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Research Article

Investigating the Effects of Twelve Biochars on the Growth of *Capsicum annuum* 'Jalapeno' Pepper, Microbial Population and Enzyme Activities in Soil

Shagufta Gaffar, Clara Riquelme, Krishnaswamy Jayachandran*

Department of Earth and Environment, Institute of Environment, Florida International University, Miami, FL, USA

ABSTRACT

In recent years biochar is being applied extensively to agricultural soils because of the potential influence on plant productivity and soil microbial activities. This study was conducted to observe the effects of twelve biochar treatments (made from six different feedstocks at 350°C and 500°C) on Jalapeno pepper (*Capsicum annuum* 'Jalapeno') growth, bacteria and fungi population, arbuscular mycorrhizal (AM) fungi root colonization and soil enzyme activities in an organic rich soil. Biochar treatments did not significantly affect the growth and yield of Jalapeno pepper plant but significant influence on soil microbial and enzyme activities were observed. There was an overall increase in the bacteria (10%) population with a decrease in fungi (8%) population in soils during the entire growing period of Jalapeno. Treatments consisting of biochars made at 350°C and 500°C from coconut husk (T7 and T8), loblolly pine (T9 and T10), cypress (T11 and T12), and pecan shell (T13 and T14) resulted in 159%, 169%, 203%, and 179%, respectively, significantly higher root colonization by arbuscular mycorrhizal (AM) fungi compared to the control (T2). Higher β-1-4-glucisidase (9 times) and alkaline phosphatase (3.5 times) enzyme activities were detected in soils treated with cypress biochars made at 350°C (T11) and Brazilian pepper biochars made at 500°C (T6), respectively, relative to the control. Among the different biochar properties, the pore structure (pore size and volume), surface behavior (SSA and CEC), pH and ash content were responsible for influencing plant growth, soil microbial population and enzyme activities. Overall, it was the type of feedstocks that had a significant effect rather than the pyrolysis temperatures.

Keywords: Biochar; Invasive plant species; Jalapeno pepper; Arbuscular mycorrhizal fungi; Soil enzymes

INTRODUCTION

Biochar is the solid byproduct from pyrolysis of carbon rich biomass or biowaste. It has the capacity to improve soil physical and chemical properties, promote soil biological properties, influence plant growth, reduce contaminant levels in soil, minimize greenhouse gas emissions, and sequester carbon to mitigate climate change [1-3]. Knowledge of biochar in agriculture dates to 'Terra Preta' soils in the Amazon [4,5] and within the past few years the scientific community has focused attention to the use of biochar as a soil amendment in agricultural settings to explore potential benefits.

Several studies have shown that biochars in combination with added nutrients either as inorganic or organic fertilizers can have a positive, neutral or even negative effect on the productivity and performance of plants [6-8]. However, the effectiveness of biochar for influencing plant production depends not only on the type of soils, crops, and climatic conditions [9,10] but also on the properties of the biochars [11,12]. The inherent variability of biochars caused by different feedstock and production conditions suggests a high variability of their effect on soil properties and plant productivity [13,14]. The application of biochars can change the physicochemical and biological properties of soils and substrates which subsequently can affect the plant growth and health [5,12]. Therefore, the effects of biochars on crop production are rather variable [12,15,16].

Soil microorganisms are crucial to maintaining soil conditions and influencing plant productivity. A great variability has been observed in the response of microbial communities to biochar addition to soil [17-19]. The effects of biochars on arbuscular mycorrhizal (AM) fungi have received greater attention. AM fungi colonize the root of plants providing them with mineral nutrients and in return receiving photosynthetically derived carbohydrates [20]. Some studies have shown positive effects of biochars on the abundance of root colonization by AM fungi [21,22] while others have found that biochars can negatively affect AM fungi [22,23].

Correspondence to: Krishnaswamy Jayachandran, Department of Earth and Environment, Florida International University, Miami, FL, USA, Tel: +1 (305) 348-6553; Email: jayachan@fiu.edu

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The AM fungi can obtain nutrients from biochar pores that may be inaccessible to roots and provide to the host plant [24]. This can ensure adequate nutrients for plant growth through the combined effect of AM fungi and biochar application. Generally, biochars can influence soil microbes by potentially supplying organic matter and nutrients for metabolism [19], the pores can provide habitat and protection from predators [25,26] and the production and/or adsorption of certain substances can stimulate or even prohibit microbial growth [27,28]. Biochar induced changes in the soil physical and chemical properties can also impact microbial activities [29,30]. Microbially produced extracellular enzymes are important for organic matter decomposition and nutrient cycling for microbial as well as plant uptake [31]. Therefore, the influence of biochars on activities of soil extracellular enzymes is important. Studies indicate that biochars have variable effects on extracellular enzyme activities in soil [17,32-35]. The influence of biochars on soil enzyme activity depends on the interaction of substrate and enzyme with biochars and is related to the porosity and surface area of biochars [33,36].

Similar to plant response, the variability in biochar properties together with the variation in soil types strongly affect microbial and enzyme activities in soil, thus, warrants intensive research to better understand the role of different biochar applications. Even though several studies have been conducted on biochar amendments in soils, the use and effect of biochars on plant and soil microorganisms in organic soils have been little studied. Therefore, this study was designed to investigate the plant and microbial response and enzyme activity in an organic soil following the addition of twelve biochars made from different feedstocks.

MATERIALS AND METHODS

Biochars used in this study

The twelve biochars used in this study were produced from six different feedstocks pyrolyzed at temperatures 350°C and 500°C. The feedstocks were a combination of plant species invasive to South Florida, USA, along with agricultural residues and native plants. Feedstocks consisted of Australian pine (*Casaurina equisetifolia*), Brazilian pepper (*Schinus terebinthifolius*), coconut husk (*Cocos nusifera*), cypress (*Taxodium distichum*), loblolly pine (*Pinus taeda*), and pecan shell (*Carya illinoinensis*). The biochars were denoted based on the feedstock and production temperature, such as AP350 indicated Australian pine derived biochars pyrolyzed at 350°C and so on. The biochars have been characterized in a previous study [37] and selected physicochemical properties are listed in (Table 1).

Table 1: Selected physicochemical properties of the twelve different biochars used as treatments for the pot experiments.

Sample†	Volatile Matter (%)	Ash(%)	pH	CEC‡ (cmol kg-1)	SSA§ (m2/g)	TPV¶ (cm3/g)	Average pore size (nm)	Carbon (%)	Nitrogen (%)
AP350	79.24	5.32	8.58	16.31	0.98	0.003	12.46	64.93	0.94
AP500	61.35	10.19	9.37	8.19	2.59	0.006	9.40	66.65	1.10
BP350	66.47	2.06	7.72	8.47	0.57	0.002	12.26	67.54	0.5
BP500	55.80	4.02	9.65	7.92	2.29	0.008	14.60	77.37	0.51
CH350	85.05	3.74	9.40	16.32	0.89	0.003	13.31	66.69	0.51
CH500	79.37	8.88	9.89	12.04	1.94	0.004	7.99	67.00	0.69
L350	71.31	1.70	7.63	8.51	0.30	0.001	12.81	67.71	0.5
L500	48.59	3.20	7.84	7.93	5.21	0.004	3.13	79.47	0.5
Cy350	72.75	0.55	7.11	10.55	0.41	0.001	10.01	76.10	0.5
Cy500	62.66	1.59	7.67	9.18	4.18	0.002	2.39	83.59	0.5
P350	68.04	2.18	7.36	6.14	0.36	0.001	14.56	68.45	0.5
P500	56.33	3.82	7.94	4.66	2.14	0.002	4.41	78.96	0.5

†Sample abbreviation are as follows; AP350 & AP500=Australian pine derived biochar pyrolyzed at 350°C and 500°C; BP350 & BP500=Brazilian pepper derived biochar pyrolyzed at 350°C and 500°C; CH350 & CH500=Coconut husk derived biochar pyrolyzed at 350°C and 500°C; Cy350 and Cy500=Cypress derived biochar pyrolyzed at 350°C and 500°C; L350 & L500 = Loblolly pine derived biochar pyrolyzed at 350°C and 500°C; P350 & P500=Pecan shell derived biochar pyrolyzed at 350°C and 500°C; ‡CEC=Cation Exchange Capacity; §SSA=Specific surface area; ¶TPV=Total pore volume

Site description and Experimental design

The potted experiment was conducted at the Organic Garden shade house (25.7540° N, 80.3801° W) located near the nature preserve at Florida International University (FIU), Miami, FL, USA, between 22 March and 25 June 2019. Treatments for this study were laid out according to a randomized complete block design (RCBD) and each treatment had five replications. Treatment abbreviations are as follows:

T1=No Biochar+No Hoagland's nutrient solution (HNS)

T2 (Control)=No Biochar+HNS

T3=AP350+HNS (Australian pine derived biochar pyrolyzed at 350°C)

T4=AP500+HNS (Australian pine derived biochar pyrolyzed at 500°C)

T5=BP350+HNS (Brazilian pepper derived biochar pyrolyzed at 350°C)

T6=BP500+HNS (Brazilian pepper derived biochar pyrolyzed at 500°C)

T7=CH350+HNS (Coconut husk derived biochar pyrolyzed at 350°C)

T8=CH500+HNS (Coconut husk derived biochar pyrolyzed at 500°C)

T9=L350+HNS (Loblolly pine derived biochar pyrolyzed at 350°C)

T10=L500+HNS (Loblolly pine derived biochar pyrolyzed at 500°C)

T11=Cy350+HNS (Cypress derived biochar pyrolyzed at 350°C)

T12=Cy500+HNS (Cypress derived biochar pyrolyzed at 500°C)

T13=P350+HNS (Pecan shell derived biochar pyrolyzed at 350°C)

T14=P500+HNS (Pecan shell derived biochar pyrolyzed at 500°C)

Soil collection and preparation

Soil used for this study was collected from the garden research plot at FIU. It is classified as a Krome loamy skeletal, carbonatic, hyperthermic lithic Udorthent, according to the USDA-NRCS Soil Series Classification Database (Velez et al., 2018). The soil had a pH of 7.52 and consisted of 9.9% carbon (C), 0.55% nitrogen (N), 15.5% organic matter (OM), 76% sand, 22% silt and 2% clay. The research plot soils were incorporated with cover crops from a previous study and compost produced onsite at an undetermined rate which attributed to the high OM content of the soil. The soil collected in March 2019 was passed through a 4 mm opening sieve, homogenized and amended with the twelve different biochars at the rate of 22.5 t/ha soil (1%, w/w). Approximately 6 kg of soil (dry weight basis) was lightly packed into 2-gallon nursery pots (7.6 L). Miracle grow (20N:8.7P:16.7K) was added as starter fertilizer at the time of planting seeds in the pots and approximately 500 ml of Hoagland's No. 2 Basal salt (with macro and micro nutrients) solution (HNS) was also added to each pot twice a week to eliminate nutrient limitations over the course of the growing period. Water was added every other day except on days when there was rain. Pots consisting of treatment T1 did not receive any starter fertilizer or HNS.

Potted plant experiment and plant parameter analysis

Jalapeno pepper (*Capsicum annuum* 'Jalapeno') was used as the plant for this study. It is one of the popular crops grown in Florida, USA. The U.S. demand for Jalapeno rises every year because of the growing popularity of ethnic cuisine [38]. The low calories and rich vitamins, minerals, fiber, antioxidants and bioactive compounds in Jalapeno are reported to have many health benefits including reduced risk of death due to cardiovascular diseases, tumor development and cancer [39,40]. Four seeds per pot were placed a few centimeters apart and about an inch deep on 22 March 2019. After seedlings emerged, the plants were thinned to one per pot. To evaluate the effects of the biochar treatments on plant, selected parameters were measured throughout the growing duration of Jalapeno using methods adapted from [41,42] Plant height (cm) and number of leaves were measured at 4, 6, 8, 10, 12 and 14 weeks after planting (WAP). Height was measured from the first cotyledon's node as a reference point to the uppermost leaf node. The average leaf chlorophyll content was measured using Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll meter at 6, 8, 10, 12 and 14 WAP. Fruits were harvested 90 days after planting (DAP). Yield was recorded from the weight (fresh) and number of fruits per treatment. The plants were removed from pots at 14 WAP and the leaves, branches and roots were collected for analysis. Roots were washed thoroughly to remove soil prior to any experiment. All samples were oven dried at 70°C for 72 hours to estimate leaf, branch, shoot and root dry biomass weight.

Enumeration of soil microbial population

Microbial population was estimated by a modified dilution spread plate method [43]. Soils collected at 40 DAP and 90 DAP were used for this experiment. Soil dilutions were made using sterile saline solution (0.85% NaCl) and vortex shaker was used for dispersion. Bacteria were cultured at three dilutions (10^{-4}, 10^{-5}, 10^{-6}) with two replicates each on tryptic soy agar (TSA) media. Fungi were cultured at three dilutions (10^{-3}, 10^{-4} and 10^{-5}) with two replicates each on corn meal agar (CMA) media containing streptomycin to limit bacterial growth. Plates containing 30-300 colonies were counted manually after 24 hours of incubation at 28°C for bacteria and after 7 days for fungi. The colony forming units (CFU) were calculated using the following formula:

$$\text{CFU/g soil} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume of culture plate (ml)} \times \text{dry weight of soil (g)}}$$

Estimation of arbuscular mycorrhizal (AM) fungi root colonization

The degree of arbuscular mycorrhizal (AM) fungi colonization in the root samples was performed following a modified method by [44]. At the end of the Jalapeno growth period, the roots of each plants were carefully washed in a 2 mm sieve to remove all remaining soil particles. 25 thin root fragments were removed from fresh root samples and submerged in micro centrifuge tubes containing 10% potassium hydroxide solution (KOH). The tubes were placed in the oven at 70°C for 2 hours prior to rinsing with deionized (DI) water. Since the roots were clean and already white, bleaching was not conducted. The roots were then stained by adding a 0.5% Trypan blue/lactoglycerol in the tube and placed in the oven at 70°C for 30 minutes. Finally, the samples were thoroughly washed to remove any excess blue stain. Each set of 25 roots was placed horizontally on a microscopic slide containing a drop of lactoglycerol solution. Each root was examined under a compound microscope and recorded for colonization which was indicated by the visual presence of any three structures: hyphae, vesicles, or arbuscules. The percentage of AM fungi colonization was calculated by the following formula:

$$\text{AM Fungi Root Colonization (\%)} = \frac{\text{Number of colonized roots}}{25} \times 100$$

Estimation of soil enzyme activity

Soil enzyme activities for β-1-4-glucisidase (C), alkaline phosphatase (P), and β-N-acetylglucosaminidase (N) enzymes were conducted using the fluorescent model substrate 4-methylumbelliferone (MUF) assay [45,46]. The soils collected at 90 DAP (during harvest) were used for this analysis. The substrates used for each of these enzyme assays were MUF-β-D-glucoside (MUF-C), MUF-phosphate (MUF-P), and MUF-N-acetyl-β-D-glucosaminide

(MUF-N). Enzyme activity was determined from the difference between the amounts of fluorescent substrate liberating during incubation time (tf) from time zero (t0). The amount of substrate liberated per gram of dry soil was determined by comparison to standard curves generated using known concentration of MUF substrates. Synergy HT Multi-Mode 96 well Plate reader was used to conduct this experiment.

Statistical analysis

Statistical analysis was performed using statistical analysis system (SAS 9.4 and JMP pro 14) and IBM SPSS Statistics 23. Two way analysis of variance (ANOVA) was carried out for the effects of different feedstocks and temperatures on plant growth parameters, microbial population and enzyme activities in soil. Significant differences between each treatment and the control as well as between treatments were also evaluated. Data was significant when $p<0.05$. Pearson multiple correlation coefficient analysis was conducted to show the relationship between biochar properties and the measured plant parameters, soil microbes and enzyme activities.

RESULTS AND DISCUSSION

Effect of treatments on plant parameters

The effect of different treatments on *Capsicum annuum* 'Jalapeno' plant growth and yield are presented in Figures 1 and 2 and Table 2. Overall, the plant height, number of leaves and SPAD values were not significantly influenced by the treatments. The biochar treatments also did not have any significant effect on the above ground (leaf, branch and shoot) biomass dry weight (g) and below ground (root) biomass dry weight (g) compared to the control (T2), however there was significant difference among the biochar treatments in their effects (Table 2). A significant influence from temperature on the above and below ground biomass weights in plants receiving treatments T6 (Brazilian pepper derived biochar at 500°C) and T11 (Cypress derived biochar at 350°C) was observed. Plants that received the treatment T6 had the highest shoot (45%) and root (43%) dry biomass weight compared to the rest of the treatments. The effect from T6 was also significantly higher than the plants receiving treatment T12. Some of the biochar treatments caused a reduction in shoot and root biomass dry weight, particularly, treatment T12 resulted in the lowest, but the difference was not significant compared to the control. An overall increase was observed in the shoot to root ratios of the plants

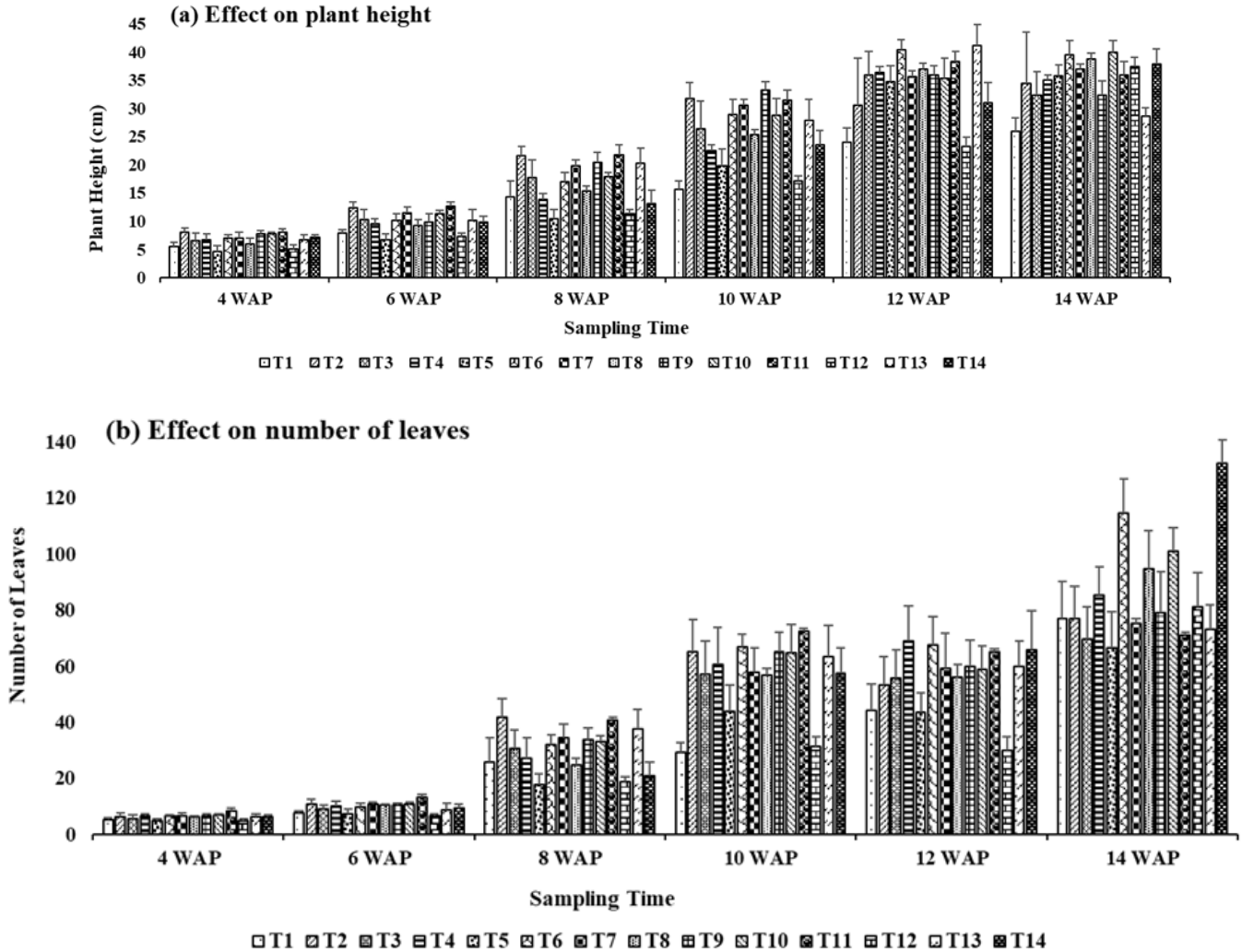

Figure 1: Effect of different treatments on (a) plant height and (b) number of leaves throughout the growing season of *Capsicum annuum* 'Jalapeno'. Error bar represents standard error of mean values. WAP = Weeks after planting

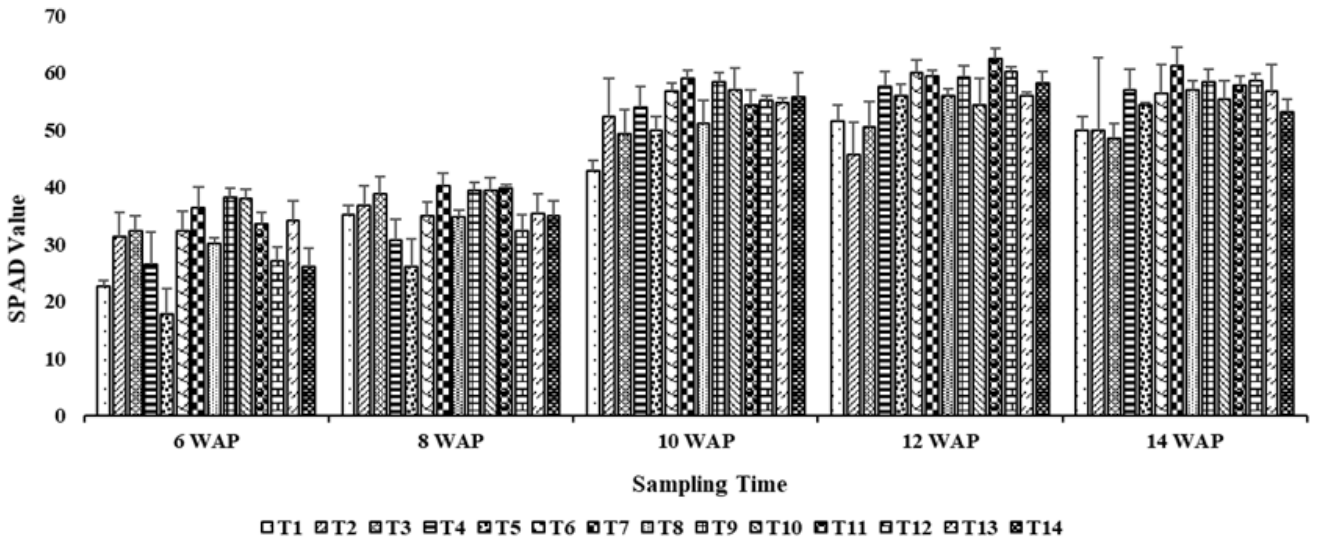

Figure 2: Comparison of soil plant analysis development (SPAD) chlorophyll meter value of the leaves throughout the growing season of *Capsicum annuum* 'Jalapeno'. Error bar represents standard error of mean values. WAP = Weeks after planting

Table 2: Effect of different treatments on plant leaf, branch, shoot and root dry weight, fruit number and fruit fresh weight.

Treatment	Leaf `Weight (g)	Branch Weight (g)	Shoot Weight (g)	Root Weight (g)	Shoot to Root Ratio	Number of Fruit	Fruits Weight (g)
T1	1.736 ± 0.28^{C}	1.55 ± 0.23^{C}	3.29 ± 0.48^{C}	1.12 ± 0.22^{C}	3.17	1.33 ± 0.29^{B}	10.96 ± 2.21^{B}
T2	6.42 ± 0.94^{AB}	4.90 ± 1.11^{ABC}	12.06 ± 2.11^{AB}	3.46 ± 0.95^{AB}	2.75	9.20 ± 1.08^{AB}	71.76 ± 13.17^{AB}
T3	4.33 ± 1.17^{ABC}	4.06 ± 1.08^{ABC}	8.39 ± 2.23^{ABC}	2.98 ± 0.70^{ABC}	2.73	10.00 ± 1.87^{AB}	84.98 ± 15.20^{AB}
T4	5.31 ± 1.29^{ABC}	4.98 ± 1.38^{ABC}	10.29 ± 2.66^{ABC}	3.48 ± 0.85^{ABC}	2.94	9.00 ± 1.96^{AB}	85.23 ± 11.84^{AB}
T5	4.00 ± 0.71^{ABC}	3.74 ± 0.72^{ABC}	7.74 ± 1.40^{ABC}	2.59 ± 0.39^{ABC}	2.92	5.75 ± 1.03^{AB}	48.35 ± 14.79^{AB}
T6	7.26 ± 0.73^{A}	6.63 ± 0.69^{A}	13.90 ± 1.34^{A}	4.71 ± 0.39^{A}	2.94	12.25 ± 1.70^{AB}	98.66 ± 8.28^{AB}
T7	5.9 ± 0.85^{AB}	5.13 ± 0.86^{ABC}	11.03 ± 1.71^{ABC}	3.74 ± 0.61^{ABC}	2.97	8.25 ± 0.75^{AB}	54.49 ± 9.26^{AB}
T8	4.76 ± 0.73^{ABC}	4.35 ± 0.92^{ABC}	9.11 ± 1.62^{ABC}	3.06 ± 0.58^{ABC}	3.02	5.40 ± 1.21^{AB}	48.16 ± 10.37^{AB}
T9	6.20 ± 0.75^{AB}	6.04 ± 0.73^{AB}	12.24 ± 1.48^{AB}	4.19 ± 0.48^{AB}	2.91	11.50 ± 1.19^{AB}	85.57 ± 16.00^{AB}
T10	5.17 ± 0.58^{ABC}	4.87 ± 0.97^{ABC}	10.04 ± 1.52^{ABC}	3.78 ± 0.66^{ABC}	2.74	9.00 ± 1.73^{AB}	71.82 ± 15.50^{AB}
T11	6.04 ± 0.77^{AB}	5.75 ± 0.62^{ABC}	11.79 ± 1.35^{AB}	4.21 ± 0.42^{AB}	2.78	11.00 ± 1.34^{A}	103.30 ± 12.84^{A}
T12	2.29 ± 0.27^{BC}	1.92 ± 0.32^{BC}	4.21 ± 0.58^{BC}	1.44 ± 0.23^{BC}	2.99	2.80 ± 0.58^{B}	22.47 ± 6.05^{B}
T13	5.14 ± 1.04^{ABC}	5.28 ± 1.20^{ABC}	10.42 ± 2.16^{ABC}	3.79 ± 0.91^{ABC}	2.85	6.40 ± 1.60^{AB}	65.74 ± 16.82^{AB}
T14	4.35 ± 0.83^{ABC}	3.67 ± 0.73^{ABC}	8.03 ± 1.54^{ABC}	2.91 ± 0.65^{ABC}	2.90	8.40 ± 1.33^{AB}	89.63 ± 13.35^{AB}

Values are expressed as mean ± standard error. Means within a column followed by the same letters are not significantly different at $p<0.05$

receiving biochar treatments than the control.

The variation among the biochar treatments significantly influenced the number and fresh weight (g) of Jalapeno fruits but the difference was not significant compared to the control. The Jalapeno yield (fruit number and weight) in plants receiving BP and Cy derived biochar treatments was significantly affected by the interactive effects of feedstock and temperature. Treatments T3 (Australian pine derived biochar at 350°C), T6 (Brazilian pepper derived biochar at 500°C), T9 (loblolly pine derived biochar at 350°C), and T11 (cypress derived biochar at 350°C) produced 9%, 33%, 25%, and 20% higher number of fruits than the control (not significant at $p<0.05$), respectively. Plants treated with T11 were found to have the maximum fresh weight of fruits which was almost double the average weight of fruits from all the remaining treatments. The effect of T11 on fruit weight was significantly higher than the plants receiving T12 (Table 2). Treatments T3 and T4 (Australian pine derived biochars at 350°C and 500°C), T6 (Brazilian pepper derived biochar at 500°C), T9 (loblolly pine derived biochar at 350°C), T11 (cypress derived biochar at 350°C) and T14 (pecan shell derived biochar at 500°C) produced fruits with fresh weight 18%, 19%, 38%, 20%, 44% and 25% higher than that produced from plants receiving the control (T2), respectively. Similar to shoot and root biomass dry weight, plants treated with T12 also resulted in the lowest number and fresh weight of fruits.

Biochars can have variable effects on plant growth [6-8]. The effect of biochars on plant growth depends on several factors including type of biochars, applicate rate, soil properties and plant species [47,48]. In this study no significant effects of the biochar treatments were observed on plant growth and yield compared to the control. A study conducted by [49] using spruce (*Picea abies* (L.) H. Karst.) Derived biochar made at 550-600°C also found no significant effects of biochars on the growth and yield of wheat. Similarly, [50] observed no distinct effect of biochar made from Eucalyptus sp. at 400°C-500°C on rice yield. Generally, improved plant growth and yield have been attributed to improved structure, pH conditions, water, and nutrient availability in soil due to biochar addition [51,52]. Even though soil nutrient levels were not measured in this study, the significant correlation between biochar pore size with the shoot and root biomass dry weights attribute towards the retention and slow release of nutrients to soil that can be conducive to the observed increase in Jalapeno biomass (Table 3) [21,53]. Additionally, biochars can serve as a fertilizer by supplying certain macro and micro nutrients to soil which may be present in the ash fraction during the production of biochars [42,54,55]. Biochars can also influence the bacterial diversity which can enhance nitrogen mineralization, thus improving plant nutrition and growth [55,56]. Lower plant biomass weight and yield can be attributed to the higher volatile matter (VM) content (>23%), C/N ratio and pH of the biochar [41,42,57,-59]. High C/N ratios can cause higher nitrogen immobilization and result in decreased nitrogen availability and uptake by plants. Availability of macro and micro nutrients needed for plant growth can be reduced by the high pH of biochars (pH>8). Toxic substances (e.g., phenols, furans, and oligosaccharides) can be present in biochars, specially biochars made from woody feedstock, and this can severely affect the plant growth [57]. The high VM content, C/N ratio and pH observed in some of the biochars may have been responsible for the reduced growth and yield in plants receiving those treatments even though no significant correlation was observed (Table 1).

Table 3: Pearson's multiple correlation coefficient values for the relationship between biochar properties and plant growth parameters.

	Biochar Average pore size	Leaf Weight	Branch Weight	Shoot Weight	Root Weight
Biochar Average pore size	1	0.603*	0.645*	0.626*	0.567*
Leaf Weight	0.603*	1	0.981	0.995	0.978
Branch Weight	0.645*	0.981	1	0.995	0.989
Shoot Weight	0.626*	0.995	0.995	1	0.988
Root Weight	0.567*	0.978	0.989	0.988	1

*=Correlation is significant at the 0.05 level

Enumeration of soil microbial population

The estimates of soil microbial population (colonies) based on dilution spread plate counts are presented in (Figure 3). The treatments significantly influenced the bacteria and fungi population in soil ($p<0.05$).

The treatments containing biochars made from coconut husk (T7 and T8) resulted in the highest number of bacteria colonies in soils collected at 40 days after planting (DAP) Jalapeno. This was 9.5% (significant at $p<0.05$) higher than the number of bacteria colonies observed in the control (T2). These treatments also resulted in 19%, 11%, 13%, 15% and 8% significantly higher bacteria colonies than the treatments containing Australian pine biochars (T3 and T4), Brazilian pepper biochars (T5 and T6), loblolly pine biochars (T9 and T10), cypress biochars (T11 and T12), and pecan shell biochars (T13 and T14), respectively. Soils treated with Australian pine biochars (T3 and T4) resulted in the lowest number of bacteria colonies, particularly treatment T4 (Australian pine derived biochar at 500°C) had the significantly lowest (14%) compared to control (T2) at 40 DAP soils. There was an overall average 10% increase (significant at $p<0.05$) in the bacteria population in soils collected at 90 DAP from soils at 40 DAP for most of the treatments including the control. Compared to the control (T2), soils treated with Australian pine

biochars (T3 and T4), Brazilian pepper biochars (T5 and T6), pecan shell biochars (T13 and T14), coconut husk biochars at 350°C (T7), and cypress biochars at 500°C (T12) resulted in marginally higher (an average 2%) bacteria colonies in 90 DAP soils. Treatment T10 (loblolly pine derived biochars at 350°C) had the lowest number of bacteria colonies at 90 DAP soils, which was significantly lower (7%) than soils treated with T5 (Brazilian pepper derived biochars at 350°C) but the difference was not significant compared to the control (T2). For different treatments it was the type of feedstocks that had significant effect on bacteria population in soil. The biochar specific surface area and pore size influenced the bacteria population as observed by the significant correlation (Table 4).

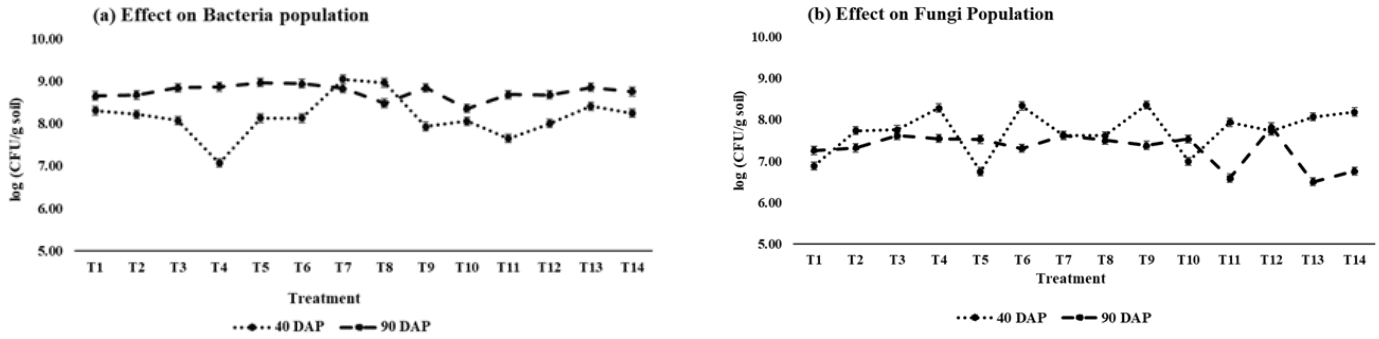

Figure 3: Effect of different treatments on (a) bacteria and (b) fungi population in soil 40 days and 90 days after plating *Capsicum annuum* 'Jalapeno' based on spread plate counts. DAP = Days after planting

Table 4: Pearson's multiple correlation coefficient values for the relationship between biochar properties and soil microbial population.

Biochar Average pore size		SSA	CEC	TPV	pH	Ash	C	N	Bacteria (40 DAP)	Bacteria (90 DAP)	Fungi (40 DAP)	Fungi (40 DAP)	AMF
Biochar Average pore size	1	0.608	0.014	0.064	0.063	0.063	0.399	0.188	0.130*	0.719**	0.392	0.305	0.322
SSA	0.608	1	0.196	0.719	0.462	0.392	0.357	0.250	0.156*	0.623*	0.189	0.608	0.200
CEC	0.014	0.196	1	0.004	0.182	0.028	0.524	0.387	0.039	0.266	0.378*	0.567*	0.112
TPV	0.064	0.719	0.004	1	0.868	0.783	0.196	0.718	0.096	0.041	0.096	0.387	0.720**
pH	0.063	0.462	0.182	0.868	1	0.853	0.385	0.788	0.434	0.039	0.091	0.354	0.673**
Ash	0.063	0.392	0.028	0.783	0.853	1	-0.531	0.852	0.270	0.158	0.098	0.175	0.739**
C	0.399	0.357	0.524	0.196	0.385	-0.531	1	0.735	0.119	0.403	0.021	0.273	0.676**
N	0.188	0.250	0.387	0.718	0.788	0.852	0.735	1	0.069	0.204	0.098	0.35	0.767**
Bacteria (40 DAP)	0.130*	0.156*	0.039	0.096	0.434	0.270	0.119	0.069	1	0.190	0.343	0.086	0.018*
Bacteria (90 DAP)	0.719**	0.623*	0.266	0.041	0.039	0.158	0.403	0.204	0.190	1	0.371	0.119	0.537*
Fungi (40 DAP)	0.392	0.189	0.378*	0.096	0.091	0.098	0.021	0.098	0.343	0.371	1	0.417	0.011
Fungi (90 DAP)	0.305	0.608	0.567*	0.387	0.354	0.175	0.273	0.35	0.086	0.119	0.417	1	0.353
AMF	0.322	0.200	0.112	0.720**	0.673**	0.739**	0.676**	0.767**	0.018*	0.537*	0.011	0.353	1

**=Correlation is significant at the 0.01 level
*=Correlation is significant at the 0.05 level

Similar to bacteria population, the fungi population was also significantly influenced by the treatments. The treatments T4 (Australian pine derived biochars at 500°C), T6 (Brazilian pepper derived biochars at 500°C), T9 (loblolly pine derived biochars at 350°C) and T14 (pecan shell derived biochars at 500°C) resulted in significantly higher number of fungi colonies in soils collected at 40 DAP when compared to the control (T2), which was 7%, 8%, 8%, and 6% higher, respectively. Treatment T9 also had 8%, 24%, 10%, 20%, and 7% significantly higher fungi colonies compared to treatments T3 (Australian pine derived biochars at 350°C), T5 (Brazilian pepper derived biochars at 350°C), T7 and T8 (coconut husk derived biochars at 350°C and 500°C), T10 (loblolly pine derived biochars at 500°C), and T11 and T12 (cypress derived biochar at 350°C and 500°C), respectively. Among the different biochar treatments T5 resulted in the lowest number of fungi colonies at 40 DAP soils, which was significantly lower (15%) than the control and the remaining biochar treatments, except T10. There was an overall average 8% decrease ($p<0.05$) in the fungi population in soils collected at 90 DAP from soils at 40 DAP for most of the treatments including control, except for T5 and T10 treated soils, where 12% and 8% increase were observed, respectively. The soils treated with T12 had a significantly higher (7%) fungi population compared to the control (T2) at 90 DAP soils. Considering the rest of the biochar treatments, T12 (cypress derived biochar at 500°C) resulted in 5%, 6% and 18% significantly higher fungi population than T5 and T6 (Brazilian pepper derived biochars at 350°C and 500°C), T9 (loblolly derived biochar at 350°C), and T13 and T14 (pecan shell derived biochars at 350°C and 500°C) at 90 DAP soils, respectively. Soils treated with pecan shell biochars, particularly T13 (pecan shell derived biochars made at 350°C) resulted in the lowest number of fungi colonies which was significantly lower (11%) than the control (T2) at 90 DAP soils. The correlation showed that the cation exchange capacity of the biochar significantly influenced the fungi population possibly by retaining and supplying necessary nutrients (Table 4).

The physicochemical properties of biochars, as well as the biochar induced changes in soil physicochemical properties can alter the activities of soil microorganisms. In this study it was observed that there was an increase in bacteria population whereas a decrease in fungi population by the effects of the different biochar treatments. A study conducted by Jones et al. (2012) using biochars made from European ash tree (*Fraxinus excelsior L.*), European beech tree (*Fagus sylvatica L.*) and European oak tree (*Quercus robur L.*) at 450°C also observed an increase in bacterial growth with an inhibition in fungal growth. Similar results were also found by [60] using biochars made from wheat (*Triticum aestivum*) at 350-550°C. Several factors can contribute to the change in microbial population in soil caused by the effect of biochars. The porous structure of biochars can potentially provide habitat for bacteria and fungi as well as protection from predators [61,62]. The relatively larger sized fungi can be restricted to live on the surface and in the macro pores of biochars, whereas relatively smaller sized bacteria can live inside the micro pores. This may result in higher chances of protection for the bacteria than the fungi from predators, especially on the smaller pores. The biochar itself or biochar modified soil can sorb toxins thus lowering the toxicity to microbes and enhancing microbial population in soil [27,63]. The addition of biochars increases the organic carbon content in soil which improves the retention and accessibility of nutrients to soil microbes [19]. The organic compounds (VM content) present in biochars during production may suppress some members of the microbial community and promote others [19,64]. The microbial growth can be influenced by the pH of the biochars or the biochar modified soil [60,65]. A neutral or slightly alkaline condition is favorable for bacterial growth but reduces fungal growth [66]. Another major component that can affect microbial population is the ash content of the biochars [19]. As previously mentioned, the ash content includes macro and micro nutrients which can be available for microbial uptake. In this study the specific surface area, pore size and cation exchange capacity of the biochars influenced changes in bacteria and fungi population.

Estimation of root colonization by arbuscular mycorrhizal (AM) fungi

The biochar treatments significantly affected the arbuscular mycorrhizal (AM) fungi colonization in the Jalapeno plant roots (Table 5). Comparing between the feedstock type and different temperatures on AM fungi growth, it was found that the feedstock types had significant influence.

Table 5: Effect of different treatments on arbuscular mycorrhizal (AM) fungi root colonization during *Capsicum annuum* 'Jalapeno' production.

Treatment	AM Fungi Root Colonization (%)
T1	34.0 ± 1.15^{BCD}
T2	21.0 ± 5.26^{D}
T3	29.0 ± 3.42^{CD}
T4	28.8 ± 3.88^{D}
T5	47.2 ± 6.74^{ABCD}
T6	44.8 ± 1.96^{ABCD}
T7	53.6 ± 6.25^{ABC}
T8	55.2 ± 3.20^{AB}
T9	57.6 ± 3.71^{AB}
T10	55.2 ± 3.44^{AB}
T11	61.6 ± 4.31^{A}
T12	65.6 ± 2.04^{A}
T13	59.2 ± 2.65^{AB}
T14	58.0 ± 4.76^{ABC}

Numbers are expressed as mean ± standard error. Means within a column followed by a different letter are significantly different at $p<0.05$.

Plant roots that received treatments containing coconut husk biochars (T7 and T8), loblolly pine biochars (T9 and T10), cypress biochars (T11 and T12), and pecan shell biochars (T13 and T14) resulted in 159%, 169%, 203%, and 179% higher (significant at $p<0.05$) root colonization by AM fungi than the control (T2), respectively. It can be noted that among the different biochar treatments, the ones containing Australian pine derived biochars had the lowest percentage of root colonization, which was significantly lower than the remaining biochar treatments, except

for treatments containing Brazilian pepper derived biochars.

There are several ways biochars could affect AM fungi colonization in plant roots including protection in the biochar pores from grazers, change in soil physicochemical properties and nutrient availability, change in soil microbial population that support AM fungi colonization, sorption of signaling compounds or detoxification of allelochemicals that inhibit AM fungi colonization [67]. The significant correlation between the total pore volume, pH, ash, carbon and nitrogen content of biochars with AM fungi explains the increase in root colonization possibly resulting from favorable habitat and beneficial nutrients (Table 4). Also, the correlation between bacteria and AMF suggests abundance of soil bacteria enhanced AM colonization (Table 4).

Estimation of soil enzyme activity

Significant changes in enzyme activities for β-1-4-glucisidase and alkaline phosphatase enzymes were observed in soils by the different biochar treatments but no significant influence in the β-N-acetylglucosaminidase enzyme activity (Figure 4). The type of feedstocks had significant influence on the β-1-4-glucisidase enzymes whereas the interactive effect of feedstock and temperature significantly influenced the alkaline phosphatase enzymes.

Soils that received treatment T11 (cypress derived biochar at 350°C) showed the highest (3.5 times) alkaline phosphatase enzyme activity compared to the control (T2) (significant at $p<0.05$). Soils treated with T13 (pecan shell derived biochars at 350°C) resulted in the lowest alkaline phosphatase enzyme activity, which was significantly lower (98%) from the soils that received biochar treatment T11 but not significant compared to the control. Treatment T6 resulted in β-1-4-glucisidase enzyme activity 9 times significantly higher than the control. A relatively low activity was observed for β-1-4-glucisidase enzyme and β-N-acetylglucosaminidase enzyme whereas higher activity for alkaline phosphatase was found in soils treated with different biochars.

A Number of factors influence the effect of biochars on soil enzyme activity. Changes in nutrient availability and microbial population by biochar addition could affect soil enzyme activities [68,69]. As a huge portion of carbon is present in a stable form in biochars it may not act as a stimulant to enzyme activity in soil, therefore, comparatively lower β-1-4-glucisidase activity can be observed by different biochar applications [70]. Soil enzymes

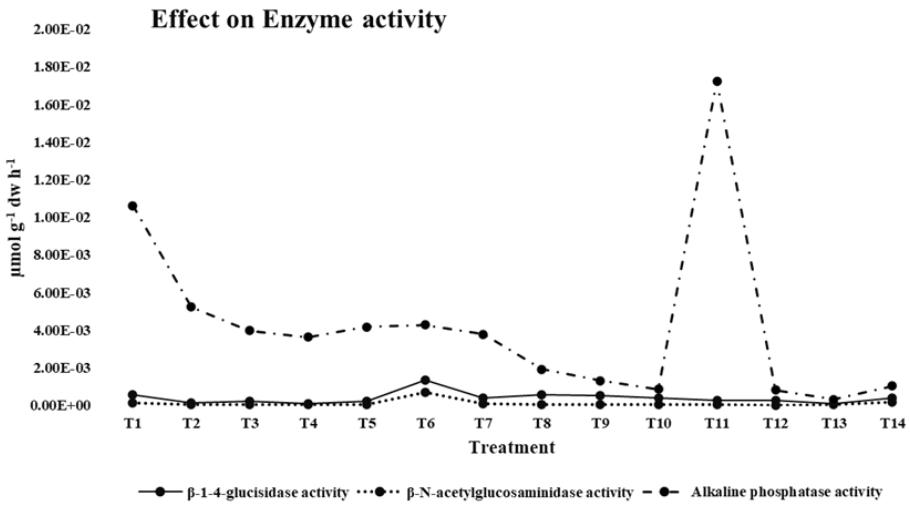

Figure 4: Effect of different treatments on β-1-4-glucisidase, β-N-acetylglucosaminidase, and alkaline phosphatase enzyme activities in soil using the fluorescent model substrate 4-methylumbelliferone (MUF) assay.

and substrates can be sorbed to biochar particles (CEC, SSA and the pore structures of the biochars), thus interfering with the rate of substrate diffusion to the active site of enzyme catalysis and reduce or even inhibit enzyme activity in soil [33,36]. Phosphatase enzyme activity is pH dependent and the high pH of biochars can influence the higher alkaline phosphatase activity in soil [71]. In this study the relatively higher phosphatase activity can be attributed to the higher pH of the biochars as observed from the correlation (Table 6). The relatively low activity observed for β-1-4-glucisidase enzyme and β-N-acetylglucosaminidase enzyme may have resulted due to the negative correlation with biochar total pore volume that caused inhibition of enzyme activity by trapping of the enzymes or even the substates to biochars (Table 6). Biochars made from Brazilian pepper and cypress also enhanced the bacteria population in soil which may have indirectly influenced the higher enzyme activities in soils treated with these biochars.

CONCLUSION

This study found that the biochars did significantly affect the soil bacteria and fungi population, AM fungi root colonization and soil enzyme activities but no significant effect on the Jalapeno pepper growth and yield were observed. Invasive plant species and agricultural wastes or residues are difficult to manage and can be expensive. We found that two common South Florida invasive plants Australian pine, Brazilian pepper were effective to enhance plant productivity and yield. We also found that biochars made from coconut husk and pecan nut shells which are considered as agricultural wastes, increased mycorrhizal fungi activity which in turn is beneficial for plant. Therefore, utilizing these invasive plants and agricultural residues to produce biochars for soil conditioning and crop production will increase the overall agricultural sustainability. As potted plant growth cycles are relatively short, it might have been a limiting factor for adequately investigating the effects of biochars on Jalapeno. Applying this study on a field

Table 6: Pearson's multiple correlation coefficient values for the relationship between biochar properties and soil enzyme activities.

	TPV	pH	Alkaline phosphatase Enzyme	β-1-4-glucisidase enzyme	β-N- acetylglucosaminidase enzyme
TPV	1	0.785	-0.113	-0.631*	-0.761*
pH	0.785	1	0.191*	0.442	0.553
Alkaline phosphatase Enzyme	-0.113	0.133*	1	-0.004	-0.019
β-1-4-glucisidase enzyme	-0.631*	0.442	-0.004	1	0.887
β-N-acetylglucosaminidase enzyme	-0.761*	0.553	-0.019	0.887	1

*= Correlation is significant at the 0.05 level

scale and measuring soil properties will allow for better evaluation of the effects of the biochars on plant growth. The results suggest that the surface behavior (CEC and SSA), pore structure (TPV and pore size) of the biochars played important role in affecting microbial population in soil along with the effects from the pH and ash content of the biochars. The enzyme activities in soil were influenced by pore volume and pH of the biochars. The characteristics of biochars vary profoundly depending on the type of feedstock and pyrolysis temperature which in turn greatly influence the biochar application outcomes. Therefore, it is important to carefully select feedstocks and pyrolysis process to produce biochars in order to meet specific goals. In this study the type of feedstocks had a greater influence on the biochar application outcomes than the selected production temperatures.

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The authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are included in the manuscript.

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Project design, execution and original draft preparation: Shagufta Gaffar; Data collection and analysis: Shagufta Gaffar and Clara Riquelme; Supervision and final manuscript review: Krisnaswamy Jayachandran.

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