POXC, ON and TC in Adentan serie compared to the other soil types could be attributed to the type of management practice (fallow) undertaken on the soil before sampling. It is well known that during fallowing, the soil regains some of its lost nutrients through decomposition of litter and roots from grasses (Sanchez, 2019). The high pH of Keta series was a result of the inherent pH characterized by high basic cations in most coastal soils (Whipkey *et al.,* 2000) as well as the management practice such as continuous application of manure on the soil (Asomaning *et al.,* 2015; Awadzi *et al.,* 2008; Dodor *et al.,* 2018; 2019).

4.5.2 Effect of BMCs on amidohydrolase activities

Amidohydrolases are enzymes that catalyze the hydrolysis of C-N bonds other than peptide bonds in amines and amino acids to generate ammonium (Tabatabai, 1994; Dodor 2002; Dodor and Tabatabai, 2003). Generally, it has been suggested that alterations in the activity of amidohydrolases can be used as an indicator of changes in management practices, including fertilizing soils with exogenous organic matter such as BMCs (Deng and Tabatabai, 1996; Marschner *et al*., 2003; Dodor and Tabatabai, 2002; Deng *et al*., 2006). This assertion was confirmed by the increased activity of the amidohydrolases in BMC and MC-amended soils compared to the soil alone. Generally, addition of BMC and MC to the soils resulted in a significant increase in SOM storage and ON containing compounds in soils (Antonangelo *et al* 2021; Yuan *et al* 2017). According to Agegnehu *et al*. (2016), application of biochar-compost increased the SOC and TN by 43-73% and 14-29%, respectively, compared to the soil alone. Yuan *et al*. (2017) also reported a significant increase in TN, TC and KCl-extractable ON contents of soils following application of biochar-chicken-manure compost and chicken-manure compost compared to soil alone. The positive relationship between the activity of the amidohydrolases and POXC, TC, pH and ON indicates that the properties of these amendments influenced the activity of the enzymes. Quyang *et al*. (2014) also attributed the increase in enzyme activities to biochar-induced pH increase in biochar amended soils.

The reduced activity of the amidohydrolases in soils amended with SD-based BMCs can be attributed to the reduced labile C and available nutrients as well as condensed aromatic structure in the SB, which increased recalcitrance and reduced biodegradability of the amendment (Quyang *et al*., 2014; Zimmerman *et al*., 2011; Pokharel *et al*., 2018; Li *et al*., 2018). Also, it is possible that SB may have absorbed the enzyme substrates and immobilized the enzymes due to its high specific surface area and porosity, as have been reported by other researchers (Quyang *et al*., 2014; Khadem and Raiesi, 2017).

The lower amidohydrolase activities in the soils amended with MCs compared to BMCs could be attributed to the reduced bioavailability of organic substrates for the enzymes to act on (Aranda *et al*., 2015). The higher activity of the amidohydrolases in Adentan compared to Denteso and Keta series could be due to the higher OM in the soil which provided substrate for enzymatic activities. This finding is consistent with a study by Khadem and Raiesi (2017) who reported a significant increase in dehydrogenase, protease, cellulose, catalase and invertase activities in organically managed soils. It is also likely that the higher clay content of the Adentan series may have stabilized the amidohydrolase enzymes, resulting in increased resistance to extreme environmental conditions such as pH, denaturing and degradation by other soil proteases, culminating in improved operational stability, durability, and higher activity of the enzymes compared to the other soils (Dodor *et al.,* 2004; 2020).

4 .5.3 Effect of BMCs on C_{mic} and N_{mic}

The increased C_{mic} and N_{mic} values in the BMC and MC-amended soils compared to the soil alone can be attributed to incorporation of C and N via the amendment which stimulated microbial activity and biosynthesis which have been reported by other workers (Yuan *et al.,* 2017, Schulz *et al*., 2013; Mackie *et al*., 2015). The higher C_{mic} and N_{mic} content of the soils amended with BMCs compared to the MCs, suggests that biochar contains readily available OC (Dodor *et al*., 2018; 2019), of which the former contain higher amounts compared to the latter. The results are in line to those of Yuan *et al*. (2017) who reported increased C_{mic} and N_{mic} in soils amended with biochar chicken manure compared to chicken manure and the unamended soils. The results further suggest that the higher labile C in biochar probably exerted considerable influence on microbial activity and cell biosynthesis as measured by the increased C_{mic} and N_{mic} contents of the BMC-amended compared to the MC (Birk *et al*., 2009). Additionally, BMC can influence microbial activity and proliferation through the supply of nutrients, provision of pores space for microbial habitation and availability of labile C (Lehmann *et al.,* 2011; Zimmerman *et al* 2011, Quyang *et al* 2014; Dodor *et al*., 2018; 2019). Furthermore, Antonangelo *et al*. (2021) reported that co-composting reduces toxic organic compounds in biochar thereby increasing microbial abundance and function when incorporated into the soil.

The feedstock type, biochar type and rate in the BMCs affect the micro-biochemical properties of amended soils (Novak and Busscher, 2013; Quyang *et al*., 2014; Antonangelo *et al*., 2021). Studies by Brtnicky *et al*. (2019) reported increased C_{mic} with increasing rate of biochar when biochar and manure were added to soils. In contrast, Dempster *et al*. (2012) observed decreased soil C_{mic} when eucalyptus biochar was co-applied with wheat straw and composted pig manure. The authors attributed this to the recalcitrance and limited labile C content of the biochar, which reduced biodegradability and microbial activity, resulting in decreased C_{mic} and N_{mic}, as observed in soils amended with SB-based compost in the present study.

Microorganisms utilize dissolved and readily available OC for cell biosynthesis and growth (Dodor *et al*., 2018; 2019). The higher POXC content of CM-based composts compared to PM-based types can be attributed to higher availability of labile OC pools in the soils amended with CM-based composts for microbial activity and growth, resulting in higher C_{mic} and N_{mic} values in the amended soils, as have been reported by other authors (Antonangelo *et al* 2021; Thiele-Brhm and Ngigi 2021; Xiao *et al*., 2017; Mishra *et al*., 2022). The results further suggested that readily available OC content of BMCs, as measured by POXC, can be used as an index of bioavailability of OC substrates to microbes in BMC-amended soils. Working with sandy soils from a dry equatorial coastal savanna region of Ghana, Dodor *et al*. (2018; 2019) reported very close association between water extractable and $KMnO_4$ oxidizable OC contents of biochar-manure amended soils and suggested that they can be used as an index of labile and important source of C for microorganisms in soils.

4.5.4 Activity of amidohydrolases in relation to C_{mic} and N_{mic}, and N_o

Soil microorganisms mediate mineralization of N in organic amendments such as BMCs, through the activity of amidohydrolases that are closely associated with the microbial biomass (Powlson *et al*., 1987; Dick 1992; Klotse and Tabatabai, 2000; Dodor and Tabatabai, 2002; 2003). Therefore, the strong positive correlation between the activity of the amidohydrolases and SMB suggests that application of the BMCs induced the production of these enzymes, resulting in increased mineralization of the BMCs and synthesis of microbial biomass. This finding is corroborated by the work of Foster *et al*. (2016) who reported increased C_{mic} values in biochar-manure amended soils which translated into higher extracellular enzyme activities.

The amidohydrolases play a vital role in cycling of N in soils through the hydrolysis of C-N bonds in linear amides associated with SOM (Dodor and Tabatabai, 2003; Dodor and Tabatabai, 2020; Dodor *et al*., 2020) to release mineral N for microbial assimilation and plant uptake. As a result, amidohydrolase activity has been assessed as a measure of soil N mineralization (Burket and Dick 1998; Dodor and Tabatabai 2002; 2003; 2007; 2020). The significant relation between the activity of the amidohydrolases and N_o observed in the present study is consistent with the above studies and suggests that the activity of these enzymes can be used as a good proxy of N_o and N availability in BMC-amended soils.

4.5.5 Amidohydrolase activity and SMB in relation to POXC, ON, pH and TOC

The observed significant and positive relation between the activity of the amidohydrolases and POXC can be attributed to the labile C in BMCs that stimulated proliferation, biosynthesis and production of amidohydrolase enzymes by the microbes. This finding is consistent with the study by Sandhu *et al*. (2019) who reported that application of diary manure and pinewood biochar increased labile C, urease activity and microbial community structure of an eroded and depositional landscape position of different sampling sites. Furthermore, the increased pH resulting from BMC application may have provided a favorable microenvironment for microbial and amidohydrolase activities, resulting in the observed positive correlation among amidohydrolase activity, SMB and pH of the amended soils (Schulz *et al*., 2013; Mackie *et al.,*2015).

The increase in ON content following incorporation of BMCs enhanced the activities of microbes and amidohydrolase catalysis leading to mineralization of N. This assertion is supported by the observed increase in ON content of BMC-amended soils, which increased biosynthesis of SMB and activity of the enzymes, resulting in the significant positive correlation among the aforementioned parameters, as has been reported by Muruganandam *et al*. (2009). To maintain the positive correlation between amidohydrolases and SMB, labile sources of C must be available for biosynthesis and growth of microbes (Rao *et al.,* 2014). This assertion was confirmed by the observed positive correlation between SMB and AA with TC in the present study. Deng and Tabatabai (1996) reported significant correlation between amidohydrolases activity and OC. Studies by Muruganandam *et al*. (2009) also found a significant positive correlation between AA and TOC in a no-till soil.

4.6 Conclusions

Overall, this study demonstrated that BMCs, comprising different manure types and biochar had significant and positive influence on SMB and its related amidohydrolase activities through the enhancement of chemical properties in three tropical soils compared to the manure compost (MC) and unamended soils. The activity of L-glutaminase was the highest among the amidohydrolases studied. The C_{mic} increased with application of amendment, but N_{mic} varied with soil type. Comparing the manure and biochar types, the RB, CB, CM based-BMCs had the highest effect on SMB and AA compared to SB, 0B, PM-based BMCs. Among the soil types, Adentan series had the highest AA, SMB and chemical properties while Keta series had the highest pH. A positive relation was established among AA, SMB and N_o. Also, there was a significant and positive relation among AA, SMB and selected soil properties.

4.7 References

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CHAPTER 5

5.0 EFFECT OF BIOCHAR MANURE CO-COMPOST ON ORGANIC N FRACTIONS AND ITS RELATION TO N MINERALIZATION AND AMIDOHYDROLASE ACTIVITIES IN THREE TROPICAL SOILS

5.1 Abstract

The sustainable use of biochar manure co-composts (BMCs) for ameliorating soil fertility constraints must start with characterization of their organic nitrogen (ON) fractions and the development of a predictive relationship between compositional factors and ON mineralization. A laboratory incubation experiment was conducted to characterize the ON fractions of BMC and manure compost (MC- control)-amended soils (Adentan, Denteso and Keta series) and assess their contribution to the potentially mineralizable N (N_o) and relationship with the activity of amidohydrolases involved in hydrolysis of ON in soils. Three soils of contrasting textures were amended with BMCs and MCs (200 mg N kg^{-1}) of varied quality and total hydrolysable N (THN), hydrolysable ammonia N (HAN), hydrolysable amino sugar N (HASN), hydrolysable amino acid N (HAAN), hydrolysable unknown N (HUN) and non-hydrolysable N (NHN) contents were determined before and after 26 weeks of incubation. The results indicated that BMC and MC-amended soils had a higher ON fraction compared to unamended soils. The ON fractions also varied in the BMCs and MCs representing between -23% to 83% of the total N (TN) content which was attributed to an increase in the extractable ON. The THN, HAN, and HAAN were negatively correlated with N_o, indicating these fractions contributed to N_o of the amended soils. A stepwise regression analysis showed that the reduction in HAN, HAAN and THN fractions could explain about 60% of the variations in N_o. A positive correlation between

HAAN, HAN, THN and amidohydrolase activities in the BMC-amended soils shows that the enzymes were hydrolyzing these ON fractions to release inorganic N.

5.2 Introduction

Co-composting of biochar and agro-municipal wastes such as manure and market wastes into nutrient enriched value-added organic amendments that can be used to ameliorate soil fertility constraints in tropical agricultural soils is being promoted as a sustainable and environmentally sound method of managing these waste streams (Antonangelo *et al.,* 2021; Lim *et al*., 2018; Li *et al*., 2011; Thiele-Bruhn and Ngigi, 2021; Dodor *et al*., 2024). Biochar manure co-compost (BMC) contains nutrients especially nitrogen (N) in organic form that must be mineralized to release mineral N for microbial assimilation and plant uptake (Schulz *et al.,* 2013; Sanchez-Monedero *et al*., 2019). Because the extent and rate of mineralization of N in organic amendments depend on the proportion of organic N (ON) fractions (Bremner, 1965; Stevenson, 1996), a precise knowledge of the compositional dynamics of ON in BMC-amended soil must be evaluated.

The nature and form of N in soils is influenced by the composition of applied organic amendment (Bird *et al*., 2002; Kaur and Singh, 2014; Yang *et al*., 2018), with hydrolysable N and active fractions being the major source of plant available N in soils. Sekhon *et al*. (2011) reported that the application of manure and press mud increased the portion of hydrolyzable amino acid N (HAAN), hydrolyzable amino sugar N (HASN) and hydrolyzable ammonia N (HAN) content of soils, contributing to grain yield and N uptake of rice and wheat crops. Similarly, Durani *et al*. (2016) found that the N derived from green and farmyard manures and NPK translated into HAAN, HASN and hydrolyzable unknown N (HUN) content of amended soils. Campbell *et al*. (1986) observed an increase and decrease in the proportion of HAAN and HUN, respectively under long-term organic fertilization. Other authors have also reported that HAAN, HASN and HAN are the main determinants of the N mineralization potential of the manured soil, therefore, they can be used as an indicator of the impact of management practices (Wander *et al.,* 2007; Li and Li, 2014).

Biochar contains recalcitrant aromatic and heterocyclic carbon (C) structures (Lehmann, 2007; Clough *et al.,* 2013) that have been reported to influence N mineralization in soils. For example, Nelissen *et al*. (2012) found that incorporating maize biochar prepared at low temperature increased the labile N pool (hydrolyzable N fraction) of the soil. Contrarily, Wang *et al*. (2012) reported a decline in hydrolysable organic N fraction especially amino acids with the application of biosolid pyrolyzed at a higher temperature. Sun *et al*. (2018) reported that applying biochar produced from cattle manure improved the labile C content of the soil hence increasing N mineralization.

Co-composting of biochar and manure has been reported to generate a higher amount of ON compared to the sole application due to the sorptive capacity of biochar and labile ON content of the manure (Kammann *et al*., 2016; Antonangelo *et al*., 2021). Biochar manure compost can act as a potential supplier of N, synchronizing N release with crop demand, thereby improving crop productivity (Agegnehu *et al*., 2016; Bass *et al.,* 2016; Teodoro *et al*., 2020). This effect of BMC on crop productivity may depend on the biochar or feedstock types used in production of the BMC, therefore, precise knowledge on the different fractions of ON and their contribution to potentially mineralizable N (N_o) is essential for the management of BMC to improve on the N use efficiency of amended soils. However, the fractions of ON contributing to the liability and mineralizable N potential of BMC has scarcely been explored.

The amidohydrolases, L-asparaginase (EC 3.5.1.1), L-glutaminase (3.5.1.2), and amidase (EC 3.5.1.4) are the enzymes that catalyze the hydrolysis of C-N bond other than peptide bonds in linear amides (Tabatabai 1994). These enzymes are involved in the hydrolysis of native and added ON in soils (Tabatabai, 1994; Dodor and Tabatabai, 2003). It is well known that a significant amount of N (15–25%) in soils which is acid hydrolyzable (6 M HCl) to release NH_4^+-N originates from the hydrolysis of amino acids in linear amides such as asparagine and glutamine residues in soil organic matter (SOM) (Sowden, 1958). Given that the amidohydrolases play a vital role in the mineralization of ON in soils (Dodor and Tabatabai, 2003; Dodor and Tabatabai, 2019) and the supply of N to plants, it follows that their activities will be crucial in the mineralization of ON in BMCs added to soils. Additionally, depending on how specific enzyme reactions are, the relationship between the ON composition and the activity of amidohydrolase could give an indication of the type of ON pools being hydrolyzed in the BMCs. The use of the linkage between enzyme type and ON type in characterising ON pools has not been explored fully in studies on BMC-amended soils.

Therefore, objectives of this research was to: (i) investigate the types and distribution of ON in BMC and manure compost (MC- control) prepared from different composting feedstocks in three soils, (ii) assess changes in the ON fractions in relation to N_o of amended soils, and (iii) investigate the relationship between ON fractions and the activity of amidohydrolases in BMC-amended soils.

5.3 Material and Methods

5.3.1 Soil and Biochar manure co-compost

The soils used in the present study are Adentan, Denteso, and Keta series. Adentan series is a Ferric Acrisol (IUSS-WRB, 2022 classification) sampled from University of Ghana farms located in the coastal savannah agro-ecological zone of Ghana. The area has a mean temperature of 28 °C and a bimodally distributed rainfall of about 800 mm (Dowuona *et al.,* 2012).

Denteso series, classified as Dystric Fluvisols (IUSS-WRB, 2022 classification), was sampled from Donkorkrom located in the transitional agro-ecological zone of Ghana. The area experiences an annual temperature of 26 °C and a bimodal distribution of mean annual rainfall of 1331.7 mm (Adu and Mensah-Ansah, 1995). Keta series were sampled from Anloga, located in the semi-arid coastal savanna zone of Ghana. The area has a dry equatorial climate, with a mean annual temperature of about 28-30^{o} °C (Dickson and Benneh, 1988). Keta series is classified as Gleyic Arenosols (IUSS-WRB, 2022 classification).

The BMCs were prepared from three biochar (B) types: ricehusk biochar (RB), coconut husk biochar (CB) and sawdust biochar (SB) at a rate of 20% (v/v). The biochars were pyrolyzed using a Kuntan kiln at 450 ºC after drying feedstocks. Two manure (M) types, cattle manure (CM) and poultry manure (PM) were sampled from the Livestock and Poultry Research Center (LIPREC, University of Ghana). Market waste was collected from the Madina market in the La-Nkwantanang Municipal Assembly (Ghana).

Manure compost (MC) was prepared by co-composting MW, CM and PM. The BMCs were prepared by mixing 20%, 40% and 40% (v/v) of B, M and MW. The MCs and BMCs were labeled based on the feedstock with the subscripts representing the percentages of the respective feedstock in the pile. For example, $RB_0CM_{40}MW_{40}$ implies 0% B, 50% CM and PM50%, while $RB_{20}CM_{40}MW_{40}$ means 20%RB, 40%CM, and 40%MW in the composting pile. (Table 3.1, see chapter 3 section 3.3.2). In all, 8 compost piles were prepared in triplicate. Feedstocks for the composting were moistened, thoroughly mixed and intermittently stirred till maturity after 90 days. Composite sampling of matured compost was taken for chemical analysis in the laboratory and the results are presented in Table 3.3 (see chapter 3 section 3.4.2).

5.3.2 Experimental procedure.

Five hundred grams of the soil samples were uniformly mixed with the BMCs and MCs to provide 200 mg N kg^{-1} soil, moistened to 60% water holding capacity, placed in a 1 kg plastic container with holes perforated on the lid and kept in the incubator for 26 weeks. A control without amendment was also set up. Sampling was done before (week 0) and after 26 weeks of incubation for the determination of ON fractions in both amended and unamended soils.

5.3.3 Laboratory procedure

5.3.3.1 Determination of organic N fraction

Organic N fractions were determined by a method described by Bremner (1965). Briefly, 2-drops of octylic acid and 20 mL 6 M HCl were added to BMC-soil mixtures containing approximately 10 mg N and refluxed for 12 hrs. The hydrolysate obtained was filtered into 100 mL volumetric, neutralized with NaOH to pH 6.5 and made up to mark. Hydrolysable Ammonia-N (HAN) was determined by steam distillation of 10 mL of the hydrolysate with 0.07 g MgO for 2 min. The distillate was collected in 2% boric acid containing mixed indicator and titrated against 0.001 M HCl. Hydrolysable Ammonium Sugar-N (HASN) was determined by steam distilling 10 ml of hydrolysate with 10 ml of phosphate borate buffer (pH 11) for 4 min and the N released was corrected for HAN ([HASN+HAN]-HAN). Hydrolysable Amino Acid N (HAAN) was determined by pipetting 5 ml of the hydrolysate into 50 mL distillation flask after which 1 mL of 0.5 M NaOH was added. The mixture was heated in a water bath, cooled, and mixed with 500 mg of citric acid and 100 mg of ninhydrin, and heated for another 10 min. The mixture was agitated for 1 min, 10 mL was steam-distilled with 10 mL of phosphate borate buffer and 1 mL of 5 M NaOH. The total hydrolysable N (THN) was determined from the neutralized hydrolysate by pipetting 5 mL into a conical flask, addition of 0.5 g of K_2SO_4-catalyst mixture (comprising of 100 g K_2SO_4, 20 g $(CuSO_4)5H_2O$ and 2 g grey selenium powder (Se), 2 mL of 18 M sulphuric acid and heated gently until water was eliminated and no white smoke evolved. Heating continued till a clear mixture was observed. The flask was cooled, and 10 mL of distilled water was added while agitating. The solution was transferred into a distillation flask and steam distilled with 10 mL of 10 M NaOH for 4 min. The hydrolysable unknown N (HUN) was calculated as the difference between the estimate of THN and known hydrolyzable N (HAN, HASN and HAAN). The non-hydrolysable N (NHN) was determined by the difference between the total N (determined using the Leco CNS analyzer) and total hydrolysable N. The reduction in the ON fractions of the BMC-amended soils at the end of 26 weeks of incubation were determined by subtracting N fractions before incubation from those after incubation. All ON fractions were expressed in mg kg^{-1} of soil as well as percentage of total N determined.

$$HASN = (HASN + HAN) - HAN \qquad 5.1$$

$$HUN = THN - (HAN + HASN + HAAN) \qquad 5.2$$

$$NHN = TN - THN \qquad 5.3$$

5.3.4 N mineralization

The method by Stanford and Smith (1972) was used to determine mineralization in the MC BMC amended soils. The cumulative amounts of N mineralized were fitted to the first-order equation proposed by Stanford and Smith (1972):

$$N_{min} = N_o[1 - \exp(-k_m t)] \qquad 5.4$$

where N_{min} (mg N kg^{-1}) is the cumulative amount of N mineralized at time, t (week), N_o (mg N kg^{-1}) is the potentially mineralizable N and k_m (week^{-1}) is the first-order mineralization rate constant.

5.3.5 Amidohydrolase activity

The activities of L-asparaginase, L-glutaminase and amidase were determined using the method by Tabatabai (1994). Summary of the enzyme assay is represented in Table 5.1.

Table 5.1 Summary of the procedures used for the assay of Amidohydrolase activities

Enzyme	Substrate concentration/ Deactivating reagent	Reaction involved	Opt. pH and temperature	References
L-asparaginase	L-asparagine (0.5 M)/ Ag_2SO_4	L-asparagine + $H_2O \rightarrow$ L-asparatic acid $+NH_3$	10(37ºC)	Tabatabai (1994)
L-glutaminase	L-glutamine (0.5M)/ Ag_2SO_4	L-glutamine + $H_2O \rightarrow$ L-glutamic acid $+NH_3$	10(37ºC)	Tabatabai (1994)
Amidase	Formamide (0.5 M)/ Uranyl acetate	Amides$+H_2O \rightarrow$ carboxylic acid $+NH_3$	8.5(37ºC)	Tabatabai (1994)

The figures in parentheses are the concentration of substrate and the optimum (opt.) temperature for enzyme reaction respectively.

5.3.5 Statistical analysis

The normality of experimental data was tested using the Shapiro-Wilk test. The data were then statistically analyzed using Genstat version 12. The means were separated using the Tukey Posthoc test. The relationship among ON fractions, N_o and amidohydrolase activities of the amended soils were evaluated using correlation (Pearson correlation analysis) and regression analysis.

5.4 Results

5.4.1 Effect of BMC and MC on the initial ON fractions.

Generally, the ON fractions in BMC and MC-amended soils were significantly higher than those of the unamended soils (soil alone) (Table 5.2, 5.3 and 5.4). The THN increased from 469 mg kg^{-1} (54% of TN) in soil alone to 545 mg kg^{-1} (28% of TN) and 1094 mg kg^{-1} (52% of TN) in Adentan series amended with $B_0PM_{50}MW_{50}$ and $SB_{20}CM_{40}MW_{40}$, respectively. In Denteso series, THN increased from 174 mg kg^{-1} (38% of TN) in soil alone to 248 mg kg^{-1} (17% of TN) in $B_0CM_{40}MW_{40}$, to 609 mg kg^{-1} (44% of TN) in $SB_{20}PM_{40}MW_{40}$, while in Keta series it increased from 73 mg kg^{-1} (11% of TN) in soil alone to 170 mg kg^{-1} (13% of TN) in $CB_{20}PM_{40}MW_{40}$ and to 866 mg kg^{-1} (56% of TN) in $SB_{20}PM_{40}MW_{40}$. The HAN in Adentan, Denteso and Keta series without amendment were 114 mg kg^{-1}, 134 mg kg^{-1} and 91 mg kg^{-1}, accounting for 13%, 30% and 14% of total N, respectively. This amount increased with the incorporation of BMCs and MCs, with the highest recorded in $RB_{20}CM_{40}MW_{40}$, $B_0CM_{50}MW_{50}$ and $RB_{20}CM_{40}MW_{40}$, corresponding to 16%, 17% and 16% of total N in Adentan, Denteso and Keta series, respectively.

The proportion of HASN in the BMC and MC-amended and unamended soils were similar and the least among the hydrolysable N measured. The proportion of HASN in Adentan, Denteso and Keta series were 7 mg kg^{-1}, 74 mg kg^{-1} and 35 mg kg^{-1}, accounting for 0.7%, 16% and 6% of total ON. Analysis of variance indicated that the content of HAAN in the soils was significantly affected by amendment application, with the highest proportion being THN especially in Denteso and Keta series. The HAAN increased from 84 mg kg^{-1} in soil alone (14% of TN) to a range of 189 mg kg^{-1} (11% of TN) to 405 mg kg^{-1} (24% of TN) in Adentan series; from 142 mg kg^{-1} (35% TN) to 377 mg kg^{-1} (29% of TN) in Denteso series. In Keta series, HAAN increased from 108 mg kg^{-1} (17% of TN) to 329 mg kg^{-1} (25% of TN). The HUN fraction ranged from 106 mg kg^{-1} (5% of TN) to 631 mg kg^{-1} (36% of TN) in Adentan series, from -430 mg kg^{-1} (-34% of TN) to 84 mg kg^{-1} (7% of TN) in Denteso series, and -293 mg kg^{-1} (-23% of TN) to 465 mg kg^{-1} (30% of TN) in Keta series. The proportion of NHN was 363 mg kg^{-1} in Adentan series, 270 mg kg^{-1} in Denteso series, 531 mg kg^{-1} in Keta series, accounting for about 41%, 58% and 83% of total N, respectively. The NHN increased proportionally with BMC and MC amendment in all the soils. Analysis of variance showed that biochar types, manure and soils types significantly influenced the ON fractions of the amended soils (Table 5.5). The highest THN was observed in the Adentan series.

Table 5.2 Content and distribution of organic N in BMC and MC-amended Adentan series prior to incubation.

Treatment	THN	% of TN	HAN	% of TN	HASN	% of TN	HAAN	% of TN	HUN	% of TN	NHN	% of TN
Soil alone	469_{a}	54	114_{a}	13	7_{a}	0.7	84_{a}	14	254_{b}	30	363_{a}	41
$B_0CM_{50}MW_{50}$	558_{b}	33	218_{f}	13	6_{a}	0.3	189_{b}	11	147_{a}	9	1028_{f}	61
$CB_{20}CM_{40}MW_{40}$	1027_{e}	71	198_{de}	14	17_{ab}	1.2	204_{bc}	26	609_{e}	42	319_{a}	22
$RB_{20}CM_{40}MW_{40}$	1077_{f}	71	250_{g}	16	32_{c}	2.1	240_{d}	18	398_{c}	29	357_{a}	23
$SB_{20}CM_{40}MW_{40}$	1094_{f}	63	215_{f}	12	11_{a}	1.0	237_{d}	21	631_{e}	36	572_{b}	33
$B_0PM_{50}MW_{50}$	545_{b}	28	186_{cd}	10	17_{ab}	0.9	237_{d}	10	106_{a}	5	1344_{g}	69
$CB_{20}PM_{40}MW_{40}$	836_{c}	53	168_{b}	11	35_{c}	2.2	230_{d}	17	403_{c}	26	689_{c}	44
$RB_{20}PM_{40}MW_{40}$	892_{c}	52	213_{ef}	12	30_{c}	1.7	405_{e}	24	245_{b}	14	764_{d}	45
$SB_{20}PM_{40}MW_{40}$	980_{d}	52	177_{bc}	9	24_{bc}	1.3	220_{cd}	18	558_{d}	30	834_{e}	44

%TN: Percentage of organic N by total N; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

Table 5.3 Content and distribution of organic N fractions in BMC and MC- amended Denteso series prior to incubation.

Treatment	THN	% of TN	HAN	% of TN	HASN	% of TN	HAAN	% of TN	HUN	% of TN	NHN	% of TN
Soil alone	174_{a}	38	134_{a}	30	74_{a}	16	142_{a}	35	-176_{c}	-39	270_{a}	58
$B_0CM_{50}MW_{50}$	248_{b}	17	252_{f}	17	165_{d}	11	261_{d}	18	-430_{a}	-34	1239_{e}	86
$CB_{20}CM_{40}MW_{40}$	330_{c}	31	175_{bc}	16	160_{d}	15	245_{d}	39	-247_{b}	-23	684_{c}	63
$RB_{20}CM_{40}MW_{40}$	502_{e}	39	194_{cd}	15	118_{bc}	9	377_{f}	29	-187_{c}	-14	749_{c}	58
$SB_{20}CM_{40}MW_{40}$	467_{d}	34	176_{bc}	13	260_{e}	19	295_{e}	29	-264_{b}	-19	892_{d}	65
$B_0PM_{50}MW_{50}$	562_{f}	37	223_{e}	15	113_{bc}	8	217_{c}	18	9_{d}	1	901_{d}	60
$CB_{20}PM_{40}MW_{40}$	448_{d}	37	168_{b}	14	84_{ab}	7	205_{bc}	28	-9_{d}	-1	755_{c}	62
$RB_{20}PM_{40}MW_{40}$	715_{h}	56	199_{d}	15	145_{cd}	11	288_{e}	24	84_{e}	7	545_{b}	42
$SB_{20}PM_{40}MW_{40}$	609_{g}	44	225_{e}	16	111_{bc}	8	198_{b}	19	75_{e}	5	752_{c}	54

%TN: Percentage of organic N by total N; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

Table 5. 4 Content and distribution of organic N fractions in BMC amd MC-amended Keta series prior to incubation.

Treatment	THN	% of TN	HAN	% of TN	HASN	% of TN	HAAN	% of TN	HUN	% of TN	NHN	% of TN
Soil alone	73_{a}	11	91_{a}	14	35_{a}	6	108_{a}	17	-161_{b}	-25	531_{a}	83
$B_{0}CM_{50}MW_{50}$	573_{f}	38	199_{ef}	13	58_{bc}	4	274_{de}	18	42_{e}	3	832_{d}	55
$CB_{20}CM_{40}MW_{40}$	506_{e}	39	189_{de}	14	87_{de}	7	291_{e}	23	-58_{c}	-4	697_{c}	54
$RB_{20}CM_{40}MW_{40}$	590_{f}	45	211_{f}	16	63_{bc}	5	329_{f}	25	-13_{d}	-1	607_{b}	47
$SB_{20}CM_{40}MW_{40}$	721_{g}	49	184_{de}	12	138_{f}	9	269_{d}	18	130_{f}	9	687_{c}	46
$B_{0}PM_{50}MW_{50}$	282_{c}	18	172_{cd}	11	101_{e}	6	261_{d}	17	-252_{a}	-16	1197_{g}	76
$CB_{20}PM_{40}MW_{40}$	170_{b}	13	159_{bc}	12	65_{bc}	5	239_{c}	19	-293_{a}	-23	1004_{f}	79
$RB_{20}PM_{40}MW_{40}$	396_{d}	29	183_{d}	13	73_{cd}	5	274_{de}	20	-134_{b}	-10	897_{e}	66
$SB_{20}PM_{40}MW_{40}$	866_{h}	56	146_{b}	9	50_{ab}	3	205_{b}	13	465_{g}	30	594_{b}	38

%TN: Percentage of organic N by total N; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

Table 5.5 Analysis of variance of distribution of organic N as affected by biochar type, manure type, their interaction (final product) and the soil type.

Soil series	Source of variation	Df	THN	HAN	HASN	HAAN	HUN	NHN
Adentan	Manure (M)	2	***	***	***	***	***	***
	Biochar (B)	3	***	***	***	***	***	***
	M*B	6	***	***	***	***	***	***
Denteso	Manure (M)	2	***	***	***	***	***	***
	Biochar (B)	3	***	***	***	***	***	***
	M*B	6	***	***	***	***	***	***
Keta	Manure (M)	2	***	***	***	***	***	***
	Biochar (B)	3	***	***	***	***	***	***
	M*B	6	***	***	***	***	***	***
All soils	Soil	2	**	***	**	***	***	***

Ns= not significant *** = $p < 0.001$, **= $p < 0.01$, $p < 0.05$

5.4.2 Reduction in organic N fractions in BMC and MC amended soils in relation to N mineralization

At the end of the 26 weeks of incubation, THN, HAN, HAAN and HUN were reduced significantly in the BMC and MC-amended soils, with values ranging from -861 mg kg^{-1} to -323 mg kg^{-1}, -150 mg kg^{-1} to -54 mg kg^{-1}, -321 mg kg^{-1} to -60 mg kg^{-1} and -673 mg kg^{-1} to -73 mg kg^{-1}, respectively in Adentan series and accounted for -58% to -19%, -10% to -3%, -21% to -5% and -46 % to -4% of the respective total N fractions. Generally, the reduction in the proportions of HASN and NHN was not significant in the amended soils. However, in Denteso series, hydrolysable N such as THN, HAN, HASN, HAAN and HUN fractions reduced significantly after 26 weeks of incubation. The reduction in THN, HAN, HASN, HAAN and HUN ranged from -517 mg kg^{-1} to -21 mg kg^{-1}, -139 mg kg^{-1} to -5 mg kg^{-1}, -254 mg kg^{-1} to -27 mg kg^{-1}, -269 mg kg^{-1} to -69 mg kg^{-1} and -224 mg kg^{-1} to -0.9 mg kg^{-1} respectively which accounted for -40% -1%, -17% to -4%, -18% to-4%, -28% to -2% and -47% to -0.1% of their respective total N. A similar trend was observed in Keta series where THN, HAN, HASN, HAAN and HUN reduced from -627 mg kg^{-1} to -13 mg kg^{-1}, -140 mg kg^{-1} to -34 mg kg^{-1}, -116 mg kg^{-1} to -6 mg kg, -225 mg kg^{-1} to -99 mg kg^{-1} and -331 mg kg^{-1} to -134 mg kg^{-1}, accounting for -30% to -2%, -10% to -1%, -8% to -0.4%, -19% to -7% and -26% to -9% of their respective total N (Table 5.6, 5.7 and 5.8).

Table 5.6 Reduced organic N fractions in BMC and MC-amended Adentan series after 26 weeks of incubation.

Treatment	THN	% of TN	HAN	% of TN	HASN	% of TN	HAAN	% of TN	HUN	% of TN	NHN	% of TN
Soil alone	-433	-46	-73	-8	17	1.9	-60	-7	-317	-36	543	62
$B_0CM_{50}MW_{50}$	-323	-19	-146	-9	4	0.2	-108	-6	-73	-4	222	13
$CB_{20}CM_{40}MW_{40}$	-844	-58	-105	-7	37	2.6	-103	-7	-673	-46	908	63
$RB_{20}CM_{40}MW_{40}$	-861	-56	-150	-10	40	2.6	-317	-21	-433	-28	875	57
$SB_{20}CM_{40}MW_{40}$	-829	-48	-102	-6	4	0.2	-110	-6	-621	-36	753	43
$B_0PM_{50}MW_{50}$	-364	-19	-91	-5	20	1.0	-170	-9	-324	-6	-10	-1
$CB_{20}PM_{40}MW_{40}$	-566	-36	-54	-3	-14	-0.9	-136	-9	-361	-23	418	26
$RB_{20}PM_{40}MW_{40}$	-637	-37	-88	-5	-21	-1.2	-321	-19	-206	-12	385	22
$SB_{20}PM_{40}MW_{40}$	-678	-36	-64	-3	-1	-0.1	-103	-5	-510	-27	325	17

%TN: Percentage of organic N by total N; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

Table 5.7 Reduced organic N fractions in BMC and MC-amended Denteso series after 26 weeks of incubation.

Treatment	THN	% of TN	HAN	% of TN	HASN	% of TN	HAAN	% of TN	HUN	% of TN	NHN	% of TN
Soil alone	-91	-20	-77	-17	-27	-6	-127	-28	-212	-47	292	66
$B_0CM_{50}MW_{50}$	-21	-1	-139	-10	-119	-8	-168	-12	-455	-32	-292	-20
$CB_{20}CM_{40}MW_{40}$	-80	-7	-59	-5	-127	-12	-116	-11	-273	-25	184	17
$RB_{20}CM_{40}MW_{40}$	-218	-17	-102	-8	-49	-4	-269	-21	-171	-13	147	11
$SB_{20}CM_{40}MW_{40}$	-129	-9	-51	-4	-254	-18	-127	-9	-224	-16	22	2
$B_0PM_{50}MW_{50}$	-439	-29	-136	-9	-60	-4	-142	-9	-82	-5	103	7
$CB_{20}PM_{40}MW_{40}$	-276	-22	-71	-6	-43	-4	-69	-6	-112	-9	123	10
$RB_{20}PM_{40}MW_{40}$	-517	-40	-129	-10	-59	-5	-157	-12	-5	-0.4	349	27
$SB_{20}PM_{40}MW_{40}$	-383	-27	-129	-9	-76	-5	-28	-2	-0.9	-0.1	127	9

%TN: Percentage of organic N by total N; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

Table 5.8 Reduced organic N fractions in BMC and MC-amended Keta series after 26 weeks of incubation.

Treatment	THN	% of TN	HAN	% of TN	HASN	% of TN	HAAN	% of TN	HUN	% of TN	NHN	% of TN
Soil alone	-13	-2	-34	-5	-21	-3	-99	-15	-183	-29	231	36
$B_0CM_{50}MW_{50}$	-448	-30	-140	-9	-6	-0.4	-168	-11	-134	-9	433	29
$CB_{20}CM_{40}MW_{40}$	-295	-23	-63	-5	-51	-4	-142	-11	-154	-12	328	24
$RB_{20}CM_{40}MW_{40}$	-315	-24	-131	-10	-13	-1	-225	-19	73	6	542	42
$SB_{20}CM_{40}MW_{40}$	-457	-31	-38	-3	-116	-8	-129	-9	85	6	569	38
$B_0PM_{50}MW_{50}$	-151	-10	-91	-6	-81	-5	-218	-14	-264	-17	83	5
$CB_{20}PM_{40}MW_{40}$	37	3	-63	-5	-30	-2	-125	-10	-331	-26	-12	-1
$RB_{20}PM_{40}MW_{40}$	-243	-18	-99	-7	6	0.4	-157	-12	-261	-19	299	22
$SB_{20}PM_{40}MW_{40}$	-627	-41	-20	-1	-28	-2	-114	-7	465	30	532	34

%TN: Percentage of organic N by total N; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

5.4.3 Relationship between the reduction in organic N fractions and N_o.

Potentially mineralization N (N_o) was positively and significantly correlated with initial proportion of THN, HAN, HAAN and HUN with *r* values ≥ 0.506 ($p < 0.001$) prior to incubation. However, after the 26 weeks of incubation, N_o related negatively ($p < 0.001$) with the reduced THN, HAN and HAAN with *r* values ranging from -0.643 ($p < 0.001$) to -0.472 (Table 5.9).

A stepwise regression analysis to assess the importance of the reduction of N fractions to N mineralization indicated that the reduction in HAN alone could explain about 23% of the variations in N_o in all the soils (Table 5.10). Inclusion of reduction in HAAN in the stepwise equation resulted in a slight increase in the percent variation in N_o explained to about 30% in the amended soils (Table 5.10). When a reduction in THN was incorporated in the equation, the proportion of variation in *No* explained increased significantly to about 60% (Table 5.10).

Table 5.9 Correlation coefficient between N fraction and N_o in amended soils.

ON F	N fractions before incubation	Reduced N fractions after incubation
	N_o (Person correlation coefficient (r)	
THN	0.700^{***}	-0.643^{***}
HAN	0.864^{***}	-0.490^{***}
HASN	0.300^{*}	0.487^{***}
HAAN	0.584^{***}	-0.472^{***}
HUN	0.506^{***}	-0.011^{ns}
NHN	0.187^{ns}	0.249^{ns}

N_o: Potentially mineralizable N; ON F: Organic N Fractions; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

Table 5.10 Multiple regression analysis of reduced organic N fractions and N_o after 26 weeks of incubation.

Regression model ($Y=b_1X_1 \ldots . b_nX_n+b_o$)	R^2	*F*
$N_o = -0.562HAN + 110$	0.23	<0.001
$N_o = -0.408HAN - 0.153HAAN + 100$	0.30	<0.001
$N_o = -0.316HAN - 0.126HAAN - 0.069THN + 82$	0.60	<0.001

5.4.4 Organic N fractions in relation to Amidohydrolase activities

The activity of amidase was correlated positively and significantly with HAAN, HAN, HASN and THN in the amended soils, with *r* values ranging from 0.300 (*p*

< 0.05) to 0.714 ($p < 0.001$). The HAAN, HAN, THN and HUN also corrected positively with L-asparaginase activities, with r values ≥ 0.425 ($p < 0.01$). Also, there was positive and significant relationship between L-glutaminase activity and HAAN, HAN, THN and HUN, with r-values ranging from 0.435 ($p < 0.01$) to 0.733 ($p < 0.001$) (Table 5.11).

Table 5.11 Pearson correlation coefficient between Amidohydrolase activities and organic N fractions

ON F/AA Act.	Am. Act.	As. Act.	Glut. Act.
HAAN	0.714^{***}	0.425^{**}	0.435^{**}
HAN	0.570^{***}	0.672^{***}	0.649^{***}
HASN	0.300^{**}	-0.402^{**}	-0.545^{***}
THN	0.353^{**}	0.674^{***}	0.733^{***}
HUN	0.069^{ns}	0.560^{***}	0.640^{***}
NHN	-0.073^{ns}	-0.220^{ns}	-0.194^{ns}

ON F: Organic N fractions; AA Act: Amidohydrolase Activity; Am. Act.: Amidase Activity; As. Act.: Asparaginase Activity; Glut. Act.: Glutaminase Activity; HAAN: Hydrolysable Amino Acid N; HAN: Hydrolysable Ammonia N; HASN: Hydrolysable Amino Sugar N; THN: Total Hydrolysable N; HUN: Hydrolysable Unknown N; NHN: Non-Hydrolysable N

5.4 Discussion

5.5.1 Initial organic N fractions in BMC and MC-amended soils

The increase in ON fractions of the BMC and MC-amended soils compared to the soil alone is consistent with the result of Sanchez-Monedero *et al*. (2019) and Yuan *et al.* (2017) who reported an increase in the extractable ON in soils after application of BMCs and MC. Kaur and Singh (2014) also observed increased hydrolysable ON fractions (THN, HAN, HAAN, HASN and HUN) in soils amended with farmyard manure, press mud and green manure, which may have contributed to the higher N_o of the BM-amended soils compared to unamended control and inorganic fertilized soils (González-Prieto *et al*.,1997). The higher THN values obtained in the present study compared to those reported by Sekhon *et al*. (2011) in manure, biochar and inorganic fertilizer amended soils could be attributed to the 26 weeks of incubation used.

The increased HAN and HAAN obtained in the BMC-amended soils compared to the control are similar to the findings of Wang *et al*. (2014) who reported ranges of 266 to 459 mg/kg and 83 to 483 mg/kg, respectively in organic and inorganic N fertilized soils. The values of HASN values obtained in the present study agree with those of Sekhon *et al*. (2011), especially in Adentan and Keta series, while those in Denteso series are consistent with those reported by Wander *et al*. (2007). However, the lower HASN observed in some BMC-amended soils compared to the control may be due to the deamination of amino sugars during hydrolysis, as has been reported by Stevenson (1994). The lowest HASN recovered compared to the other hydrolysable fractions may be due to its lower turnover rate in soils (Wu *et al*., 1986). The highest proportion of HAAN in the THN recorded in the present study is consistent with the results by Xu *et al*. (2003) who reported that about

30-32% of THN was recovered in manured soil. The HUN values obtained in the BMC-amended soils are comparable to those reported by Sekhon *et al*. (2011) and Kaur and Singh (2014) on manure and inorganic fertilizer amended soils. The high hydrolysable N recorded in RB-based BMC and BMC without biochar could be due to the high liability of N based on the biochar type used in the BMC as emphasized by Wang *et al*. (2012). The highest THN in Adentan series may be attributed to the high ON in the soil due to the management practice before sampling which may have resulted in high N mineralization, as reported by González-Prieto *et al*. (1997).

5.5.2 Reduction of organic N in relation to N mineralization

The significant decrease in HAN, HAAN and THN in most of the soils indicates that these fractions of ON contributed to N mineralization. This finding is consistent with those of Li and Li (2014) who identified a significant reduction in THN, HAN, HASN, HAAN and HUN when the soils were amended with manure. The authors concluded that these fractions contributed to N mineralization. Sun *et al*. (2018) also reported an increase in THN fraction in cattle manure amended soils which contributed to N mineralization. The increase in NHN at the end of the incubation could be attributed to the recalcitrancy of biochar in the BMC which probably contributed less to N mineralization in the soils. According to Wang *et al*. (2012) charring of animal manure mixed with wood chips decreased THN but increased NHN fraction of lower libality, which contributed less to N mineralization. The authors also attributed the occurrence to the formation of aromatic and heterocyclic structures and the degradation of labile N fractions in biochar during charring. This probably accounted for the lower N mineralization in BMC-amended soils, especially the CB and SB-based BMCs.

5.5.3 Relationship between reduced organic N fraction and N_o.

The negative relation between HAN, HAAN; THN and N_o after incubation shows that mineralized N was mainly from these ON fractions. This result is in line with those of Li and Li (2014) who reported a negative relationship between N mineralized and reduction of HAN and HAAN in a manure-amended soil. On the contrary, results from Mulvaney and Khan (2001) identified HASN as the main source of mineralizable N which was attributed to the high turnover rates of these ON fractions.

A stepwise regression analysis using all the ON fractions showed that HAN, HAAN and THN were the sole contributors to N mineralized in the amended soils, with a predictive value for N_o. The positive and predictive relation between the ON fractions and N_o indicates that these N fractions can be used to predict the variability in N mineralization from BMC.

5.5.4 Relationship between organic N fractions and Amidohydrolase activity

The positive relationship between HAAN, THN and HAN and the amidohydrolase activities in all the MC and BMC-amended soils suggests the involvement of these enzymes in hydrolyzing these ON fractions. Studies by Tabatabai *et al*. (2010) showed that L-asparaginase, urease, L-glutaminase, amidase, L-aspartase, and arylamidase were involved in the hydrolysis of the bonds in the specific amino acids released from the organic matter in an organically managed soil.

5.6 Conclusions

Generally, the types and distribution of hydrolysable and non-hydrolysable ON fractions were higher in BMC and MC-amended soils compared with the unamended soils. The decrease in HAN, HAAN and THN at the end of the 26 weeks of incubation indicated that these fractions contributed to N_o. Also, the positive correlation between amidohydrolase activities, HAAN, THN and HAN in the amended soils suggests that the enzymes were involved in the hydrolysis of these ON fractions to release inorganic N which contributed to N mineralization in the soils.

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CHAPTER 6

6.0 GENERAL CONCLUSION AND RECOMMENDATIONS

This study sought to investigate the effect of biochar manure co-compost (BMC) on N mineralization (N releasing capacity of soil), microbial and biochemical properties, (soil microbial biomass and amidohydrolase activities), ON fractions contributing to N mineralization and the relationships among these parameters in three soils (Adentan, Denteso and Keta series) using manure compost (MC) and soils alone as controls.

The results indicated that application of BMCs to the soils resulted in lower N_o values compared with MCs. In all, CB- and SB-based composts had the lowest N_o values when amended with the three tropical soils. Also, the BMC-amended soils had low k values compared with the MC. Among the soils used, Adentan series had the highest N_o. The results also showed a negative correlation between C: N ratio, lignin content, TC and N_o, while positive correlation was observed between NH_4^+-N, cellulose content and N_o values. In all, BMC properties such as lignin, cellulose, C: N and NH_4^+-N were identified as good predictors of N_o.

Overall, the study demonstrated that BMCs, comprising different manure types, biochar types and rates had significant and positive influence on SMB and its related amidohydrolase activities through the enhancement of chemical properties of three soils compared to the MC and unamended soils. The activity of L-glutaminase was the highest among the amiodohydrolases studied. The C_{mic} increased with application of amendment, but N_{mic} varied with soil type. Comparing the manure and biochar types, the RB, CB, CM based-BMCs had a higher effect on SMB and AA compared to SB, 0B, PM based-BMCs. Among the soil types, Adentan series had the highest AA, SMB and chemical properties while Keta series had the highest pH. A positive relation was established among AA, SMB and N_o. Also, there was a asignificant and positive relation among AA, SMB and selected soil properties.

Generally, the types and distribution of hydrolysable and non-hydrolysable ON fractions were higher in BMC and MC-amended soils compared with the unamended soils. The decrease in HAN, HAAN and THN at the end of the 26 weeks of incubation indicated that these fractions contributed to N_o. Also, the positive correlation between amidohydrolase activities and HAAN, THN and HAN in the amended soils suggests that these enzymes were involved in the hydrolysis of these ON fractions to release inorganic N which contributed to N mineralization in the soils.

Finally, BMC application to soils can stabilize ON and control N release, enhance microbial and biochemical activities and increase ON fractions, which contribute to mineralizable N capacity of the soils.

According to the findings, various recommendations were made:

1. The preparation and use of BMC should be adopted by the Ministry of Food and Agriculture (MoFA) to make it a policy for farmers to convert available wastes for sustainable crop production and yields
2. Although available organic materials are encouraged to produce BMC, for long term nutrient sustainance of the soil, sawdust, coconut husk and poultry manure should be a preferred choice for compost preparation.
3. For increase microbial properties of the soil, ricehusk, coconut and cattle manure should be used for compost preparation.

Possible future research

Although some progress has been done to provide knowledge on the N release and microbial properties of BMC-amended soils in Ghana, other works can be conducted to improved nitrogen management and crop productivity of this amendment, which include:

1. A greenhouse experiment should be conducted to investigate the synchrony between N release pattern in BMC-amended soils and crop N uptake.
2. A field experiment should be conducted to ascertain the effect of BMCs on crop yields.
3. Different biochar rates should be used for co-composting to investigate its effect on N mineralization, microbiological and biological properties of soils.
4. A long-term incubation studies (about 1 year) should ne done to assess the long term nutrient release patterns of BMC.