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Effects of biochar and manure on soil properties and growth of *Casuarina equisetifolia* seedlings at the coastal region of Kenya

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This study investigated the effects of biochar and manure applied singly or together on soil biochemical properties, *Casuarina equisetifolia* seedling growth and seedling quality. Therefore, a nursery experiment consisting of *Prosopis juliflora* biochar and cattle manure treatments at 0% (control), 10% biochar, 20% biochar, 10% manure, 10% biochar + 10% manure, and 20% biochar + 10% manure was conducted. Generally, biochar containing treatments recorded higher P concentrations; where the combination of 20% biochar and 10% manure gave the highest phosphorus concentration, which was 137.3% above the control. Nitrate concentrations were higher in manure treatments than the control by 122.9%. As a result of *C. equisetifolia's* ability to fix nitrogen, nitrates concentration in the control increased from 0.016 ppm at the onset of the experiment to 2.18 ppm at the end the experiment. There was also a general increase in pH with biochar and manure application. Seedlings treated with 10% manure recorded the highest collar diameter and height which was 36.4 and 27.4% above the control respectively while treatments with biochar alone recorded the lowest seedling collar diameters. Seedling quality as observed using the shoot/root ratio showed that sole biochar treatments had the recommended range of <3:1. These results suggest that the use of biochar and manure enhances nitrogen and phosphorus availability which are crucial for production of seedlings that grow significantly faster than seedlings raised from forest soil only.

Key words: Biochar, manure, Casuarina, seedling quality, soil properties.

INTRODUCTION

Casuarina equisetifolia is an evergreen, actinorhizal tree that thrives well in semi-arid to sub-humid climates (GISD, 2020). The species is mainly restricted to a narrow strip of land adjacent to sandy coastal region and rarely extends towards the inland (Orwa et al., 2009). There are more than two million hectares of *C. equisetifolia* plantations raised throughout the tropics which provide several socio-economic, environmental and ecological services (Mbuvi et al., 2014). Due to its fast growth rate, adaptability to a range of ecological zones, different edaphic and climatic conditions, ability to fix nitrogen in soils together with many other uses such

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Table 1. Treatments for the nursery experiment.

Treatment	Biochar and manure rates (% of biochar and manure in relation to weight of soil used)
T1	0%
T2	10% biochar
T3	20% biochar
T4	10% manure
T5	10% biochar + 10% manure
T6	20% biochar + 10% manure

as for building, firewood, and for commercial purposes, it makes *C. equisetifolia* to stand out as an ideal tree species for farmers along the Coastal region of Kenya (Diagne et al., 2013; Kandioura et al., 2013; Mbuvi et al., 2014).

The coastal region of Kenya where *C. equisetifolia* is grown is often characterized by poor soils coupled with low and unreliable rainfall which have contributed to low agricultural productivity and perennial food shortages (Mwangi et al., 2010; Karuku, 2018). As such, farmers have in the recent past started engaging themselves in agroforestry practices using *C. equisetifolia* where some farmers are growing pure *C. equisetifolia* for commercial purposes as woodlots, which is an alternative source of livelihood that is easily adaptable to climate change (Mbuvi, 2010).

In Kenya, research on *C. equisetifolia* has been carried out on soils with low Phosphorus (Nyamai and Juma, 1996), on degraded limestone quarries for rehabilitation (Gathuru, 2011), on survival and growth performance at different spacing (Balozi et al., 2013), on species adoption rates at the Coastal region (Mbuvi et al., 2014) and on economic evaluation (Wekesa and Mwalewa, 2015). However, no research seems to have been done on using soil amendments such as biochar and manure on soil fertility enhancement for improved Casuarina performance at different growth stages. Among many amendments, the use of biochar and manure offer a cost-effective means of improving soil fertility and water holding capacity of soils (Imoro et al., 2012; Carvalho et al., 2013). Biochar is a solid and porous product made from pyrolysis of organic materials. Numerous studies have reported the benefits accrued to biochar and manure as soil amendments especially for soil moisture storage and crop yield improvement worldwide (Adeyemi and Idowu, 2017; Al-Wasfy and El-Khawanga, 2008; Pühringer, 2016). However, results on the effect of biochar and manure on tree growth are inconclusive. In spite of numerous studies done on the effect of biochar and manure on soil properties and crop yields in other parts of the world, the use of biochar and manure for improving tree growth in Kenya has not been extensively carried out, more especially at the coast region. Moreover, knowledge and data on soil fertility requirements for growing *C. equisetifolia* both at seedling and field establishment levels is still inadequate.

Based on the above arguments, there is need therefore to examine the effect of some organic amendments on soil fertility enhancement for improved Casuarina growth in the region. This study therefore addressed three specific objectives: (i) the effects of biochar and manure on *C. equisetifolia* seedling growth; (ii) the effect of biochar and manure on soil properties; and (iii) the effect of biochar and manure on on *C. equisetifolia* seedling quality. The results of this study are crucial to researchers and extension officers tasked to advice farmers on appropriate *C. equisetifolia* tree farming approaches for optimum performance and productivity.

MATERIALS AND METHODS

Study site

The study was conducted at Kenya Forestry Research Institute (KEFRI) Tree Nursery site situated at Gede, Kilifi County in the coastal region of Kenya. The Kilifi county lies between latitude 2°20´ and 4°0´ South, and between longitude 39°05´ and 40° 14´ East (Figure 1). Neighbouring counties include Taita Taveta, Kwale, Tana River and Mombasa. Kilifi County also borders the Indian Ocean to the east (www.kilifi.go.ke). According to the 2009 Kenya Population and Housing Census, the county had a population of approximately 1,109,735 people. This accounted for 2.9% of the total population in Kenya (CRA, 2014). The total land area of the county is roughly 12,609.7 km^2, with a terrain that generally slopes towards the sea. The mean annual rainfall ranges between 300mm in the hinterland and 1,300 mm along the coastal belt of the County. The County is divided into five Agro- Ecological Zones (AEZs); which are categorized using annual rainfall, mean temperatures, vegetation and humidity. The County experiences high evaporation rates varying from 1800mm along the coastal strip to 2200 mm in the hinterland. Over half of the land area in the County is arable, with maize and cassava being the key subsistence crops mainly grown by small-scale farmers. Other crops grown but mostly by large-scale farmers as cash crops include coconuts, sisal, cashew nuts and citrus fruits.

Experimental design

The study was conducted between October 2018 and May 2019. The experiment was set up in a randomized complete block design (RCBD) with six treatments replicated three times. The six treatments are as shown in Table 1. Biochar was prepared from

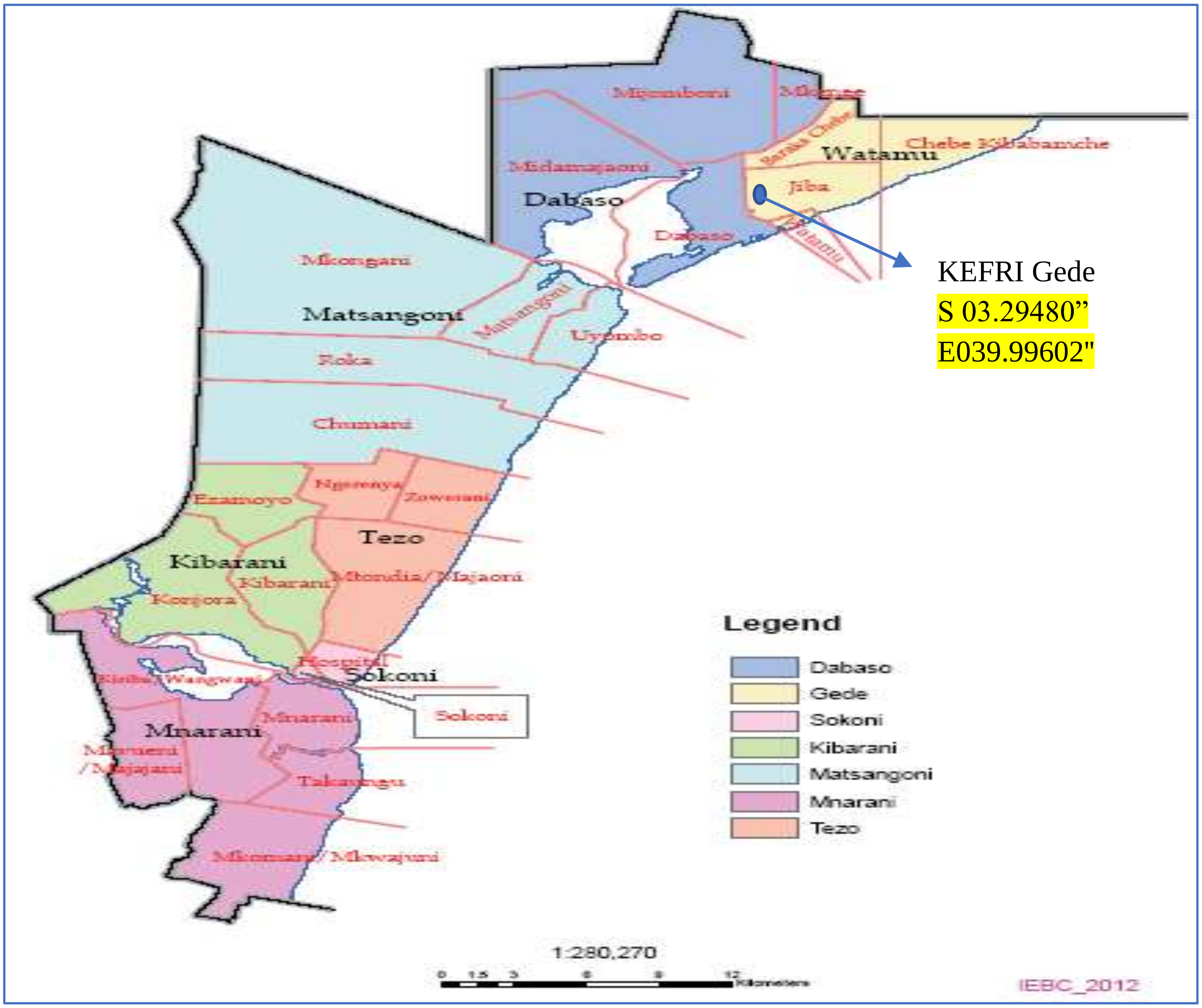

Figure 1. Map of Kilifi County showing Gede site and its environs.
Source: www.kilifi.go.ke

Prosopis juliflora branches through slow pyrolysis procedure while for manure, cattle manure was used. Forest soil used for potting was obtained from Arabuko Sokoke forest which is adjacent to the KEFRI-Gede tree nursery. Certified *C. equisetifolia* seeds were obtained from KEFRI seed Centre.

Casuarina seedlings growth and quality assessment

Growth assessment was done fortnightly throughout the seedlings' growth period (177 days). Height (cm) of *C. equisetifolia* seedlings was measured using a metre rule while diameter at ground level/collar diameter (mm) was measured using a Vernier caliper. Other parameters measured included Sturdiness Quotient and Shoot/Root ratio. The shoot/root ratio (S:R) measures the balance between the transpiration area (shoot) and the water absorbing area (root) of the seedlings. It is an important measure for seedling survival and relates the transpiring area (shoot) to the water absorbing area (roots). Sturdiness quotient was calculated using the following formulae by Jaenicke (1999):

$$\text{Sturdiness Quotient} = \frac{Shoot\ length\ (cm)}{Collar\ diameter\ (mm)}$$

Shoot/Root ratio was calculated using the following formula by Takoutsing et al. (2014):

$$\text{Shoot/Root ratio} = \frac{Shoot\ dry\ weight\ (g)}{Root\ dry\ weight\ (g)}$$

Soil sampling and analysis

Soil sampling and analysis were undertaken at the on-set of the experiment to determine the baseline soil fertility status. Nutrients concentrations in the manure and biochar used in the experiment were also analysed. At the end of the experiment, soil sampling and analysis were also done to determine the effects of treatments on soil properties. The following parameters were analysed using

Table 2. Baseline status of materials used for experiment.

Material	Soil nutrients									
	pH	EC (mS/cm)	P (ppm)	K (me /100g)	Na (me /100 g)	$NO3^-$ (ppm)	Ca (me /100 g)	TC (%)	TN (%)	C:N
Forest soil	7.1	0.10	1.45	1.5	2.0	0.02	0.5	0.45	0.31	2
Biochar	8.7	1.39	2.99	26.6	4.3	1.73	1.0	48.02	0.84	57
Manure	8.5	0.83	9.49	38.4	8.2	18.12	2.0	5.54	0.79	7

mS/cm, Milli siemens per centimetre), EC (Electrical Conductivity), K (Potassium), P (Phosphorus) Na (Sodium), NO_3^-(Nitrates), Ca (Calcium), TC (Total Carbon), TN (Total Nitrogen), C:N (Carbon Nitrogen ratio), me/100 g (milliequivalent per 100 g soil).

standard procedures as outlined by Okalebo et al. (2002). Soil pH and Electro Conductivity (EC) were determined using portable glass electrode; available Phosphorus (P), Nitrates (NO_3^-) and Magnesium (Mg) were analysed using UV spectrophotometer method; Potassium (K), Calcium (Ca) and Sodium (Na) were determined using Flame photometer method (Krotz et al., 2013; Okalebo et al., 2002) and microbial population analysis was determined using the plate count method (Ogunmwoyi et al., 2008).

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using R software for windows at 95% confidence level. Means were separated using Duncan's multiple comparison tests and growth trends were presented in MS Excel line graphs.

RESULTS AND DISCUSSION

Baseline data for soil, manure and biochar

The baseline chemical data for forest soil, manure and biochar analysed at the onset of the experiment are shown in Table 2 above. The pH for the three materials used for this study laid between 7.1 and 8.7 which are slightly alkaline to moderately alkaline. Manure among the three materials used had the highest nutrient concentrations (P, K, Na, Ca and NO_3^-) this was followed by biochar and lastly forest soil which was used as the primary nursery soil mixture and control of the study because of its least nutrient concentrations. The inherent low soil fertility of the forest soil in this area is a characteristic of the Coastal soils in Kenya (Karuku, 2018).

Effect of biochar and manure on growth of *Casuarina* seedlings

Casuarina seedlings collar diameter

The effects of biochar and manure on seedling collar diameter, that is" Diameter at Ground Level of trees (DGL)" over a period of 177 days was measured periodically and found as shown in Table 3. There was a gradual increase in DGL across the growth period as indicated by the various treatments. Collar diameter was significantly different for the various treatments at day 135 *(p=0.05)* and day 177 (p=0.00005) as indicated in Table 3. Seedlings treated with 10% manure had the highest DGL across the growth periods while seedlings treated with 10% biochar had the lowest DGL across the growth periods by a difference of 29% at day 135 and lower than the control across the three growth periods (135, 147 and 177 days) by 7.7, 11.5 and 11.2%, respectively. The effect of biochar on seedling collar is a subject for further studies to understand biochar characteristics that may lower growth of seedling collar diameter.

Seedlings treated with a combination of 20% biochar and 10% manure; and those treated with 10% manure had higher DGL compared to the control by 23 and 30.7%, respectively at day 135. At day 147, there was no significant difference in DGL across treatments (*p=0.09*) but seedlings treated with 10% manure recorded the highest DGL (<0.27 cm). At day 177, seedlings treated with 10% manure recorded the highest DGL of 0.487 cm, 36.4% higher than the control and 53.6% higher than seedlings treated with 10% biochar (Table 3). This increase in collar diameter with manure application could be attributed to increased availability of nutrients through mineralization of the manure (Biederman and Harpole, 2013; Khaitov et al., 2019). Some studies had also reported similar results where there was an increase in collar diameter of *Acacia senegal* seedlings with manure application compared to the control (Daldoum and Hammad, 2015). The results of this study also show that seedlings treated with biochar had a lower collar diameter than the control (Table 3). This could be attributed to the inherent characteristics of this biochar for example the wide C/N ratio (57:1) of biochar produced from feedstock materials as reported by Gao et al. (2019) which might have slowed the mineralization process. The wide C/N ratios of substrates always lead to decreased soil plant nutrients due to low mineralization rates of substrates which lead to low release of available mineral N in soil for both microbial metabolism and plant growth, Eventually, the available N in soil is immobilized faster by microbes for their metabolic activities than uptake by plants hence hindering plant growth (Masakazu and Tomohirio, 1993;

Table 3. Effect of Biochar and manure on collar diameter of Casuarina seedlings.

Seedling DGL (cm) ± SE	Growth period (days)		
Treatments	**135**	**147**	**177**
Control	0.13±0.02^{b}	0.26±0.01^{ab}	0.357±0.03^{c}
10% Biochar	0.12±0.15^{b}	0.23±0.001^{b}	0.317±0.02^{d}
20% Biochar	0.12±0.02^{b}	0.24±0.15^{b}	0.407±0.01^{b}
10% Manure	0.17±0.02^{a}	0.27±0.03^{a}	0.487±0.04^{a}
10% Biochar+10% Manure	0.12±0.04^{b}	0.26±0.03^{ab}	0.420±0.03^{b}
20% Biochar+10% Manure	0.16±0.03^{ab}	0.25±0.01^{ab}	0.420±0.02^{b}
$F_{(5,12)}$	3.161	2.432	20.378
p<0.05	0.05*	0.09 n.s.	0.00005***

Values denoted by the same letter along the column are not significant different at p<0.05.

Gao et al., 2019).

Casuarina seedlings height (cm)

The effect of biochar and manure on height of Casuarina seedlings is shown in Table 4 and Figure 1. There was gradual increase in seedling height from day 35 to day 177 for all treatments. There was a significant difference in seedling height from day 77 across the various treatments (p=0.005, p=0.0004, p=0.0001, p=0.00005, p=0.0006, p=0.0000006 for day 77, 91, 105, 119, 133, 147 and 177 respectively). The results show that seedlings treated with 10% manure recorded the highest heights than all the treatments across the measurement periods. It was followed by treatments of 20%Biochar +10% Manure and 10% Biochar +10% Manure in that order while the control recorded the lowest heights across the growth periods except on day 177 where seedlings treated with 10% biochar recorded the lowest (47.7 cm) height, even lower than the control by 6.53% (Table 4). For seedlings treated with 10% manure, the heights were significantly higher compared to the control by 27.9, 30.8, 22, 29.5, 32.2, 27.4 and 46.8% for day 77, 91, 105, 119, 133, 147 and 177, respectively, while the seedlings treated with a combination of 20% Biochar and 10% Manure recorded the second highest seedling heights across the growth periods (Table 4). Also observed is that there were no significant differences in heights across treatments from day 35 to 63. Generally, biochar and manure treated seedlings recorded higher Casuarina seedling height than the control (Table 4). This could be as a result of increased nutrients such as nitrogen and phosphorus with manure and biochar application and enhanced soil moisture holding capacity (Khaitov et al., 2019; Bierderman and Harpole, 2013). Availability of nitrogen is essential for plant vegetative growth while phosphorus plays a crucial role in root development (Razaq et al., 2017).

From this study results, it is evident that application of manure enhanced shoot growth of *C. equisetifolia* compared to application of biochar alone and the control. The increase in *C. equisetifolia* seedling height with manure application can be attributed to increased available nitrogen with manure application. Manure used for the experiment had a narrow C/N ratio which led to net N mineralization compared to biochar which had a wider C/N ratio (Table 2) (Truong and Marschner, 2018; Daldoum and Hammad, 2015). Such increase in plant height with manure application had also been reported by Prakash et al. (2014) who used seedlings of *Calendula officinalis.* This study also showed that biochar increased seedling height compared to the control as indicated in Table 4. This increase in height with biochar application may be as a result of increased pH, Cation Exchange Capacity (CEC), C, N, P and water holding capacity which enhanced nutrient availability for *C. equisetifolia* seedlings (Uddin et al., 2012, Berek and Hue, 2013; Drabkin and Weinfuether, 2014; Trupiano et al., 2017; Gao et al., 2019). However, there was no significant increase in height with increased rates of biochar from 10 to 20%. Similar findings had also been reported by Carter et al. (2013), Hafeez et al. (2017) and Mohan et al. (2018) who reported increased plant growth due to biochar application as compared to the control. The study further indicated that biochar-amended soils resulted in highest increase in plant height where the average plant height improved from 8.3 cm on day 7 to 20 cm (day 49) versus 6.0 cm (day 7) to 9.5 cm (day 49) in case of the control. The combination of biochar and manure resulted to higher *C. equisetifolia* seedling height than the control treatment. The increase in seedling height as a result of combining biochar and manure can also be attributed to Phosphorus increase from biochar (Gao et al., 2019) and Nitrogen release from manure (Uddin et al., 2012).

Effect of biochar and manure on selected biochemical soil properties

The effects of biochar and manure on soil chemical properties were found as shown in Table 5. There was a significant difference in available phosphorus and nitrates at the end of the experimental period (p=0.0003 for

Table 4. Effect of Biochar and manure on height (cm) of Casuarina seedlings.

Seedling height ± SE (cm)	Growth period (Days)									
Treatments	**35**	**49**	**63**	**77**	**91**	**105**	**119**	**133**	**147**	**177**
Control	5.51±0.71^{b}	7.20±0.56^{b}	9.79±0.55^{b}	12.98±0.90^{d}	15.77±1.12^{c}	17.62±1.05^{c}	19.13±0.68^{e}	21.22±0.50^{c}	25.50±0.71^{b}	51.03±3.04^{d}
10% Biochar	5.91±0.45^{ab}	7.96±0.56^{ab}	10.70±1.02^{ab}	14.45±0.50^{c}	18.29±0.42^{b}	19.16±0.75^{b}	20.61±1.14^{de}	22.13±1.09^{c}	26.04±1.48^{b}	47.70±2.17^{d}
20% Biochar	5.93±0.18^{ab}	8.35±0.67^{a}	11.51±0.59^{a}	15.58±0.51^{abc}	18.62±0.66^{b}	19.52±0.53^{b}	21.49±0.93^{cd}	22.78±0.88^{c}	26.86±1.58^{b}	59.77±0.75c
10% Manure	6.15±0.29^{ab}	8.48±0.35^{a}	10.86±1.47^{ab}	16.60±0.79^{a}	20.62±0.80^{a}	21.61±1.26^{a}	24.77±0.82^{a}	28.06±2.0^{a}	32.48±2.97^{a}	74.93±6.02^{a}
10% B+10% M	6.05±0.26^{ab}	8.08±0.38^{ab}	11.08±0.44^{ab}	15.22±0.83^{bc}	18.60±0.89^{b}	19.62±0.48^{b}	22.51±0.90^{bc}	25.21±0.40^{b}	30.25±1.51^{a}	65.20±1.11^{b}
20% B+10% M	6.49±0.28^{a}	8.42±0.70^{a}	11.40±0.57^{ab}	15.92±0.47^{ab}	19.40±0.97^{ab}	20.56±1.05^{ab}	23.48±1.20^{ab}	26.54±1.18^{ab}	32.03±1.23^{a}	68.50±0.56^{b}
$F_{(5,12)}$	1.939	2.225	1.579	10.223	10.865	8.214	13.373	16.894	9.791	37.589
$p<0.05$	0.1612 n.s.	0.119 n.s.	0.239 n.s.	0.0005***	0.0004***	0.001**	0.0001***	0.00005***	0.0006***	0.0000006***

Values denoted by the same letter along the column are not significant different at p<0.05; B. Biochar ; M, Manure.

phosphorus and p=0.007 for nitrates). For phosphorus, the combination of manure and biochar resulted in the highest soil P. The combination of 20% biochar and 10% manure gave the highest concentration of Phosphorus (21.40 ppm), which was 137.3% higher than the control. Generally, all treatments recorded higher P concentration than the control (Table 5). Biochar containing treatments had higher P concentration than control and manure alone plots. Treatment with 10% biochar and 20% biochar recorded higher soil P than the control by 42.7% and 140.2%, respectively (Table 5). The increase in P with biochar application can be attributed P availability in the biochar as a result of the feedstock used for biochar production (woody feedstock) and the high pyrolysis temperature used for biochar production (Glaser and Lehr, 2019). Previous studies have shown that P concentration in biochar increased with increased pyrolysis temperature (Fayong et al., 2019). Similar findings had been found by Gao et al. (2019) who reported an increase in soil P with biochar application. The increase in P with biochar application is essential for enhancing plant root development. Approximately half of the P in biochar is released as orthophosphates and pyrophosphates (Glaser and Lehr, 2019); these are P forms that can be taken up by the plant to enhance root development (Fayong et al., 2019).

Manure treatment at 10% recorded the highest nitrates concentration of 4.86 ppm and was followed by the combination of 20% biochar and 10% manure treatment (4.43 ppm). In terms of percentage, the nitrate concentrations above the control were 122.9 and 103.2% for 10% manure and the combination of 20% biochar and 10% manure, respectively. The high nitrates concentration in manure treatments can be as a result of N mineralization resulting from the narrow C/N ratio of the manure used for the experiment (Ezlu et al., 2019). Comparable results had been reported by Ewulo et al. (2008) and Han et al. (2016) who observed an increase in soil nitrogen with manure application. Combining biochar and manure has also been reported to increase soil nitrogen (Yunilasari et al., 2020). The increase in nitrates with manure application was crucial for vegetative growth of *C. equisetifolia* seedlings (Leghari et al., 2016).

Interestingly, nitrate concentration tended to increase in the control towards the end of the experiment compared to nitrates concentration at the beginning of the experiment. This could probably be due to biological nitrogen fixation by *C. equisetifolia* under nitrogen deficiency conditions as demonstrated by the number of nodules formed in the control treatment (Figure 2). *C. equisetifolia* has been reported to play an important role in biological nitrogen fixation through their symbiotic relationships with Frankia bacteria (Kandioura et al., 2013).

Generally, there were no significant differences in soil Potassium, Sodium and magnesium as influenced by the various treatments (Table 5). The combination of 10% biochar and 10% manure, however, recorded the highest potassium concentration (1.33 me/100 g) compared to other treatments which was 52.9% above the control. The concentration of Magnesium was also high in the combination of 20% biochar and 10% manure (2.06 me/100 g). Treatments with manure that is

Table 5. Effect of Biochar and Manure on selected chemical soil properties.

Parameter	Soil nutrients ± SE				
Treatments	**P (ppm)**	**K (me/100 g)**	**Na (me/100 g)**	**NO_{3-} (ppm)**	**Mg (me/100 g)**
Control	9.02±0.81[b]	0.87±0.29[ab]	2.33±0.38[a]	2.18±0.89[b]	1.90±0.05[ab]
10% Biochar	12.87±3.84[b]	0.67±0.06[ab]	1.63±0.32[ab]	1.43±0.42[b]	1.78±0.20[ab]
20% Biochar	21.67±2.74[a]	0.80±0.46[ab]	2.23±0.29[ab]	2.30±1.12[b]	1.68±0.07[b]
10% Manure	12.82±3.81[b]	0.53±0.15[b]	1.50±0.60[b]	4.86±0.55[a]	1.99±0.19[ab]
10% Biochar+10% Manure	20.77±2.62[a]	1.33±0.31[a]	2.23±0.38[ab]	2.11±0.88[b]	1.95±0.19[ab]
20% Biochar+10% Manure	21.40±2.73[a]	0.57±0.12[b]	1.63±0.25[ab]	4.43±1.78[a]	2.06±0.27[a]
$F_{(5,12)}$	11.302	2.108	2.81	5.49	2.067
p<0.05	0.0003***	0.135 n.s.	0.06 n.s.	0.007**	0.141 n.s.

Values denoted by the same letter along the column are not significant different at p<0.05.

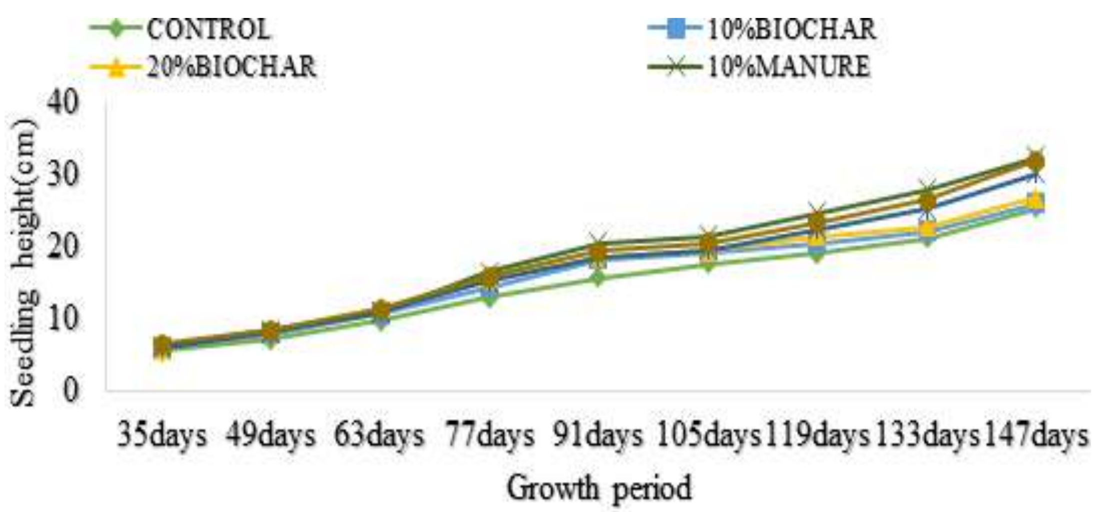

Figure 2. Trend of Casuarina seedling growth across various treatments.

10% manure, 10% biochar + 10% manure and 20% biochar +10% manure had higher Mg concentration than the control by 4.8, 2.8 and 8.6%, respectively. Organic manure has been reported to significantly influence phosphorous, potassium, and magnesium, content in the soil (Kariithi et al., 2017). In a study conducted in Brazilian Savannah by Carvalho et al. (2013), soil pH, and Mg were observed to increase linearly with biochar application (Figure 3).

Table 6 shows the effect of biochar and manure on soil Total N, Total C and its influence on C/N ratio. Biochar treated soil had higher Carbon content than the control or sole manure treatment. Combination of 20% biochar and 10% manure recorded the highest carbon content of 0.77%. This was 71.1% higher than the control and 33.8% higher than manure treatment. This can be attributed to the fact that biochar is a carbon-rich residue (Kazmi et al., 2010). This trend of increase in Carbon content with biochar application was reported by Abdullaeva (2014) and Mohan et al. (2018). There was a significant difference (p<0.004) among treatments in terms of Total N with soil treated with 10% manure recording the highest Total N (0.33%). This was also observed by Ewulo et al. (2008) where they reported manure of up to 50 tonnes per hectare improved soil chemical properties as indicated by increased nutrient content, especially nitrogen and phosphorus. Biochar treatments recorded low Total N as shown in Table 6. Treatments with a combination of biochar and manure had higher C/N ratio than other treatments. The higher C/N ratio of biochar treatments can be attributed to the feedstock used for pyrolysis (Prosopis wood chips); the biochar used had a C/N ratio of 57:1.

Effect of biochar and manure on pH and electrical conductivity (EC)

There were no significant differences in soil pH and electrical conductivity (EC) among the treatments. There

Figure 3. Nodulation (nodules circled in red) in nitrogen deficient (control) and nitrogen rich (10% manure) treatments.

Table 6. Effect of biochar and manure on soil total C, total N and C/N ratio.

Treatments	Soil nutrients ± SE		
	Total C (%)	**Total N (%)**	**C/N Ratio**
Control	0.45±0.02[b]	0.32±0.08[a]	1.46±0.47[b]
10% Biochar	0.66±0.19[ab]	0.32±0.01[a]	2.05±0.64[b]
20% Biochar	0.62±0.14[ab]	0.31±0.06[a]	2.00±0.37[b]
10% Manure	0.51±0.08[b]	0.33±0.11[a]	1.69±0.70[b]
10% Biochar+10% Manure	0.62±0.11[ab]	0.11±0.06[b]	7.17±3.81[a]
20% Biochar+10% Manure	0.77±0.14[a]	0.17±0.03[b]	4.55±1.21[ab]
$F_{(5,12)}$	2.56	6.41	5.33
$p<0.05$	0.08 n.s.	0.004**	0.008**

Values denoted by the same letter along the column are not significant different at p<0.05.

was, however, general increase in pH with the addition of biochar and manure especially towards the end of the experiment which ranged from 8.87 to 9.24, an increase of 24.9 and 30.1% respectively (Table 7). This increase of pH could have been contributed by biochar itself for its pH level was alkaline (8.7; Table 2). The increase in pH was around 1.6 units. Biochar has been reported to increase pH buffering capacity of the soil and help mitigate decrease of pH over time which also enhanced P uptake by plants (Trupiano et al., 2017). Similar trend of pH increases with biochar application had also been reported in a study by Mohan et al. (2018) where pH increases by different biochar types lay between 0.3 to 0.8 units. They attributed this increase in pH to the rise in soil cation exchange capacity (CEC) due to the biochar's large surface area and porosity. Other studies have also reported slight increase in soil pH with manure application (Kariithi et al., 2017). Table 7 shows that there was increase in soil EC at day 35 with biochar application. However, the EC declined towards the end of the experiment in biochar-amended soils. This could be attributed to release of weakly bound nutrients of biochar into the soil solution which became available for plant uptake thus leading to the decline in EC towards the end of the experiment (Chintala et al., 2013). Similar studies have observed an increase in soil EC with biochar

Table 7. Effect of biochar and manure on soil pH and electro-conductivity.

Treatments	Soil pH± SE		Soil EC ($mScm^{-1}$) ± SE	
	35 days	147 days	35 days	147 days
Control	8.69±0.06[b]	8.95±0.21[a]	0.09±0.01[b]	0.12±0.06[a]
10% Biochar	9.25±0.19[a]	9.02±0.21[a]	0.42±0.27[a]	0.11±0.05[a]
20% Biochar	8.84±0.43[b]	8.87±0.25[a]	0.25±0.19[ab]	0.08±0.07[a]
10% Manure	8.78±0.07[b]	9.24±0.23[a]	0.13±0.02[ab]	0.16±0.006[a]
10% Biochar+10% Manure	9.02±0.07[ab]	9.13±0.19[a]	0.14±0.09[ab]	0.08±0.03[a]
20% Biochar+10% Manure	8.79±0.14[b]	9.10±0.17[a]	0.36±0.19[ab]	0.12±0.006[a]
$F_{(5,12)}$	2.953	1.223	2.17	1.251
p<0.05	0.06 n.s.	0.356 n.s.	0.126 n.s.	0.346 n.s.

Values denoted by the same letter along the column are not significant different at p<0.05.

Table 8. Effect of Biochar and manure on soil microbial population.

Treatments	Bacteria × 10^5 ± SE (CFU)	Fungi × 10^4 ± SE (CFU)
Control	3.04±0.52[a]	3.97±2.63[a]
10% Biochar	2.36±1.06[a]	3.03±1.64[a]
20% Biochar	2.69±0.34a	4.17±4.29[a]
10% Manure	2.83±0.73[a]	3.07±1.10[a]
10% Biochar+10% Manure	2.40±1.01[a]	2.63±1.67[a]
20% Biochar+10% Manure	3.39±0.40[a]	3.97±2.46[a]
$F_{(5,12)}$	0.85	0.19
p<0.05	0.54	0.96

Values denoted by the same letter along the column are not significant different at p<0.05. CFU, Colony Forming Units.

application at the onset of the experiment (Chintala et al., 2013; Shah et al., 2017). Studies by Abu-Zahra and Tahboub (2008), however, reported no significant changes in soil pH and soluble salts concentrations (EC) at the end of the experiment and attributed this lack of changes in soil pH to the high buffering capacity of the soil based on its high carbonate content (22-28%).

The effect of biochar and manure on microbial biomass

The effects of biochar and manure on microbial population as shown in Table 8 indicates that there was no significant difference in soil bacteria and fungi population across treatments. The combination of 20% biochar and 10% manure, however, recorded 11% higher bacteria population than the control while 10% biochar treatment alone recorded the lowest bacteria population of 2.36×10^5 Colony Forming Units (CFUs). The high bacteria population in the treatment with 20% biochar and 10% manure can be attributed to increased organic matter which enhanced porosity and aeration (Bhattarai et al., 2015). Fungi population was highest in the treatment with 20% biochar and was 5.04% higher than control and the combination of 20% biochar and 10% manure. Generally, bacteria population was higher than the fungi population across all treatments. This can be attributed to presence of favourable conditions for bacterial growth. Studies have shown that low pH is associated with fungal dominance while higher soil pH favours bacteria dominance (Claassens, 2019). The pH variations could explain the bacteria dominance as observed in this study. The results of this study are in agreement with the study by Zhang et al. (2014) who reported limited seasonal fluctuation in Microbial Biomass Carbon (MBC) for soils ameliorated with biochar as compared to control treatments. This is an indication that biochar does provide favourable soil environment conditions for microorganisms more especially for bacteria.

Effect of biochar and manure on Casuarina seedling quality

The treatment with a combination of 10% biochar and 10% manure recorded the highest shoot/root ratio of 4.83 and was followed closely by 10% manure treatment with

Table 9. Effect of biochar and manure on Casuarina seedling quality.

Treatments	Seedling quality indicator	
	S:R± SE	SQ ± SE
Control	3.92±1.56[a]	14.32±1,06[b]
10% Biochar	3.43±0.44[a]	15.08±0.65[ab]
20% Biochar	3.57±0.54[a]	14.70±0.28[ab]
10% Manure	4.66±0.24[a]	15.39±0.23[ab]
10% Biochar+10% Manure	4.83±0.78[a]	15.57±1.18[ab]
20% Biochar+10% Manure	3.87±0.14[a]	16.33±0.71[a]
$F_{(5,12)}$	1.65	2.48
p<0.05	0.219 n.s	0.09 n.s.

Values denoted by the same letter along the column are not significant different at p<0.05. S: R, Shoot: Root; SQ, sturdiness quotient.

a ratio of 4.66. The high shoot/root ratio can be attributed to enhanced height growth with manure application as a result of enhanced nutrient availability especially nitrogen (Khaitov et al*.,* 2019). Seedlings treated with 10% biochar and 20% biochar had the lowest S:R ratio of <3.6 :1 as shown in Table 9. This was within the recommended quality ratio range of 3:1 according to Jaenicke (1999) and Kung'u et al. (2008). The use of biochar therefore led to balanced growth of *C. equisetifolia* seedlings in terms of height and root growth. This can be attributed to enhanced P availability with biochar application thus improving root development (Fayong et al., 2019).

The *C. equisetifolia* seedlings had high sturdiness quotient (SQ<16.33) due to the morphological characteristics of *C. equisetifolia* (Orwa et al., 2009). A small quotient indicates a sturdy plant with a higher expected chance of survival, especially on windy or dry sites. A sturdiness quotient higher than 6 has been considered undesirable. A sturdiness quotient greater than 6 has been reported as an indication of physiological imbalance resulting in tall spindly seedlings while an extremely small sturdiness quotient implies difficulty in seedling establishment (Jaenicke, 1999). However, in the case of *C. equisetifolia* further studies are required to determine the appropriate SQ for the species due to its morphology (grows tall with slender stems).

CONCLUSION AND RECOMMENDATIONS

The aim of the study was to determine the effects of biochar and manure on selected soil properties, growth of *C. equisetifolia* seedlings and seedling quality. The physical and chemical properties of the different treatments were found to affect soil properties, seedling height, collar diameter, and shoot/root ratio. Generally, biochar containing treatments (biochar alone or in combination with manure) recorded higher P concentrations than other treatments, where a combination of 20% manure and 10% biochar gave the highest phosphorus concentration which was 137.3% above the control. Nitrates concentration was higher in manure containing treatments than the control by 122.9%. However, as a result of *C. equisetifolia's* ability to fix nitrogen, nitrates concentration in the control increased from 0.016ppm at the onset of the experiment to 2.18 ppm at the end of the experiment which was a reflection of higher nodule numbers in the control than other treatments. In the case of pH, there was a general increase with biochar and manure application while with soil microorganisms, the effects were not significant.

In terms of seedling growth, 10% manure treatment recorded the highest (0.487 cm) collar diameter while treatment with biochar alone recorded the lowest (0.317 cm). The mechanism under which biochar influences seedling collar diameter is a subject for further studies. With height, seedlings treated with 10% manure recorded the highest height (74.9 cm), which was 46.8% above the control. Combining manure and biochar was also seen to enhance seedling height growth compared to the control. Seedling quality as observed using the shoot/root ratio indicated that seedlings treated with 10% biochar and 20% biochar had the lowest shoot/root ratio (3.6:1). In terms of seedling Sturdiness Quotient, there was no significant difference observed. Further studies are required to determine the optimal SQ for *C. equisetifolia* as a seedling quality indicator owing to the morphology of the species. Forest soil as the control in this study was found neither to enhance seedling growth nor collar diameter due to its low soil fertility especially in nutrients contents.

The results of this study therefore have shown that biochar and manure do affect seedling growth in various ways. The combination of 20% biochar and 10% manure evidently enhanced both soil nutrients and seedling growth. This suggests that the use of organic matter with high nutrient levels such as cattle manure and biochar can produce good quality seedlings than seedlings raised from forest soil alone. Farmers in the coastal region of Kenya are therefore advised to incorporate manure and biochar into forest soils in order to improve the nutrients contents as growing media in their seedlings' nurseries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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