EFFECT OF DIFFERENT PARTICLE SIZES OF RICE HUSK BIOCHAR ON SOIL PROPERTIES, NUTRIENT LEACHING, AND *BRASSICA ALBOGLABRA* PRODUCTIVITY IN SANDY LOAM SOIL

KARMA WANGDI

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in Agricultural Science
2021

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has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science in Agricultural Science of Naresuan University

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Title	EFFECT OF DIFFERENT PARTICLE SIZES OF RICE HUSK BIOCHAR ON SOIL PROPERTIES, NUTRIENT LEACHING, AND *BRASSICA ALBOGLABRA* PRODUCTIVITY IN SANDY LOAM SOIL
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ABSTRACT

Soil health enhancement with biochar application is well documented. However, the influence of particle sizes of biochar coapplied with synthetic fertilizers on soil properties (physical, chemical, and biological), hydrological properties, nutrient leaching, and nutrient use efficiency are not well understood, particularly for sandy loam soil. There is still a knowledge gap regarding the effects of varying particle sizes of biochar on soil characteristics and the microbial community. Therefore, the influence of biochar particle size along with fertilizer was investigated in this study. We examined the short-term interactive effect of particle sizes of rice husk biochar (RHB) with fertilizer. For this study, RHB was produced at 300 to 550 ^{o} C using the traditional kiln method and was conducted at Naresuan University, Thailand. Morphology of RHB was classified using Field Emission Scanning Electron Microscope and element analysis by Energy Dispersive X-ray Spectrometer (FESEM-EDS). Three different sizes; of RHB: (i) less than 0.25 mm, (ii) 0.25 – 1 mm, and (iii) 1 – 2 mm were used to mix with the sandy loam soil, tested in the soil column, and sampled for four-time periods at 0, 8, 18, and 29 days after transplanting of *Brassica alboglabra*. Treated soils were analyzed for pH, electrical conductivity (EC), organic carbon (OC), total nitrogen, available phosphorus, and exchangeable potassium. BIOLOG EcoPlate was used to investigate the effect of biochar and fertilizer on soil microbial activity at harvesting time. Biochar amendment without fertilizers significantly increased soil pH and organic carbon but did not affect EC, while co-application with fertilizers significantly increased nutrient concentrations. The soil water and nutrient retention increased with decreasing biochar particle sizes. However, large size biochar decreased bulk density more significantly. The smallest biochar size (<0.25 mm) was 20% greater potential in leaching reduction as compared to the control. Besides, medium (0.25 – 1 mm) and large (1 – 2 mm) sizes of RHB reduced leaching by 11%, and 5%, respectively. Adding biochar (regardless of size) increased microbial activity in the metabolization of phenolic compounds. The large size of biochar (1-2 mm) provided the greatest microbial activity on carboxylic acids. Results suggest that biochar improved soil properties and reduced water and nutrient leaching, increase nutrient use efficiency and microbial diversity and activity, and the beneficial effects were enhanced when coapplied with fertilizer. No significant effect on *Brassica alboglabra* height and biomass was observed.

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CHAPTER I

INTRODUCTION

1.1 Background and Significance of the Study

Agriculture is critical for food security, self-sufficiency, and economic (Cervantes-Godoy & Dewbre, 2010). It provides most of the food, raw materials, revenue, and jobs (Praburaj et al., 2018). However, as the global population continues to grow, the demand for food increases, driving greater crop production. As a result, the soil is a critical resource for agricultural production systems. However, soils have become increasingly vulnerable to degradation because of overexploitation of land and water resources to pick up the growing demand for food and fiber from an ever-expanding population. Soil health and fertility are critical for effective crop production since it measures the soil's capacity to deliver nutrients to plants (Jones Jr, 2012). It functions as a 'SINK' for nutrients, allowing plants to grow and develop optimally, resulting in optimum production (Wiedenhoeft, 2006). Low soil fertility will jeopardize agricultural systems' sustainability. Intensive usage of chemical fertilizers and overexploitation of land due to intensive farming has resulted in soil degradation. Additionally, nitrogen leakage from agricultural soils is a significant contributor to soil fertility depletion, resulting in decreased crop yields, increased fertilizer expenditures for farmers, and deteriorated surface and groundwater quality (Laird et al., 2010). As a result, effective soil and crop management procedures must be used. In sandy soil, the problem is exacerbated. Sandy soils are found worldwide and are frequently acidic due to their low water holding capacity and low cation exchange capacity (CEC) of the soil (Aprile & Lorandi, 2012; Olorunfemi et al., 2016). Furthermore, sandy soils continue to pose a problem for water and nutrient management at the landscape and field scales. The inability of sandy soils to fully retain water and nutrients to excessive leaching and water quality. Moreover, sandy soils don’t have to be 100% sand, and in fact, it is any soil material with 85 or more percent of sand. Therefore, being predominantly rich in sand, sandy loam soils are classified as coarse in texture. Sandy loam is a soil material that contain less than 7%clay, less than 50% silt, and a sand range of 43-52% (Kettler et al., 2001). The size of sandy loam grains varies from very fine to very coarse and is mainly dependent on the size of the component sand particles. This implies that sandy loam soils cannot retain water well, which lead to insufficient nutrition in plants. They require frequent fertilization and water to boost plant growth. The application of biochar has been proved to reduce nutrient leaching and after incorporation into the soil, biochar improves soil fertility (Lehmann et al., 2003; Lehmann & Joseph, 2009; Steiner et al., 2007). Biochar application significantly decreased soil bulk density (Omondi et al., 2016); increased soil cation exchange capacity (Jien & Wang, 2013); neutralized soil acidity and improved soil nutrients availability (Atkinson et al., 2010; Igalavithana et al., 2016) increased overall net soil surface area (Chan et al., 2008) and improved soil water retention capacity (Basso et al., 2013; Toková et al., 2020). The porous structure of biochar was also suitable as a habitat for beneficial soil organisms and enhanced their activities (Cao et al., 2017). Biochar as a soil amendment can improve crop yields and the quality of degraded soils. Biochar generated from black carbon biomass has been shown to increase yields (Filiberto & Gaunt, 2013; Lehmann & Joseph, 2009). Hence, with necessary seek, biochar serves as an alternative option by rehabilitating this threat in agriculture.

The idea of biochar application in the agriculture field originated from Amazonian dark earth or Terra preta research (Glaser & Birk, 2012). Biochar is a carbon-rich material produced from the thermal decomposition of biomass produced by a process called pyrolysis (Tripathi et al., 2016). According to Lehmann and Joseph (2009), the application of biochar its application is towards environmental management, and productivity benefits the soil. Biochar is used as a soil amendment for improving soil health and productivity and enhancing carbon sequestration in the soil for a long in an attempt to mitigate climate change (Igalavithana et al., 2016; Woolf et al., 2010). In recent years, biochar has gained popularity in the field of agriculture due to the global issues of climate change, environment, and recognition for its ability to amend the sustainable use of the soil (Kong et al., 2014; Lehmann & Joseph, 2009; Sohi et al., 2009). Therefore, biochar is a good alternative to manage crop residue burning. Biochar is prepared in a controlled environment that prevents emissions of harmful gases into the atmosphere. Rice is a staple food for many people with annual global paddy rice production being about 580 million tons.

Approximately 140 million tons of rice husk are produced each year as a by-product of rice processing, which presents a considerable waste management challenge (Kalderis et al., 2008; Moraes et al., 2014). This conversion of rice husk into values added to biochar may result in secondary carbon benefits through the avoidance of field burning and bio-resource recycling. Pyrolysis of wastes such as rice husk can result in a variety of benefits, including energy production, sustainable waste recycling, carbon sequestration, soil quality improvement, and improved plant development (Abrishamkesh et al., 2015). Moreover, the high volatile matter content (70.2–78.5 percent) and carbon content (35.2–44.7 percent) of rice husk favor the pyrolysis-based generation of biochar. Rice husk has high levels of potassium and silicon, both of which have the potential to improve soil fertility (Masulili et al., 2010). Numerous research has demonstrated the potential of rice husk biochar as a soil amendment to improve the soil properties. (Abrishamkesh et al., 2015; Masulili et al., 2010).

The type of feedstock used to produce biochar, pyrolysis temperature, and time duration, as well as the particle size of biochar, affect the influence of soil and biochar interaction on biochar properties. In light-textured soils, biochar's ability to improve its attributes is heavily dependent on its features, namely its particle size (Alghamdi et al., 2020). Biochar physical properties have been found to have a significant impact on its performance as a soil amendment. The properties of the soil matrix will be affected using varied biochar particle sizes. The biochar particle size has a significant impact on the way it interacts with the soil matrix (Esmaeelnejad et al., 2016; He et al., 2018). It was determined that the particle size of biochar plays a critical role in soil bulk density, water and nutrient retention and availability as well as pore size distribution and carbon sequestration in soil (de Jesus Duarte et al., 2019; He et al., 2018; Jaafar et al., 2015). However, particle size is a critical feature of biochar "design" that has gotten far less attention in the literature. The particle size of biochar is expected to have a significant effect on the interactions between soil and biochar, as smaller biochar particles will inevitably come into physical contact with soil particles. Numerous investigations on the relevant properties of biochar have been conducted to determine its potential for improving soil physical properties, soil pH adjustment, nutrient availability and retention, and soil microbiota. Biochar has been shown in research to significantly increase crop productivity when applied to the soil. However, an assessment of the effect of biochar particle size on soil characteristics is absent. A study on soil physiochemical characteristics, nutrient leaching, crop growth, and yield is necessary to have a better understanding of the limitations and opportunities associated with the use of various particle sizes of biochar as a soil amendment. The study's primary objective is to get an understanding of the particle size parameters that influence biochar's effect on soil characteristics, nutrient leaching, and crop growth and yield in sandy loam soil.

1.2 Objective

To investigate the effects of biochar particle sizes on soil physical-chemical and biological properties, leaching loss of soil nutrient element, nutrient use of efficiencies, and crop growth and biomass of *Brassica alboglabra* in sandy loam soil

1.3 Conceptual Framework

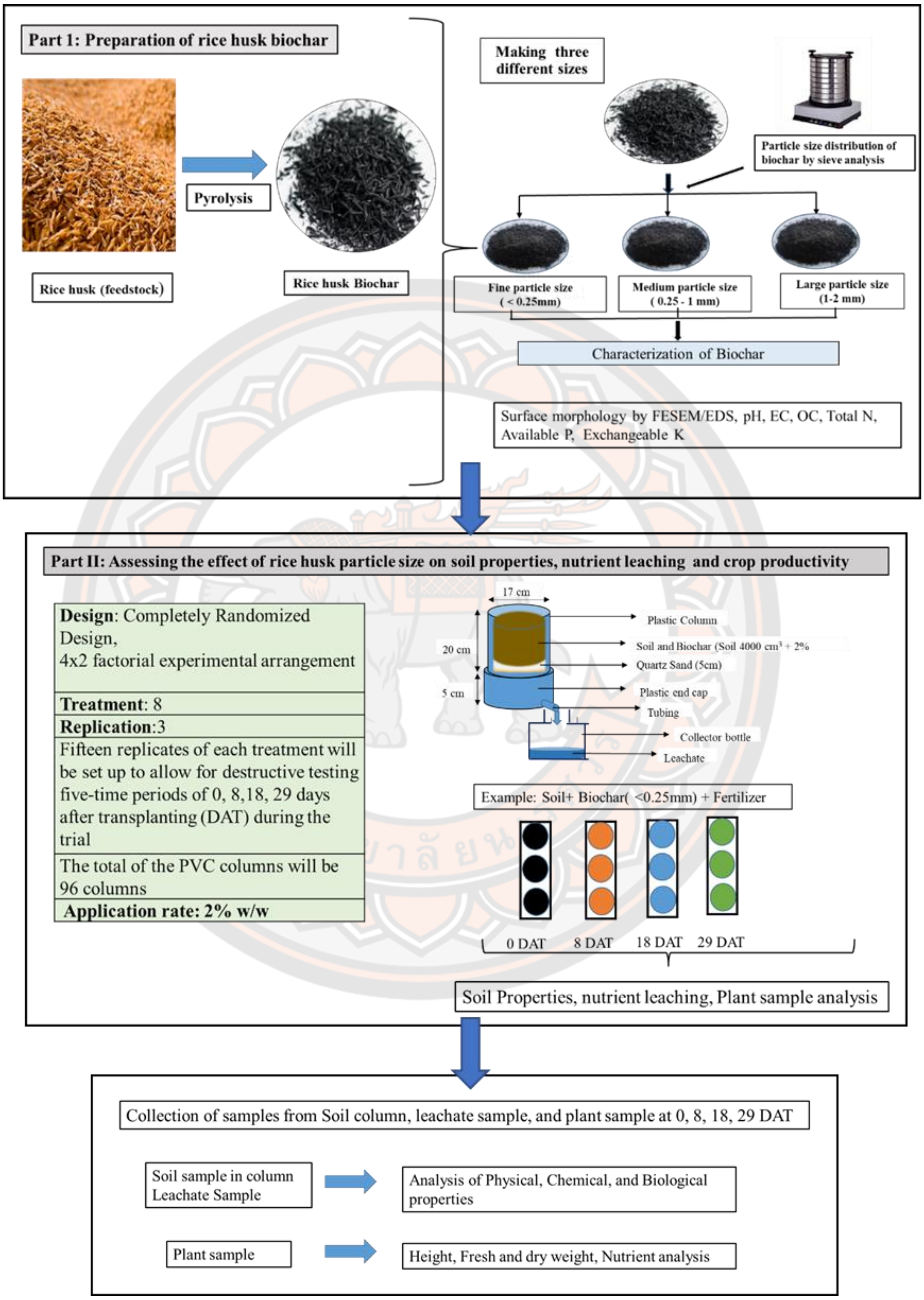

Figure 1 Conceptual Framework of the Study

1.4 Scope of the Study

The study's primary objectives and scope were to advance scientific knowledge and understanding of the effects of different particle sizes of rice husk biochar on soil characteristics, nutrient leaching, and crop productivity in sandy loam soil. Rice husk biochar was created using a typical charring pyrolysis reactor for two hours and then ground and sieved into three particle sizes (< 0.25 mm, 0.25-1 mm, and 1-2 mm). A soil column with pot experiments was be established to quantify nutrient leaching, soil characteristics, and crop productivity in sandy soils with various particle sizes of biochar. *Brassica alboglabra* were sown in plastic tray in the potting mixture and transplanted to soil columns at three weeks after sowing.

The experiment was a completely randomized design (CRD), with a 4x2 factorial experimental arrangement.

Factor 1: three rice husk biochar particle sizes (< 0.25 mm, 0.25-1 mm, and 1-2 mm) plus no biochar

Factor 2: with and without chemical fertilizer

Soil samples in the column, leaching samples, and plant samples were destructively collected in each treatment from the PVC columns (n=3) at time increments of 0, 8, 18, 29 days after transplanting (DAT) and harvesting. The total of the PVC columns were 96 columns.

1.5 Hypotheses of the Study

The effect of biochar on nutrient leaching in sandy soil is believed to be more substantial. When applied to heavily worn tropical soils, biochar has been shown to improve soil quality and reduce nutrient loss. We hypothesized that treating soils with biochar with small particle sizes can aid in nutrient retention and limit leaching potential compared to large particle sizes. Additionally, when combined with a chemical fertilizer, rice husk biochar with the smallest particle size (less than 0.25 mm) can improve the soil's physical, chemical, and biological properties while also enhancing crop growth and development. Further, addition of fertilizer with biochar is expected to improve soil properties and increase crop productivity.

CHAPTER II

LITERATURE REVIEW

The following sections summarize the literature on the influence of different particle sizes of biochar on soil characteristics, nutrient leaching, and *Brassica alboglabra* production in sandy soil.

- Biochar production
- Characterization of biochar
- Rice husk biochar
- Effect of biochar on soil properties
- Effect of biochar on nutrient leaching
- Effect of different particle sizes of biochar
- Effect of biochar on crop growth and yield

2.1 Biochar Production

Biochar has been produced by several burning methods such as auger pyrolysis reactors (industrial level), traditional kiln method (local level), and muffles furnace (laboratory level). Carbonizing wood to produce biochar has been used in the past since ancient times (Emrich, 1985). The technology used in biochar production ranges from simple pits to sophisticate industrial plants. There are several methods for producing biochar, but they all include heating biomass with little or no oxygen to eliminate volatile gases and leave carbon behind. Biochar is a carbon-dense substance formed by pyrolysis (300-800°C), the thermochemical breakdown of biomass under oxygen-deficient circumstances (Chan et al., 2008; Lehmann & Rondon, 2006). Pyrolysis is a high-temperature (>350°C) thermal treatment of biomass in a low-oxygen environment to create biochar, syngas, and bio-oil (Brown, 2009; Li et al., 2017). Pyrolysis converts aliphatic carbon to more stable aromatic carbon, releasing combustible gases (H_2, CH_4, CO). The biochar production structure of different feedstocks is presented in Figure 2.

As pyrolysis temperature rises, biochar output decreases as the carbon content of the biochar increases. Biochar produced at a lower temperature (250 to 400℃) has a higher yield and contains more ion-exchange functional groups with diversified organic character. As an alternative, biochar made at high temperatures (400 to 700°C) contains a substantial amount of carbon in aromatic structures, but it also has fewer functional groups that can exchange ions with other elements (Glaser et al., 2002). Similarly, biochar yield and total nitrogen were found to decrease with increasing pyrolysis temperature by Nwajiaku et al. (2018) but ash content to increase along with pH and EC as well as total carbon and the minerals extractable calcium, magnesium, and sodium as well as available phosphorus, potassium, and silica.

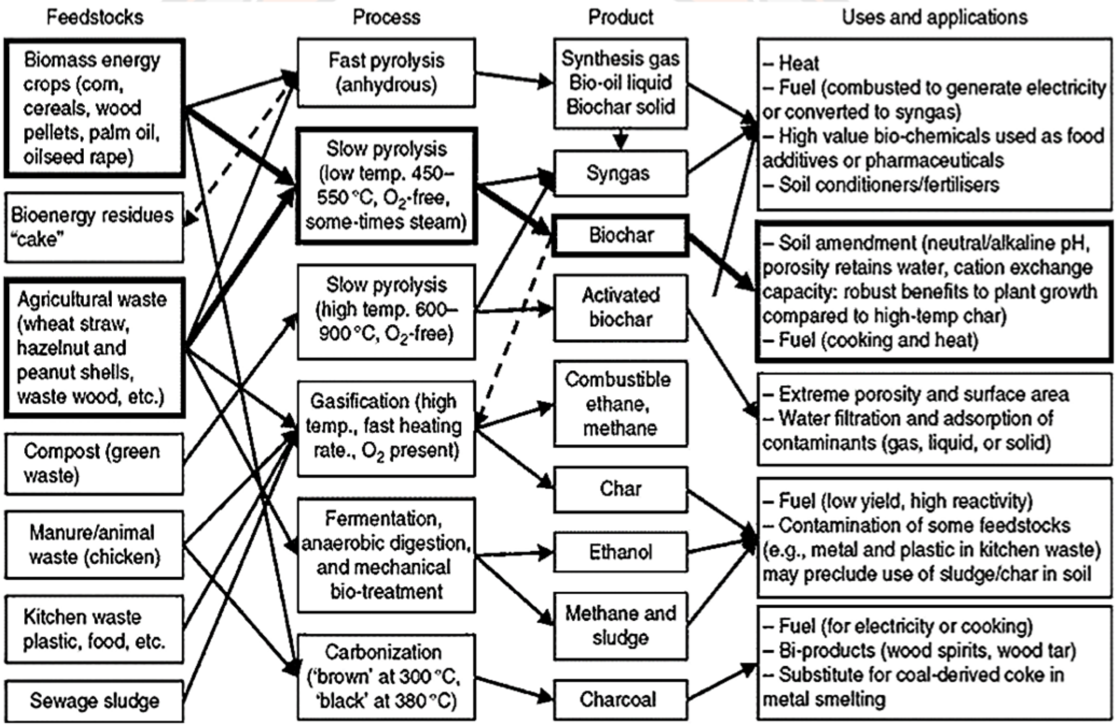

Figure 2 Biochar and other Thermally Converted Biomass Products Based on Existing Technology and Feedstocks

Source: Nsamba et al., 2015

2.2 Characterization of Biochar

The effect of biochar as soil amendment depends on several factors. The effect and characteristics of biochar depend on the source of feedstocks, pyrolysis duration, and temperature. The pH values of biochar at different pyrolysis temperature ranges at alkaline and increased with pyrolysis temperature (Cantrell et al., 2012). Rice husk biochar was found to exhibit somewhat alkaline characteristics, with a pH of 8.7, a CEC of 17.57 cmol kg^{-1}, carbon content of 18.72 percent, and potassium content of 0.20 percent (Masulili et al., 2010). Similarly, Zhao et al. (2013) evaluated the characteristics of biochar created from a variety of waste sources (animal waste, wood waste, crop waste, food waste, aquatic plant waste, and municipal waste) and discovered that the pH of all biochar is in the alkaline range.

2.2.1 Nutrient Content in Biochar

The nutritional and trace mineral content of biochar varies significantly depending on the source of the biomass feedstock, which is critical for soil fertility and plant growth. Sánchez et al. (2009) determined that rape biochar includes 0.76 percent nitrogen, 0.36 percent phosphorous, and 4.40 percent potassium, whereas sunflower biochar has up to 1.19 percent nitrogen, 0.44 percent phosphorous, and 7.26 percent potassium. Shenbagavalli and Mahimairaja (2012) investigated the properties of biochar made from various biological wastes. They reported that the properties of the biochar varied significantly depending on the feedstock. The NPK content of biochar ranged between 8.5 and 1.12 g kg^{-1}, 0.6 and 3.2 g kg^{-1}, and 2.4 to 29 g kg^{-1}. The coconut shell biochar has a greater concentration of P than the maize stover biochar. Additionally, the concentrations of Na, Ca, and Mg varied significantly between biochar samples. While the sodium content varied between 5.2 and 38 g kg^{-1}, the calcium amount varied between 1.8 and 11 g kg^{-1}. The Prosopis biochar contained a comparatively high concentration of sodium and calcium. The magnesium content of biochar samples varied between 0.36 and 6.2 g kg^{-1}, with paddy straw biochar having the greatest concentration and Prosopis biochar having the lowest. Zhao et al. (2013) investigated biochar generated from a variety of biowastes and discovered that nutrients (N, P, K, Ca, and Mg) were present in abundance, ranging from 0.18 to 5.62 percent, 0.12 to 10.8 percent, 0.079 to 13.7 percent, 0.12 to 41.8 percent, and 0.058 to 2.86 percent, respectively.

2.3 Rice Husk Biochar

Rice husk, also called rice husk is an abundantly available farm waste that can be derived into value-added biochar. Masulili et al. (2010) characterized the properties of rice husk biochar and found out that it contains 4.96% water content, 8.7 pH, 17.57 cmol kg^{-1} CEC, 18.72% C, 0.12% P, 0.2% K, 0.41% Ca, 0.42% Mg and 1.4% Na, respectively. Ghorbani et al. (2019) derived rice husk biochar at 500℃ and recorded 9.18 pH, 0.347 dSm^{-1} EC, 17.57 $cmol^{-1}$ CEC, 0.84 g cm^{-1} BD, and 478 g kg ^{1} total carbon.

In Sri Lanka, Gamage et al. (2016) investigated the impact of four different rice-husk biochar rates (0, 0.1, 0.5, and 1 percent) on the soil characteristics of sand and sandy loam. A significant rise in the soil's pH, cation exchange capacity, and organic carbon (OC), as well as a decrease in bulk density, were found at greater rates of biochar application (0.5 percent and 1 percent). Similarly, Pratiwi and Shinogi (2016) combined rice husk biochar with soil at rates of 0% (control), 2%, and 4% (biochar weight/soil weight). Rice shoot height was substantially greater in soil supplemented with 4% biochar than in control soil. Additionally, it was discovered that treating the soil with biochar enhances biomass output.

2.4 Effect Of Biochar on Soil Properties

2.4.1 Soil Physical Properties

2.4.1.1 Bulk Density

Bulk density serves as a proxy for soil compaction. Numerous studies have demonstrated and established that the application of biochar influences the bulk density of the soil. Basso et al. (2013) conducted an incubation experiment to determine the capacity of biochar to increase the water holding capacity of sandy soils. They discovered that biochar treated soils had a bulk density up to 9 % lower than control soils. Similarly, Omondi et al. (2016) quantified the impacts of biochar on soil hydrological parameters and found that it lowered bulk density by an average of 7.6 percent.

According to a study conducted by Abrishamkesh et al. (2015) to determine the influence of rice husk biochar on the soil characteristics and lentil productivity. They reported a significant decrease in soil bulk density from 1.39 g cm^{-3} to 1.14 g cm^{-3} when 3.3 weight percent rice husk biochar was applied. Similarly, when rice husk biochar was applied at a rate of 2% (w/w) to sandy loam soil, the bulk density decreased dramatically from 1.65g cm^{-3} to 1.45g cm^{-3} (Esmaeelnejad et al., 2016). Toková et al. (2020) also observed that biochar applied at a rate of 20 t ha^{-1} without fertilizer considerably reduced bulk density by 12% and enhanced porosity by 12%.

2.4.1.2 Soil Porosity

To examine the impact of biochar on soil physical properties, Mukherjee and Lal (2013) conducted a greenhouse experiment in sandy soil noticed a significant increase in porosity from 56.1% control to 62.1% treated with eucalyptus wood biochar.

The influence of biochar on soil physical properties has been studied by Aslam et al. (2014) where they led a laboratory experiment in sandy soil and noticed an increase of porosity from 0.50 cm^{-3} (control) to 0.78 cm^{-3} by application of peanut hull biochar. Later, Esmaeelnejad et al. (2016) reported that application of applewood biochar at 1:1 soil noticed increasing soil porosity from 37 cm^{-3} (control) to 49 cm^{-3} in sandy loam soil. In another study, biochar was amended at a different rate in sandy loam soil. The result showed porosity increasing from 0.50 to 0.77 cm^{-3} with increased biochar (Githinji, 2014).

2.4.1.3 Water Holding Capacity

Biochar has been well documented for increasing the water retention capacity of soils. Basso et al. (2013) investigated the effect of biochar on the water retention capacity of sandy loam soil by incubating it at various rates with hardwood biochar. According to the study, adding biochar increased the gravity-drained water content by 23% when compared to a control. Similar benefits were observed in several soil types, O. Y. Yu et al. (2017) discovered that 10% switchblade grass biochar increased the water retention capacity of loamy sand by 228%, compared to 133% for hemlock. The switchblade grass biochar alone retained 448.69% of its weight in water, while the hemlock biochar held 268.34 % of its weight in water.

2.4.1.4 Hydraulic Conductivity

Hydraulic conductivity is different in each type of soil and is normally low in sandy soil. Biochar is one of the solutions to increase hydraulic conductivity. Githinji (2014) experimented on sandy loam soil amended with distinct rates of peanut hull biochar and its physical and hydraulic properties have been examined. The results showed a linear decrease in saturated hydraulic conductivity at 0.49, 0.31, 0.23, 0.20, and 0.18 cm min^{-1} with excelling rates of 25%, 50%, 75%, and 100% of biochar has been noticed.

Shenbagavalli and Mahimairaja (2013) did a field experiment on wetland rice field of clay loam soil texture applied Prosopis wood biochar at 5 t ha^{-1}, this experiment showed improvement in the hydraulic conductivity of rice from 6.5 to 18.5 cm hr^{-1}. Jeffery, Meinders, et al. (2015) conducted a field experiment in sandy soil to study the effect of biochar in soil hydrological characteristics by applying herbaceous feedstock biochar at 10 t ha^{-1} and reported no significant effects of biochar application on soil hydraulic conductivity.

2.4.2 Chemical Properties of Soil

2.4.2.1 Soil pH

Biochar treatment on soil parameters and lettuce and cabbage growth was studied by Carter et al. (2013) in a pot culture experiment on sandy loam soil. The study found a 0.6-unit increase in pH from 5.5 to 6.1 and a 1.2-unit increase from 5.5 to 6.7 when biochar was applied at 50 g kg^{-1} and 150 g kg^{-1}, respectively. According to Jien and Wang (2013), an incubation experiment on the influence of waste wood biochar on the physical parameters of the acidic ultisol found that the pH increased from 3.91 to 5.01 when 5% of biochar was applied to the soil. In their greenhouse pot experiment on sandy soil, de Sousa Lima et al. (2018) found that increasing the application of coffee husk biochar from 4 to 16 Mg ha^{-1} generated a linear rise in soil pH from 5.5 to 7.0.

2.4.2.2 Electrical Conductivity

EC of soil is a good indicator of the number of salts present in the soil (salinity of soil). It serves as a vital indicator of the state of the soil's fertility. Using five different rates of biochar (0, 2.5, 5.0, 7.5, and 10 t ha^{-1}), Elangovan and Sekaran (2014) examined the effects of biochar application on cotton growth, yield, and soil fertility status in clay soils of a Tamil Nādu agricultural college farm and found that the EC increased with increased rates of biochar application. Biochar application at 10 t ha^{-1} resulted in the highest EC (0.67 dSm^{-1}) than the control (0.32 dS m^{-1}). While Chathurika et al. (2016) studied the effects of biochar on sandy clay loam soil, they also conducted a two-season field experiment. EC of the soil rose from 136.5 S cm^{-1} to 150 eV s^{-1} after the addition of sawdust biochar, according to the researchers' findings. According to Conz et al. (2017), soil EC increased from 1.13 dS m^{-1} to 1.35 dS m^{-1} when sugarcane straw biochar was incubated in clay textured soils.

2.4.2.3 Oxidisable Organic Carbon

Many soils have lost significant amounts of carbon since they were cultivated for crop production; as a result, these soils may be less capable of supplying nutrients to meet plant demand. Organic carbon contributes to agricultural production by promoting soil health, increasing fertility, reducing erosion, and promoting soil biota (Han et al., 2016). Abrishamkesh et al. (2015) observed a substantial increase in the organic carbon value of alkaline soil with increasing rates of biochar application (0.4 percent, 0.8 percent, 1.6 percent, 2.4 percent, and 3.3 percent). With 3.3 percent biochar applied as a control, the maximum organic carbon content (9.95 g kg^{-1}) was obtained (7.30 g kg^{-1}). Additionally, Ndor et al. (2015) conducted a field experiment at the college of agriculture's research farm in Lafia, Nigeria to determine the effect of biochar on soil characteristics and sesame yield. According to the study, applying rice husk and sawdust biochar at a rate of 10 t ha^{-1} considerably enhanced soil organic carbon from 0.31 percent (control) to 0.68 percent and 0.75 percent, respectively.

2.4.2.4 Cation Exchange Capacity (CEC)

The texture of the soil has a considerable effect on the cation CEC. Sandy soils are always deficient in CEC. Numerous studies have been undertaken on the application of biochar to improve CEC in the soil to promote cation nutrient absorption. Masulili et al. (2010) conducted a field experiment in West Kalimantan, Indonesia, on acid sulfate soil. They discovered that applying rice husk biochar at a rate of 10 t ha-1 raised CEC considerably from 6.64 cmol (p+) kg^{-1} (control) to 8.03 cmol (p+) kg^{-1}. Nigussie et al. (2012) showed that applying biochar at a rate of 10 t ha^{-1} raised the CEC of the soil from 27.22 meq 100 gm^{-1} (control) to 33.69 meq 100 gm^{-1} in chromium unpolluted clayed soils and from 26.58 meq 100 gm^{-1} (control) to 34.48 meq 100 gm^{-1} in chromium contaminated clayed soils. Another similar study by Jien and Wang (2013) found that when 5% biochar was applied to acidic ultisol, the CEC increased dramatically from 7.41 to 10.8 cmol (p+) kg^{-1}. Likewise, Kamara et al. (2015) observed an increase in soil CEC from 7.4 cmol (p+) kg^{-1} (control) to 10.2 cmol (p+) kg^{-1} when 15 g rice straw biochar was applied to the soil.

2.4.2.5 Some Soil Nutrients

In various investigations, it has been found that biochar, when used as a soil amendment, can also boost the levels of some soil nutrients. Dume and Ayele (2017), from the incubation study of acid soil, reported that application of rice husk biochar at 15 t ha^{-1} and corn cob biochar at 5 t ha^{-1} significantly increased total nitrogen by 0.50% and 0.51% from the initial value of 0.32%. Also, Hien et al. (2017) through a glasshouse study with clay loam soil observed a slight increase in soil total nitrogen from 0.22% (control) to 0.26% by application of 2% bamboo biochar.

Coffee husk charcoal incorporation at 15 t ha^{-1} in acid clay loam soil results in an increase in accessible phosphorus from 3.64 mg kg^{-1} to 23.21 mg kg^{-1} (Dume & Ayele, 2017). Oladele et al. (2019) found the maximum level of accessible P (114 mg kg^{-1}) at a soil depth of 0–10 cm at 12 t ha^{-1} application of rice rusk biochar, which was 78 percent greater than other treatment combinations and the control. Masulili et al. (2010) found that the amount of potassium accessible in West Kalimantan acidic soil increased from 0.20 to 0.51 milligrams per kilogram of soil after the addition of 10 tons of rice husk biochar per ha.

2.4.3 Soil biological properties

Biochar amendments have been shown to boost microbial biomass in several investigations. Biochar application affects soil microbial biomass and microbial activity (Steinbeiss et al., 2009). Soil microbial biomass is influenced by the type of feedstock, the type of biochar used, and the rate at which it is applied (Cao et al., 2017; Muhammad et al., 2014). Water hyacinth (Eichornia crassipes) biochar was researched by Masto et al. (2013) for its effect on soil biological activity. Echornia biochar increased DHA and catalase (CAT) activity significantly in the research. At the 20 g kg-1 Echornia biochar treatment dose, they also discovered a rise in acid phosphatase enzyme activity (+32%), alkaline phosphatase enzyme activity (+22.8%), and fluorescein hydrolase activity (50%) Similarly, biochar at 65 Mg ha^{-1} plus compost at 50 Mg ha^{-1} increased phosphatase activity in clay textured soils from 250 nanomoles g^{-1} (control) to 1000 nanomoles g^{-1} (treatment) (Trupiano et al., 2017).

The application of biochar at 6 t ha^{-1} + compost at 15 t ha^{-1} to watermelon crop in loamy textured soils have shown a significant increase in bacteria population from 1.23 x 10^{6} (CFE g^{-1} soil), fungi population from 5.36 x 10^{6} to 7.38 x 10^{6} (CFE g^{-1} soil), actinomycetes population from 1.65 x 10^{6} to 4.81 x 10^{6} (CFE g^{-1} soil) and alkaline phosphatase activity from 47.85 μ g^{-1} hr^{-1} to 123.06 μ g^{-1} hr^{-1} over the control plots (Cao et al., 2017).

Similarly, Devika et al. (2018) reported on the use of biochar at 5 t ha^{-1} increase bacteria population from 26.31 x 10^6 (CFE g^{-1} soil) to 31.66 x 10^6 (CFE g^{-1} soil) and actinomycetes from 3.90 x 10^6 (CFE g^{-1} soil) to 7.32 x 10^6 (CFE g^{-1} soil). They also noticed a significant increase in soil urease from 86 to 156 µg NH_4^+ g^{-1} 2 n hrs^{-1}, alkaline phosphatase from 33.31 to 63.56 µg PNP g^{-1} hr^{-1} and dehydrogenase from 126 to 185 µg TPF g^{-1} hr^{-1} by application of biochar at5 t ha^{-1} + 75% RDF + Azophos to sweet corn crop in clay loam soil.

2.5 Effect of Biochar on Nutrient Leaching

Biochar's accessible nutrients and minerals, as well as its unique nutrient retention capabilities, boost soil fertility and nutrient availability to crops. Glaser et al. (2002) stated that amending soil with biochar helps retain nutrients, improves the amount of accessible P and N to plants, and so increases plant growth and yield. According to Lehmann et al. (2003), adding biochar into the soil increases the ratio of absorption to leaching for all nutrients. Similarly, Lehmann and Rondon (2006) observed an increase in plant uptake of P, K, Ca, Zn, and Cu when a high rate of biochar was applied in a tropical environment. B. P. Singh et al. (2010) evaluated the effect of four different types of biochar on two contrasting soil types (alfisol and vertisol) and found that the application of poultry manure biochar reduced NH_4^+N by 87 percent in alfisol and 94 percent in vertisol. Similarly, Laird et al. (2010) evaluated the effect of biochar on nitrogen leaching from agricultural soils in the Midwest. In this investigation, biochar was applied at various rates (0, 5, 10, and 20 g kg-1 soil). According to the laboratory data, the addition of biochar significantly reduced nutrient leaching. The overall amount of nitrogen, phosphorus, magnesium, and silicon leached from the manure-amended columns decreased dramatically as biochar rates increased. It is stated that treatments with 20 g kg 1 biochar reduced total nitrogen and total dissolved phosphorus leaching by 11% and 69%, respectively.

Nitrogen loss and retention in biochar amended soils fertilized with NH_4^+–N and NO_3^-–N were studied by Zheng et al. (2013) using leaching and pot experiments. The leaching of NO_3–N from soils fertilized with NH_4^+–N and NO_3–N was dramatically reduced with the addition of biochar. However, biochar considerably reduced NH_4^+–N leaching from the NO_3–N fertilized soil, whereas no effect was detected for the NH_4^+–

N fertilized soil. The effect of rice husk biochar on nitrogen leaching and retention in riparian soils was investigated by Bu et al. (2017). Increased rate of biochar application decreased cumulative levels of leachate NH_4^+-N by 28 to 63%, NO_3-N by 23% to 84%, and dissolved nitrogen by 15% to 46%, while increasing leaching of PO_4^3-P by 43% to 108%.

2.6 Effect of Different Particle Size of Biochar

Several factors influence the effect of biochar properties on soil properties including the type of feedstock to produce biochar, pyrolysis temperature, and time duration, the particle size of biochar affects the influence of soil and biochar interaction. Biochar's potential to improve the attributes of light-textured soils is strongly dependent on its features, notably its particle size (Alghamdi et al., 2020). In a short-term incubation investigation, Jaafar et al. (2015) investigated the potential interactions between biochar’s from diverse sources and with varying particle sizes in terms of soil microbial characteristics. Three particle size fractions (sieved) were used: 0.5–1.0, 1.0–2.0, and 2.0–4.0 mm. On biochar surfaces and in larger charcoal pores, hyphal colonization and transient changes in soil microbial biomass were detected.

According to He et al. (2018), the longitudinal and transverse variability of biochar, as well as the dominating cleavage during the preparation process, may account for the large changes in characteristics caused by particle sizes. A variation in the qualities of biochar particles smaller than 5 μm confirmed the existence of a distinct property of superfine powder, which demonstrated a significant difference in physicochemical properties when compared to other particle sizes. Additionally, the continuous particle size range of 75–150 μm has been identified as a turning point. The features of various biochar particle sizes identified in this study may assist in selecting the most appropriate particle size for a particular environmental application.

Similarly, Verheijen et al. (2019) mixed large (2-4 mm) and fine (0.05–1.00 mm) particle size mixed woody biochar in sandy and sandy loam soil to determine the influence of particle size on bulk density and water retention capacity in a laboratory column. They said that small biochar particles lowered bulk density in sandy soils more than in sandy loam soils, but big biochar particles reduced bulk density in sandy loam soils more than in sandy soils. Further small particles at 20% volumetric concentration increased water holding capacity by 60% in sandy loam soil.

Likewise, de Jesus Duarte et al. (2019) investigated the effect of biochar particle size on the soil's quality attributes. The results established that the particle size of biochar has a critical role in water retention, water availability, pore size distribution, and carbon sequestration. Biochar has been shown to significantly improve soil water retention in the finest fraction of loamy and sandy soils. The smaller particle size of 0.15 mm resulted in an increase in water retention in both soil types, but mainly in the loamy soil. Bulk density reduced marginally, particularly in loamy soils with biochar > 2 mm and in sandy soils with 0.15–2 mm biochar. The addition of biochar enhanced the porosity of both soils by 0.15–2 mm. The total carbon content increased primarily in sandy soils when compared to the control treatment; the highest carbon content was obtained with biochar particle sizes of 0.15–2 mm in loamy soil and 0.15 mm in sandy soil, while the TN content and C: N ratio increased slightly in both soils with reduced biochar particle size.

2.7 Effect of Biochar on Crop Growth and Biomass

Biochar application shows promising results in crop growth and yield. Numerous studies have discovered significant increases in crop growth and yield when a variety of soil biochar combinations are used (Filiberto & Gaunt, 2013; Jeffery, Abalos, et al., 2015). Jiang et al. (2020) assert that with the proper mix of biochar and nutrients and proper application, even higher yields. Kraska et al. (2016) reported that the addition of biochar improved the grain production of winter rye, which they attributed to the nutrient administration via biochar.

Rondon et al. (2007) showed a 46 percent increase in bean yield and a 34% increase in biomass output when 60 g kg^{-1} biochar was applied. Similarly, Chan et al. (2008) observed a 96 percent yield increase in radish after applying chicken litter biochar (up to 50 t/ha) in an Alfisol. Arif et al. (2012) investigated the influence of biochar on maize crop yield. The field experiment was done at the Agricultural University Peshawar's New Developmental Farm. They observed that plots treated with biochar at a rate of 30 t ha^{-1} had a higher grain yield of 4194 kg ha^{-1} than control plots (2042 kg ha^{-1}). Cornelissen et al. (2013) discovered that applying maize cob biochar and wood biochar at a rate of 4 t ha^{-1} enhanced maize grain production significantly from 0.9 t ha^{-1} (control) to 3.8 t ha^{-1} and 3 t ha^{-1}, respectively. These yields were 444 percent and 352 percent, respectively, of control yields. Carter et al. (2013) reported that biochar treatments increased end biomass, root biomass, plant height, and leaf number in lettuce and cabbage across all cropping cycles when compared to no biochar treatments. Genesio et al. (2015) conducted a four-year field experiment in Tuscany, Italy to determine the effect of biochar application on vine yield and grape quality. They discovered that treated plots produced up to 66% more than control plots, while no significant difference in grape quality parameters was observed. According to Abrishamkesh et al. (2015), rice husk biochar application influenced both the growth and yield of lentils. There was an increase in the biomass of lentils in the soil. Soils amended with 3.3% biochar had the highest below-ground dry biomass, while soils not altered with biochar had the lowest. Paddy crop factors such as panicle length, the number of tillers, and grain production were studied by Singh et al. (2018). RHB and commercialized bio-formulation treated plots were shown to have higher agronomic parameters compared to untreated (control) plots. In RHB + CSR-BIO treated plots, the percentage increase in panicle length, tiller number, rice grain, and paddy straw yields was 50.96, 80.91, 121.01, and 66.71 percent, respectively, over the control pl.

CHAPTER III

MATERIALS AND METHODS

3.1 Study Location

This study was carried out at the training field of the Faculty of Agriculture, Natural Resources, and Environment, Naresuan University, Thailand (Latitude: 16.4406, Longitude: 100.1136) (Figure 3). The experiment was done under greenhouse conditions between September to November. The weather condition in the greenhouse during vegetable growth was depicted in Figure 4. The average maximum temperature was 36 ^{o}C with an average minimum of 26 ^{o}C. Relative humidity was ranging from 60 – 95%.

Figure 3 Experimental Location Map

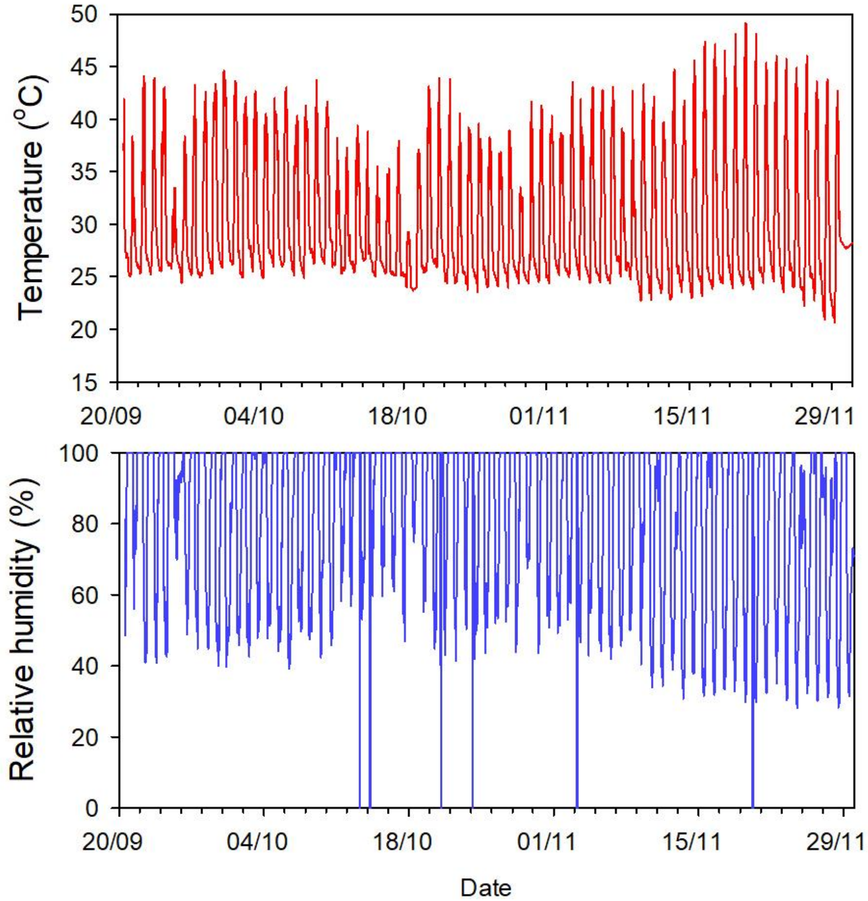

Figure 4 Weather Condition in the Greenhouse during Vegetable Growth

3.2 Biochar Production and Particle Size

The present study locally available rice husk was utilized to produce rice husk biochar. Rice husk collected from a nearby rice mill was dried before the preparation of biochar. Following drying, the rice husk was carbonized by the pyrolysis process in a low-cost pyrolysis production unit made in the Renewable Resources School of Naresuan University for the preparation of rice husk biochar. A metal sheet was used to make a biochar lid of 50 cm x 56 cm (height x diameter). The schematic layout of a biochar kiln is depicted in Figure 5. The kiln consisted of three parts: i) an inner chamber, ii) an exterior chamber, iii) a lid. The inner chamber, which has a diameter of 20 cm, was created to generate heat to pyrolyze the biomass put in the outer chamber. The interior chamber was constructed from a thicker metal sheet (10 mm) that was firmly fastened. The anterior chamber was filled with fuel supplies used for lighting purposes. The biochar kiln was sealed by a lid. The rice husk was placed between the space between and outer chamber. The heat was generated in the inner chamber and the lid was tightly closed. After 30 to 40 min the fuel material was burnt hotter; After 60 minutes, the outside chamber began to burn. It began to emit gases at that point, and the flame became blue with minimal smoke. This meant that the gasoline had been completely used. Simultaneously, thermocouple probes were set up inside the kiln in radial and longitudinal positions. The temperature recorded ranges from 250 to 550 °C (Figure 6). The RHB was produced by burning the firewood at the bottom of the biochar kiln to trigger the pyrolysis process for 4 hours. Rice husk biochar was further grounded and sieved into three sizes: < 0.25 mm (fine), 0.25 – 1 mm (medium), and 1 – 2 mm (large) biochar.

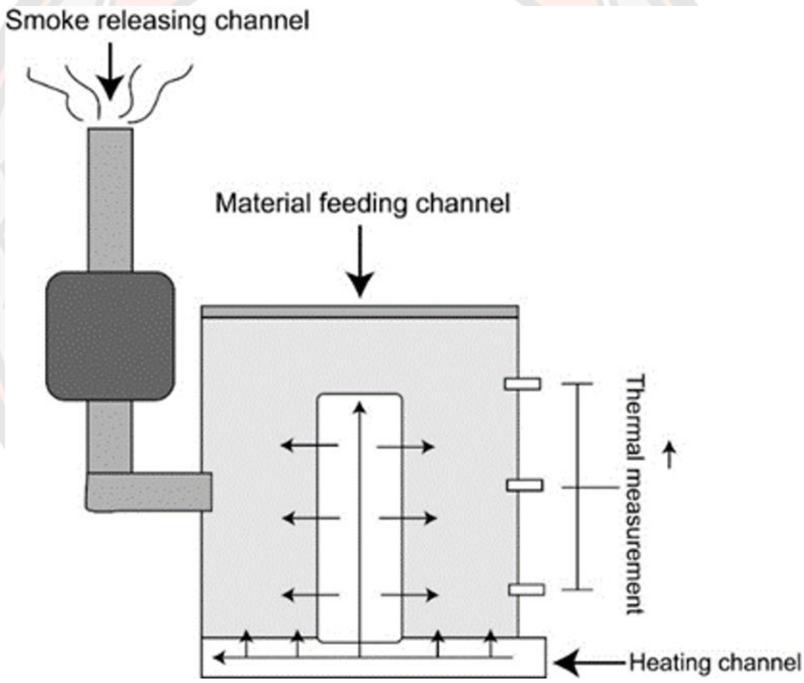

Figure 5 Schematic Layout of Traditional Kiln Reactor

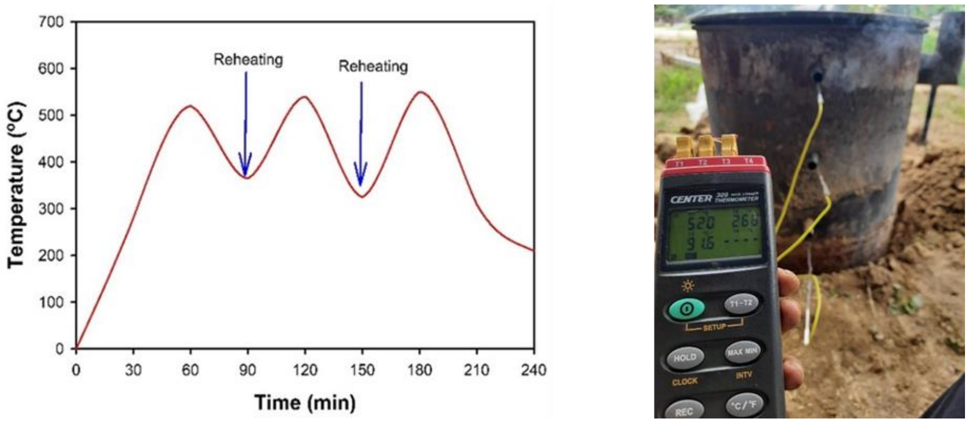

Figure 6 Temperature Profile of Rice Husk Biochar Production

Figure 7 Rice Husk Biochar Production

3.3 Characterization of Rice Husk Biochar

3.3.1. Rice Husk Biochar's Morphological Properties

To analyses, the structure of the biochar particles, a small number of samples was positioned in the sample holder using copper conductive tape. After that, the surface morphology of the rice hush biochar samples was investigated by using a High-Resolution FE-SEM (Thermo Scientific Apreo S, USA). The microscope was equipped with an

Energy Dispersive X-ray detector that was used to explore the elemental composition of biochar.

3.3.2. Rice Husk Biochar's Chemical Characteristics

Chemical characteristics of rice husk biochar were determined. The pH and EC of biochar were determined in a weight ratio of 1:20 with deionized water (biochar: water) (Rajkovich et al., 2012). Moreover, primary nutrients including total nitrogen, available phosphorus, exchangeable potassium were determined through the following methods illustrated in Table 1.

Table 1 Rice Husk Biochar's Chemical Characteristics

Parameters	Methods
pH	pH meter (1: 5 biochar: water) (Thomas, 1996)
EC	EC meter (1:20 biochar: water) (Rhoades, 1996)
Organic Carbon	Walkley and Black method (Díaz-Zorita, 1999)
Total Nitrogen	Kjeldahl digestion and Distallation Jackson (1973)
Available Phosphorus	Diacid digestion and Vanadomolybdate reagent method Jackson (1973)
Exchangeable Potassium	Ammonium acetate (Pratt, 1965)

3.4 Soil Sample Used in the Experiment

3.4.1 Soil Sample Used in the Experiment and Its Characterization

The soil for this experiment was gathered from Mueang Phitsanulok District, Phitsanulok province, Thailand, at a depth of 0-20 cm. Before being used in the experiment, soil samples were air-dried, homogenized, and sieved (2 mm). The soil was classified as a Coarse-loamy, mixed, isohperthermic Typic Paleustults (Phichai soil series; Pch) according to Soil Survey Staff, 2014.

The soil's physical and chemical qualities were determined. The dry weight of soil per unit volume (g cm^{-3}) was used to calculate the bulk density (BD) using the soil core method (Blake, 1965) .Soil electric conductivity (EC) was determined by EC meter, the proportion of soil per water as 1:5 (Rhoades, 1996). By using a pH meter, the pH of the soil was measured electrometrically in a 1:1 (soil: H_2O) ratio (Peech, 1965). The hydrometer technique was used to determine the soil texture (Gee & Or, 2002). Water holding capacity (WHC) of the soil will be estimated by droplet counting method (Brischke & Wegener, 2019). Walkley and Black method was used to determine the amount of organic matter (OM) (Díaz-Zorita, 1999). The Kjeldahl technique was used to calculate total nitrogen (Page et al., 1982). The Bray II extraction technique was used to assess the amount of accessible phosphorous (P) (Bray & Kurtz, 1945) . The exchangeable potassium was analyzed with ammonium acetate (NH_4OA_c) and then measured by atomic absorption spectrophotometry. The soil property is presented in Table 2.

Table 2 The Physical and Chemical Parameter of Soil

Soil Physical Properties	
Parameters	**Values**
Sand (%)	71.1
Silt (%)	23.7
Clay (%)	4.6
Textural Class	Sandy Loam
Bulk Density (g/cm^3)	1.67
Water Holding Capacity (%)	16.64
Soil Chemical Properties	
Parameters	**Values**
pH	5.7
EC (µS/cm)	29.2
Organic Carbon	0.7
[1/]N_{tot} (%)	0.14
[2/]P_{ava} (mg/kg)	8.49
[3/]K_{exc} (mg/kg)	99

[1/]Total Nitrogen; [2/] Available Phosphorus; [3/] Exchangeable Potassium

3.5 Experimental Design and Preparation of Soil Columns

The experiment is a completely randomized design (CRD), with a 4x2 factorial experimental arrangement consisting of:

Factor 1: three rice husk biochar particle sizes (0.25 mm, 0.25-1 mm, and 1-2 mm) plus no biochar

Factor 2: with and without chemical fertilizer (NPK fertilizer)

The treatments were: 1) soil with chemical fertilizer, 2) soil without chemical fertilizer, 3) soil+ rice husk biochar at <0.25 mm particle size with chemical fertilizer, 4) soil+ rice husk biochar at <0.25 mm particle size without chemical fertilizer, 5) soil+ rice husk biochar at 0.25-1 mm particle size with chemical fertilizer, 6) soil+ rice husk biochar at 0.25-1 mm particle size without chemical fertilizer, 7) soil+ rice husk biochar at 1-2 mm particle size with chemical fertilizer and 8) soil+ rice husk biochar at 1-2 mm particle size without chemical fertilizer.

The application rate of biochar applied at 2% w/w with 4 kg of sandy loam soil were used in each column. Fifteen replicates of each treatment were set up to allow for destructive testing 4-time periods (0, 8, 18, and 29 days after transplanting (DAT) during the trial. (The total of the PVC columns was 96 columns).

We developed soil plastic columns with a height of 20 cm and an external diameter of 17 cm. Each column is equipped with a plastic end cap with a 3 mm drain hole and a connected tube for collecting water that drains from the columns' bottoms. At the bottom of each column, a little amount of coarse sand (5 mm) was deposited. Depending on the treatment, soil and biochar mixes were put in PVC columns. Tap water was used to irrigate the soil columns. Depending on the treatment, soil and biochar mixture were put in PVC columns (Figure 7).

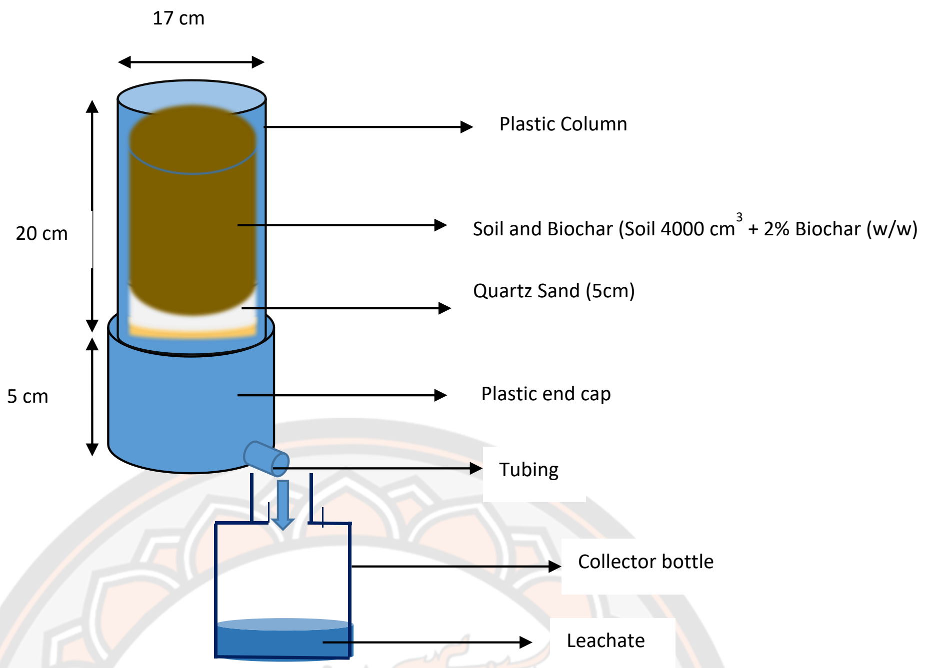

Figure 7 Schematic of the Soil Column Constructed for the Leaching Experiment

All columns were wetted with water to 40% of their water holding capacity (WHC) and pre-incubated for two weeks. Following pre-incubation, all columns of each treatment were wetted with extra water to 60% of their WHC, and only fertilized treatments were fertilized with the same quantity of water containing dissolved fertilizer (5 g of fertilizer) (Kuo et al., 2020). Figure 8 depicts the overview of the experiment setup.

Soil samples in the column, leaching samples, and plant samples were destructively collected in each treatment from the PVC columns (n=3) at time increments of 0, 8, 18, 29 DAT.

Figure 8 Overview for the Experiment Setup

3.6 Incubation of Soil Columns and Analysis of Nutrient Leaching

3.6.1 Soil Column Incubation Analysis

Soil samples in the column were destructively collected in each treatment from the PVC Columns (n=3) at time increments of 0, 8, 18, and 29 DAT and harvesting. Before further chemical analysis, the soil from each column was collected, air-dried, and powdered to pass through a 2-mm screen. Wet oxidation was used to test soil samples in the column for OM (Nelson & Sommers, 1996). Soil pH and electrical conductivity (EC) were measured in 1:1 and 1:5 soil-to-water solutions. The Bray II extraction procedure was used to assess the available phosphorous (P). The exchangeable potassium (K) was measured using atomic absorption spectrophotometry after being examined with ammonium acetate (NH_4OAc).

3.6.2. Nutrient Leachate Analysis

To investigate nutrient leaching via the soil column, a total of 700 mL of water was used for each flushing process. Leachate was collected after each irrigation event and combined into samples at 0, 8, 18, 29 DAT and harvesting that were stored in -20 ^{o}C in a freezer until analysis (Figure 9). Leachates were subjected to chemical analyses. Finally, leachate samples were analyzed for total nitrogen.

Figure 9 Collection of Leachates from the Soil Column

3.7 Plant Growth, Nutrient in the Plant, and Yield

The *Brassica alboglabra* were grown from transplants after three weeks of sowing. Only one plant was grown in the soil column. Height, fresh and dry weight of plants, number of leaves, the nutrient analysis was measured at 8, 18, and 29 DAT. The total N of the plant sample was analyzed by the Kjeldahl method. Plant samples were digested with a mixture of sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids to evaluate total N, P, and K contents. Colorimetrically, the chlorostannous phosphomolybdic acid method established by Jackson (1973) was used to determine the digests' P concentration. The K content was analyzed by Atomic Absorption Spectrophotometry.

Nutrient uptake was calculated for each nutrient by multiplying the plant dry weight by the nutrient concentration as shown in Eq 1. The nutrient use efficiency (NUE) in plants was calculated in terms of the nutrient efficiency ratio (NER) as presented in Eq. 2 (Baligar et al., 2001).

(1) N, P and K uptake (mg/plot) =

$$\frac{\text{N, P and K concentrations (mg/kg) in plant part x dry biomass (mg/plot)}}{1000}$$

(2) Nutrient efficiency ratio (NER) =

$$\frac{\text{Units of yield (g)}}{\text{Unit of nutrients in tissue (g)}}$$

3.8 Microbial Communities- Biolog EcoPlate^{TM}

The soil microbial population was identified using the Biolog EcoPlateTM (Biolog Inc., Hayward, CA, USA) (Figure 10) with the aid of a microplate reader (BioTek Synergy H1 Hybrid Reader). Substrates are classified into six biochemical classes which are Amines, Carbohydrates, Complex carbon sources, Carboxylic acids, Amino acids and Phosphate Carbon (Figure 11). A soil sample was taken after 29 DAT. Five grams of dry soil weight were suspended in 45 mL of 10 mM phosphate buffer (pH 7) and agitated for 30 minutes at 25°C, 200 rpm (Sigler & Zeyer, 2004). The soil suspension was then applied in a volume of 100 μl to each microplate well and incubated at 25 C for 144 hours. The intensity of color development was determined spectrophotometrically at = 590 nm (Insam & Goberna, 2004) at 0 h and 72 h intervals using a microplate reader. Thirty-one substrates were produced according to Insam and Goberna (2004) classification of substrates into six biochemical classes. Since the Biolog EcoPlateTM contains 31 carbon sources and control wells (blank) in three replicates, all measurements were done three times, one–one series from each of the three copies of the experimental setup. As shown in Table 3.3, the average metabolic response (AMR), richness, Shannon diversity (H'), and evenness (E) indices were calculated using the collected data.

Table 3 Formula Used to Calculate Indices Based on Biology Microplates Data

Index	Formula and Description	Source
Average metabolic response (AMR)	$AMR = \frac{\Sigma(O.D.well - O.D.neg)}{31}$	
Shannon-Wiener diversity (H')	H' $= -\sum_{i=1}^{N} pi(lnpi)$ Pi: proportional color development of the well over the total color development of all wells (96) of a plate N: number of substrates on an EcoPlate™ (n = 31)	(Hill et al., 2003)
Evenness (E)	E = H'/lnR R: substrate utilization richness (the number of wells with color development)	(Zak et al., 1994)
Margalef index (Dmg)	$Dmg = \frac{n-1}{ln(N)}$ Where: n = number of individuals of each species N = total number of individuals of all species	(Schleuter et al., 2010)

Figure 10 Physiological Profiling of Microbial Communities Using Biolog Ecoplate

Biolog EcoPlate	1	2	3	4
A	Water	β-methyl-D-glucoside	D-galactonic acid γ-lactone	L-arginine
B	Pyruvic acid méthyl ester	D-xylose	D-galacturonic acid	L-asparagine
C	Tween 40	i-erythritol	2-hydroxy benzoic acid	l-phenylalanine
D	Tween 80	D-mannitol	4-hydroxy benzoic acid	L-serine
E	α-cyclodextrin	N-acetyl-D-glucosamine	γ-hydroxy butyric acid	L-threonine
F	Glycogen	D-glucosamic acid	Itaconic acid	Glycyl-L-glutamic acid
G	D-cellobiose	Glucose-1-phosphate	α-ketobutyric acid	Phenylethyl-amine
H	α-D-lactose	D,l-α-glycerol phosphate	D-malic Acid	Putrescine

Amines
Carbohydrates
Complex carbon sources
Carboxylic acids
Amino acids
Phosphate-carbon

Figure 11 Carbon Sources in Biolog Ecoplate Wells. Codification Matrix and Groups of Carbon Source Isolated

Source: Chazarenc et al., 2010

3.9 Statistical Analysis

The statistical investigation of the influence of different particle sizes of rice husk biochar on soil characteristics, nutrient leaching and use efficiency, and crop productivity in sandy loam was carried out in a factorial arrangement with three replications in a randomized design. The mean separation analysis was performed on triplicate data of chosen soil parameters, the quantity of leachate nutrient, and crop development and yield were analyzed by factorial analysis of variance (ANOVA) to test for the interactive and main effect of different sizes of rice husk biochar and fertilizer at a significance level of $p < 0.05$, least-squares means were used to determine whether there were significant differences between treatments. Results were analyzed by using R and statistics 10 software.

CHAPTER IV

RESULTS AND DISCUSSION

The current study, dubbed "Effect different particle sizes of rice husk biochar affect soil properties, nutrient leaching, and crop productivity in sandy loam soil" involved a soil column experiment which was carried out at Naresuan University training farm. The results obtained from the experimental and laboratory analysis are presented, tabulated, analyzed, interpreted, and discussed below with relevant research references and evidence under the following heads.

4.1 Characteristics of Rice Husk Biochar

A representative biochar sample was made using rice husk that was readily available in the area. The rice husk biochar was ground to pass through a 2 mm sieve and analyzed for different parameters and results are presented in Table 4. The results showed that the traditional kiln method was able to produce RHB of about 40% of the total fresh weight. The pH of biochar was noticed to be 7.9 with an EC of 113.2 µS/cm. The concentration of organic carbon, total nitrogen was found at 13.33 and 0.75 %, whereas available phosphorus and exchangeable potassium were 73.13 and 536.6 mg/kg, respectively. Comparing the biochar with sandy loamy soil, the biochar is alkaline with high organic matter and carbon content. This difference can contribute to increasing the soil properties in sandy loam soils. The physical, chemical, morphological and spectral properties of biochar are influenced by the type of feedstocks, pyrolysis time, and temperature (Peng et al., 2011; Zhao et al., 2013). Probably biochar had a higher content of basic cation, carbonates, ash, cation anions capacity which might have contributed to alkalinity and high EC. An alkaline pH of biochar was found to be much higher than the soil pH in this study. The pH and EC of the biochar were lower than the values reported previously for the rice husk biochar (Ghorbani et al., 2019; Masulili et al., 2010) but similar to Abrishamkesh et al. (2015). The overall yield of 40 % was also reported by (Abrishamkesh et al., 2015; Gamage et al., 2016). Total Organic Carbon, Nitrogen, phosphorus, potassium was comparable to the value observed for rice husk biochar by Gamage et al. (2016),Pratiwi and Shinogi (2016) and Singh et al. (2018).

Table 4 Properties of Rice Husk Biochar Use for Study

Parameters	Units	Values
OM	%	22.93
OC	%	13.33
Moisture	%	2.88
pH	-	7.9
EC	µS/cm	113.2
N_{tot}	%	0.75
P_{ava}	mg/kg	73.13
K_{exc}	mg/kg	536.6
Yield	%	40

Note: OM = Organic Matter; OC = Organic Carbon; EC = Electrical Conductivity; N_{tot} = Total Nitrogen; P_{ava}= available Phosphorus; K_{exc}= Exchangeable Potassium; Yield of biochar = mass yield of biochar/mass of raw biomass x 100

4.2 Morphology, Elemental Components, and Elemental Distribution Maps of Rice Husk Biochar Through FESEM/EDS

The morphological characteristics of various forms of biochar were identified using FESEM. Furthermore, the use of EDS and FESEM provides useful information on the spectrum and elemental components of surface rice husk biochar analysis. Figure 12. illustrates the fresh rush husk under FESEM/EDS where no pores formation has been observed. The element composition shows comparatively less carbon and other nutrient elements as compared to rice husk biochar. For elemental distribution maps, there is an increase in over 300 % in carbon, 70 % in Nitrogen, 10% in Phosphorus and around 70 % in Potassium which may result from the pyrolysis process for making rice husk biochar.

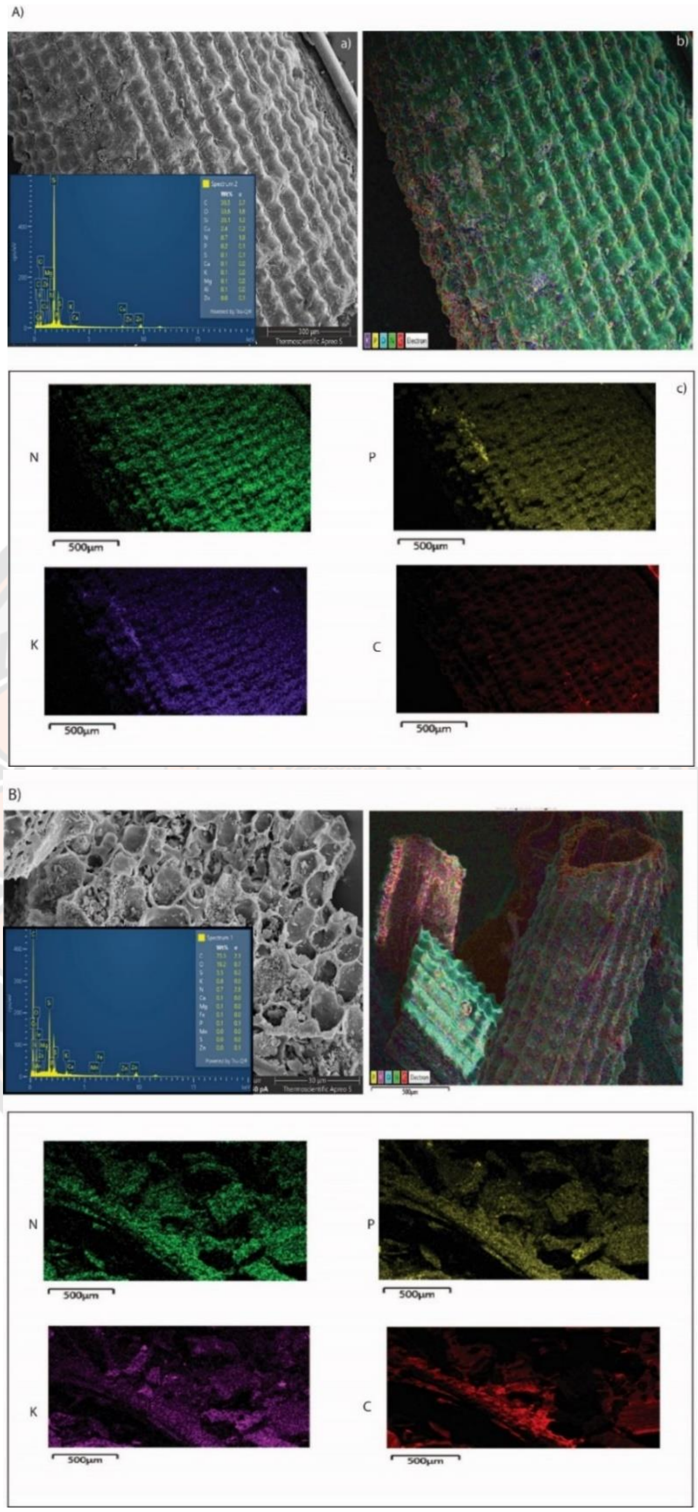

Figure 12 FESEM/EDS Maps and Spectra of (a) Fresh Rice Husk and (b) Rice Husk Biochar

Morphology of RHB showed in Figure 13 at low magnification images (100x) showed a similar structure of smooth lateral surfaces and long channels of RHB. At high magnification (1000x), biochar's pores and channel formations were displayed. FESEM revealed a honeycomb structure of biochar with a high specific surface area due to the abundance of holes concealed beneath the biochar surface. Biochar produce conventionally retains its surface and interior structures perfectly. Pores size and distribution did not vary between different biochar particle sizes. The average pore size was 10.64 µm. Even though this traditional kiln method cannot keep the temperature constant at 500^{o} C, it can produce RHB with many pores and a large quantity of biochar. EDS analysis the results are presented in mass percentage of the samples. EDS of RHB indicates that C (> 60%). and O (~20%) silica (~15%) were the major elements including some amount of mineral such as P, K, Mg, Ca, Fe (Figure 13). In addition to carbon and nitrogen, biochar's include significant amounts of silica, calcium, magnesium, sodium, potassium, and phosphorus. Other elements are found in trace amounts. There is no difference in the elemental and mineral composition of different sizes of biochar.

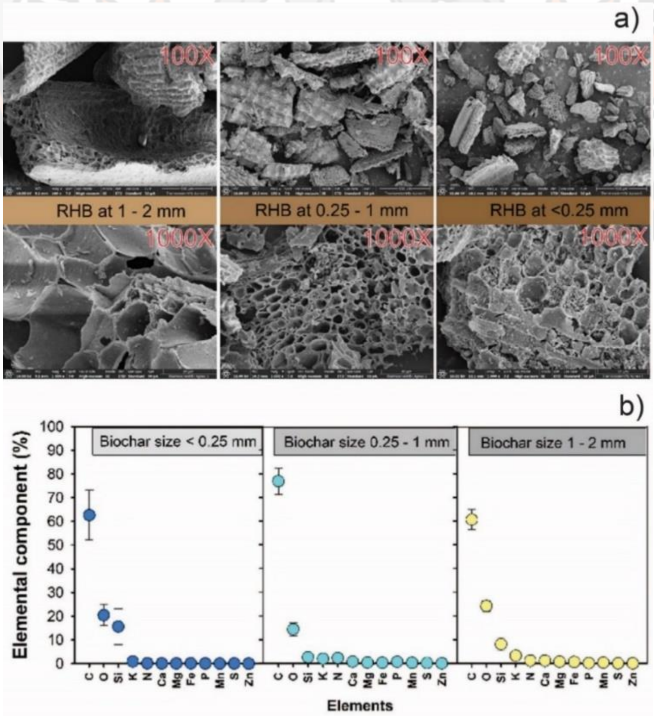

Figure 13 FESEM / EDS of Different Sizes of Rice Husk Biochar

Carbon was found to be the most abundant skeleton component in the RHB, followed by oxygen and silica (Figure 12). Depicts the elemental distribution mapping in rice husk and rice husk biochar using Field emission scanning electron microscope with energy-dispersive X-ray spectroscopy attached facilities (FESEM-EDS), where it shows that rice husk itself contains less carbon and potassium in contrast to rich rice biochar which is highly dominated in carbon and other elements. In regards to our study Claoston et al. (2014) also discovered that when RHB is exposed to a 500°C pyrolysis temperature, its shape changes to a honeycomb-like structure, and numerous pores appear on the RHB surface. RHB pores pyrolyzed at 350°C were not fully developed, whereas the RHB's regular pattern was destroyed at 650°C. This distinct porous structure of biochar act as a sponge to retain water and nutrient which is made available to plants at a later stage. It also creates a favorable environment and acts as a habitat for soil microbial communities (DeLuca et al., 2015; Lehmann & Joseph, 2009). Furthermore, rice husk biochar containing high contains SiO_2, Al_2O_3 and Fe_2O_3 was confirmed and reported by Claoston et al. (2014) and Gamage et al. (2016)

4.3 Influence of Biochar on Soil Properties

4.3.1 Bulk Density

The data obtained on the effect of rice husk biochar on soil bulk density is presented in Table 5 which indicated the significant effect of different sizes of biochar on soil bulk density. The significant effect of biochar was noticed in all stages of sampling time. The bulk density of the control soil increased slightly from 1.70 to 1.72 g cm^{-3} at the end of the experimental period. Biochar, on the other hand, lowered the bulk density of soil and biochar combinations at all biochar particle sizes. The decrease in bulk density of the soil and biochar mixes was greater with larger biochar particles, reaching 7%, 5.81%, and 4% respectively for large, medium, and small sizes, from the bulk density of the control soil. Overall, the treatment with biochar showed an improvement in BD. The highest was noticed in control (1.72 g cm^{-3}) and the lowest bulk density was observed in treatment with the large (1.60 g cm^{-3}), medium (1.62 g cm^{-3}) and small particle sizes (1.65 g cm^{-3}) of biochar at the end of the experiment. It is evident from the data that there is a significant difference in the particle size factor. The addition of fertilizer does not show any significant difference. There is no interaction effect between the two factors. Our results herein indicate that the larger particle size of biochar help to decrease significantly than the finer particle size.

Table 5 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Soil Bulk Density

Factors	Bulk Density (g cm $^{-3}$)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	1.70^{a}	1.73^{a}	1.70^{a}	1.72^{a}
Soil + SB (<0.25mm)	1.64^{ab}	1.63^{bc}	1.63^{b}	1.65^{b}
Soil + MB (0.25-1mm)	1.62^{b}	1.66a^{b}	1.62^{bc}	1.62^{bc}
Soil + LB (1-2mm)	1.60^{b}	1.60^{c}	1.57^{c}	1.60^{c}
Fertilizer				
Without Fertilizer	1.62	1.65	1.61	1.64
With Fertilizer	1.65	1.66	1.65	1.66
F- Test				
Particle size (P)	*	**	**	**
Fertilizer (F)	ns	ns	ns	ns
P * F	ns	ns	ns	ns
CV (%)	3.12	3.31	2.96	1.86

Note: a, b represents significant difference among treatments at p<0.05 according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with p < 0.05, p < 0.01, p < 0.001. and non-significant (p > 0.05), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

A significant reduction of soil bulk density was noticed in soil treated with biochar. Bulk density is a proxy for soil structure, compaction, and aeration, affecting water infiltration, plant roots depth, and nutrient transport (Alghamdi, 2018). Several findings have stated that the application of biochar influences the bulk density of soil (Chen et al., 2011; Omondi et al., 2016; Verheijen et al., 2019). The biochar amended directly leads to a drop in bulk density because of the lower density and high porosity of biochar. According to Lu et al. (2015), a dilution effect caused by the addition of lightweight, low-density material like biochar to soil might partly result in a decrease in soil density. The significant effect in bulk density is consistent with the findings of Omondi et al. (2016) who found that amending soil with biochar can reduce bulk density by 7.6%. Abrishamkesh et al. (2015) also stated a substantial reduction in soil bulk density from 1.39 g cm $^{-3}$ to 1.14 g cm $^{-3}$ through the application of rice husk biochar. Likewise, Basso et al. (2013) evaluated biochar's capacity to increase the water holding capacity of sandy soils, revealing that biochar-treated soils had a bulk density up to 9% lower than control soils. Another study, Toková et al. (2020) found that biochar applied at a rate of 20 t ha^{1} without fertilizer considerably reduced bulk density by 12% and enhanced porosity by 12%. This occurs because the density of the biochar is lower than soil and with high porous nature containing macro and micropores retain air, water, and nutrients, greatly reducing the BD. We found out that large size biochar decreases the soil bulk density much superior compared to other treatments. As we increased the particle size of biochar, bulk density was decreased significantly. The rise in bulk density with decreasing particle sizes of biochar could attribute to an arrangement of biochar particles in the volume of the soil, where finer particle size can occupy the pores in the soil with this configuration, resulting in a reduction in total porosity and an increase in soil bulk density which is not feasible if the biochar particle is largest. Large size biochar has a far lower particle density, assuming the biochar does not have poor mechanical strength, its application can result in a reduction in overall density. Due to the replacement of soil particles with biochar particles, a decreased particle density of biochar reduces the bulk density of soil (Liu et al., 2016). On the other hand, smaller biochar increases soil aggregation and tortuosity, making it more compact. It makes the individual soil particles together, can occupy the inter pores of the soil which is not possible for large size biochar. With this configuration, small biochar is incorporated into the soil pores, resulting in a decrease in total porosity and an increase in bulk density. Similar to our finding, Verheijen et al. (2019) reported that in sandy loam soil large size particles decrease more than small particles of biochar by 5.5 %. The effect of particle sizes of biochar and physical properties of soil were also similarly observed by Alghamdi et al. (2020) and de Jesus Duarte et al. (2019). Thus, this study suggests that soil bulk density characteristics depended on biochar particle size.

4.3.2 Soil pH

The data on the effect of different particle sizes of biochar application and recommended dose of fertilizer on soil pH are presented in Table 6. A perusal of the data concerning soil pH at the end of the experiment reveals that there was a significant increasing trend in pH at biochar amended soil compared to the control. Nonetheless, no significant variations in pH values were observed between large, medium, and small biochar-soil mixtures, even though all biochar-amended substrates had pH values greater than the control. The pH of the soil (control) was 5.5 and was noticed to increase by 0.6 units in biochar amendment treatments. In general, the application of biochar without fertilizer results in higher pH of the soil over the unamended soil. On the other hand, the effect was significant at the whole phase of the growth period with the application of fertilizer. It is also evident from the result that the decrease in the pH was significant in the treatment with the application of fertilizer with biochar. The average value of pH with biochar with fertilizer is 5.5 whereas for non-fertilized treatment is 6.3 at the end of the experiment. There is no interaction effect between the two factors.

Table 6 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Soil pH

Factors	pH			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	5.7	5.7	5.5	5.5[b]
Soil + SB (<0.25mm)	5.9	5.8	5.7	6.0[a]
Soil + MB (0.25-1mm)	5.8	5.8	5.7	6.0[a]
Soil + LB (1-2mm)	5.8	5.8	5.7	6.1[a]
Fertilizer				
Without Fertilizer	6.23[a]	6.2[a]	6.1[a]	6.3[a]
With Fertilizer	5.45[b]	5.4[b]	5.2[b]	5.5[b]
F- Test				
Particle size (P)	ns	ns	ns	**
Fertilizer (F)	***	***	***	***

Factors	pH			
	Transplanting	8 DAT	18 DAT	Harvesting
P * F	ns	*	ns	ns
CV (%)	4.49	3.45	4.63	4.06

Note: a, b represents significant difference among treatments at p<0.05 according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

The pH of the soil determines acidic and alkalinity which influences the movement and availability of various nutrients and chemical components. The significant effect of biochar on soil pH is mainly due to the alkaline nature of biochar and high exchangeable bases, upon application to soil caused a rise in soil pH. Biochar is well-known for its high liming equivalency, which helps acidic, base-deficient soils raise their ph. Additionally, Khanna et al. (1994) noted that ashes might be used to neutralize acidic soil. Another possible reason for soil pH increases is the huge surface area and porous nature of biochar, which increases the cation exchange capacity of the soil. de Sousa Lima et al. (2018) discovered that biochar treatments had a significant effect on soil pH, causing a linear increase in soil pH from 5.5 to 7.0. Other studies corroborate the conclusions of this study, Carter et al. (2013) conducted a pot culture experiment in sandy loam soil to determine the effect of biochar treatment on soil characteristics and the development of lettuce and cabbage crops. The study discovered that applying 50g kg^{-1} biochar improved the pH of the soil by 0.6, from 5.5 to 6.1, and that applying 150 g kg^{-1} biochar increased the pH of the soil by 1.2, from 5.5 to 6.7. Rice husk biochar has been demonstrated to enhance the pH of soils. As predicted, the increase in pH caused by biochar was less when biochar was combined with fertilizer since biochar and fertilizer have opposing effects on soil pH. Similar findings were found on acidic soils (Van Zwieten et al., 2010), where the pH rise was larger when biochar was applied without fertilizer than when fertilizer was applied. Our results were in close proximity with Liao and Thomas (2019). They noticed that there was no significant difference among particle size (small, medium, large) ($p > 0.05$), though all biochar showed higher pH values than did the control.

This conclusion is consistent with previous research that established alkalinity as a critical factor affecting biochar's liming capacity.

4.3.3 Electrical Conductivity (EC)

The data on the effect of different particle sizes of biochar application and recommended dose of fertilizers on soil EC during the experiment are presented in Table 7. There was no significant effect but a slight increase in soil EC with the biochar at all sampling stages. The interaction effect of particle size of biochar and fertilizer had statistically no significant effect on soil EC. The application of biochar alone did not show significant changes ($p > 0.05$) in EC relative to the control treatment in the soil. The data indicate that applying fertilizer in conjunction with biochar considerably raised the soil EC over no application of fertilizer. The average EC value of treatments with fertilizer at different sampling stages was 618.3 µS/cm, 449.77[a] µS/cm, 419.75 µS/cm 362.21 µS/cm which was decreasing overtime to at the end of the experiment. Whereas for no application of fertilizer treatments the average values of treatments were 53.29 µS/cm, 96.02 µS/cm, 102.47 µS/cm, and 125.09 µS/cm at different sampling stages.

Table 7 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on soil EC

Factors	EC (µS/cm)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	374.93	282.14	235.92	236.58
Soil + SB (<0.25mm)	283.39	267.44	235.92	230.70
Soil + MB (0.25-1mm)	366.79	267.44	300.50	272.12
Soil + LB (1-2mm)	317.55	274.04	239.82	235.20
Fertilizer				
Without Fertilizer	53.29[b]	96.02[b]	102.47[b]	125.09[b]
With Fertilizer	618.03[a]	449.77[a]	419.75[a]	362.21[a]
F- Test				

Factors	EC (µS/cm)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle size (P)	ns	ns	ns	ns
Fertilizer (F)	***	***	***	***
P * F	ns	ns	ns	ns
CV (%)	28.06	29.64	36.45	34.33

Note: a, b represents significant difference among treatments at $p < 0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

Electrical conductivity is a measure of soluble salt concentration in soil. A higher amount of salt in the soil restricts nutrient uptake and thus affects plant growth. The changes in EC due to the application of biochar were not significant. In the literature, some research observed a considerable rise in soil EC with biochar application (Elangovan & Sekaran, 2014; Masulili et al., 2010), while others found no significant increase in soil EC with biochar application (Jien & Wang, 2013; Singh et al., 2018). The increase in EC following biochar application may be attributed to ash containing a high concentration of carbonates of alkali and alkaline earth metals (B. Singh et al., 2010). In our study, biochar had a minimal and insignificant effect on soil EC. This could be related to the displacement of exchangeable acidity, the high buffering capacity, or the conditions under which biochar is produced. (Dume et al., 2015; Wang et al., 2012). Moreover, flushing of the column at every sampling stage could result in the leaching of nutrients and minerals might have affected. On contrary, as expected, fertilizer enhanced the EC in soil due to the addition of cations and anions.

4.3.4 Soil Total Organic Carbon

The data on the effect of different particle sizes of biochar application and recommended dose of fertilizer on total organic carbon are presented in Table 8. It is evident from the results that the application of fertilizer had no statistically significant effects on soil organic carbon. However, the application of biochar was found to significantly increase the organic carbon over no application of biochar. The organic carbon of control was recorded to be 0.73%, whereas for small, medium, and large was 1.40%, 1.30%, 1.40%, respectively. It was noticed a 91.7% increase in soil organic carbon after the application of biochar over the unamended soil. Nonetheless, no significant variations in total organic carbon values were observed between large, medium, and small biochar-soil mixtures, even though all biochar-amended substrates had organic values greater than the control. There was no significant ($p > 0.05$) interactive effect of different sizes of biochar and fertilizer on soil total organic carbon during the entire growth phase.

Table 8 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Soil Total Carbon

Factors	Total Organic Carbon (%)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	0.70	0.83[b]	0.74[b]	0.73[b]
Soil + SB (<0.25mm)	1.01	1.42[a]	1.36[a]	1.40[a]
Soil + MB (0.25-1mm)	0.84	1.46[a]	1.50[a]	1.30[a]
Soil + LB (1-2mm)	0.90	1.49[a]	1.60[a]	1.40[a]
Fertilizer				
Without Fertilizer	0.90	1.31	1.30	1.14
With Fertilizer	0.82	1.30	1.31	1.25
F- Test				
Particle size (P)	ns	***	**	***
Fertilizer (F)	ns	ns	ns	ns
P * F	ns	ns	ns	ns
CV (%)	25.51	15.61	27.85	19.23

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size. TOC by Organic matter (%) = Total organic carbon (%) x 1.72

Biochar enriched soil samples had a significant increase in soil organic carbon compared to unamended soil. Our results align with the finding of Jien and Wang (2013);Masulili et al. (2010);Trupiano et al. (2017). This increase in soil organic carbon might be due to biochar's high organic carbon content, upon its application to soil, it releases carbon into the soil system, and due to the mineralization of organic matter absorbed by the biochar particles, the organic carbon content in the soil gets increased. This rise in SOC could be attributed to soil enrichment with organic carbon, the sequestering of a considerable amount of carbon in soil supplemented with biochar, and the recalcitrance of OC in biochar. Qadeer et al. (2014) published and validated similar findings, stating that the application of biochar in soil significantly enhances the organic pool of the soil. According to Trupiano et al. (2017), biochar, as a rich source of carbon, improves carbon uptake and sequestration in the soil, greatly increasing the TOC of the soil system. Our results were presented and supported by Ndor et al. (2015) where they conducted a field experiment to assess the effect of biochar on soil characteristics and sesame yield. According to the study, applying rice husk biochar and sawdust biochar at a rate of 10 t/ha raised soil organic carbon from 0.31 percent (control) to 0.68 percent and 0.75 percent, respectively.

4.3.5 Effect of Biochar on Total Nitrogen in the Soil

The data on the effect of different particle sizes of biochar and recommended dose of fertilizers on soil total nitrogen is presented in Table 9 which indicated that there was a significant difference between particle size as well as combined application of fertilizers and biochar. The Total nitrogen at all 4 states was highest in Treatment with biochar mixed with fertilizer and the lower corresponding values in control were. Biochar amendment and fertilizer interaction significantly ($p< 0.05$) affect the total Nitrogen content in the soil. The lowest total nitrogen was recorded in control (0.0705%) whereas for small, medium, and large biochar was 0.0823%, 0.0835%, and 0.0822 %, respectively at the end of the experiment. In comparison to the control, the biochar treatments regardless of size significantly increase the quantity of total nitrogen in soil samples by 19 %. Fertilizer application recorded a significant increase in total nitrogen by 43% over the treatment without fertilizer.

Table 9 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Total Nitrogen in the Soil

Factors	Total Nitrogen (%)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	0.0623[b]	0..0623[b]	0.0672[b]	0.0705[b]
Soil + SB (<0.25mm)	0.0692[a]	0.0692[a]	0.0763[a]	0.0823[a]
Soil + MB (0.25-1mm)	0.0693[a]	0.0698[a]	0.0790[a]	0.0835[a]
Soil + LB (1-2mm)	0.0630[b]	0.0623[a]	0.0777[a]	0.0822[a]
Fertilizer				
Without Fertilizer	0.057[b]	0.057[b]	0.062[b]	0.065[b]
With Fertilizer	0.075[a]	0.076[a]	0.087[a]	0.093[a]
F- Test				
Particle size (P)	***	***	***	***
Fertilizer (F)	**	**	***	***
P * F	ns	**	***	***
CV (%)	4.86	4.58	4.14	3.6

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

The difference in soil total nitrogen from NPK fertilizer applied treatment was significantly higher compared to control in all the stages of crop growth. When biochar and fertilizer are used together, their impact on soil nutrients can vary greatly depending on the features of both their underlying soil and the biochar. (Lehmann et al., 2012). Our findings are in close agreement with previous studies conducted by (Butnan et al., 2015; Dong et al., 2016). This could be attributed to the biochar directly supplying nitrogen, as well as an increase in nitrogen retention and mineralization due to increased microbial activity. Although biochar does not contain high nitrogen, it helps to trap the added fertilizer inside the pores of biochar which are made available at later stages of the crop. Biochar also reduces leaching losses of N from the soil. Studies and findings of Nelissen et al. (2012) also supported the increase in nitrogen content by application of biochar due to enhance nitrification rate because biochar absorbs the potential inhibitors of nitrification. Such increase in nitrogen was with the addition of biochar was also reported by Oladele et al. (2019), Rajkovich et al. (2012), Zheng et al. (2013).

4.3.6 Available Phosphorus

The data on the effect of different particle size of biochar and fertilizer application are presented in table 10. It is evident from the data that the application of fertilizer with biochar increases the available phosphorous over no application of fertilizer. Application of biochar had a statistically significant effect on soil available phosphorus. The interaction effect of particle size of biochar and fertilizer also significantly influences the soil extractable P after harvesting. In the fertilizer application biochar, the average available phosphorus was 129 mg/kg compared to 82.44 mg/kg to non-fertilizer treatment at the end of the experiment. Among different particle size treatment combinations, it was observed that the Phosphorus (mg/kg) was significantly affected by the treatments while the Phosphorus (mg/kg) was lowest in control (97.50 mg/kg) whereas it was 10% more in other treatments.

Table 10 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Available Phosphorus in Soil

Factors	Available Phosphorus(mg/kg)			
	Transplanting	8 DAT	18 DAT	Harvesting
Particle sizes				
Soil (control)	40.55	76.53b	89.43b	97.50[b]
Soil + SB (<0.25mm)	41.04	88.30a	94.60ab	108.28[a]
Soil + MB (0.25-1mm)	41.00	90.10a	98.64a	108.94[a]
Soil + LB (1-2mm)	41.00	90.60a	87.22b	108.16[a]
Fertilizer				
Without Fertilizer	35.50[b]	72.31[b]	78.95[b]	82.44[b]
With Fertilizer	46.24[a]	100.47[a]	106.00[a]	129.00[a]
F- Test				

Factors	Available Phosphorus(mg/kg)			
	Transplanting	8 DAT	18 DAT	Harvesting
Particle size (P)	ns	***	*	**
Fertilizer (F)	***	***	***	***
P * F	ns	*	ns	*
CV (%)	4.70	5.78	7.68	5.11

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

Hence the data obtained noticed a significant level showing that there is a gradual increase of available phosphorus concerning fertilizer and biochar application. This might be due to the fact of co-application of fertilizer and biochar increased the concentration of available phosphorus in sandy loam soil due to a direct result of the fertilizer and biochar supplying available phosphorus. This finding has implications for increasing the availability of phosphorus in sandy loam soil, which initially had a relatively low phosphorus content. The addition of biochar materials altered the pH of the soil, which may have influenced the rate of P release. As a result, biochar materials were expected to include a high concentration of bonding sites or other co-precipitation components, resulting in dramatically improved P retention efficiency levels (Kuo et al., 2020). Another probable mechanism is that biochar contains soluble and exchangeable phosphate, which acts as a buffer for P-complexing metals ($Al^{3+,}$ $Fe^{3+)}$ and promotes microbial activity, hence expediting P mineralization. The finding and results are in close consonance with the findings of Laird et al. (2010), Dume and Ayele (2017), Ghorbani et al. (2019) reporting an increasing trend of available phosphorus with biochar application.

4.3.7 Soil Exchangeable Potassium (K)

The data on the effect of different particle sizes of biochar and fertilizer application on soil exchangeable K are presented in Table 11 which shows the significant difference among the treatments concerning control. It is evident from the data that application of fertilizer with biochar significantly influences the soil exchangeable potassium which showed an increase in average means by 472.9 mg/kg over nonfertilizer treatment with 162.24 mg/kg at the end of the experiment. The lowest Potassium content was recorded in the control (205.62 mg/kg). The potassium content for small. Medium and large size was 342.72 mg/kg, 360.26mg/kg, and 361.67 mg/kg, respectively. Application of biochar significantly increased the soil exchangeable potassium after harvesting by over 75 percent over no application of biochar. There was no interactive effect between the two factors.

Table 11 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Exchangeable Potassium

Factors	Exchangeable Potassium (mg/kg)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	364.72	131.32[b]	256.49[b]	205.62[b]
Soil + SB (<0.25mm)	357.72	262.02[a]	301.67[b]	342.72[a]
Soil + MB (0.25-1mm)	387.11	280.62[a]	382.72[a]	360.26[a]
Soil + LB (1-2mm)	372.19	291.84[a]	300.62[b]	361.67[a]
Fertilizer				
Without Fertilizer	143.25[b]	158.73[b]	167.98[b]	162.24[b]
With Fertilizer	597.46[a]	324.17[a]	452.76[a]	472.9[a]
F- Test				
Particle size (P)	ns	***	***	***
Fertilizer (F)	***	***	***	***
P * F	ns	ns	ns	ns
CV (%)	28.9	12.31	17.04	14.01

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

We discovered that biochar and biochar plus fertilizer significantly increased the exchangeable potassium content of the soil. This might be due to the fact of co-application of fertilizer and biochar increased the concentration of exchangeable Potassium concentration in sandy loam soil due to a direct result of the fertilizer and biochar acting as a Potassium source. Moreover, owing to the high K and high ash content in rice husk biochar which could release immediate potassium resulting in higher K availability in biochar amended soil. According to Yao et al. (2012) to the increased exchangeable K+ in comparison to the control could be due to electrostatic attraction forces on the surface of the biochar-soil matrix, which aid in potassium retention. Liu et al. (2013) reported that the application of biochar at 40 t/ha reliably enhanced potassium availability for five seasons. Likewise, similar results were reported by Filiberto and Gaunt (2013), Aslam et al. (2014), Elangovan and Sekaran (2014).

4.4 Effect on Water Leachate

The data on the effect of different particle sizes of biochar application and recommended dose of fertilizer on water leachate are presented in Table 12. It was noticed that biochar-treated samples exhibited significantly smaller leachate volumes than that of the control for each flushing event. The smaller particle sizes of biochar held water more strongly than the large particle size. The reduction in irrigation leaching reached the soil mixed with the particle size of small, medium, and large were lower than that observed for the control by 20%, 11%, 5%, respectively at the end of the experiment. It is evident from the results that the application of fertilizer has not statistically significant. There was no significant ($p > 0.05$) interactive effect of different sizes of biochar and Fertilizer on soil water leachate through the column.

Table 12 Effect of biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Water Retention in Soil

Factors	Water leachate (%)			
	Transplanting	8 DAT	18 DAT	Harvesting
Particle sizes				
Soil (control)	69.80^{a}	65.64^{a}	62.02^{a}	58.00^{a}
Soil + SB (<0.25mm)	55.10^{c}	51.54^{b}	47.57^{c}	46.31^{d}
Soil + MB (0.25-1mm)	57.02^{b}	51.00^{b}	53.57^{b}	51.88^{c}
Soil + LB (1-2mm)	58.04^{b}	55.54^{b}	55.97^{b}	55.33^{b}
Fertilizer				
Without Fertilizer	60.02	55.61	55.43	52.58
With Fertilizer	59.95	56.40	54.14	52.18
F- Test				
Particle size (P)	***	***	***	***
Fertilizer (F)	ns	ns	ns	ns
P * F	ns	ns	ns	ns
CV (%)	2.41	6.97	8.57	2.92

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size

Biochar increased soil water retention, as demonstrated by our findings. This could be a result of an increase in the porosity of the soil. Increased porosity and porous nature of the biochar help to absorb and retain water, resulting in an increase in water retention. A shift toward more mesopores, particularly in sandy soils, may also result in a loss of macropores and a decrease in hydraulic conductivity. When biochar is added to soil, it typically enhances its porosity, pore count, and pore connectivity (Alghamdi et al., 2020; Obia et al., 2016). This effect, when combined with a decrease in bulk density and an increase in macroaggregate formation and aggregate stability, can result in a decrease in soil water flow and an increase in water retention (Pituello et al., 2018; Speratti et al., 2017; Wang et al., 2017). Our findings corroborate those of several prior research (Alghamdi et al., 2020; Kuo et al., 2020; Liao & Thomas, 2019; Obia et al., 2016), all of which reported higher soil water retention following biochar amendment.

We discovered that smaller biochar fractions retained a lot of water over other treatments, similar to results reported by Liu et al. (2016) and Esmaeelnejad et al. (2016). Ibrahim et al. (2017) confirmed that as particle size decreases, water retention increases. This increase is due to the enormous specific surface area of the particles, which increases as particle size decreases. These microscopic particles could contribute to the creation of pores between biochar and soil particles (interpore). Intrapore (pores inside biochar) systems are crucial for soil water retention. This is because small biochar particles have more micropores than large biochar particles, they can hold more water than larger biochar particles. The fine biochar particles can dwell in the pore spaces between soil particles due to the biochar's small particle size, limiting water routes, and lowering water infiltration in the soil. The small particle size of biochar is more likely to fill the space between soil particles than coarse biochar particles, retaining more water via capillary pressure. de Jesus Duarte et al. (2019) explored the influence of biochar particle size on soil characteristics. The findings show that biochar particle size has a significant impact on water retention, water availability, and pore size distribution. Because biochar improves the volume of water held in the soil, it may be possible to reduce irrigation frequency. Biochar may have amplified the influence on soil water content, resulting in beneficial effects on plant development during periods of water scarcity.

4.5 Total Nitrogen Leachate

Table 13 illustrates the cumulative amount of total nitrogen leached from soil columns. Biochar's influence on nutrient leaching is clearly dependent on its particle size and fertilizer application. When compared to the control, biochar considerably reduced nitrogen leaching by up to 73% during the harvesting period. In comparison to the control, the medium and large treatments significantly reduced the quantity of total N leached from soil samples by 58% and 48%, respectively. The control treatment leached the most inorganic nitrogen from the soil, whereas the finer biochar leached the least (73%). The leaching of total nitrogen was much higher during the first growth period compared to the lateral one. However, the leaching sequence among different treatments was similar for both growth periods increasing in the order control to biochar treated treatment.

Table 13 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Total Nitrogen Leaching from the Soil

Factors	Nitrogen Leachate (mg/l)			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	23.83[a]	20.70[a]	11.60[a]	9.72[a]
Soil + SB (<0.25mm)	6.892[d]	4.93[c]	4.03[d]	2.60[c]
Soil + MB (0.25-1mm)	8.74[c]	9.80[b]	5.64[c]	4.04[b]
Soil + LB (1-2mm)	12.42[b]	10.03[b]	7.28[b]	5.00[b]
Fertilizer				
Without Fertilizer	1.62[b]	1.49[b]	1.10[b]	1.22[b]
With Fertilizer	24.32[a]	21.22[a]	13.17[a]	9.40[a]
F- Test				
Particle size (P)	***	***	***	***
Fertilizer (F)	***	***	***	***
P * F	***	***	***	***
CV (%)	8.39	14.95	16.43	20.29

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

The findings of our study show that incorporating rice husk biochar into sandy loam soil samples can boost the soil's ability to absorb nutrients and prevent nutrient leaching. Higher nitrogen leaching during the initial phase of the experiment can be ascribed to the presence of greater nitrogen at the start of the experiment. Additionally, enhancements to soil physical qualities, such as soil aggregation and water holding capacity, may have a beneficial effect on nutrient leaching. Increased water retention may be necessary to avoid nutrient leaching. Yoo et al. hypothesized that boosting aggregate formation with the use of biochar might effectively enhance nitrogen retention. Additionally, they indicated that higher water holding capacity following charcoal addition contributed to N leaching reduction. Enhanced cation exchange capacity, increased soil water holding capacity, and microbial nitrogen immobilization because of biochar application are all plausible drivers of nitrogen absorption and retention in soil, according to Liu et al. (2017). Additionally, the mechanisms underlying the reduction in NO_3^- N leaching caused by biochar amendment may be connected to the intrinsic features of biochar, such as its negatively charged surface area, porous structure, and high ion exchange capacity (Lehmann et al., 2003). Yao et al. (2012) showed that adding peanut hull biochar (2 % of the soil, w/w) reduced NO_3-N and NH_4^+-N leaching by 34% and 14%, respectively, in a column experiment. By enhancing soil N retention, reducing ammonia (NH_3) volatilization, or converting it to NO_3 via nitrification, biochar can significantly reduce nitrogen leaching (Sun et al., 2017). The reduction in NO_3 and NH_4^+ leaching could be a result of enhanced adsorption of these ions onto the surface of biochar, increased immobilization of these ions by the increased microbial biomass caused by biochar addition, or a combination of the two (Alkharabsheh et al., 2021; Rubin et al., 2020). Additionally, according to Xu et al. (2016), biochar reduced NO3, NH4+, and total N leaching between 19 and 28 %, 16 and 19 %, and 19 to 20 % when compared to the unamended soil (control). Leaching of the nutrients is lower in small size biochar treatment because small size biochar treatment has more water retention capacity than other treatments. Soil water and nutrient retention generated by biochar may have a beneficial effect on nutrient and water conservation as well as soil quality. Reduced water and nutrient leaching from soils could improve nutrient utilization efficiency, resulting in reduced fertilizer consumption. As a result, biochar can help conserve both soil and water. Additional research should be conducted to see whether these favorable benefits are applicable in the field and to downstream water bodies at the watershed level.

4.6 Nutrient Use Efficiency

The data concerning on effect of different particle sizes of biochar application and recommended dose of fertilizer on nutrient use efficiency through nutrient use ratio is illustrated in Table 14 which shows a significant effect of Nitrogen and Potassium use efficiency. There is a significant interaction between biochar and fertilizer application on nitrogen and potassium use efficiency. The average mean percentage of fertilizer treatments is 47% higher than the non-fertilizer treatments. The nutrient use ratio recorded for the control, small, medium, and large treatment are 33.1%, 24%, 22.50%, and 23.10 %, respectively. Regarding Potassium use efficiency, the average mean percentage of fertilizer treatments is 14.9 % higher than the average means of non-fertilizer treatments. The highest nutrient ratio was recorded in control (36.60%), followed by treatment with large particle size (32.14%), small and medium with 30.1%and 30.03%, respectively.

Table 14 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Nutrient Use Efficiency

Factors	Nutrient use efficiency (NUE)		
	Total N	**Phosphorus**	**Potassium**
Particle sizes			
Soil (control)	33.31^{a}	20.71	36.60^{a}
Soil + SB (<0.25mm)	24.00^{b}	20.70	30.1^{b}
Soil + MB (0.25-1mm)	22.50^{b}	17.23	30.03^{b}
Soil + LB (1-2mm)	23.10^{b}	19.20	32.14^{ab}
Fertilizer			
Without Fertilizer	31.03^{a}	21.10	34.80^{a}
With Fertilizer	20.40^{b}	17.80	29.63^{b}
F- Test			
Particle size (P)	***	ns	*
Fertilizer (F)	***	ns	**
P * F	***	ns	*
CV (%)	6.34	28.86	11.81

Note: a, b represents significant difference among treatments at p<0.05 according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with p < 0.05, p < 0.01, p < 0.001. and non-significant (p > 0.05), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size. NUE= Units of yield (g) / units of nutrients in tissue (g)

Biochar treatment in conjunction with fertilizers boosted the nutrient use efficiency of the fertilizer while minimizing nutrient losses. This might be due to unique and distinct advantageous biochar features such as high surface area, porosity, cation exchange capacity and availability of acidic and basic functional groups all contribute significantly to soil nutrient loss reduction and increase the nutrient use efficiency (Alkharabsheh et al., 2021; Rubin et al., 2020). Overall, significant increase in nitrogen and potassium use efficiency through biochar application could be attributed due to biochar's native nutrient content, soil physical properties improvements (i.e., bulk density, water retention, and nutrient retention), promotion of microbial activity and function, increased mineralization of native soil nutrients. Moreover, the application of biochar could contribute to nutrient use efficiency both directly by increasing nutrient uptake and indirectly by reducing nutrient loss through leaching and gaseous emission. According to Oladele et al. (2019) and L. Yu et al. (2017) application of biochar increase nitrogen uptake, hence enhancing nitrogen use efficiency in crop. Similarly, Zhang et al. (2020) recorded biochar boosting nitrogen use efficiency to 20-53 percent in a rice-wheat cycle in a six-year field trial. Increased nutrient uptake and utilization by plants increases the effectiveness of applied fertilizers, lowers input costs, and prevents nutrient loss to ecosystems (Baligar et al., 2001)

4.7 Biochar on Plant Height and Biomass

Biochar effects on plant height and biomass in the experimental soils were recorded and parented in Table 15 and Table 16. Analysis of variance didn’t show a significant (p < 0.05) interaction of rice husk biochar and fertilizer on plant height and biomass. The main effect of biochar did not exert any significant effect. The addition of biochar irrespective of particle size did not significantly influence plant biomass and plant height. Additionally, the application of biochar in combination with fertilizer at different particle sizes had no beneficial effect on plant biomass and height compared to treatment without biochar. Additionally, the application of biochar in combination with fertilizer at different particle sizes didn't show a beneficial effect on plant biomass compared to treatment without biochar even though the plant biomass and height increase over the growth period.

Table 15 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Plant Height

Factors	**Plant height (cm)**			
	Transplanting	**8 DAT**	**18 DAT**	**Harvesting**
Particle sizes				
Soil (control)	7.5	11.06	15.93	19.26
Soil + SB (<0.25mm)	7.5	12.13	16.55	20.50
Soil + MB (0.25-1mm)	7.5	13.05	15.85	21.01
Soil + LB (1-2mm)	7.4	12.43	16.40	20.35
Fertilizer				
Without Fertilizer	7.50	12.07	16.24	20.05
With Fertilizer	7.45	12.28	16.12	20.45
F- Test				
Particle size (P)	ns	ns	ns	ns
Fertilizer (F)	ns	ns	ns	ns
P * F	ns	ns	ns	ns
CV (%)	8.01	10.54	10.66	8.41

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

Table 16 Effect of biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Plant Biomass

Factors	Plant biomass (g)		
	8 DAT	18 DAT	Harvesting
Particle sizes			
Soil (control)	0.26	0.50	1.15
Soil + SB (<0.25mm)	0.25	0.60	1.14
Soil + MB (0.25-1mm)	0.25	0.60	1.16
Soil + LB (1-2mm)	0.24	0.64	1.10
Fertilizer			
Without Fertilizer	0.23	0.54	20.05
With Fertilizer	0.26	0.60	20.45
F- Test			
Particle size (P)	ns	ns	ns
Fertilizer (F)	ns	ns	ns
P * F	ns	ns	ns
CV (%)	17.17	15.48	39.22

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

As the majority of research suggests that biochar improves the agronomic qualities of several crops, while others claim negative or no effects on agricultural productivity (Spokas et al., 2012). In the present study, *Brassica alboglabra* height and biomass were not significantly increased due to the application of biochar or biochar and fertilizer. Possible reasons for the non-significant effects of biochar could be because of the short duration and time of vegetables where the effect of biochar was not refund. Biochar might have a significant influence in long term. Another possible reason might be due to flushing of the column at different sampling stages which might have leachate the nutrient and disturbed the soil's physical properties. Moreover, the application of biochar provided immediate labile organic matter to the soil which increased microbial biomass and thus caused immobilization of nutrients. This yield drop associated with biochar treatment could be attributed to nitrogen immobilization as a result of high C/N ratios (Rondon et al., 2007). Another possible explanation is that the 2% rate of biochar application is too low in sandy soils where the effects of biochar are negligible within short period regarding plant growth and development. Similar biochar induced deployed response to yield improvements with negative or no impact on the first crop and followed by yield increase has been reported in the literature (Asai et al., 2009; Haefele et al., 2011; Kulmatiski & Beard, 2006).

The result is in consonance with the finding of Gholizadeh et al. (2020), Mclennon et al. (2020).Hansen et al. (2016). Gholizadeh et al. (2020) reported no significant difference in maize yield following biochar treatment compared to control. Additionally, Mclennon et al. (2020) stated that biochar had no effect on *Schedonrus arundinacea* and *Poa pratensis L* whether used alone or in combination with nitrogen fertilization. Similarly, Hansen et al. (2016) in a pot experiment discovered that the application of straw or wood gasification biochar did not influence barley growth and productivity.

4.8 Soil Microbial Community

4.8.1 Average Metabolic Response

The data regarding the effect of biochar, fertilizer, and interaction between biochar and fertilizer on the use of different carbon sources of a microbial community through average metabolic response is illustrated in Table 17 and Fig 14. The six types of carbon substrates (amines, amino acids, carbohydrates, phenolic compounds, carboxylic acids, and polymers) were utilized in a variety of ways by different treatments (Fig.11). It is evident from the data that biochar without combined fertilizer application shows an increase in amines, carbohydrates, and polymers carbon sources. There was the significant effect to average metabolic response to amines, carbohydrates, and polymer between fertilizer treatment and non-fertilizer treatment. The average metabolic response values of non-fertilizer treatments recorded at 1.60, 1.22, 1.36 for amines, carbohydrates, and polymers, respectively. whereas those of fertilizer treatments were 0.64, 0.53, and 0.78, respectively. The greater microbial activity was observed in non-fertilizer treatment especially with amines, carbohydrates, and polymers. Furthermore, microbial activity to metabolize phenolic compounds and carboxylic acids were lowest in control (AMR = 0.60 for phenolics and AMR = 1.20 for carboxylic compounds) compared to biochar treated treatments. Microbial activity in soil incorporated with biochar treatments was higher than control even though no significant difference was noticed between three sizes. There was no interaction effect between two factors (particle size of biochar and fertilizer).

Table 17 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer on Different Carbon Sources of a Microbial Community Through Average Metabolic Response

Factors	Average Metabolic Response (C sources)					
	Amines	**Amino Acids**	**Carbohydrates**	**Phenolic compounds**	**Carboxylic acids**	**Polymers**
Particle sizes						
Soil(control)	0.70	1.13	0.74	0.60[b]	1.20[b]	0.95
Soil + SB (<0.25mm)	1.44	1.31	0.93	1.15[a]	1.50[ab]	1.15
Soil + MB (0.25-1mm)	1.30	1.51	0.87	1.32[a]	1.43[ab]	1.10
Soil + LB (1-2mm)	1.20	1.50	0.93	1.07[a]	1.60[a]	1.10
Fertilizer						
Without Fertilizer	1.60[a]	1.36	1.22[a]	0.11	1.52	1.36[a]
With Fertilizer	0.64[b]	1.35	0.53[b]	0.93	1.30	0.78[b]
F- Test						
Particle size (P)	ns	ns	ns	*	*	ns
Fertilizer (F)	*	ns	**	ns	ns	*
P * F	ns	ns	ns	ns	ns	ns
CV (%)	71.32	33.68	42.93	37.43	21.68	39.07

Note: a, b represents significant difference among treatments at p<0.05 according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

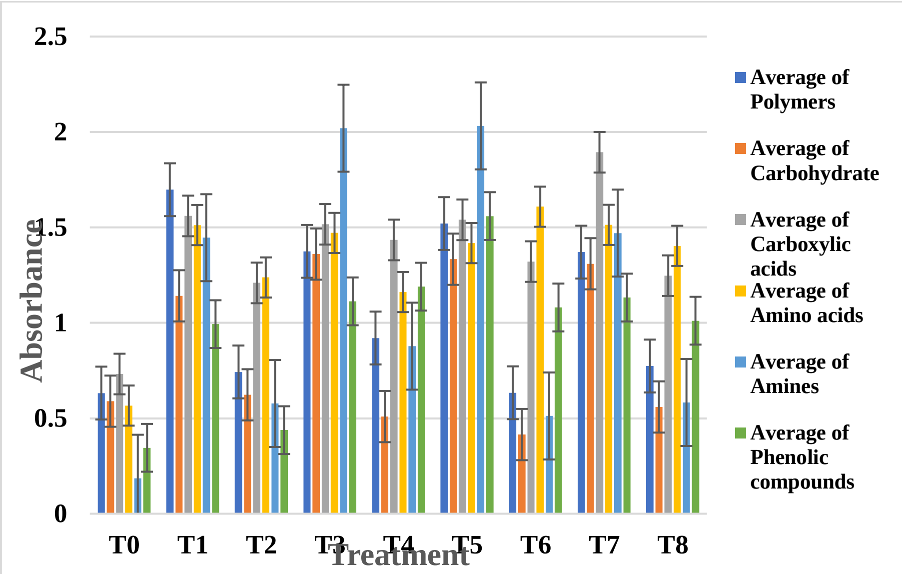

Figure 14 The average metabolic response (AMR) values of different treatments on six categories of carbon substrates.

As previously stated, the carbon substrates of the Biolog EcoPlate can be classified into six categories based on their biochemical properties. The greater microbial activity was observed in non-fertilizer treatment especially with amines, carbohydrates, and polymers. In terms of phenolic compounds and carboxylic acids and other substrates, microbial activity was greater in the biochar amendments than those in the control. This finding demonstrated that biochar could enhance the growth of microorganisms that prefer these types of substrates, implying that the addition of biochar may have reshaped the soil environment for microorganisms, with some species spreading more rapidly than others. Because of this finding demonstrating that biochar can enhance the growth of microorganisms that thrive on such substrates, the addition of biochar may have changed the soil habitat for microorganisms, allowing some species to spread more rapidly than others (Bamminger et al., 2016; Jin, 2010). Soil microbial growth could be aided application of biochar by improvements in soil properties, such as an increase in soil pH, soil–water retention capacity, and nutrient availability. Furthermore, biochar contributes to the health of microbial communities by providing labile carbon substrates for degradation. Additionally, the porous structure of biochar may alleviate competition between microorganisms, enhancing their capacity to utilize carbon substrate.

4.8.2 Microbial Metabolic Activity and Diversity

Microbial metabolic activity and diversity were assessed through the measurement average metabolic response, Shannon-Wiener diversity index (H'), Shannon's evenness, and Margalef index in the biology plate which is presented in Table 18. There was no significant interaction of different sizes of biochar and fertilizer on the effect of microbial metabolic activity and diversity indices. However, there was a significant effect between biochar combined fertilizer treatments and non-fertilizer treatments. The average means of the Shannon-wiener diversity index (H') was over 8% higher in non-fertilizer treatments (H'=3.15) compared to non-fertilizer biochar applications (H'=2.90). The lowest, Shannon-wiener diversity index (H') was recorded in control (H'=2.94) whereas the highest was recorded in small size (H'=3.06), followed by medium (H'=3.05) and large particle size of biochar (H'=3.02). Similarly, Shannon's evenness was also found to be higher in non-fertilizer treatments 0.10 over fertilizer biochar combined treatments 0.09. Regarding Margalef index it was higher in fertilizer combined biochar treatment than the non-fertilizer combined biochar treatments. In contrast, the average metabolic response was greater in non-fertilizer treatment than in fertilizer treatment.

Table 18 Effect of Biochar (RHB), Fertilizer and Interaction of Biochar and Fertilizer Diversity Indices and Total Average Metabolic Response

Factors	Diversity indices			
	Shannon-Wiener index (H)	**Shannon's evenness**	**Margalef index**	**Average Metabolic Response (AMR)**
Particle sizes				
Soil (control)	2.94	0.09	9.22	1.13
Soil + SB (<0.25mm)	3.06	0.09	8.42	1.21
Soil + MB (0.25-1mm)	3.05	0.09	8.50	1.20
Soil + LB (1-2mm)	3.02	0.09	8.50	1.21
Fertilizer				
Without Fertilizer	3.15^{a}	0.10^{a}	8.20^{b}	1.45^{a}
With Fertilizer	2.90^{b}	0.09^{b}	9.09^{a}	0.93^{b}
F- Test				
Particle size (P)	ns	ns	ns	ns
Fertilizer (F)	*	*	*	*
P * F	ns	ns	ns	ns
CV (%)	7.82	8.66	10.51	10.79

Note: a, b represents significant difference among treatments at $p<0.05$ according to the LSD test. Levels of significance. *, **, ***, and ns indicate significant levels with $p < 0.05$, $p < 0.01$, $p < 0.001$. and non-significant ($p > 0.05$), respectively; SB= Small biochar size. MB= Medium biochar size, LB= large biochar size.

Soil microbes are critical for organic matter decomposition, soil structure maintenance, nutrient recycling, pest and disease suppression and secretion of plant growth stimulants (Kirchman, 2018). In the present study, microbial community was increased due to application of biochar. The significant biochar effect on soil microbial activity and community due to its key properties such as pore space, surface area, porosity, minerals, surface volatile organic compounds, functional groups, free radicals, and pH (DeLuca et al., 2015). The average metabolic response for four carbon sources and microbial diversity were observed more compared with fertilizer addition. It is possibly due to the fact that in the initial sandy loam soil with the presence of sufficient nutrients the addition of fertilizer could retard the microbial activity. Soil chemical properties, particularly pH, are a determinant of soil microbial abundance and activity, which can be significantly altered by biochar when a sufficient amount is added to the soil whereas application of fertilizer decrease the soil ph. However, species richness was higher with the fertilizer treatments. This might be due to fertilizer supplying carbon sources than other treatments which may lead to increased number of dominant groups. The increase in microbial community abundance could be due to increase availability of nutrients or labile organic matter on the biochar surface, decreased competition, improved habitat suitability and refuge and increased water, nutrient retention, and air circulation (Chen et al., 2017; Lehmann et al., 2011; Muhammad et al., 2014; Thies & Rillig, 2009). There was a rise in microbial abundance from 366.9 (control) to 730.5 gCg^{-1} (biochar 30 t ha^{-1}) in the study area, according to Domene et al. (2014). Similarly, when maize stover biochar rates were increased (from 0% to 14%) for preincubation times (2–61 days), microbial abundance climbed by 5–56% (Domene et al., 2015).

CHAPTER V

CONCLUSION

The present study demonstrates that different rice husk biochar particle sizes at the rate of 2 % w/w in sandy loam soil can have a profound impact on soil pH, bulk density, water and nutrient retention, soil fertility, nutrient use efficiency, and microbial community activities and diversity of soil quality due to its intrinsic structure and physicochemical properties of rice husk biochar. Rice husk biochar (RHB) with high carbon and minerals content is excellent for improving soil health and fertility. The high, surface area, pore-volume, and porosity of biochar ameliorate the water and nutrient retention. The pores distribution and sizes of RHB did not vary between different biochar particle sizes. The average pore size was 10.64 µm. RHB indicates that C, O, and Si were the major elements including some amount of minerals such as N, P, K, Mg, Ca, Fe. Since biochar has high pH (alkaline), there is also the potential for them to be used as an acidic soil amendment. The application of rice husk biochar was noticed to increase soil pH by 0.6 units in biochar amendment treatments over control treatment. Biochar's physical properties have been found to have a significant impact on its performance as a soil amendment. Additionally, soil physical properties and biochar effects were generally dependent on particle sizes. The soil bulk density was significantly decreased in large particle size over the control and finer particle sizes of biochar in sandy loam soil. The soil bulk density decreased at 7%,5.81%, and 4 % compared with non-amended soil when incorporated with large, medium, and small sizes of biochar, respectively. On contrary, water and nutrient leaching decreased with the finer particle sizes of biochar. The smallest biochar size (<0.25 mm) was 20% greater potential in leaching reduction as compared to control. Besides, medium (0.25 – 1 mm) and large (1 – 2 mm) sizes of Rice husk biochar reduced leaching by 11%, and 5%, respectively. Biochar's influence on nutrient leaching is clearly dependent on its particle size. When compared to the control, biochar considerably reduced nitrogen leaching by up to 73% during the harvesting period. In comparison to the control, the medium and large treatments significantly reduced the quantity of total N leached from soil samples by 58% and 48%, respectively. The control treatment leached the most inorganic nitrogen from the soil, whereas the finer biochar leached the least (73%). There was an interaction effect on the particle size of biochar and fertilizer applied on decreasing total nitrogen leachate. Our research found that biochar size should be one of the design factors for maximizing the benefits of biochar in the field. In contrast to charcoal feedstocks and production processes, end users can readily change particle size. To maximize the advantages, the methodology for applying biochar to degraded landscapes should consider soil and biochar particle size distribution. Manipulation of particle size (fine-to-coarse fraction) in biochar products might thereby enhance benefits while reducing unforeseen negative outcomes. Moreover, the application of biochar with synthetic fertilizer has increased the nutrient content and nutrient use efficiency with the positive interaction between particle size and fertilizer. The interaction effect between particle sizes of biochar and fertilizer was observed only in soil chemical properties. Microbial metabolic activity and diversity were assessed through the measurement of total average metabolic response, the average metabolic response by chemical groups, Shannon-Wiener diversity index (H’), Shannon’s evenness, and Margalef index. Adding biochar (regardless of size) increased microbial activity on metabolization of phenolic compounds. The large size of biochar (1-2 mm) provided the greatest microbial activity on carboxylic acids. Fertilizer treatments (regardless of biochar amendment) provided greater species richness. However, no fertilizer treatment (regardless of biochar amendment) provides greater diversity, evenness, and total AMR. Finally, we noticed that the application of biochar in combination with fertilizer at different particle sizes shows no significant effect on plant height, plant biomass, and microbial activity compared to treatment without biochar even though the plant height, plant biomass, and microbial activity increase over the growth period. These results and findings indicated that biochar has the potential to manage and enhance soil properties (Figure. 15)

The findings of this study demonstrate that the particle size of biochar plays a critical role in soil bulk density, water, and nutrient retention in a sandy loam soil. This suggest that the particle size of biochar is a critical factor to consider when using it as a soil amendment. The size of biochar particles can have a direct impact on biochar–soil interactions, influencing changes in soil physical properties. Small biochar particles mixed or interact more easily with soil particles to form aggregates than large biochar particles. If we compare small biochar particles to large biochar particle sizes in sandy loam soil, small biochar particles improved soil compaction and aggregation and retains more water and nutrients. Despite the growing interest in using biochar to manage soils, there are still several research gaps and ambiguities such as the dependency of particle size of biochar. Further relevant investigations, the application rate of rice husk biochar, cultivation of other crops, long-term effect, and field-scale application need to be assessed.

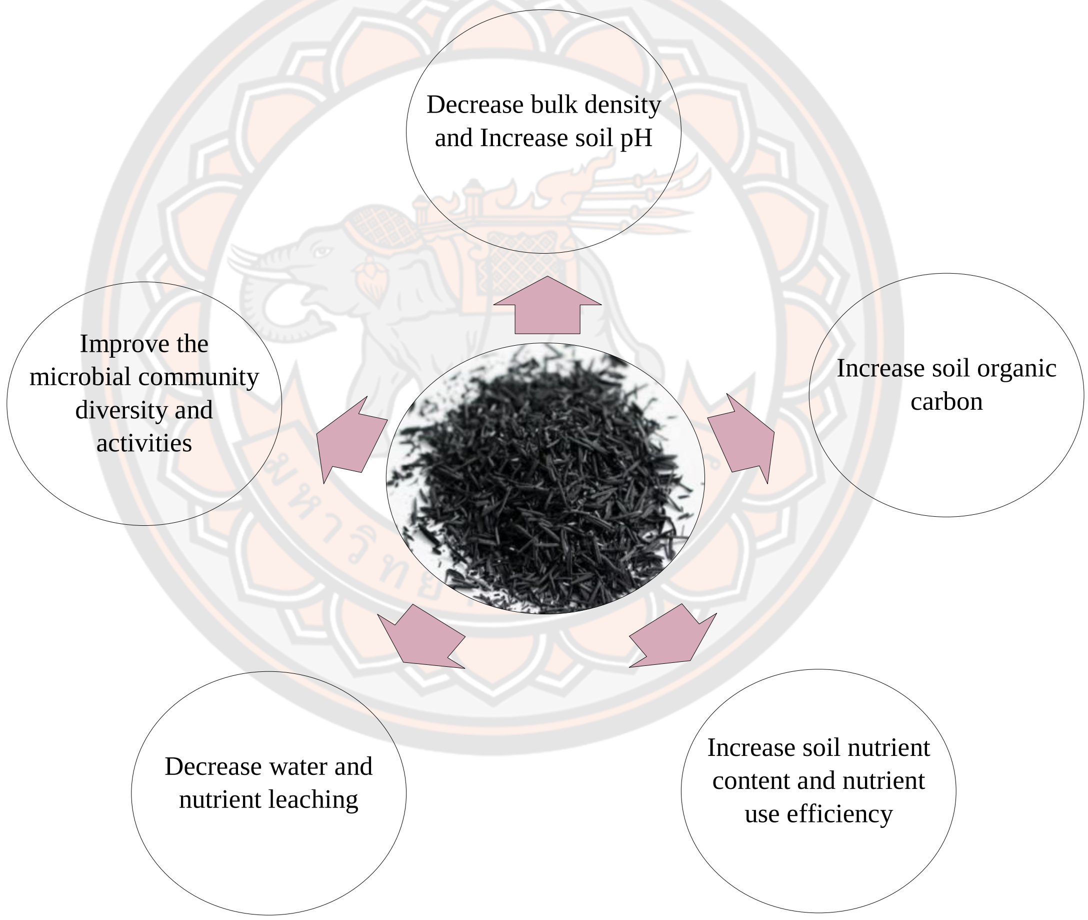

Figure 15 Conceptual Framework of our Findings

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