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Response of Lettuce (*Lactuca sativa* L.) Inoculated With *Rhizophagus irregularis* to Charcoal Amended Tabela Sand

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Abstract

The presence of marginal or degraded soils across the hinterland regions of Guyana has stymied efforts to expand agriculture. Charcoal can be used as an organic soil amendment to improve soil quality and agricultural productivity. In a greenhouse study, four charcoal (derived from *Dimorphandra conjugate* Splitg.) application rates of 0, 10, 20 and 30 t/ha were used to amend Tabela sand (acidic and infertile soil from the intermediate savannahs of Guyana). *Lactuca sativa* L. (lettuce) was used as an indicator crop to evaluate the optimum charcoal application rate for plant growth when inoculated with an arbuscular mycorrhizal fungus (AMF), *Rhizophorus irregularis*. Evidence from this study showed that as charcoal application rates increased, root length, yield of lettuce, root colonisation and the abundance of AMF spores increased. Soil pH, organic carbon, cation exchange capacity and water holding capacity increased significantly ($p < .05$), while soil bulk density and exchangeable acidity showed marked decrease as charcoal application rates increased. In addition, considerable increases were observed in total nitrogen, and available phosphorus, potassium, magnesium, manganese and zinc. In contrast, notable decreases were observed for available iron and copper, also. Therefore, charcoal amended Tabela sand in combination with AMF has the potential to increase the productivity of soils.

***Keywords*:** Soil amendment, charcoal, *Rhizophorus irregularis, Lactuca sativa* L.

1. Introduction

Population growth and increasing urbanisation are transforming the global landscape at a rapid pace. Such transformation requires increased food production to attain food security. This implies the need for a shift from traditional agriculture to contemporary means of agriculture. Current practices include the use of synthetic agrochemicals, high energy input and genetically modified germplasm, and mechanisation (Gomiero et al., 2011). These methods are not without shortcomings and may result in reduction in soil fertility owing to soil compaction, loss of organic matter, water retention capacity and biological activity, and a large source of water pollutants derived from sediment salts, fertilisers, and pesticides (Gomiero et al., 2011).

The~~se~~ negative impact~~s~~ associated with conventional agriculture have encouraged farmers to shift towards sustainable agriculture which protects the integrity of the environment, human health, and soil productivity. To achieve these results farmers experimented with soil amendments to restore degraded lands as well as utilise marginal soils (Hue & Silva, 2000). These innovations of sustainable agricultural practice align well with the sustainable land management trajectory of Sustainable Development Goal 15.

Over the years, agriculture has played a key role in the growth of Guyana's economy by contributing 25% to the gross domestic product (GDP) (International Trade Administration, 2023). This success in the agricultural sector propelled the Government of Guyana to expand and diversify agriculture beyond the coast (where conventional agricultural practices are prevalent) to hinterland locations such as the intermediate and southwest savannahs. These efforts are executed by the National Agricultural Research and Extension Institute (NAREI) to enhance the traditional and non-traditional crop sectors (Ministry of Agriculture, 2016). Some of these crop diversification initiatives include the increased exportation of coconut (*Cocos nucifera* L.), cassava (*Manihot esculenta* Crantz*)*, plantain (*Musa paradisiaca* L.), pineapple (*Ananas comosus* L.), pepper (*Capsicun sp.* L.), corn (*Zea mays* L.), and soybean (*Glycine max* L.) (Ministry of Agriculture, 2016). Lettuce (*Lactuca sativa* L.) was used as the test crop, since it is in keeping with the agenda outlined by the Government of Guyana for crop diversification.

However, the major limitation of diversification and expansion of agriculture in the hinterland is the presence of low fertility, highly leached, acid sand and sandy loam soils (Ahmad, 1989; Simpson, 1988). Various studies on the relationship between soil fertility and sustainable agricultural potential of the hinterland have shown that the acidic nature of soil in the intermediate savannahs negatively affects agricultural sustainability (Ahmad, 1989; Bullen, 1996; Bullen, 1989; Bullen et al., 1983; Bullen et al., 1982; Chesney et al., 2010; Chesney, 1975; Chesney, 1973; Chesney, 1972; Simpson, 1988). Therefore, organic soil amendments could be introduced in these soils to improve soil conditions and better sustain agriculture.

Biochar and charcoal are two methods used to improve soil fertility of marginal and degraded soils. The application of biochar could enhance soil fertility of marginal soil by improving soil organic matter content and soil moisture. Biochar production is comparable to charcoal production, which is among the oldest and most established procedures developed by humans to improve soil fertility. Biochar is a carbon-rich product made through thermal decomposition of various biomass and biomass residue (organic material) under no or limited oxygen. However, charcoal is traditionally made from wood (Hornung et al., 2024). Despite the considerable benefits of biochar as a soil amendment, most of the marginal and degraded soils in Guyana are in the Soesdyke Linden area and the intermediate savannahs where the local population primarily uses the traditional pit method to produce charcoal. Charcoal made from the utilisation of simple pits using wood biomass is considered an effective option as it is widely practiced in most rural areas (Bayabil et al., 2015). Charcoal produced by this method is done in a low oxygen-controlled environment than those produced from sophisticated kilns, which influences the quality of charcoal (Bayabil et al., 2015). Historically, charcoal has had the potential to effectively amend soil, thereby improving soil fertility through significant improvement of the soil's chemical, physical and microbial properties (Siregar, 2007).

Plant materials contain copious amounts of nutrients that could be subjected to pyrolysis to produce organic amendments. Plant residues are another source of organic amendment that contains generous amounts of plant nutrients such as carbon (C), nitrogen (N), potassium

(K), phosphorus (P), calcium (Ca) and magnesium (Mg) (Laird, 2008). The transformation of plant residues via pyrolysis to produce charcoal has the potential to return the majority of these nutrients to the soil (Laird, 2008). Longstanding rewards of charcoal applications on nutrient availability to plants are derived from enhanced stabilisation of organic matter, slow nutrient release from organic matter, and a greater cation exchange capacity (Ricigliano, 2011). Alterations in the availability of nutrients to plants may also be justified by changes in the chemical and physical parameters of the soil. These parameters include fluctuation in pH, elevated soil cation exchange capacity, increased water holding capacity, and reduction in bulk density (Gale et al., 2017). The availability of P and base cations is prevalent due to increases in soil pH around neutral values and cation exchange capacity (Glaser et al., 2002). Studies have shown that charcoal application up to 20% increased organic C and total N in marginal soils. However, available P increased significantly when charcoal was applied at a rate of 10% (Siregar, 2007). Ding (2010) reported that a charcoal application rate of 0.5% at a depth of 0-10cm limited the leaching of NH_4^+-N and the total loss of NH_4^+-N was reduced by 15% when charcoal was added. Cation exchange capacity was higher at kiln sites which increased by 15.2% because of the large surface area and intricate pore structure of charcoal residues at kiln sites. Sohi et al. (2010) also reported that high cation exchange capacity is indicative of the soil's ability to retain vital nutrient cation in a form accessible to plants and reduce leaching. It is evident that charcoal application at different rates improve the fertility of marginal and degraded soils.

Low water retention in many unproductive soils is linked to low soil organic matter, which could be improved with the addition of charcoal. It can enhance physical soil properties such as water retention and aggregation, thereby increasing water availability for crops and decreasing erosion (Gale et al., 2017). Similarly, Glaser et al. (2002) reported that water retention was 18% higher in soils when charcoal was added as compared to soils where no charcoal was added. Charcoal acts as a bulking agent which improves soil structure after application. A reduction in the bulk density of charcoal amended soil enhances water holding capacity, root development and microbial activity (Hussain et al., 2017; Zhang et al., 2010). Studies have also shown that rice hull charcoal decreased bulk density by 7% and 14% when it was applied at 2% and 4% respectively (Mishra et al., 2017). Therefore, the addition of charcoal to unproductive soils improves water holding capacity and aids in enhancing crop production.

Rhizophagus irregularis, which is characterised by the production of arbuscule, vesicles, and spores found within the plant root and soil, has proven to be an effective growth enhancer for various agriculturally important crops (Tisserant et al., 2013). As a symbiont, *R. irregularis* is very effective in the mobilisation, uptake, and transfer of mineral nutrients such as inorganic orthophosphate ions, nitrogen (N) and sulphur (S) from the soil to plants (Tisserant et al., 2013). Studies have shown that *R. irregularis* significantly increased the growth of wheat (*Triticum aestivum* L.), with aboveground biomass increases greater than 22% and a positive correlation between mycorrhizal root colonisation and mycorrhizal soil infectivity (Wahbi et al., 2015).

Charcoal has various benefits as a soil amendment in agriculture since it improves soil health and fertility, but these functionalities have been understudied in Guyana. Knowledge about the use of charcoal in agriculture within the hinterlands of Guyana and its agronomic value as it relates to crop response and soil benefits is limited. Despite the foregoing studies, the influence of charcoal soil amendments on plant growth inoculated with *R. irregularis* within the Guyana context is still unknown. Lettuce has also been documented as a good test crop for *ex-situ* experiments that use charcoal treatment as a soil amendment and *R. irregularis* as an inoculant (Abdelaziz, 2018; Wahbi, 2015). Lettuce also provides a good mimic to other crops needed in the hinterland and can grow in sandy soils of the hinterland; however, application test is needed on a larger scale *in-situ* to better evaluate effect. This study assessed the response of lettuce (*Lactuca sativa* L.) inoculated with *R. irregularis* to charcoal amended Tabela sand. The objective was to investigate the effect of physicochemical properties of charcoal amended Tabela sand on the growth of lettuce inoculated with *R. irregularis*.

2. Methodology

2.1 Sample collection

Soil samples were collected from the National Agricultural Research and Extension Institute (NAREI) research station (6° 9' 19" N and 58° 14' 21" W), situated at Kairuni along the Soesdyke-Linden Highway, Region 4, Guyana. Within this area of sample collection, soils were collected from an area covered with natural vegetation where no crops were previously grown. Tabela sand (Guyana Soil Series Mapping Unit 800) samples were collected at a depth of 0-20cm for this experiment. The samples were stored in labelled Ziploc® bags and transported to NAREI, Mon Repos, East Coast Demerara, Guyana for experimental set up under shaded conditions. The maximum temperature of the shade-house during the experimental phase of the study (November 2017 to April 2018) was 32℃ inside and 30℃ outside compared to hinterland temperature of 29℃ – 32℃. The roof of the shade-house was enclosed with ultraviolet plastic and a black shade mesh, and the sides were enclosed with white shade netting. This facilitated an environment with gentle to moderate wind, similar to that of the hinterland. Each experimental unit was a 71cm x 71cm wooden frame with a depth of 15.3cm. Units were fitted with fine mesh at the bottom to prevent soil loss and placed on elevated platforms to mitigate flooding.

2.2 Charcoal production and sowing of seeds

Charcoal was produced from the *Dimorphandra conjugate* Splitg. (Dakama) wood species—which is readily available in the hinterland and not a threatened species within the Guyana context—under slow pit pyrolysis at 500℃. This slow pit pyrolysis process was done in a low oxygen-controlled environment to remove water and volatile constituents from the vegetative biomass of *D. conjugate*. The produced charcoal contained a carbon content of 77.1%, ash content of 1.3%, water holding capacity of 65.5%, bulk density of 0.7 g/cm^3, and volatile matter of 14.7%. Each unit was filled with 100kg of autoclaved (121℃ for 30 minutes to eradicate bacteria, fungi, and soil insects, thereby standardising treatment) Tabela sand and charcoal (sieved using 4mm mesh). Seeds were sown in seedling trays and seedlings were transplanted after three weeks. Lettuce seedlings were inoculated with AMF powder (3.8g per plant) at transplant and transferred to the experimental units at a plant spacing of 20cm in rows and 20cm between rows, which was equivalent to a planting density of 250,000 plants/ha. Plants were watered daily using a sprinkler water hose method to reflect natural precipitation, and the shade-house created a similar environment to the hinterland. Four cropping cycles were done, with each cropping cycle considered the time from transplanting of seedlings to harvesting under four treatment rates of 0, 10, 20, and 30 t/ha.

2.3 Experimental design and application rate

This research was experimental in nature with a focus on the *ex-situ* application of treatments to ascertain plant growth responses to soil amendments. A completely randomised design was used to conduct this study. The experiment contained four treatments and four replicates, for a total of 16 experimental units. The four treatments were: T1 - no application of charcoal (control, 0 t/ha); T2 - application of charcoal at 1 kg/m^2 (10t/ha); T3 - application of charcoal at 2 kg/m^2 (20t/ha); and T4 - application of charcoal at 3 kg/m^2 (30t/ha).

2.4 Soil analysis

Baseline soil samples were collected from all replicates within each treatment at a depth of 0-6cm to analyse physical and chemical parameters. Soil samples were also collected at the end of each cropping cycle, air dried, and analysed for physical and chemical properties and quantity of spores.

The NAREI manual of standard analytical procedures for soil chemistry laboratory was used to guide physiochemical soil analysis. A soil-to-water ratio of 1:2.5 using 10g of sieved soil was prepared to measure pH and electrical conductivity (EC) with a digital pH metre and an electrical conductivity metre respectively. Exchangeable acidity (EA) was determined using the potassium chloride (KCl) method by adding 10g of sieved soil to the extractant of KCl (NAREI, 2008). Cation exchange capacity (CEC) was calculated by adding the exchangeable cations (Ca, Mg, K, Na) derived from the atomic absorption spectroscopy and the EA values (NAREI, 2008). Organic carbon (OC) was measured using the Walkley-Black method by adding concentrated H_2SO_4 to a mixture of 10g sieved soil and aqueous $K_2Cr_2O_7$ to oxidise organic matter, followed by a chromic acid titration with ferrous sulphate (NAREI, 2008).

The Guyana Sugar Corporation (GuySuCo) manual of standard operating procedures for soil analysis was used for nutrient analyses. Nutrient analyses were done for pure charcoal and soil samples prior to transplanting to obtain baseline data and after each cropping cycle. Total N was analysed using the total Kjeldahl digest and measured spectrophotometrically (GuySuCo, 2013). Phosphorous (P) was analysed using the Bray Exchangeable Phosphorus method and measured spectrophotometrically (GuySuCo, 2013). Ammonium acetate (1 molar) was used to extract K, Ca, Mg, Zn, and Mn and measured using atomic absorption spectroscopy (NAREI, 2008). The ash content of charcoal was analysed by the Guyana Sugar Corporation Central Laboratory, La Bonne Intention Estate, East Coast Demerara, Guyana. Particle size distribution analysis of Tabela sand was done using the hydrometer method by thoroughly mixing 50g sieved soil with 100ml 5% Calgon solution and letting mixture sit for a minimum of 12 hours to effectively disperse the soil into clay, silt, and sand fractions (GuySuCo, 2013).

Bulk density (BD, g/cm^3) was done utilising the core method (core 5cm in diameter and 5cm in length) and calculated using Equation 1 (Walter et al., 2016):

$$Bulk\ density = \frac{Oven\ dry\ weight\ of\ soil}{Volume\ of\ soil} \tag{1}$$

Water holding capacity (WHC) was measured directly by adding 50g of soil to a sample container followed by a slow addition of water to the container. The mixture was agitated gently until excess water was observed. The mixture was left to stand for 24 hours to ensure the homogeneity of the water throughout the sample. The mixture was drained by gravity through filter paper. Samples were weighed using a digital scale and placed in a convection oven at 110°C for 24 hours. Samples were weighed repeatedly until a constant weight was obtained. Water holding capacity (%) was calculated using Equation 2 (Yu et al., 2013):

$$Water\ holding\ capacity = \frac{Weight\ of\ wet\ soil - Weight\ of\ dry\ soil}{Weight\ of\ dry\ soil}\ x\ 100 \qquad (2)$$

2.5 Plant growth analysis

Plants were harvested 40 days after transplanting and growth parameters were recorded at harvest. Fresh weight (g) was recorded, the number of leaves was quantified by visual counting, and root length (cm) was measured. Leaf surface area was measured using the Image J software (Rasband, 2012). Three mature leaves from each plant were used to assess the leaf surface area by fully expanding leaves for analysis (Hunter et al., 2015).

The root-to-shoot ratio was determined by removing all soil debris from the plant. The roots were separated from the above ground part of the plants at the soil line and weighed separately. Plants were dried at 80°C for 24 hours then weighed. The root-to-shoot ratio was calculated using Equation 3:

$$Root\ to\ shoot\ ratio = \frac{Dry\ weight\ for\ roots}{Dry\ weight\ for\ top\ of\ plant} \qquad (3)$$

Plant shoots were dried at 80°C for 24 hours, ground, passed through a 0.5mm sieve, and placed in bags to be analysed for macronutrients (N, P, K, Ca and Mg) and micronutrients (Fe, Cu, Mn and Zn). Total N, P, K, Ca, Mg, Zn, and Mn were measured using the methods described above to measure these parameters in soil (NAREI, 2008).

2.6 AMF spore analysis in soil

Soil was sampled randomly from the active feeding zone (depth of 0-6cm) in each replicate at the end of each cropping cycle to count AMF spores. The wet sieving and decanting technique were used to quantify spores in the soil (Gerdemann & Nicolson, 1963). A hemocytometer was used to count the fungal spores in a liquid suspension. The suspension (1ml) was pipetted into the engraved counting chamber and allowed to stand for 2 minutes for the settlement of spores at the bottom. A cover glass was placed over the grid of the counting chamber to eliminate air bubbles between the slide and cover glass. The number of spores (n) was quantified using a microscope (40x) (Brundrett et al., 1996). The number of spores per ml of suspension was calculated mathematically using Equation 4:

$$Spores/ml = (n)\ x\ 10^4 \qquad (4)$$

2.7 Colonisation of roots

Root samples were collected from 10 plants per replicate (40 plants per treatment), washed with distilled water, and cut into 1cm segments. The AMF root colonisation percentage was estimated according to the gridline intersect across 100 root intersections observed under a microscope (40x) as outlined by Phillips and Hyman (1970).

2.8 Statistical analysis

All data were analysed using the statistical programme Statistix version 9.0 (Analytical Software, 2008), at an acceptable α-level of 0.05 (95% confidence level). A complete randomised design analysis of variance (ANOVA) was used to assess significant differences in relation to the performance of treatments within each cropping cycle. Cropping cycles were then combined to give an overall assessment of the treatments in all cropping seasons. This was done to increase the power of the ANOVA test in the study. Means separation was done using the least significant difference (LSD) tested by all-pairwise comparisons.

3. Results and Discussion

3.1 Physicochemical soil analysis in relation to treatments and cropping cycles

Statistical analysis revealed significant increases in soil pH as charcoal application rates increased from 0t/ha to 30t/ha in each cropping cycle (Table 1). The highest increase in pH was recorded for cropping cycle four. These findings corroborated that of Mishra et al. (2017), which reported soil pH increases by 0.1 and 0.2 units when rice hull charcoal was applied at application rates of 2% and 4% respectively. Increases in soil pH in relation to treatments within each cropping cycle may have been due to the high pH (8.3) of the charcoal used. Additionally, increases in soil pH may also be derived from the presence of ash (1.80%) contained in charcoal. The alkaline nature of charcoal can support its use as a liming agent in acidic soils as reported by Siregar (2007), where charcoal increased the pH by 0.4-0.6 units compared to the control.

Organic carbon increased significantly ($p < .02$) as charcoal application rates increased (0 t/ha to 30 t/ha) within each cropping cycle (Table 1). Even though organic carbon increases in this study were lower compared to that of Siregar (2007), charcoal amended soil at application rate of 30 t/ha increased organic carbon up to 1.2% (Table 1). Siregar (2007) reported that charcoal application at a rate of 10% significantly increased organic carbon up to 20% in marginal soil. The increase in organic carbon is attributed to the high carbon content of charcoal.

Soil EA ranged from 0.20 – 1.50 meq/100g across the four cropping cycles, and significant differences ($p < .05$) were recorded for EA in cropping cycles two and three (Table 1). These differences may be due to the higher CEC of charcoal, which has the capacity to bind Al and Fe with the soil exchange sites (Hayawin et al., 2014). Soil EC ranged from 0.15 – 0.30 mmhos/cm, with minimal variability among treatments and significant difference ($p < .05$) recorded for EC in cropping cycle two only. Increases in EC are linked to oxygen content reduction and particle size changes (Kane et al., 2021). Adinaveen et al. (2016) also reported that EC of wood materials subjected to a thermal process result in the conversion of the cellulose component into carbon. The resultant carbon powder is subjected to pressure, which increases the conductive phase and lead to increased EC. Such a mechanism may in part contribute to the EC levels recorded in charcoal amended soil. Soil CEC varied among treatments, ranging 2.34 – 6.50 meq/100g across the four cropping cycles, with significant differences ($p < .05$) in cropping cycle three only (Table 1). The CEC levels recorded in this study was lower compared to those found by Hayawin et al. (2014) for charcoal induced treatment. The recorded CEC values depict the number of exchangeable cations that are Ca^{+}, K^{+}, Mg^{+}, and Na^{+}. The highest CEC recorded in this study at 30 t/ha for cropping cycle four will produce the highest negative charge, suggesting that the soil can hold more cations. This negative surface charge of organic materials creates retention sites for nutrient cations (Hayawin et al., 2014).

Table 1

Comparison of selected soil physicochemical parameters in relation to treatments within each cropping cycle

Treatment	CEC (meq/100g)	EC (mmhos/cm)	EA (meq/100g)	pH	WHC (%)	BD (g/cm³)	OC (%)
			Cropping cycle one				
0 t/ha	2.80^{a}	0.30^{a}	0.20^{a}	5.20^{d}	20.81^{c}	1.15^{a}	2.20^{d}
10 t/ha	2.50^{a}	0.30^{a}	0.30^{a}	5.40^{c}	22.57^{b}	1.10^{a}	2.80^{c}
20 t/ha	3.10^{a}	0.30^{a}	0.30^{a}	5.50^{b}	23.18^{b}	1.04^{a}	3.20^{b}
30 t/ha	3.40^{a}	0.30^{a}	0.20^{a}	5.70^{a}	25.95^{a}	0.98^{b}	3.30^{a}
			Cropping cycle two				
0 t/ha	2.34^{a}	0.19^{ab}	0.24^{ab}	5.30^{d}	26.20^{c}	1.20^{a}	2.40^{d}
10 t/ha	2.83^{a}	0.22^{a}	0.22^{b}	5.62^{c}	29.30^{b}	1.10^{a}	3.04^{c}
20 t/ha	2.43^{a}	0.15^{b}	0.32^{a}	5.84^{b}	31.80^{b}	0.90^{b}	3.22^{b}
30 t/ha	2.72^{a}	0.16^{ab}	0.22^{b}	6.16^{a}	35.80^{a}	0.90^{b}	3.59^{a}
			Cropping cycle three				
0 t/ha	5.20^{a}	0.20^{a}	1.50^{a}	5.30^{d}	29.70^{b}	1.20^{a}	2.50^{d}
10 t/ha	4.20^{ab}	0.20^{a}	0.60^{ab}	5.80^{c}	31.10^{b}	1.00^{b}	3.20^{c}
20 t/ha	3.80^{b}	0.20^{a}	0.30^{b}	6.10^{b}	37.70^{a}	0.80^{c}	3.10^{b}
30 t/ha	4.20^{ab}	0.20^{a}	0.20^{b}	6.40^{a}	38.60^{a}	0.80^{c}	3.60^{a}
			Cropping cycle four				
0 t/ha	6.20^{a}	0.20^{a}	0.20^{a}	5.50^{d}	29.10^{b}	1.10^{a}	2.80^{d}
10 t/ha	5.70^{a}	0.20^{a}	0.20^{a}	5.90^{c}	31.20^{b}	0.70^{a}	3.20^{c}
20 t/ha	5.30^{a}	0.20^{a}	0.20^{a}	6.30^{b}	36.10^{a}	0.80^{b}	3.60^{b}
30 t/ha	6.50^{a}	0.30^{a}	0.20^{a}	6.60^{a}	37.70^{a}	0.80^{b}	3.70^{a}

Note. Different letters (a - d) indicate statistically significant differences ($p < .05$) using LSD test between each treatment per variable. CEC – cation exchange capacity, EC – electrical conductivity (EC), EA – exchangeable acidity (EA), WHC – water holding capacity, BD – bulk density, OC – organic carbon

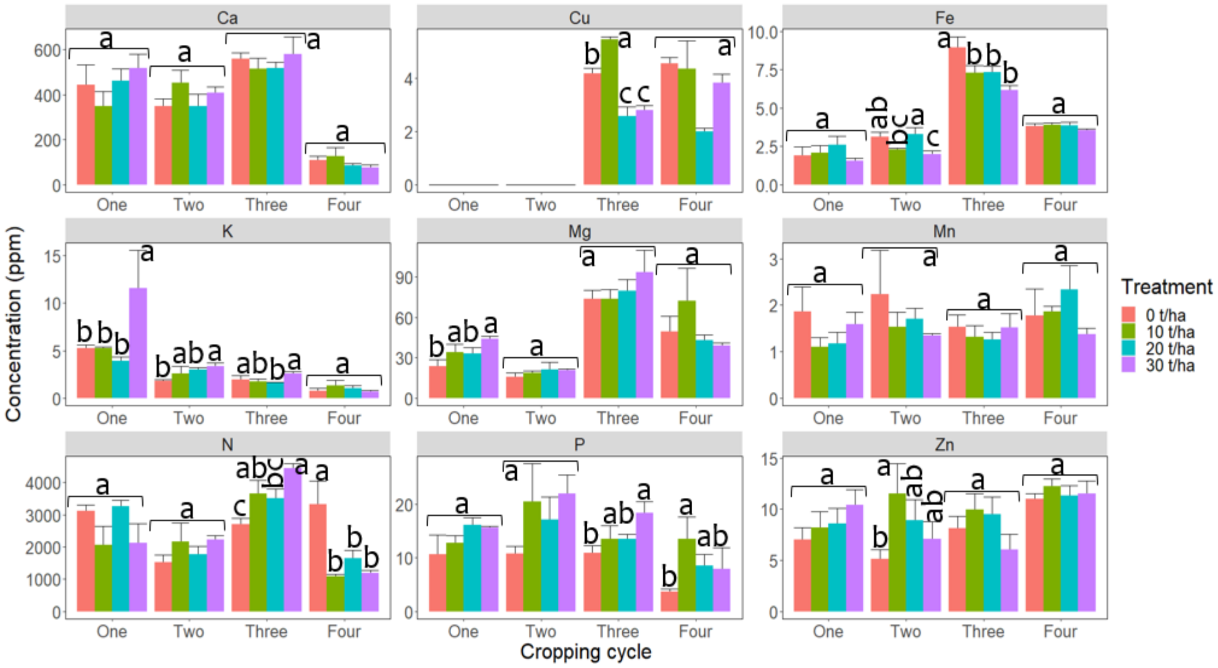
There were significant increases ($p < .01$) in water holding capacity of Tabela sand, while significant decreases ($p < .01$) were recorded in bulk density for each cropping cycle as the rate of charcoal application increased (Table 1). To diversify and expand agriculture into the hinterland, improving the water holding capacity of the sand and sandy loam soils at those locations is imperative. Water holding capacity needs to be improved since hinterland soils are drained excessively; thus, these soils would not provide sufficient water for crop production and would also contribute to the leaching of nutrients. The charcoal used in this experiment has a water holding capacity of 65.5%. The presence of small pores in charcoal increases its ability to improve soil water-holding capacity. This study shows that charcoal can be used to effectively improve the water holding capacity of excessively drained soils, thus increasing water use efficiency, reducing the occurrence of nutrients leaching from the soil, and increasing water availability for crops (Glaser et al., 2002). Although the increases in water holding capacity for this study was lower compared to other studies (Dugan et al., 2010; Glaser et al., 2002), a maximum of 9.6% increase in water holding capacity was evident for charcoal induced treatment from 0 – 30 t/ha. Such increases in water holding capacity had a positive effect on lettuce. Glaser et al. (2002) showed that water retention was 18% higher in soils when charcoal was added as compared to soils without charcoal. Dugan et al. (2010) also reported increased water retention (349 – 481%) at 5, 10, and 15 t/ha application of sawdust biochar and maize stover biochar.

The reduction in bulk density in this study with increased application of charcoal, is possible linked to the porous nature of charcoal, which aids in retaining more air and water in the soil. The high sand fraction of Tabela sand (96%) with a bulk density of 1.35 g/cm^3 and the low bulk density of charcoal (0.7 g/cm^3) contributed to the reduction of soil bulk density in the amended soil. Charcoal acts as a bulking agent, which improves soil structure after application (Piniwisat, 2014). Mishra et al. (2017) and Oguntunde et al. (2008) also reported decreased bulk density in charcoal sites compared to non-charcoal sites. A reduction in the bulk density of charcoal amended soil enhances water holding capacity, root development, and microbial activity (Hussain et al., 2017; Zhang et al., 2010).

Even though concentrations varied among certain nutrients based on treatment, significant differences ($p < .05$) were observed in available Cu, Fe, K, Mg, P, N, and Zn (Figure 1), where treatment 10 t/ha, 20 t/ha, and 30 t/ha showed higher concentrations. These differences may be attributed to the significant increase in soil pH as charcoal application increases from 0 t/ha to 30 t/ha within cropping cycles. Ding (2010) reported that a charcoal application rate of 0.5% at a depth of 0 – 10cm limited the leaching of NH_4^+-N. The total loss of NH_4^+-N was reduced by 15% when charcoal was added. Generally, micronutrients availability tends to decrease as soil pH increases. Additionally, the increase in soil pH reduces the strong bonds of aluminium oxides and iron oxides, resulting in the increased availability of nutrient elements to plants (Miller, 2016). In practice, continuous cultivation of the amended soil will require inputs of nutrients at a suitable rate to maintain plant growth and a healthy microbial population.

Figure 1

Comparison of available soil nutrients level in relation to treatments within each cropping cycle. Different letters indicate statistically significant differences (p < .05) using LSD test between each treatment per variable



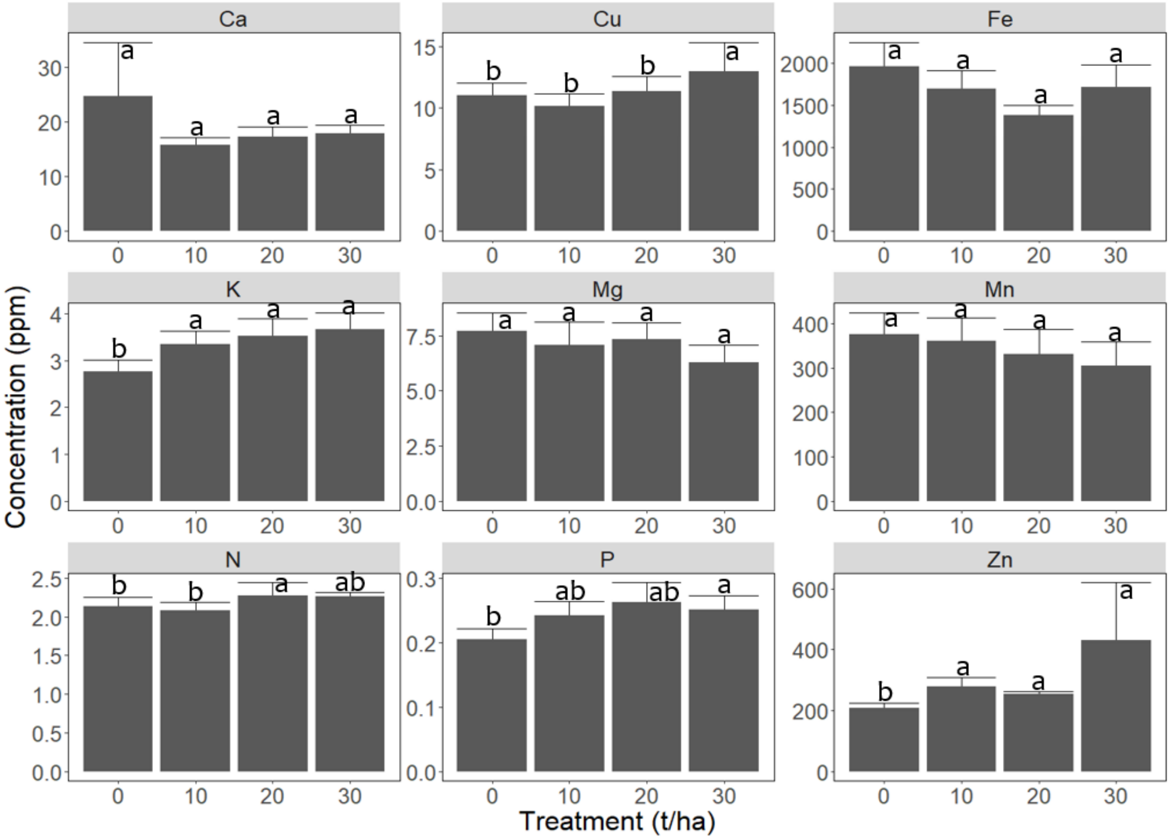
3.2 Treatment effect on plant tissue nutrient level of lettuce

The increasing application rates of charcoal from 0 t/ha – 30 t/ha significantly (*p* < .05) increased Cu, K, N, P, and Zn in plant tissue as it relates to the average treatment of all cropping cycles (Figure 2). This increasing trend based on application rate was not evident for nutrients such as Ca, Fe, Mg, and Mn, which showed no significant difference (*p* < .05) among treatment (Figure 2). The increases in Cu and Zn in the plant tissue may be attributed to the pH levels (between 5.3 and 6.6 at 0 t/ha and 30 t/ha respectively), which are suitable for the uptake of these micronutrients (Miller, 2016). The amount of nutrients and elements in the plant tissue is highly dependent on their availability in the soil environment. The amount of nutrients in the plant tissue may also be attributed to the ability of charcoal to hold and release nutrients slowly into the soil to be accessible to the plants. It should also be noted that AMF increases the uptake of N, P, K, Ca, Mg, and Mn, as well as the trace elements Cu and Zn (Abiala et al., 2013).

Interactions between charcoal and AMF may account for increased nutrient levels in plant tissue. Plants form a mutualistic relationship with AMF to increase the plant's efficiency of mineral uptake in soils where they may be deficient or less available, while the fungi receive photosynthetic carbohydrates from the plant (Garcia & Racsko, 2018). This mechanism results in enhanced plant growth and increased yield. Studies by Gundale and De Luca (2006) and Matsubara et al. (2002) have suggested that the addition of charcoal has the potential to alter soil nutrient availability by affecting the soil's physicochemical properties. Improved soil nutrient availability results in enhanced plant growth as well as higher colonisation by AMF. Charcoal can also increase the performance of AMF to help their host in resisting infection by plant pathogens (Matsubara et al., 2002). Detoxification of allelochemicals is another property of charcoal which leads to alteration in the root colonisation by AMF. However, Warnock et al. (2010) reported a decline in AMF abundance with charcoal addition. Abdelaziz (2018) showed that inoculation of *L. sativa* L. by *R. irregularis* improved plant growth by 10.4%, where the N, P, and K contents in *L. sativa* L. leaves increased. The colonisation of host plants by AMF contributes to an increase in the mineral content of the plant due to increased absorption surface of the plant root system, which enhances nutrient uptake (Smith & Read, 2008). Similarly, Wahbi (2015) also recorded increases in P concentration in shoot biomass (18%) of wheat plants mycorrhized by *R. irregularis*.

Figure 2

Treatment effect (average of all cropping cycles) on plant tissue nutrient level of lettuce. Different letters indicate statistically significant differences (p < .05) using LSD test between each treatment per variable

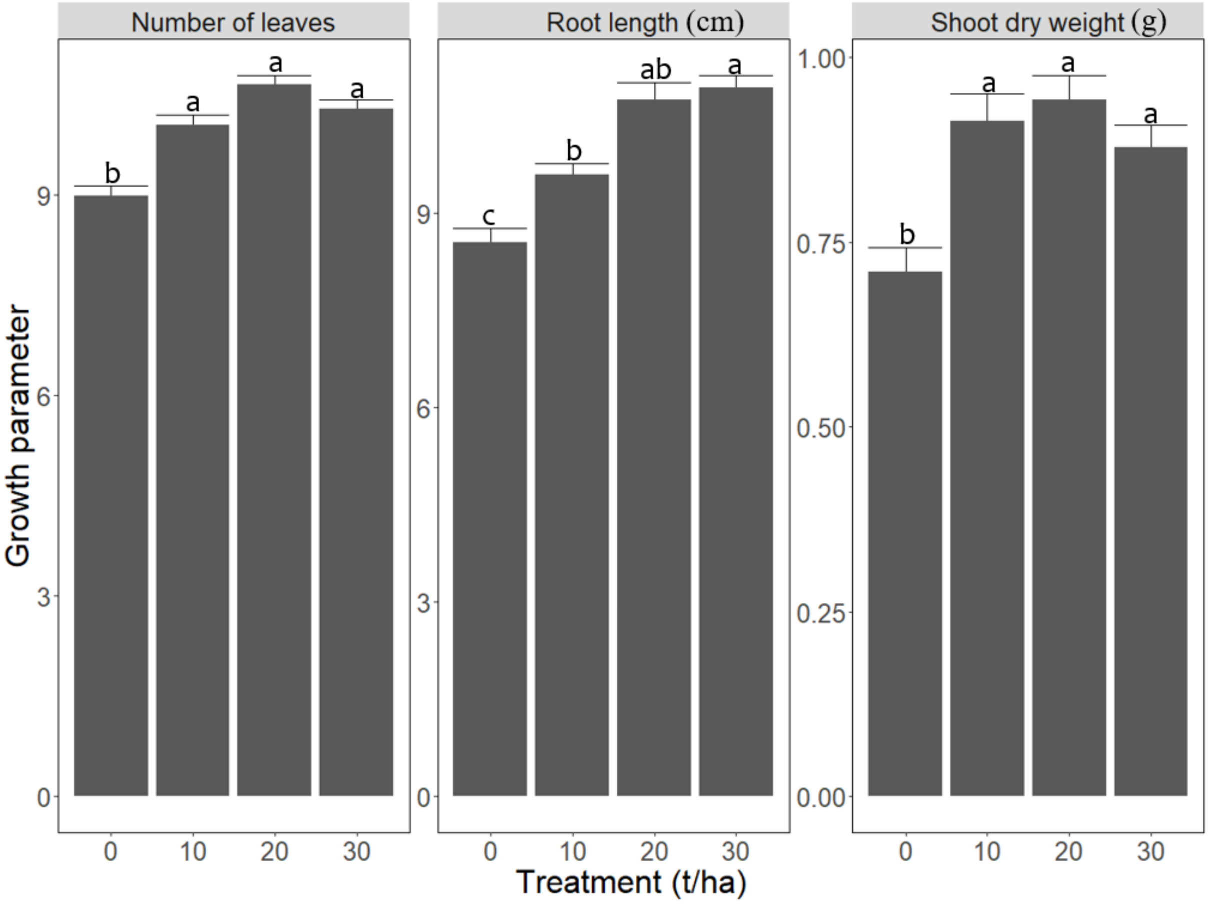


3.3 Treatment effect on growth performance of lettuce

Lettuce yield and other growth parameters increased in response to increased charcoal application rates as well as inoculation with AMF at a standard. Control treatment (no charcoal and AMF) was compared with treatment induced with charcoal and AMF. Shoot weight, number of leaves, and root length showed significant ($p < .05$) increases as charcoal application rates increased across treatments from 0 t/ha – 30 t/ha in each cropping cycle (Figure 3). The use of charcoal as a soil amendment promotes the growth of crops in marginal sandy soils since it enhances chemical, physical, and microbial soil parameters. Carter et al. (2013) reported similar increases in final biomass, number of leaves, and plant height of lettuce and Chinese cabbage when charcoal was applied at a rate from 25 g/kg to 150 g/kg. Gaydaybu et al. (2019) reported a significant increase in plant height and leaf width of local pepper when loamy sand was amended with charcoal at 2 t/ha and NPK (15:15:15) at a rate of 150kg/ha. Abdel-Wahab (2018) found that mycorrhizal inoculation significantly increased the number of leaves, leaf fresh weight, and yield of lettuce grown in sandy soils. Previous studies emphasised that charred amendments have the potential to increase AMF root colonisation percentage in acidic soils, which in turn improves crop growth (Ezawa et al., 2002; Matsubara et al., 2002).

Figure 3

Treatment effect on growth performance of lettuce. Different letters indicate statistically significant differences (p < .05) using LSD test between each treatment per variable



4. Conclusion

Charcoal as a soil amendment could be used to increase the productivity of low fertility and acid sandy soils such as those found at the Linden/Soesdyke Highway, Region 10, and the Rupununi Savannahs, Region 9, Guyana. Charcoal can accelerate the release of key crop nutrients and enhance availability when needed by the crop. It should be noted that in practice, marginal soils will require inputs of nutrients at a suitable rate and time to maintain plant growth and a healthy microbial population in nutrient limited soils. The interaction of charcoal and soil can moderate the availability of nutrient with time. Adopting the application of charcoal to marginal sandy soils increases the pH of these acidic soils, therefore reducing the amount of limestone usage. Although a significant increase in CEC was not observed, the increases observed in this study indicate charcoal's potential to hold nutrients in marginal soils. Significant increases were noted in crop yield with increased charcoal application rates within cropping cycles and when cropping cycles were combined; however, there were no apparent differences between application rates at 20 t/ha and 30 t/ha. Therefore, the charcoal rate for amending Tabela sand to increase AMF activity and achieve optimal crop yield appears to be 20 t/ha.

Ethical Considerations

There are no conflicts of interest.

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