IMPROVING SOIL PROPERTIES AND GROWTH OF *Casuarina equisetifolia* THROUGH USE OF BIOCHAR, MANURE AND INORGANIC FERTILIZER IN KILIFI COUNTY, KENYA

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DECLARATION

I **Riziki Umazi Mwadalu** declare that this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

Signature:__________ Date: 12/05/2023

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Supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors.

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DEDICATION

This work is dedicated to God for His grace and protection throughout the research period and to my late brother Mr. Jumaa Mwadalu who passed on before the completion of this study. His support in ensuring I got education will forever be appreciated. Special dedication to my husband Mr. Antony Mute for his immense support and love throughout the study period.

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ABBREVIATIONS AND ACRONYMS

ANOVA	-	Analysis of Variance
ASALs	-	Arid and Semi-Arid Lands
C:N	-	C/N ratio
CEC	-	Cation Exchange Capacity
Cm	-	Centimetres
$cmol_{(+)}/kg$	-	centimoles of charge per kilogram
DBH	-	Diameter at Breast Height
DGL	-	Diameter at Ground Level
EC	-	Electrical conductivity
FYM	-	Farm Yard Manure
g/kg	-	Grams per kilogram
$g\ cm^{-3}$	-	Grams per cubic centimeter
ha	-	Hectares
KEFRI	-	Kenya Forestry Research Institute
m	-	Meters
MAE	-	Months after the establishment
m.a.s.l	-	Meters above sea level
mg/kg	-	Milligrams per kilogram
NPK	-	Nitrogen: Phosphorus: Potassium
RCBD	-	Randomized Complete Block Design
S:R	-	Shoot/Root ratio
SE	-	Standard Error of mean
SQ	-	Sturdiness Quotient
$t\ ha^{-1}$	-	Tons per hectare
TOC	-	Total organic carbon
v/v	-	Volume -to- volume ratio
w/w	-	Weight -to- weight ratio

ABSTRACT

Soil fertility challenges coupled with frequent droughts in arid and semi-arid lands (ASALs) have led to massive crop failures thereby increasing the problem of food insecurity. As climate change unfolds, there is a need for technologies that boost soil moisture while enhancing soil fertility. Biochar has been reported to be an effective soil amendment with the capability of enhancing soil productivity. However, the impact of biochar on tree growth is still outstanding. Therefore, the present study aimed at evaluating the potential of biochar, manure, and NPK to enhance *Casuarina equisetifolia* (Casuarina) growth at different growth stages through nursery and field experiments using randomized complete block experimental design. The nursery experiment evaluated the effects of 0% biochar (control), 10% biochar, 20% biochar, 10% manure, 10% biochar + 10% manure, and 20% biochar + 10% manure on Casuarina growth at the seedling stage. The field experiment evaluated the effects of four biochar rates (0, 2.5, 5.0, and 7.5 t ha^{-1}), 5 t ha^{-1}manure, 50 kg ha^{-1} of NPK, and a combination of the different biochar rates with manure and NPK on Casuarina growth and soil physical-biochemical properties. All treatments were replicated thrice. At the seedling stage, seedlings treated with 10% manure significantly enhanced Casuarina height by up to 46.8% and collar diameter by up to 30.7% compared to the control ($p<0.05$). For the field experiment, 7.5 t ha^{-1} biochar treatment yielded a higher Casuarina height of < 23.4% than the unamended treatment. The lowest biochar rate of 2.5 t ha^{-1} yielded a higher collar diameter of < 30.2% in comparison with the unamended treatment. Largely, soil bulk density decreased with biochar application by <23.2% compared to the unamended treatment after utilization of the highest biochar rate. The use of 7.5 t ha^{-1} biochar yielded the highest soil moisture content, which increased by up to 108% compared to the untreated control across the assessment periods. In terms of biochar's impact on soil chemical properties, there was substantial soil pH improvement of up to 21.3% after biochar application of 7.5 t ha^{-1}; there was an increase of up to 1.3 pH units. Total nitrogen increased by 32.4% after biochar addition while total carbon increased by four-fold. Available phosphorus and Cation exchange capacity (CEC) increased by up to 263.7% and up to 95.2%, respectively, following biochar application. Soil bacteria increase by up to 32.6% and fungi by up to 47.6% after biochar utilization in comparison with the unamended treatment. Ameliorating soil with 7.5 t ha^{-1} biochar yielded higher Casuarina height than the sole application of NPK. These results strongly suggest optimal growth of Casuarina seedlings requires the application of manure with high nutrient contents at the seedling stage while the application of biochar at field establishment has the potential to enhance the growth of Casuarina and improve soil physical-biochemical properties.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Casuarina *(Casuarina equisetifolia*) is an actinorhizal tree that grows well in semi-arid to sub-humid environments (Orwa et al., 2009). The tree is mainly grown along the Coastal region and is not adapted to the hinterland regions (Ngom et al., 2016; Jin et al., 2021). There are more than two million hectares of Casuarina plantations that are grown all over the tropics which provide several environmental, socio-economic, and ecological benefits (Mbuvi, 2010). In Africa, Casuarina is grown in over 20 countries where it plays a crucial role in livelihood improvement (Diagne et al., 2013). Casuarina's adaptability to various ecological, edaphic, and climatic conditions; fast growth; numerous uses, and symbiotic nitrogen-fixing ability, make it an ideal tree for commercial forestry in the Coastal region (Diagne et al., 2013; Mbuvi et al., 2014).

In Kenya, Casuarina is mainly grown along the Coast by both smallholder and large-scale farmers majorly for the production of poles for construction purposes (Mbuvi, 2010). Small-holder farmers at the Coast account for 81.8% of total Casuarina production (Mbuvi et al., 2014). Government records show that Casuarina was first propagated in the Jilore tree nursery in the 1960s (Konuche & Haller, 1984; Mbuvi, 2010). Casuarina is the most preferred source of construction material in the Coastal region of Kenya. Farmers are currently practicing Casuarina farming as a livelihood source due to poor climatic conditions that have rendered other crops' farming unproductive (Mbuvi et al., 2014). The species has also been widely used by cement companies for the rehabilitation of exhausted limestone quarries (Gathuru, 2011). Casuarina is also widespread in other tropical and sub-tropical regions where it plays a significant role in biological nitrogen fixation through its symbiotic relationships with Frankia bacteria and enhances phosphorus acquisition from the soil through its symbiotic association with Mycorrhizal fungi (Diagne et al., 2013; Wang et al., 2013).

With many farmers now engaged in tree farming in the Coastal region of Kenya, in particular Kilifi County, there is increasing concern about climatic vagaries that have affected Casuarina stands with some stands drying up during the dry seasons (Researcher's observation). This can be attributed to poor water-holding capacity of sandy soils that dominate the Casuarina growing zones, and the declining of soil fertility resulting from inadequate soil nutrients replenishment (Chianu et al., 2012; Ortega et al., 2016). The deteriorating of soil fertility due to poor soil management is the main cause of diminishing land productivity in the

African continent (Vanlauwe et al., 2017). The main reason leading to declining of soil fertility is nutrient depletion through soil erosion and the leaching of nutrients such as nitrogen and phosphorus (Mirriam et al., 2022; Nyawade et al., 2021; Kisaka *et al*., 2023). Nutrient loss accrued to erosion in African soils is approximated to be 45 kg of NPK ha^{-1} yr $^{-1}$ (Obalum et al., 2012; Bashagaluke et al., 2018).

Recurrent prolonged dry periods have become a feature of the Kenyan Coast due to climate change and have led to poor tree growth. With climate change unfolding, climate unpredictability is likely to worsen thus leading to more temperature increases and erratic and unreliable rainfall (Ochieng et al., 2016). Temperature rise in Africa has been noted to be around 3ºC to 4ºC which is almost twice the global mean (Thornton et al., 2010; Altieri et al., 2016). The increase in temperature as highlighted by Ochieng et al. (2016) and Thornton et al. (2010) is expected to result in low and highly erratic rainfall hence hindering tree production. To mitigate these changes, Kenya's Vision 2030 highlights the importance of increasing the forest cover from the current 6% to 10% through the rehabilitation of degraded forests and on-farm tree farming (KFS, 2014).

For farmers to realize Casuarina farming's full economic potential in the Coastal region while contributing towards attaining the 10% forest cover, there is a need for implementing adaptable measures at the farm level that solves the problems brought by climate unpredictability and diminishing soil fertility (Ochieng et al., 2016; Karanja et al., 2021; Vanlauwe et al., 2017; Nyawade et al., 2020). Among many technologies, biochar and manure offer a cost-effective means of enhancing soil fertility and soil moisture storage (Imoro et al., 2012; Faridvand et al., 2021; De Melo Carvalho et al., 2013). Several studies have reported benefits ensued from biochar and manure as soil amendments, particularly for soil moisture retention and crop yield improvement globally (Al-Wasfy & El-Khawaga, 2008; Pühringer, 2016; Adeyemi & Idowu, 2017). Use of mineral fertilizer for tree growing is not new in developed countries globally (Adebayo et al., 2017; Sida et al., 2020; Soratto et al., 2021; Heydarzadeh et al., 2023). Nevertheless, research findings on the impact of biochar, inorganic fertilizer, and manure on tree growth are inconclusive. Regardless of numerous studies investigating the impact of biochar, manure, and inorganic fertilizer on soil characteristics and yield of crops, utilization of biochar, manure, and inorganic fertilizer for improving tree growth in Kenya has not been extensively carried out, especially at the coast region.

1.2 Statement of the Problem

The Coast region of Kenya where Casuarina is grown is characterized by poor soils which often leads to low agricultural output hence resulting in constant food shortages (Mwangi et al., 2010; Mwadalu et al., 2020). Based on the foregoing, farmers have started engaging themselves in agroforestry practices using Casuarina with some farmers in the region growing pure Casuarina woodlots (Mbuvi, 2010). More than two million hectares of Casuarina plantations grown throughout the tropics offer numerous environmental, socio-economic, and ecological benefits. Casuarina's adaptability to a range of climatic and edaphic conditions, fast growth, several end uses, and symbiotic nitrogen-fixing capability makes it the utmost preferred tree by farmers at the Coast (Diagne et al., 2013; Mbuvi et al., 2014).

In Kenya, research on Casuarina has focussed mainly on its suitability for rehabilitation of degraded limestone quarries (Gathuru, 2011); on performance on soils with low Phosphorus (Nyamai & Juma, 1996); species adoption rates at the Coast region (Mbuvi et al., 2014); survival and growth performance at different spacing (Kirongo et al., 2013); and economic evaluation (Wekesa and Mwalewa, 2015). Despite the massive economic and environmental benefits accrued to the species, there is limited research in Kenya solely focussing on this species. Data on factors affecting the growth of Casuarina is still lacking despite the challenges faced by Casuarina farmers during dry spells when several Casuarina trees dry up due to moisture stress and nutrient deficiency. Based on the foregoing, there is a need to explore soil amendment technologies that boost soil moisture retention and enhance soil fertility for improved Casuarina growth in the region. The use of biochar, manure, and inorganic fertilizer are some of the innovations that this study seeks to validate as a soil amendment with great potential for improving physical-biochemical soil properties thus leading to increased *C. equisetifolia* growth.

1.3 Objectives

1.3.1 Overall objective

The present study aimed at enhancing soil properties and growth of *Casuarina equisetifolia* through the utilization of biochar, manure, and inorganic fertilizer in Kilifi County, Kenya.

1.3.2 Specific objectives

The specific objectives include:

i. To assess the effect of sole biochar application on soil physical (soil moisture content and bulk density), chemical (CEC, pH, nutrient levels), and biological (microbial population) properties.

ii. To determine the effect of biochar application on Casuarina (*Casuarina equisetifolia*) growth performance.

iii. To assess the effect of biochar combined with manure and inorganic fertilizers application on Casuarina (*Casuarina equisetifolia*) growth.
iv. To evaluate the effect of soil moisture content and soil nutrients on the growth of Casuarina.

1.3.3 Hypotheses

The study sought to validate the following alternative hypotheses:

i. Biochar application significantly improves soil physical and biochemical properties (i.e., lowers soil bulk density and increases pH; CEC, nutrient levels, microbial population, and soil moisture).
ii. Biochar application significantly increases *C. equisetifolia* growth (Height, DGL & DBH).
iii. Biochar application combined with manure and inorganic fertilizers significantly increases *C. equisetifolia* growth (Height, DGL & DBH).
iv. Soil moisture content and soil nutrients accrued to biochar application significantly increased Casuarina growth.

1.4 Justification and Significance of the Study

Deteriorating soil fertility and unreliable rainfall are the main constraints to smallholder farming and sustainable food production in drylands (Thornton et al., 2010). This has forced farmers to engage in tree farming as an alternative source of livelihood. It is noted that changing temperature and precipitation patterns due to global warming will have a strong direct influence on both natural and forest plantations (Kirilenko & Sedjo, 2007). These adverse effects are already apparent in the Coastal region of Kenya where Casuarina plantations often dry up during the dry season. Water stress limits the productivity of forest plantations by affecting tree metabolic processes such as turgor and carbon gain (Teskey & Hinckley, 1986). Maintenance and improvement of soil quality is therefore paramount for enhancing environmental quality and land productivity (Reeves, 1997; Sairaam et al., 2023; Otieno et al., 2021).

Research on Casuarina has shown that the species provides a viable alternative source of livelihood for smallholder farmers due to its market potential in the Coast region (Mbuvi et al., 2014; Wekesa & Mwalewa, 2015). Nevertheless, with diminishing soil fertility in addition to the adverse impact of climate change, massive failures are expected, particularly during dry periods (Goher et al., 2023). This, therefore, necessitates the adoption of affordable technologies that boost soil moisture storage while enhancing soil fertility.

Biochar is an effective soil amendment with capabilities of boosting soil moisture storage and increasing soil fertility (Velez, 2012; Libutti et al., 2016;). Manure and mineral fertilizer use have also been explored in afforestation programmes in other parts of the world (Gilman & Marshall, 2014; Prakash et al., 2014). Numerous studies have documented the positive impact of biochar, manure, and mineral fertilizer in enhancing carbon sequestration, available soil nutrients, lowering bulk density, enhancing soil moisture storage, and boosting microbial activities in the soil thus enhancing nutrient cycling (Mcelligott & Coleman, 2011; Hardie et al., 2014; Adeyemi & Idowu, 2017).

This study, therefore, sought to address the moisture stress and soil fertility challenges that affect Casuarina growth through the application of biochar, manure, and inorganic fertilizer. These technologies despite their positive impacts on the soil are rarely applied for tree growing in Kenya and Sub-Saharan Africa in general (Gwenzi et al., 2015; Pühringer, 2016). The findings of this research will provide information on the most appropriate technology for tree growing in drylands. The findings are also key information to extension officers and researchers tasked to provide advisory services to farmers on suitable tree-farming technologies for optimal productivity. The current research will also provide tree nursery managers with information on soil amendments required for optimal seedling production.

1.5 Conceptual Framework

Climate change effects are mostly demonstrated in depressed and sporadic rainfall, frequent and prolonged droughts, shortened rainfall periods with unpredictable rainfall onsets, and frequent flooding (Ochieng et al., 2016). The impact of climate change has led to massive crop failures along the Coastal region of Kenya. Coupled with the already infertile soils in the region, the frequent droughts have forced many farmers to find alternative sources of livelihood (Mbuvi et al., 2014). Tree farming has now been widely adopted in the region. Casuarina is the ideal tree species due to its fast growth rate, economic value, and nitrogen-fixing capabilities (Wekesa & Mwalewa, 2015). In the recent past, harsh climatic conditions have led to its slow growth and occasionally drying up.

For the sustainability of Casuarina trees, technologies aimed at maintaining soil fertility while boosting water retention capacity in sandy soils dominant in the region, are of great importance. These include the use of biochar, manure, and mineral fertilizers which offer the potential for enhancing soil physical-biochemical characteristics (Kazmi et al., 2010; Gao et al., 2017; Razzaghi et al., 2020). The technologies enhance soil microbial population, soil moisture, organic carbon, pH, potassium, nitrogen, phosphorus, and CEC and decrease bulk density, thus leading to improved *C. equisetifolia* growth (Figure 1.1).

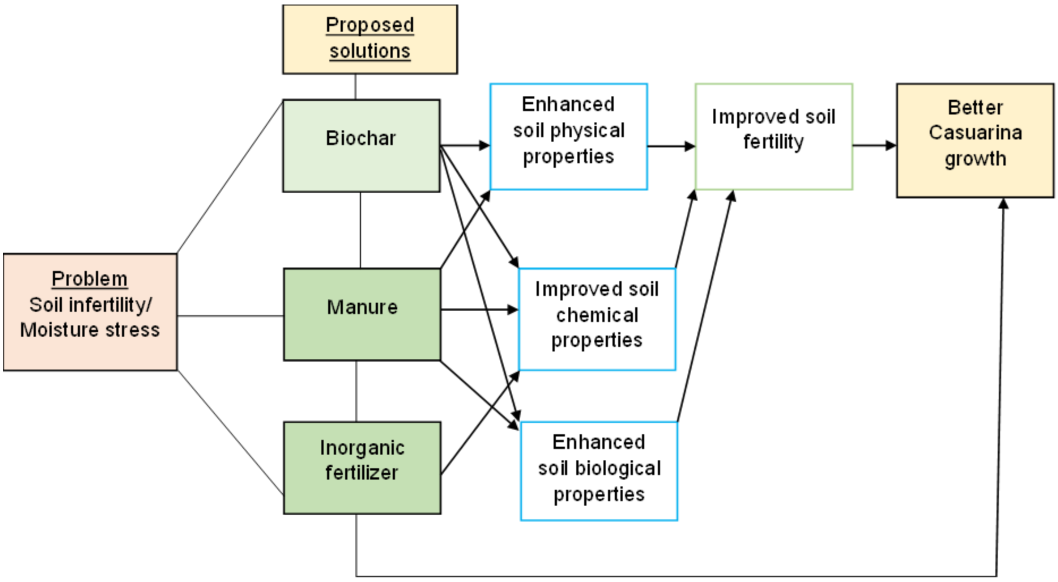


Figure 1.1: Conceptual Framework (Source: Researcher)

CHAPTER TWO

LITERATURE REVIEW

2.1 *Casuarina equisetifolia* Botany and Ecological Requirements

Casuarina equisetifolia is an evergreen dioecious tree species that grows in open coastal habitats such as sand beaches, rocky coasts, and sand dunes. The trees are capable of attaining a height of over 30.5m. Casuarina grows naturally in Australia and Southeast Asia. *Casuarina equisetifolia'*s crown is finely branched with a conical shape at the nascent stages but tends to flatten with age. Casuarina bole is usually straight, with a cylindrical shape, that is usually branchless. The outer bark of Casuarina is light greyish-brown, while the inner bark is usually slightly reddish or deep dirty brown. The species thrives in semi-arid to sub-humid climates (Orwa et al., 2009).

Casuarina equisetifolia is a woody species that thrives in salt-rich environments. The species usually grows in altitudes (0-1400 m.a.s.l.) where average temperatures are 10-35°C and mean annual rainfall of 200-3500 mm. Casuarina flourishes well in well-drained coarse-textured soils predominantly sands and sand loams (Orwa et al., 2009). The tree also tolerates both calcareous and slightly alkaline soils. The tree performs poorly in areas with prolonged waterlogging and has been reported to have stunted growth on poor sands where the subsoil moisture is inadequate. The root nodules containing the actinorhizal symbiont Frankia are prolific in soil which enables *C. equisetifolia* to fix atmospheric nitrogen. C*asuarina. equisetifolia* also possesses proteoid roots that form associations with vesicular-arbuscular mycorrhizae (Orwa et al., 2009; Diagne et al., 2013).

These trees play a vital role in increasing soil N content, land reclamation, and agro-forestry (Saravanan et al., 2012). In Kenya, research on *C. equisetifolia* has focussed mainly on: suitability for rehabilitation of degraded limestone quarries (Gathuru, 2011); survival and growth performance at different spacing (Kirongo et al., 2013); performance on soils with low Phosphorus (Nyamai & Juma, 1996; Mwakidoshi al., 2023); economic evaluation (Wekesa & Mwalewa, 2015) and species adoption at the Coastal region (Mbuvi et al., 2014). Mbuvi et al. (2010) reported that many farmers in the coastal region of Kenya grow casuarina for improved livelihood. The study estimated that on average, one out of four farmers in the region cultivates Casuarina as a source of income. The study further estimated that there is over 2000 ha of casuarina grown at the coast with 82% being grown by smallholder farmers.

2.2 Biochar Properties, Production, and Application

2.2.1 Definition and properties of Biochar

Kazmi et al. (2010) defined biochar as fine-grained carbon-rich residue which is produced through pyrolysis processes. Biochar has also been defined as a by-product of slow pyrolysis which is fine-grained, carbon-enriched, and porous where feedstock is broken down at low to moderate temperatures under a limited oxygen supply (Joseph et al., 2010; Oshunsanya & Aliku, 2016). Biomass for biochar production may include woody materials, crop residues, and dairy manure among other farm wastes. Biochar is in a more biologically and chemically stable form compared to the original feedstock from which it is produced due to its molecular configuration, thus making it more difficult to break down (Kazmi et al., 2010).

The use of biochar has been considered to apply the same principle as the traditional slash-and-burn systems in the world. Although the addition of charcoal to the soils existed as a practice, it was, however, not explicit but remained part of traditional best practices in many parts of the globe (Tel, 2018). Biochar has been reported to outperform other organic soil amendments in its capability to absorb and retain water and nutrients (Mekuria & Noble, 2013). Previous studies have highlighted enhanced crop productivity after biochar use. Biochar also reduced the total fertilizer required in a cropping season (Joseph et al., 2010). Table 2.1 shows the selected physico-chemical properties of biochar.

Table 2.1: Selected physico-chemical characteristics of biochar produced under varying conditions and feedstocks

Feedstock	**Pyrolysis Temp.**	**SSA (m2/g)**	**% C**	**% H**	**% N**	**% O**	**% Ash**
Maize cob	250 °C	1.86	61.16	4.96	0.82	27.82	3.92
Maize cob	300 °C	2.42	70.54	4.19	0.81	19.06	4.1
Maize cob	350 °C	3.36	72.92	3.79	0.79	16.86	4.35
Maize cob	400 °C	4.70	75.23	3.37	0.82	14.11	5.12
Maize cob	450 °C	7.79	77.84	2.95	0.86	11.45	5.55
Maize cob	500 °C	17.08	80.85	2.5	0.97	8.87	5.56
Wood chip	450 °C	12.96	70.44	2.67	1.11	13.86	10.23

Abbreviations: H-hydrogen; SSA-specific surface area; C-carbon; O-Oxygen and N-nitrogen; **(Source: Adeyemi & Idowu, 2017)**

The vital characteristics of biochar are high adsorptive capability and porosity; large surface area; low bulk density; high moisture storage, and reduced root penetration resistance of soil (Oshunsanya & Aliku, 2016). These properties improve available soil moisture critical for crop growth. Additionally, a substantial association exists between biochar's surface area and pore volume (Mekuria & Noble, 2013).

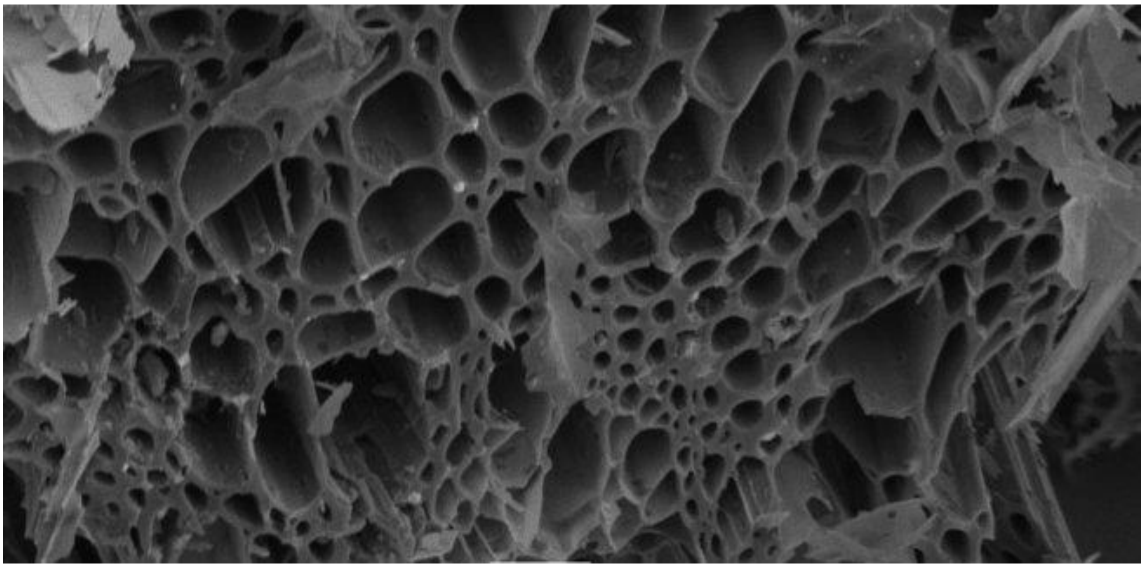


Figure 2.1: Biochar's surface area and porous structure; Photo source: www.carbolea.ul.ie via Biochar Ireland

Biochar's adsorption can be accredited to its large surface area, chemical properties, and porous nature. Biochar's adsorption capacity influences the uptake and binding effect of nutrients from the soil. The aggregate stability of soil amended with biochar determines the susceptibility of biochar to microbial activities (Kelly et al., 2015). Low biochar susceptibility to microbial processes enhances the stability of SOM down the soil profile while enhancing water and nutrient availability to crops and reducing erosion (Brantley et al., 2015; Tel, 2018).

Biochar largely contains Nitrogen, Phosphorus, and basic cations like Potassium, Magnesium, and Calcium (Poitras & Straubing, 2009; Clough et al., 2013). Biochar produced from plant feedstock contains lower nutrient concentrations such as nitrogen, potassium, and calcium but higher organic carbon in comparison to biochar derived from manure. The concentration of C and N in plant-based biochar increases with increasing pyrolysis temperature while in biochar obtained from manure, declines with increasing pyrolysis temperature (Adeyemi & Idowu, 2017). Generally, biochar's nutrient content and its bioavailability depend on the feedstock and pyrolysis conditions (Poitras & Straubing, 2009; Joseph & Taylor, 2014).

2.2.2 Production and application of Biochar

Agricultural and forestry production systems have been known to produce significant amounts of waste like forest residues, field crop residues, and animal manure (Adeyemi & Idowu, 2017). In many instances, these wastes limited value, and their disposal is often costly. These waste materials can nevertheless be converted to biochar. The ideal materials for biochar production should contain high lignin concentrations thus yielding large quantities of biochar; such as wastes from sawmills and forest residues (Lane, 2016; Adeyemi & Idowu, 2017).

There are several thermochemical technologies used for biochar production; these include gasification, pyrolysis, and hydrothermal conversion. Nevertheless, pyrolysis is the main method used for biochar production (Mekuria & Noble, 2013). Pyrolysis has been defined as the direct thermal breakdown of feedstock in oxygen-free conditions to obtain a range of products namely; gas (syngas), solid (biochar), and liquid (bio-oil). The main product of pyrolysis is dependent on the process conditions (Hovi et al., 2010; Adeyemi & Idowu, 2017). Gasification on the other hand is a thermochemical process where biomass is heated with a limited air supply to produce syngas as the key product and biochar as a by-product. Gasification produces approximately 10% of biochar (Adeyemi & Idowu, 2017). Another technique used for biochar production is Hydro-thermal carbonization (HTC); this process involves steaming biomass together with acid as a catalyst. The main by-products of this process are bio-oil and limited amounts of biochar (Mekuria & Noble, 2013).

Biochar's physico-chemical characteristics and its quality are dependent on production conditions like temperature and residence time, biomass chemical composition, and pyrolysis system (Poitras & Straubing, 2009; Mekuria & Noble, 2013; Pühringer, 2016). Biochar produced from varying pyrolysis temperatures has different impacts on its ability to adsorb nutrients. Biochar adsorption capacity is influenced by changes in its surface properties as pyrolysis temperatures change (Adeyemi &Idowu, 2017). Previous studies noted that thermal treatment of feedstock during biochar preparation results in its large surface area and its ability to persist in soils with limited decomposition (Poitras & Straubing, 2009; Xie et al., 2015). The key biochar characteristics include low bulk density; large surface area; high porosity, stability, and high cation exchange capacity (CEC) (Mohan et al., 2018); high carbon content and pH (Berek et al., 2014). These characteristics make biochar a viable soil amendment in tropical parts of Africa (Gwenzi et al., 2015).

2.3 Impact of Biochar on Physico-chemical Soil Properties

2.3.1 Biochar's influence on soil moisture content

Studies investigating the impact of biochar on soil moisture storage in Kenya have not been accorded much focus while at the same time studies conducted across the globe have recorded conflicting results. A study by Pühringer (2016) conducted in Siaya reported a slight increase in soil moisture content with increasing biochar doses. Soil moisture content differed substantially after the use of biochar (5.0 and 10 t ha^{-1}) in comparison with the unamended treatment. According to Asai et al. (2009), as quoted by De Melo Carvalho et al. (2013), biochar has high total porosity, an attribute that enables it to hold moisture in micropores thereby enhancing water-holding capacity. It also enables water to rise the soil profile through capillary after excess rainfall. In a study by Åslund (2012), soil water content at field capacity was substantially higher after the additions of biochar than the unamended treatment. Glaser (2002) as quoted by Pühringer (2016) also observed enhanced moisture retention by 18% after biochar amendment.

According to Libutti et al. (2016) application of 2%, 4%, or 8% biochar to the soil improved soil moisture content at field capacity in comparison with the unamended treatment. Libutti et al. (2016) further observed variations in soil moisture content between biochar-amended soils and unamended treatment at near wilting point. The capacity of biochar to enhance moisture retention at higher soil water potential ensures the optimum growth of plants. Novak et al. (2012) concluded that generally, biochar amendments enhance soil moisture storage. However, they further noted that soil moisture enhancement depends on biomass and pyrolysis temperature used for biochar production. The study also noted that sandy soils retained more moisture despite their coarse texture. The study by Novak et al. (2012) also reported improvement in soil moisture storage in two silt loam soils ranging between 0.5 and 0.8 cm of water per 15 cm soil depth. However, other studies observed a lack of substantial soil moisture changes as a result of biochar amendment even with increasing rates and recommended more research to be undertaken on the subject (Abdullaeva et al., 2014; Brantley et al., 2015).

2.3.2 Soil bulk density as influenced by biochar amendment

Bulk density (BD) has been used as a determinant of soil compaction and impacts on soil health. It also impacts soil moisture, nutrient availability, infiltration, rooting depth, soil porosity, and soil microorganism processes (USDA, 2020). Bulk density has been defined as the mass of dry soil per unit volume which is often expressed in g cm^{-3}. It is influenced by the organic matter content, texture, and density of the mineral soil (Ma et al., 2016). Generally, well-aggregated, loose, porous soils with adequate SOM have lower bulk density. Soils with high sand content have higher bulk density than silt and clays. Bulk density is influenced by soil management techniques that impact compaction, porosity, and soil cover (USDA, 2020). Table 2.2 below shows the interaction between bulk density and root development as influenced by texture.

Table 2.2: Interaction between bulk density and root development as influenced by texture

Soil texture	Ideal BD for plant growth (g/cm^3)	BD to root growth (g/cm^3)	BD that restricts plant growth (g/cm^3)
Sands/ loamy sands	<1.60	1.69	>1.80
Sandy loams/ loams	<1.40	1.63	>1.80
Sandy clay loams/ clay loams	<1.40	1.60	>1.73
Silt/ silt loams	<1.40	1.60	>1.73
Silt loams/ silt clay loams	<1.40	1.55	>1.65
Sandy clays/ silt clays/ clay loams	<1.10	1.49	>1.58
Clays (>45% clay)	<1.10	1.39	>1.47

Source: (USDA, 2020)

Studies have observed decreased bulk density after biochar utilization (Aslam et al., 2014; Haider, 2017; Lane, 2016; Oshunsanya & Aliku, 2016; Humberto, 2017). Humberto (2017) observed decreased density of 3 to 31% after incorporating biochar into the soil. According to Oshunsanya & Aliku (2016), the decrease in bulk density of biochar-amended soils could be resulting from improved soil structure and aeration. Omondi et al. (2016) also reported a decrease in BD by 7.6% following biochar addition. The decrease in BD was substantial as the amount of biochar used increased. Biochar has been observed to lower bulk density in coarse-textured soils more than in fine-textured soils. This is estimated at 14.2% in course textured soils and 9.2% in fine-textured soils (Omondi et al., 2016).

Low BD (1.4 mg m^{-3}) was observed in biochar-amended plots; the unamended treatment had a BD of 1.5 mg m^{-3} (Hseu et al., 2014). The plots ameliorated with 10% biochar recorded a reduction of BD of up to 25% compared to the unamended treatment. The decline in bulk density following biochar utilization may be a result of biochar's lower bulk density (0.63g cm^{-3}) than soil (1.25g cm^{-3}). Biochar application reduces BD probably through mixing or dilution effect (Humberto, 2017). Soil types have been reported to substantially influence the impact of biochar on BD. Studies have shown that reduction in BD with biochar utilization is a result of enhanced soil porosity with biochar addition thus enhancing aeration and root development. Biochar has a porosity of 70 to 90% (Aslam et al., 2014; Are, 2019). Higher bulk density has been observed to restrict root growth resulting from soil compaction (Kormanek et al., 2015).

Abrishamkesh et al. (2015) reported an insignificant impact of biochar on BD, although, with increasing rates, some changes were observed. The study attributed the decrease in soil BD to the low bulk density of biochar used compared to that of mineral soils; similar observations were described by Ma et al. (2016).

2.3.3 Carbon (C) and Soil Organic Matter (SOM as influenced by biochar utilization

Following a study that evaluated the impact of biochar on the fertility of saline and alkaline soils, Abdullaeva et al. (2014) observed enhanced SOM content as a result of increased doses of biochar. A Biochar dose of 30 g kg^{-1} recorded substantially higher SOM. Soil carbon values differed substantially in biochar-amended samples in comparison with the unamended treatment. The study by Mohan et al. (2018) revealed that amending soil with biochar resulted in substantial enhancement of soil organic carbon on the 107th day of the growing period while application of biochar at 3.0% enhanced carbon content by 328% above the unamended treatment after 107 days of the experiment period. Xie et al. (2015), observed that biochar utilization is a sustainable approach for carbon neutralization ensuing from acting as a carbon sink and the reduction of greenhouse gas emissions.

2.4 Impact of Biochar on Soil Chemical Characteristics

Understanding the physico-chemical characteristics of biochar is crucial for ensuring pathways under which biochar enhances soil productivity are understood (Ding et al., 2016). Biochar's influence on soil productivity has been widely explored by different researchers in the recent past since the discovery of Terra preta soils (Oshunsanya & Aliku, 2016). Numerous studies have evaluated soil chemical properties as influenced by biochar utilization. However, the results are still inconclusive due to conflicting results reported by different scientists. Soil nutrient transformations are dependent on the type and biochar quality (Page-Dumroese et al., 2016). While some studies have observed enhanced availability of nutrients after biochar amendment (De Melo Carvalho et al., 2013; Abdullaeva et al., 2014; Mohan et al., 2018), some studies have, however, not observed any significant effect on some experiments as revealed in literature (Bonanomi et al., 2017). Velez (2012) noted that the addition of 5% biochar substantially lowered nutrient availability while the utilization of 2% biochar enhanced virtually all soil nutrients. Biochar's chemical composition differs due to production conditions such as pyrolysis temperature and the feedstock materials used (Li et al., 2019).

Previous studies observed enhanced soil pH following biochar utilization (Mensah & Frimpong, 2018; Mwadalu et al., 2020). The trend of enhanced pH with biochar utilization was also observed by Mohan et al. (2018) where pH improved after the utilization of varying biochar types. It further revealed that in biochar-treated soils, pH increases ranged between 0.3 to 0.8 units. Mohan et al. (2018) attributed enhanced pH to cation exchange capacity (CEC) improvement resulting from biochar's porosity and large surface area. Biochar enhanced pH buffering capacity and aided in alleviating pH decline over time (Trupiano et al., 2017). Zhang et al. (2019) observed a substantial improvement in soil pH by 0.5 to 1 unit after biochar utilization. Gao et al. (2017) also observed enhanced soil pH after biochar utilization. Dai et al. (2014) observed that utilization of 3% biochar enhanced pH by up to 2.52 units with the application of swine manure biochar. The increase in pH is attributed to carbonates and organic anions (-O- and COO-) in the biochar that led to its alkalinity (Dai et al., 2014). Biochar liming effect depends on the feedstock used for its production. Biochar maintains pH for optimal plant development as observed by Leghari et al. (2016) and Yunilasari et al. (2020).

Biochar utilization influences soil electrical conductivity (EC). The enhanced EC following biochar application was attributed to the release of weakly bound nutrients on biochar surfaces into the soil solution thereby availing them for plant utilization (Chintala et al., 2013). Generally, EC improved as the rates of biochar application increased. This could be a result of the salts in biochar used (Agegnehu et al., 2015). Mensah and Frimpong (2018) observed increased soil pH in plots amended with sole and combined application of biochar and manure with sole biochar application resulting in higher pH.

In a study conducted in Brazilian Savannah by De Melo Carvalho et al. (2013), biochar application led to a linear increase of exchangeable Mg, exchangeable Ca, and CEC while a linear decrease of Al was observed. The study also revealed that biochar's influence on potential acidity had a quadratic tendency with the highest point at 16 mg ha^{-1}. The CEC of soils determines the adsorption capacity of the cationic nutrients, those available for plant utilization, and prevented from leaching to surface and ground waters. Mohan et al. (2018) reported that biochar utilization enhanced Na^+, K^+, $Ca^{2+,}$ and Mg^{2+} availability in the soil. The study further reported an increase in CEC of up to 362% following the application of higher rates of biochar compared to the control. The enhanced CEC as reported by De Melo Carvalho et al. (2013), Pühringer (2016), and Mohan et al. (2018) can be attributed to low biochar oxidation which increases char's carboxyl groups thus enhancing soil CEC. The relatively high CEC values accrued to biochar application explain its capacity to retain soil nutrients. This is contrary to the findings by Abdullaeva (2014) where no substantial increase in CEC after biochar application was observed.

The Phosphorus (P) increase with biochar application contributes to greater root growth thus enhancing nutrient and water uptake by plants. Application of biochar with liming properties on P-deficient soil enhanced phosphorus uptake by 73% and plant growth by 59%. Biochar's impact is dependent on the type of feedstock used during preparation (Shen et al., 2016). Feedstock used for biochar preparation largely affects the total P content in biochar (Li et al., 2019). Woody biochar has higher P content than biochar from manure. It has also been noted that biochar prepared from high pyrolysis temperatures >550ºC has a larger surface area. The high temperatures alter the contents of P in the biochar (Li et al., 2019). Generally, P concentration steadily increases as pyrolysis temperature increases (Li et al., 2019; Glaser & Lehr, 2019). It has been noted that during pyrolysis, P content in biochar can be enhanced by two to three folds.

Approximately 50% of P from biochar is released in form of orthophosphates and pyrophosphates at pH <9 to the soil (Li et al., 2019). Phosphorus adsorbed by biochar can be released and utilized by plants for growth as part of biochar's aging process. Mensah & Frimpong (2018) observed enhanced P following sole biochar application at 2%. They attributed this to reduced iron (Fe) and Aluminium (Al) activity as a result of the increase in pH. Higher P is also a result of biochar's capacity to retain and exchange phosphate ions due to its positively charged surface (Mensah & Frimpong, 2018).

Truong & Marschner (2018) observed improved nitrogen after biochar utilization; this trend could be resulting from biochar's large surface area which enhanced retention of NH_4^+ thus leading to improved N nutrition. Contrary findings indicating a decrease in soil N with increased biochar amount applied were reported by Gao et al. (2017). Biochar's effect on K^+ was influenced by the N application rate; higher N rates required more biochar to achieve equivalent K^+ concentration. De Melo Carvalho et al. (2013) also observed lack of substantial impact of biochar on P and soil organic carbon (SOC) levels. This contradicts a study conducted by Abdullaeva (2014) which recorded a substantial improvement in SOC after biochar amendment. Among micronutrients, Manganese increased linearly while there was a substantial impact on Cu and Zn after biochar utilization (De Melo Carvalho et al., 2013).

2.5 Impact of Biochar on Soil Microbial Characteristics

Biochar's long-term impact on soil microbial characteristics is largely not understood. Studies have mainly focussed on short-term changes in microbial biomass through lab incubation experiments. Research shows that beneficial fungi and biological nitrogen fixation bacteria interactions are enhanced after biochar utilization (Adeyemi & Idowu, 2017). A study conducted for four years revealed that the microbial biomass significantly increased with biochar application (Zhang et al., 2014). Periodic variation in Microbial Biomass Carbon (MBC) for soils amended with biochar was minimal in comparison with unamended treatment. This showed that biochar utilization provided an ideal environment for microbial activity during the season (Zhang et al., 2014). A positive correlation between soil water content

(SWC) and MBC was observed, however, no substantial correlation between soil temperature and MBC was observed. Utilization of biochar may reduce temporal unpredictability thereby reducing temporal variations in N and C dynamics.

According to Gao et al. (2017), soil bacteria composition mainly consisted of Proteobacteria, Actinobacteria, and Acidobacteria, where the Proteobacteria proportion represented >50% of the bacterial community after biochar use. The study further revealed that fungi in tobacco planting soils were mainly Ascomycota (>75%), although the fungi population declined as the biochar rate increased. Biochar utilization could improve soil nutrient status and positively impact microbial community structure (Gao et al., 2017).

2.6 Impact of Biochar Utilization on Tree Growth

Studies on the effects of biochar on tree growth are still limited (Wilson, 2015). The growth of crops as influenced by biochar has however received significant focus in the recent past with studies being conducted to evaluate the effect of biochar on yields (Poitras & Straubing, 2009; Haider, 2017; Purhinger, 2016). Mohan et al. (2018) reported enhanced eggplant growth resulting from biochar utilization in comparison with the unamended treatment. Additionally, it revealed that biochar-ameliorated soils (3%) yielded the highest growth rate in terms of plant height and the number of leaves. The average height improved from 8.3 cm in the first week to 20 cm (7th week) versus 6.0 cm (1st week) to 9.5 cm (7th week) in the unamended treatment. Similar results on enhanced plant growth resulting from biochar utilization were observed by Carter et al. (2013).

According to Rahim (2018), there was a substantial increase in height of Glycine max (L.) following biochar utilization. The study attributed this to alleviating the physiochemical stresses, particularly water and nutrient scarcity as a result of biochar application. These findings concur with the observations by Berek et al. (2014) who observed enhanced plant biomass by 2 to 4 folds following biochar amelioration. A study by Drabkin & Weinfuether,(2014) reported that tree growth showed substantial variations on sites ameliorated with biochar. The study, however, stated that it was too early to make a conclusion using the results.

Helliwell (2015) reported an insignificant influence of sole biochar use on plant growth. Its integration with mineral fertilizer and OM resulted in noticeable plant growth enhancement. Blair et al. (2014) also observed lack of substantial influence on maize growth. With such limited data on the effect of biochar on tree growth, more research is crucial to ascertain existing trends.

2.7 Impact of Inorganic Fertilizer and Manure on Physico-biochemical Soil Characteristics

Fertilization is a vital soil management strategy that impacts soil productivity and sustainable utilization (Haile et al., 2023). Limited utilization of organic soil amendments and disproportionate use of inorganic fertilizers has led to a substantial decrease in carbon contents, thereby leading to deterioration in agricultural soil productivity (Zhang et al., 2015). Numerous studies have focused on mineral fertilizer and organic manures’ effects on soil physical-biochemical parameters, such as SOC. Arriaga & Lowery (2003) reported that manure application improved total carbon content substantially to a depth of 25 cm.

The study by Arriaga & Lowery (2003) revealed that long-term manure utilization substantially lowered bulk density in the top soil layers. Shirani et al. (2002) also observed a substantial soil bulk density decrease by up to 16% with 60 kg/ha manure application. Amusan et al. (2013) noted that the application of 5 to 10 tons/ha manure improved soil porosity and soil moisture retention while bulk density decreased. In comparison with the unamended treatment, application of manure from poultry at 2.5, 5.0, 7.5, and 10 tons/ha led to a decline in BD by 4.2%, 13.9%, 22.9%, and 31.3% respectively. Ewulo et al. (2008) further revealed that the application of manure of up to 50 tonnes per hectare improved soil physical properties as indicated by a reduction in soil bulk density. Josphinos (2016) observed a reduction in bulk density by 32% in the topsoil after poultry manure utilization in comparison with the unamended treatment. Rayns & Rosenfeld (2010) reported an improvement in soil structure accrued to manure utilization.

Manure additions also improved soil moisture retention capacity at all depths with the surface having the greatest increase (Arriaga & Lowery, 2003; Rahimi et al., 2023; Ewulo et al., 2008; Karažija et al., 2015). The enhanced soil moisture retention can be accrued to increased organic matter with manure application (Ewulo et al., 2008). Josphinos (2016) however, reported lack of significant effect of organic manures on moisture retention at field capacity and permanent wilting point. The study further revealed that plots without manure retained the highest mean water content in comparison with the other treatments.

Substantial enhancement of organic matter (OM) two years after manure application was observed by Shirani et al. (2002). Manure rates of 30-60 kg/ha enhanced OM by threefold and fivefold, respectively. Manure utilization was also reported to improve infiltration rate by <3.6% when compared to the unamended treatment.

Organic fertilizer application substantially increased available potassium, magnesium, phosphorous, iron, calcium, and zinc content (Kariithi et al., 2018; Maitra et al., 2020).

Additionally, nitrogen content increased from 0.02%, 0.13%, and 0.17% when applied at 0, 8.45, and 16.9 t/ha, respectively, in season one to 0.03%, 0.33%, and 0.44% when organic manure was applied at 0, 8.45, and 16.9 t/ha respectively, in season two. Further, the study revealed that calcium was enhanced substantially from 2.93 me% in the first cropping season to 3.93 me% in the subsequent season when the rate of 16.9 t/ha was used. Soil pH enhancement equally increased with increased manure application. Ewulo et al. (2008) observed substantial nitrogen and phosphorus improvement after the use of higher rates of poultry manure.

On the contrary, the findings by Abu-Zahra & Tahboub (2008) reported that there were no substantial changes in soil pH and soluble salts (EC) at the end of the experiment. The study attributed the lack of changes in soil pH to the high buffering capacity of the soil based on its high carbonate content (22-28%). The study, however, reported enhanced potassium content resulting from organic manure utilization in comparison with the unamended treatment.

Previous studies had pointed out that soil chemical properties were significantly influenced by mineral fertilizer utilization (Kariithi et al., 2018). Application of 500 kg/ha NPK enhanced manganese content slightly from 0.26me% to 0.46me% in the first and second seasons respectively (Kariithi et al., 2018). Phosphorous content, however, declined from 13.67 to 12.67 mg/kg when 250 kg/ha NPK was applied. Li et al.(2012) also observed a decline in available P where N fertilizer was applied. The study further revealed that manure combined with inorganic fertilizer increased total N, K, and P concentrations.

The growth and activity of microorganisms are influenced by soil characteristics like nutrient availability, temperature, pH, texture, and soil moisture. Microbial population dynamic changes may be attributed to the improvement of soil quality resulting from the application of various types and doses of organic materials. Various microbial parameters can potentially be used as soil quality indicators. These parameters include microbial diversity and biomass (Zhang et al., 2015). Al-Wasfy & El-Khawaga (2008) reported an increase in microbial activity with manure application. Appropriate utilization of manures within soil management systems can potentially enhance plant growth resulting from the availability of nutrients. Manures are also key to improving soil microbial activities (Zhang et al., 2015).

2.8 Impact of Manure and Inorganic Fertilizer on Tree Growth

Manure and inorganic fertilizer utilization for boosting tree growth has been explored in many afforestation programmes in the world. Imoro et al. (2012) evaluated the use of organic manure on the growth of *Moringa oleifera*. The study revealed that the shoot height of seedlings ameliorated with poultry manure was greater compared to those treated with cattle manure and the unamended treatment. Those treated with cow manure also out-performed the control. The study attributed the influence on shoot height to nutrients added into the soil through the manure that enhanced plant growth. Enhanced plant height due to the application of manure was also observed by Prakash et al. (2014) and Josphinos (2016). The study reported that application of manure significantly influenced *Calendula officinalis* which was evident that manure plots recorded higher tree height in comparison with the unamended treatment.

A study on the growth of *Acacia Senegal* seedlings as influenced by manure and NPK revealed that manure enhanced height growth of up to 6 cm per month in comparison with NPK and unamended treatment. Growth vigour of *A. Senegal* was enhanced with increased rates of manure. The increase in plant vigour was attributed to improved soil moisture retention and increased aeration thereby stimulating growth (Daldoum & Hammad, 2015). The weak response by NPK fertilizer can be attributed to the loss of nutrients from porous sand media by irrigation and hence seedlings could have less available nutrients to absorb.

A study by Blackmon (1977) that focussed on the effect of nitrogen fertilizer on tree growth revealed that fertilization with N led to substantial enhancement of the diameter, height, and volume of the trees. Stem diameter increased by 7mm in the control compared to the treatment with 336 N which had a stem increase of 18 mm in the first season. The height of the eucalyptus was significantly enhanced by N application in the first 24 months after planting. Nitrogen and Potassium also increased the above-ground biomass (Laclau et al., 2009). However, few studies have observed lack of substantial impact of inorganic fertilizer on tree growth (Hoque et al., 2004; Parecido et al., 2021); the study further revealed that phosphorus (TSP) and urea application of varying doses had no substantial impact on height growth and relative growth rate.

The combined impact of manure and mineral fertilizer on the growth parameters of different tree species has also been studied. Application rates of 50% NPK through inorganic fertilizer and 25% N through Farm Yard Manure (FYM) yielded the highest plant height in comparison with other treatments and unamended treatments (Reddy et al., 2017). Substantial plant height differences were also observed between plots amended with fertilizers (organic manure and NPK) and the unamended treatment (Adebayo et al., 2017). The study further highlighted that plant height improved in all growth phases after the application of different types of fertilizers in comparison with the control.

Navi (2013) reported that the utilization of manure and mineral fertilizer substantially influenced *C. equisetifolia* height. Application rates of N, P_2O_5, and K_2O (200:100:200) recorded higher heights and diameters throughout the study period. Minimum height was recorded in the control treatment. Saravanakumar & Shanthinipriva (2017) also reported a positive impact of manure and mineral fertilizer on the growth of *Populus deltoids*; the highest collar diameter and plant height were recorded with the application of 75:125:75 g NPK per plant followed by 100:125:75 g NPK per plant in comparison with the unamended pots. The increase in height was attributed to better utilization of nutrients by the tree.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research trials were undertaken at the Kenya Forestry Research Institute (KEFRI) research site situated at Gede, Kilifi County in the coastal region of Kenya. The research site was situated at S 03.29470 and E 038.99602. Kilifi county lies between latitude $2^o20'$ and $4^o0'$ South, and between longitude 38^o $05'$ and $39^o14'$ East. 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 2019 Kenya Population and Housing Census, the county had a population of approximately 1,443,787 people. This accounted for 3.1 percent of the total population in Kenya (KNBS, 2019). The total land area of the county is roughly 12,609.7 km^2.

The county receives low rainfall (110 mm in the hinterland) and 1,110 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 1800 mm 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 (*Cocos nucifera* (L.)), sisal (*Agave sisalana*), cashew nuts (*Anacardium occidentale*), and citrus fruits (*Citrus sinensis*). The dominant soil type in the Gede area where the experiment was conducted is considered Arenosols according to FAO classification (Campbell, 2015).

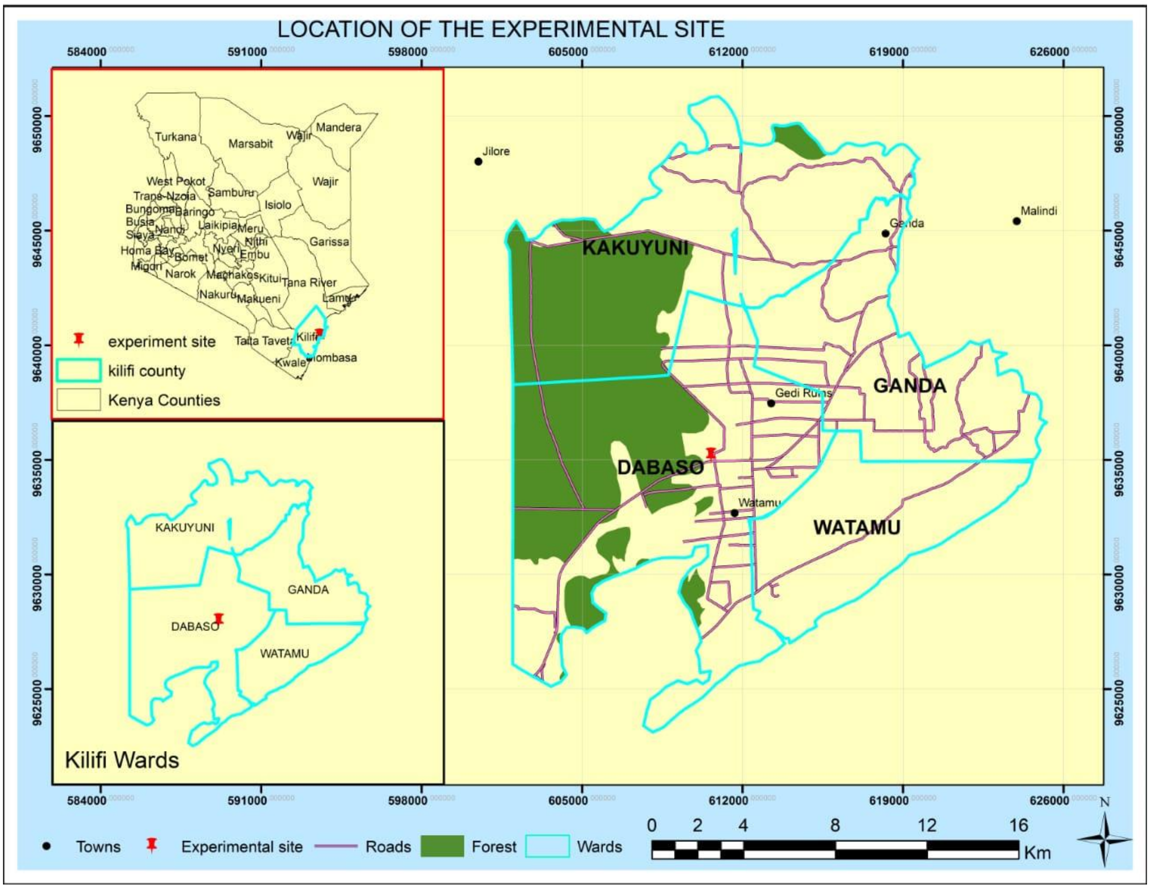


Figure 3.1: Map of Kilifi County showing the location of the field experimental site (red mark)

3.2 Experimental Design

The study consisted of two experiments, that is in the nursery and the field. The field experiment assessed the impact of the sole application of biochar on Casuarina growth performance, soil moisture content, and physicochemical properties. It also evaluated the combined impact of biochar, manure, and NPK on Casuarina growth performance.

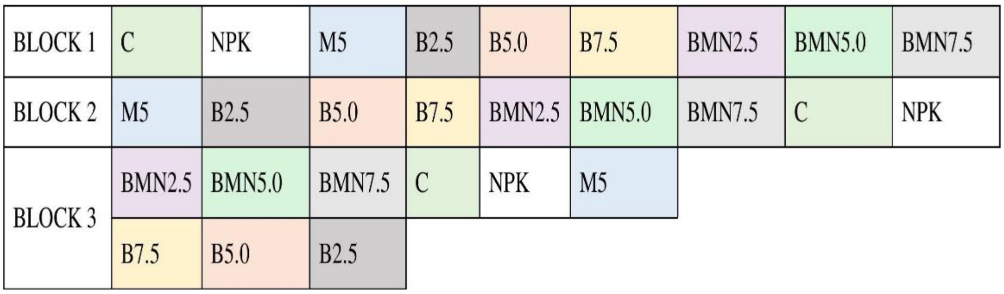
3.2.1 Field experiment

The field study consisted of nine treatments as shown in Table 3.1. The experiment was set using a randomized complete block design with each treatment replicated thrice. The test crop was *Casuarina equisetifolia* with plots measuring 12m by 12m for the field experiment. The recommended spacing of 2m by 2m (within and between rows) was used for the field experiment (Mbuvi, 2010). Seedlings measuring 45cm in height were used for the field trial. Biochar was produced using slow pyrolysis of dried *Prosopis juliflora* branches. Manure (sourced from Malindi Prisons) and inorganic fertilizer (N17: P17: K17) were used at a flat rate of 5 tons/ha and 50kg/ha, respectively. Biochar and manure (as per treatment) were mixed thoroughly with the soil removed from the planting hole measuring 30cm length, 30cm width, and 30cm depth during transplanting; the soil mixed with biochar/ manure or its combination was then returned to the planting pit and firmed up. Inorganic fertilizer was top-dressed 30 days after transplanting.

Table 3.1: Treatments for the field experiment

Treatment Code	Treatments Description
C	Control
NPK	NPK at 50 kg ha^{-1} (17-17-17)
M5	Manure (5 t ha^{-1})
B2.5	Biochar (2.5 t ha^{-1})
B5.0	Biochar (5 t ha^{-1})
B7.5	Biochar (7.5 t ha^{-1})
BMN2.5	Biochar (2.5 t ha^{-1}) + manure (5.0 t ha^{-1})+ NPK 50 kg ha^{-1} (17-17-17)
BMN5.0	Biochar (5.0 t ha^{-1}) + manure (5.0 t ha^{-1}) + NPK 50 kg ha^{-1} (17-17-17)
BMN7.5	Biochar (7.5 t ha^{-1}) + manure (5.0 t ha^{-1}) + NPK 50 kg ha^{-1} (17-17-17)

Figure 3.2 shows the layout of the field experiment where Casuarina growth (Height, DGL and DBH) as influenced by sole biochar application and integration of biochar manure and NPK was tested.



BLOCK 1	C	NPK	M5	B2.5	B5.0	B7.5	BMN2.5	BMN5.0	BMN7.5
BLOCK 2	M5	B2.5	B5.0	B7.5	BMN2.5	BMN5.0	BMN7.5	C	NPK
BLOCK 3	BMN2.5	BMN5.0	BMN7.5	C	NPK	M5			
	B7.5	B5.0	B2.5						

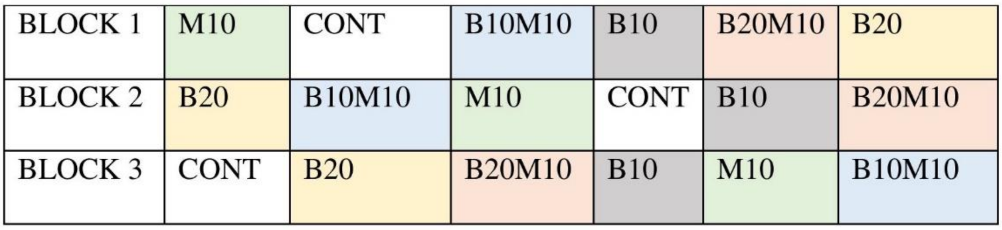
Figure 3.2: Field layout of Casuarina experiment

3.2.2 Nursery experiment

For the nursery experiment, the experiment was equally set using a randomized complete block design. The nursery experiment comprised six treatments each replicated thrice as shown in Table 3.2. Exactly 30 seedlings per replicate were used to evaluate the effect of biochar and manure on Casuarina growth at the seedling stage. The Casuarina germinants for the nursery experiment were raised by KEFRI Gede tree nursery using certified *C. equisetifolia* seeds. The germinants were transferred to the potting tubes containing treatments at the pricking-out stage. Biochar and manure (as per the treatments) were mixed thoroughly with the soil before potting and routine nursery management of watering twice daily was maintained. The collar diameter (DGL) and height of the seedlings were observed for 177 days. Figure 3.3 shows the layout of the nursery pot experiment where the growth of Casuarina seedlings under different rates of biochar and manure was evaluated.

Table 3.2: Treatments for nursery experiment

Treatment Code	Treatments Description (volume-to-volume ratio (v/v))
CONT	0%
B10	10% biochar
B20	20% biochar
M10	10% manure
B10M10	10% biochar + 10% manure
B20M10	20% biochar + 10% manure



BLOCK 1	M10	CONT	B10M10	B10	B20M10	B20
BLOCK 2	B20	B10M10	M10	CONT	B10	B20M10
BLOCK 3	CONT	B20	B20M10	B10	M10	B10M10

Figure 3.3: Nursery experiment layout

3.3 Soil Sampling and Analysis

3.3.1 Soil sampling

Periodic soil sampling was undertaken; at 0 (study onset), 3, 6, 9, and 12 months after the establishment (MAE) of the field experiment. Soil sampling was done using the Grid sampling method across the experimental field and one sampling depth (0-20cm) for nutrient analysis using a soil auger; five sampling points (10 cm from the Casuarina trees selected in the grid) for each treatment were used to obtain composite soil sample. Consequently, samples obtained were placed in zip-lock bags to prevent contamination, then clearly labelled to show; treatment, replicate, sampling depth, and date of sampling. The soil samples were transported to the lab in ice-cooled boxes to prevent further dynamic changes and taken to the lab for analysis. At the onset of the study also, other soil samples were sampled for soil characterization of the area. Soil physical properties (bulk density and soil texture); soil chemical properties (CEC, exchangeable bases, Total nitrogen, Total Carbon, and pH), and biological (microbial population) properties were analyzed.

3.3.2 Soil analysis

Soil analysis was undertaken using standard analysis procedures. Bulk density, soil texture, and gravimetric soil moisture content were determined as described by Okalebo et al. (2002); pH and electrical conductivity (EC) were determined using the method described by Anderson & Ingram (1993); Total nitrogen was determined using Kjeldahl method (Anderson & Ingram, 1993; Okalebo et al., 2002). Available phosphorus was determined using the procedure by Olsen & Sommers (1982). Extractable potassium was determined spectrophotometrically (Anderson & Ingram, 1993). Walkey Black method was used for the determination of organic carbon (Okalebo et al., 2002). Exchangeable sodium, calcium, and magnesium were determined using the procedure described by Okalebo et al. (2002). The microbial population was determined using the dilution plate count method (Ogunmwoyi et al., 2008). Cation exchange capacity was calculated using the formula described by Gao et al. (2017).

3.4 Casuarina Growth Measurements at Seedling Stage

Casuarina seedlings' growth assessment was done fortnightly throughout the seedlings’ growth period (177 days). The height (cm) of *C. equisetifolia* seedlings was measured using a one-meter ruler from the collar diameter to the tip of the Casuarina seedling while the diameter at the ground level/collar diameter (mm) was measured using a Vernier calliper. Other parameters measured included Sturdiness Quotient and Shoot/Root ratio. The Sturdiness quotient was calculated using the following formulae by (Jaenicke & International Centre for Research in Agroforestry., 1999):

$$\text{Sturdiness Quotient} = \frac{Shoot\ length\ (cm)}{Collar\ diameter\ (mm)} \qquad \text{(Eq.1)}$$

The shoot/root ratio (S: R) measures the balance between the transpiration area (shoot) and the water-absorbing area (root) of the seedlings. At the end of the experiment, destructive sampling was undertaken for the determination of the S: R ratio. For each replicate, five seedlings were randomly obtained and subdivided into roots (from collar diameter to the root tip) and shoot (from the collar diameter to the tip of the seedling). The roots were cleaned to remove all soil particles. The samples were then oven-dried at 60 ºC for 48 hours and the weight was determined using an analytical balance. The average for each replicate was calculated by dividing the dry weight by the number of seedlings sampled. The S: R ratio was calculated using the following formula by (Takoutsing et al., 2014):

$$\text{Shoot/Root ratio} = \frac{Shoot\ dry\ weight\ (g)}{Root\ dry\ weight\ (g)} \qquad \text{(Eq.2)}$$

3.5 Casuarina Growth Measurements after Field Establishment

In the field experiment, height below two meters was measured using a grade rod while height above that was measured using a Suunto clinometer. The height of Casuarina trees was measured from the base to the tip of the tree. Collar diameter or Diameter at Ground Level (DGL) and Diameter at Breast Height (DBH) (estimated at 1.3m from the ground) were measured using a Vernier calliper.

3.6 Statistical Analysis

Analysis of variance (ANOVA) was conducted for data obtained from the field and lab using R statistical software (Version 4.1.0) for windows at a 95% confidence level. The level of significance between means was evaluated using the Tukey HSD test. Regression analysis was also undertaken using R software to determine existing relationships. Table 3.3 shows a summary of the statistical analysis conducted.

Table 3.3: Data analysis methods utilized

Hypothesis	**Variable of interest**	**Analysis conducted**
[1] Biochar improves physical and biochemical soil properties	Soil bulk density, soil moisture content, pH, K, Ca, total N, available P, Mg, CEC, and microbial population	• One-way ANOVA • Pearson's correlation • regression
[2] Biochar significantly increases *C. equisetifolia* growth	Casuarina height, DGL, and DBH	• One-way ANOVA • Pearson correlation • Regression
[3] Biochar combined with manure and inorganic fertilizers significantly increases *C. equisetifolia* growth	Casuarina height, DGL, and DBH	• One-way ANOVA
[4] The combined effect of soil moisture content and soil nutrients significantly increases *C. equisetifolia* growth (Height, DGL & DBH)	Soil moisture content, soil nutrients, Casuarina DGL, Height, and DBH	• Two-way ANOVA • Pearson's correlation • Regression

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Overview

This chapter is subdivided into five sections. Section one (4.1) is an overview. The second section (4.2) presents changes in physical soil (soil moisture content, bulk density) and biochemical properties (soil pH, CEC, EC, microbial population, and nutrients- NPK Na, Ca, Mg levels) after 12 months of field study. The third section (4.3) presents and discusses, findings of sole biochar application's effect on the growth performance of Casuarina in terms of height, DGL, and DBH at different rates. This section reports findings on the impact of four biochar rates (0, 2.5, 5.0, and 7.5 t ha^{-1}) on Casuarina growth after field establishment.

The fourth section (4.4) deals with the results of the impact of biochar combined with manure and inorganic NPK fertilizer on Casuarina growth. This section presents two sets of findings: the nursery experiment results, which evaluated the effect of biochar and manure on Casuarina growth at the seedling stage, and a field experiment which evaluated the effect of different biochar rates combined with 5 t ha^{-1} manure and N: P: K (17:17:17) fertilizer (50 kg ha^{-1}) on Casuarina growth performance after field establishment. Finally, section five (4.5), presents and discusses the results of the correlation analyses between soil moisture content and plant nutrients availability, and Casuarina growth results after biochar application. In this section, the relationships between available soil nutrients (N, P, K), soil moisture content, and Casuarina growth parameters (height, DGL, and DBH) were evaluated.

4.2 Biochar's Impact on Physical and Biochemical Soil Characteristics

4.2.1 Baseline soil status

The baseline soil status of the field experimental site, forest soil obtained from Arabuko Sokoke, biochar, and manure characteristics determined at the onset of the experiment are shown in Table 4.1. The soil pH was 6.65 which is near neutral. The soil had low EC, total C, N, SOM, exchangeable K, available P, and exchangeable Ca that exhibits the characteristic of the Coastal soils (Arenosols) which have low inherent fertility (NAAIAP & KARI, 2014; Mwadalu et al., 2020). The site however had adequate exchangeable Mg of up to 149 mg kg^{-1} (NAAIAP & KARI, 2014). The soil was generally categorized as having a sandy texture (90% sand, 6% clay, and 4% silt). The soil bulk density (<1.3 g cm^{-3}) was within the recommended range for plant growth (USDA, 2020). Bulk density plays a crucial role in understanding soil compaction; lower bulk density means an optimal condition for root development thus enhancing plant growth and nutrient uptake from the soil (Amusan et al., 2013).

Table 4.1: Baseline characteristics of site and materials used for the experiments at the onset of the experiments

Parameter	Field site	Biochar	Manure	Forest soil	Optimal levels
pH	6.65	8.7	8.5	7.1	6.5-7.5
EC (mS/cm)	0.052	1.39	0.83	0.10	<0.15
Total O.C (%)	0.34	48	9.54	1.45	>2.7
Ash (%)	-	3.95	88.9	-	-
Total N (%)	0.16	0.84	0.89	0.31	>0.25
Soil Organic matter (%)	1.12	-	-	-	-
P (mg kg^{-1})	6.8	26.6	10.5	9.7	>20
K (mg kg^{-1})	62	1166.1	1497	585	>100
Ca (mg kg^{-1})	200	746	400	100	>400
Mg (mg kg^{-1})	149	354	-	-	>120
Na (mg kg^{-1})	-	989	1886	460	-
Texture (90% sand, 6% clay, 4% silt)	Sand	-	-	Sand	-
Bulk density (g cm^{-3})	1.3	-	-	-	-
C/N ratio	2.1	57	8.7	5	<25
CEC (cmol $_{(+)}$ kg^{-1})	-	11.2	-	-	>9

Note: *Optimal soil nutrient levels were obtained from NAAIAP & KARI, 2014.*

4.2.2 Impact of biochar on physical soil characteristics

4.2.2.1 Bulk density (BD)

Results from this study show substantial variations in soil bulk density following the utilization of different biochar application rates at 3 MAE and 9 MAE assessment period; ($p < 0.02$; 0.001, respectively) as shown in Figure 4.1. A gradual decline in bulk density upon increasing biochar rates was observed. Application of the highest biochar rate (7.5 tons/ha) yielded the lowest BD of 0.99g cm^{-3} for both sampling periods. The unamended treatment recorded the highest bulk density of between 1.29 and 1.32 g cm^{-3}. Bulk density declined by 14.7%, 15.5%, and 23.2% for biochar doses of 2.5, 5.0, and 7.5 t ha^{-1}, respectively, in comparison with unamended treatment at the 3 MAE assessment period. At 9 MAE, bulk density decreased by 15.2%, 17.4%, and 25% after the use of 2.5, 5.0, and 7.5 t ha^{-1} of biochar, respectively in comparison with the unamended treatment (Figure 4.1).

The results of Pearson's correlation conducted to determine the relationship between biochar application rates and soil bulk density revealed a substantial negative relationship (r = -0.87, p<0.0003) for 3 MAE and (r = -0.79, p<0.002) for 9 MAE as shown in Figure 4.2. Soil bulk density declined as the biochar rates increased. Figure 4.2 also shows that there was a strong association between biochar application rates and soil bulk density. Between 63 and 5% of the observations made in soil bulk density can be attributed to changes made in the biochar application doses.

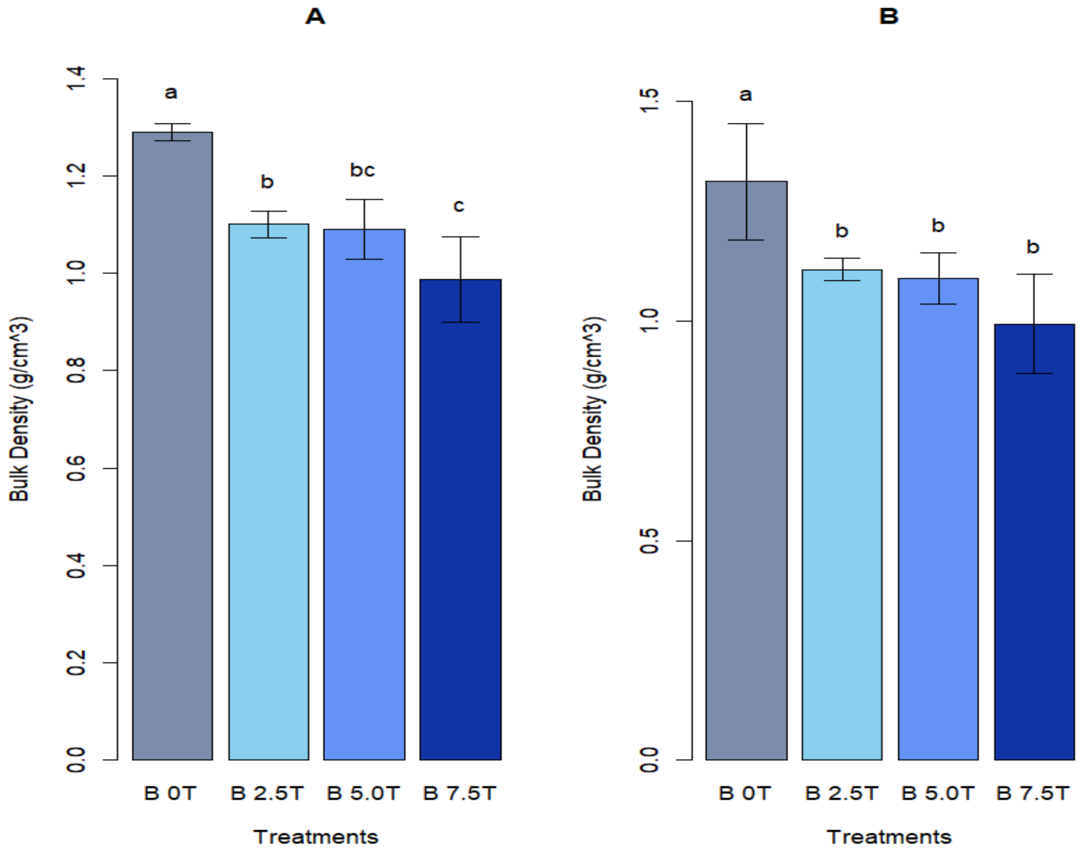


Figure 4.1 Soil bulk density as influenced by biochar application

Note: *(i) Means presented by different alphabets in each graph differ substantially; "B" means Biochar, treatment rates are in tons/ha; 3 MAE (A) and 9 MAE (B)*

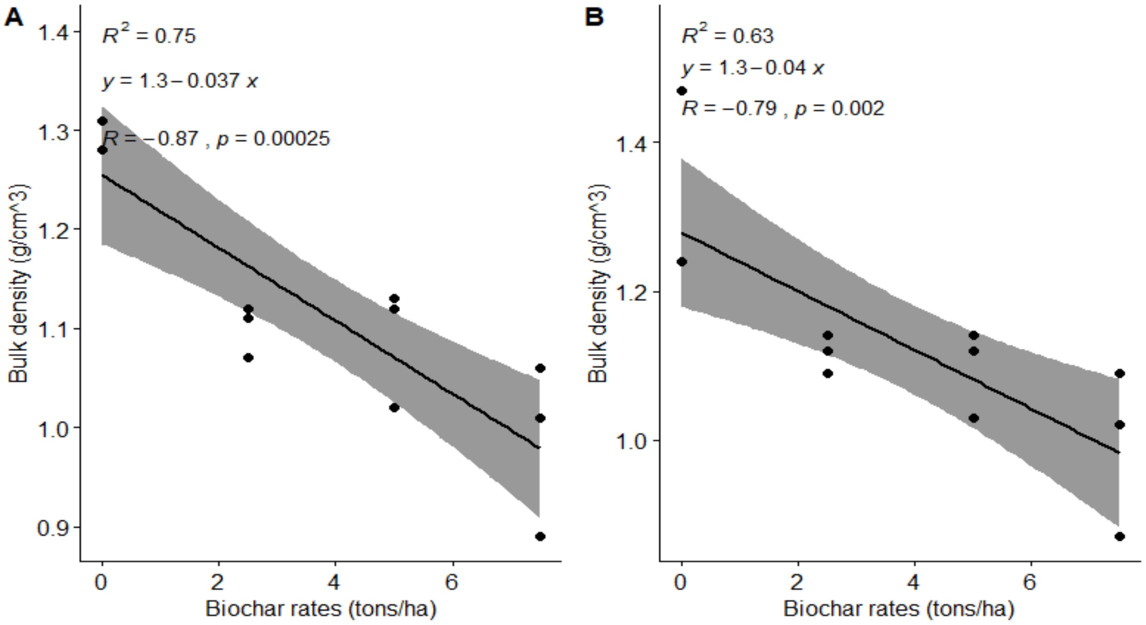


Figure 4.2: Relationship between biochar application rates and soil bulk density at different assessment periods.

Note: (A: 3 MAE, B: 9 MAE)

The reduction in BD following biochar utilization could be a factor of biochar's porosity. Studies have shown that biochar application reduces bulk density probably because of its porous nature or dilution effect due to mixing with other materials (Humberto, 2017). The porosity of biochar has been estimated to be 70 to 90% which substantially alters soil porosity (Are, 2019; Aslam et al., 2014). The decrease in bulk density following biochar application may also be a result of biochar's lower BD (0.63g cm^{-3}) than soil BD (1.25g cm^{-3}) as reported by Hseu et al. (2014).

The decline in BD resulting from increased soil porosity enhances aeration and root development which are crucial for optimal productivity. Higher BD limits root growth and development resulting from compaction (Kormanek et al., 2015). Bulk density is an indicator of soil compaction and soil health (soil structure and organic content). Ma et al. (2016) observed the dependency of soil bulk density on mineral soil density, organic matter, and texture. Bulk density affects plant nutrient availability, infiltration, available water capacity, rooting depth, soil porosity, and soil microorganism activity (Page-Dumroese et al., 2016; USDA, 2020).

Comparable studies observed decreased BD following biochar utilization (Aslam et al., 2014; Haider, 2017; Lane, 2016; Oshunsanya and Aliku, 2016). Humberto (2017) observed decreased BD by 3 to 31% after biochar utilization. Bulk density reduction in soils ameliorated with biochar could be a result of improved soil structure and aeration (Humberto, 2017). Hseu et al. (2014) also reported a reduction in BD from 1.5 mg m^{-3} in the control to 1.4 mg m^{-3} in biochar-treated soils; the plots with 10% biochar recorded a reduction in BD of up to 25% compared to the control. A study by Omondi et al. (2016) further reported a decrease in BD by 7.6% following biochar addition; soil bulk density decreased with the increasing amount of biochar applied; the same trend was also observed in the present study as shown in Figure 4.2.

Studies have also shown that biochar can reduce BD in coarse-textured soils more than in fine-textured soils, this could explain the trend observed in this study which was dominated by sandy soils (Figure 4.1). This is estimated at 14.2% in coarse-textured soils and 9.2% in fine-textured soils (Omondi et al., 2016.

4.2.2.2 Gravimetric soil moisture content

Gravimetric moisture content differed substantially at 0-20cm depth following utilization of various biochar rates (p <0.0001, 0.0004, 0.0001, and 0.0001) at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively, as shown in Figure 4.3. The highest soil moisture content was observed in plots ameliorated with the highest biochar rate (7.5 t ha^{-1}). Generally, soil moisture content was substantially higher in plots ameliorated with biochar than in the unamended treatment (Figure 4.3).

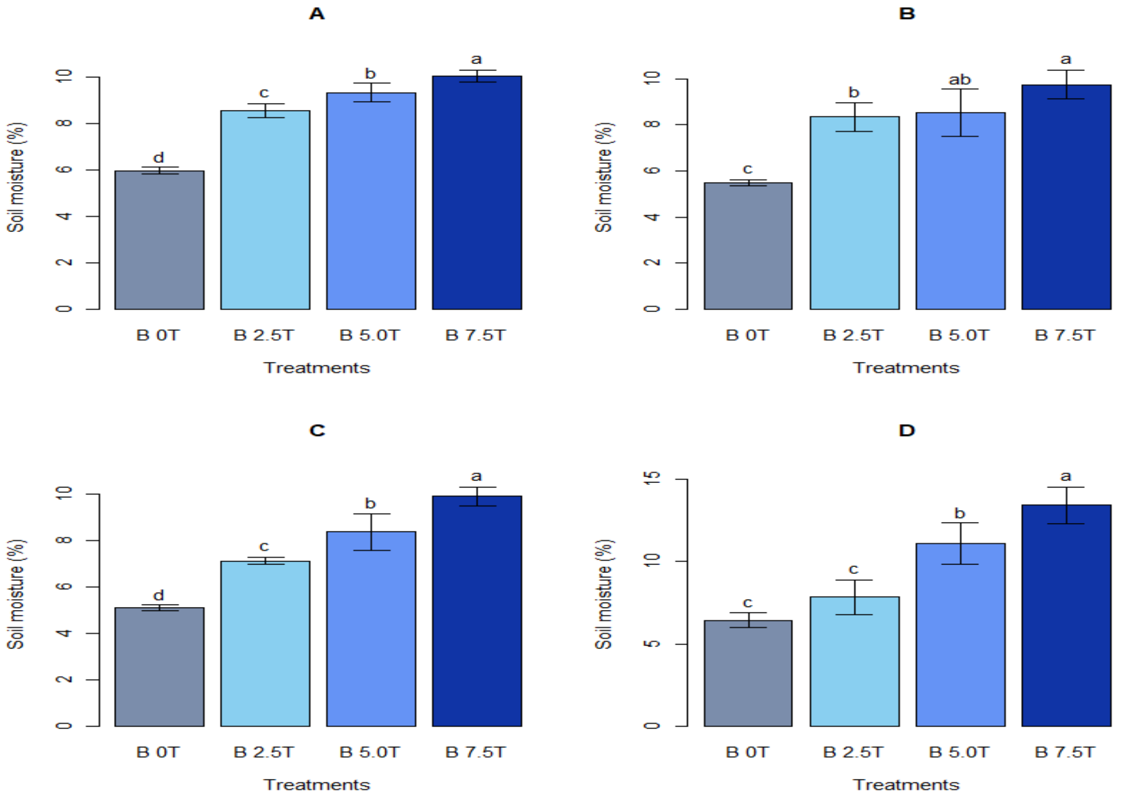


Figure 4.3: Soil moisture content under different biochar application rates

Note: *(i) Means presented by different alphabets in each graph differ substantially; "B" means Biochar, "C" means Control treatment; treatment rates are in tons/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

At 3MAE there was an increase in soil moisture content of 43.1%, 55.8%, and 67.6% in comparison with the control following biochar application at the rate of 2.5 t ha $^{-1}$, 5.0 t ha$^{-1,}$ and 7.5 t ha^{-1}, respectively. At 6 MAE there was soil moisture enhancement of 52.0%, 55.6%, and 77.3% when compared to the control when biochar rates of 2.5 t ha $^{-1}$, 5.0 t ha$^{-1,}$ and 7.5 t ha^{-1}, respectively were used. Similarly, at 9MAE there was an increase in soil moisture content of 39.6%, 64.1%, and 94.1% in comparison with the control following biochar application at the rate of 2.5 t ha $^{-1}$, 5.0 t ha$^{-1,}$ and 7.5 t ha^{-1}, respectively. At the end of the experiment (12MAE), soil moisture content in biochar-amended plots increased by 21.7%, 71.9%, and 108.2% in comparison with the control following biochar application at the rate of 2.5 t ha $^{-1}$, 5.0 t ha^{-1} and 7.5 t ha^{-1}, respectively.

All biochar application rates significantly increased soil moisture in comparison with the control across three assessment periods except 12MAE where soil moisture content in plots ameliorated with 2.5 t ha $^{-1}$ was not significantly different from the control. Increasing biochar application rates from 2.5 t ha^{-1} to 7.5 t ha^{-1} significantly increased soil moisture content at three sampling periods (3MAE, 9MAE & 12 MAE). At 6MAE, the moisture content in plots treated with 5.0 t ha^{-1} and 7.5 t ha^{-1} was not significantly different.

Figure 4.4 shows a significant positive linear relationship between biochar application rates at a soil moisture content at the four sampling periods (r = 0.93, 0.87, 0.97, and 0.95); respectively, for the four assessment periods ($p<0.001$). The results of this study further revealed a strong association between biochar application rates and soil moisture content as shown in Figure 4.4 which was between 76 and 95% of observations made in the soil moisture. This can be attributed to changes in the biochar application rates. The present study shows that soil moisture content increased with increasing biochar application rates whereby also biochar’s porosity and surface area were increased in relation to biochar application rates.

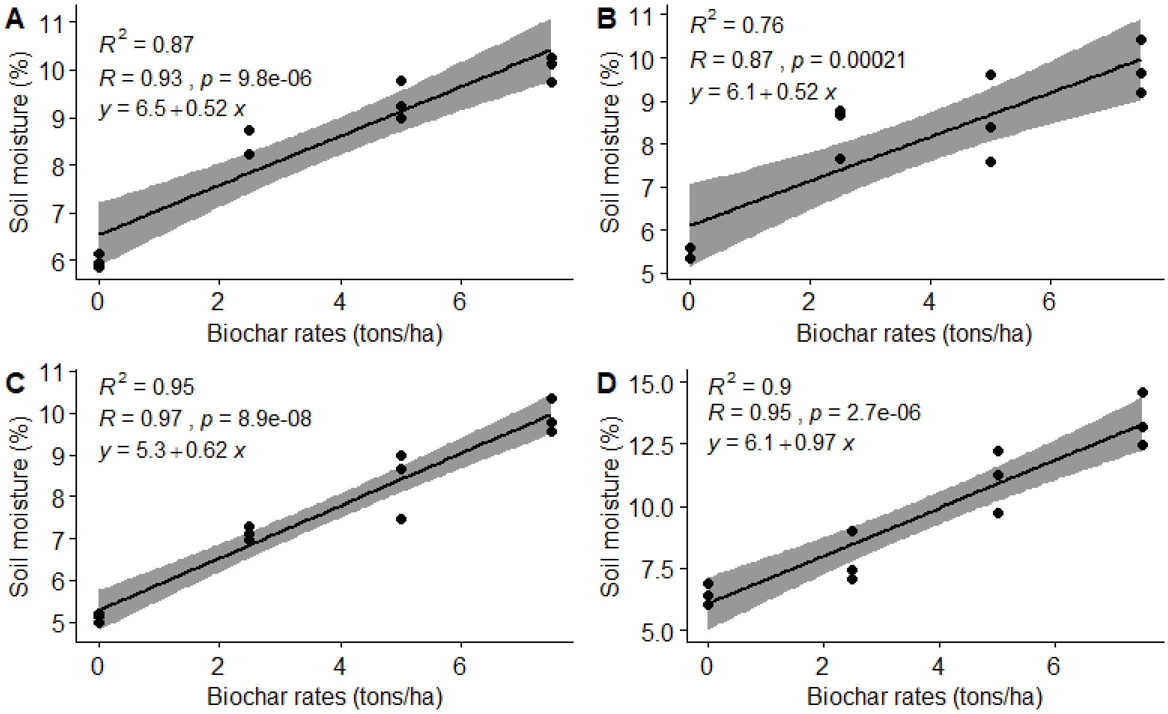


Figure 4.4: Relationship between biochar application rates and soil moisture content at different assessment periods

Note: *(A: 3 MAE, B: 6 MAE, C: 9 MAE, D: 12 MAE)*

The enhanced soil moisture content resulting from biochar utilization is attributed to biochar's large surface area and porosity which enables the retention of water in the soil micro-pores and the rise of water from lower soil horizons through capillary action (Hardie et al., 2014). Biochar has a large surface area which enhances the adsorptive capacity of soil while improving its pore size distribution, and bulk density and consequently leading to an increase in available soil moisture crucial for plant growth and development. In addition, there exists a strong direct relationship between biochar's surface area and the pore volume of the soil (Mekuria & Noble 2013; Li et al., 2019).

Novak et al. (2012) and Basso et al. (2013) observed similar trends where soil moisture content increased in sandy soils following the application of biochar. A study by Wilson and Major (2010) also reported enhanced soil moisture content in sandy loam soil following biochar application whereby the soil moisture in biochar-treated plots was 18% higher than the unamended treatment. Studies have shown that the addition of biochar in sandy soil can reduce the volume of large space between soil particles and increase the portion of micropores as a result of the intra-pores of biochar (De Melo Carvalho et al., 2013; Li et al., 2021; Ndede et al., 2022). Soil water retention accrued to biochar application is crucial for plant productivity due to its role in water uptake and transport by plants with affects plant physiology (Razzaghi et al., 2020).

Comparable studies observed the positive impact of biochar on soil moisture content (Wilson and Major, 2010; Novak et al., 2012; Pühringer, 2016). The study by Pühringer (2016) conducted in Siaya, Kenya reported a slight increase in soil moisture content with increasing biochar doses. In the study, substantial differences in soil moisture were observed for biochar rates of 5 and 10 t ha^{-1} in comparison with the unamended treatment. Similarly, Razzaghi et al. (2020) reported an increase in soil moisture of up to 45% in comparison with the control treatment. Ndede et al. (2022) observed enhanced soil moisture content by up to 191% with the application of biochar derived from the woody feedstock. A study by Novak et al. (2012) further noted that soil moisture enhancement depends on the biochar material used and the pyrolysis temperature used during biochar production. The study by Novak et al. (2012) also reported improvement in soil moisture storage in two silt loams ranging between 0.5 and 0.8 cm of water per 15cm soil depth.

4.2.3 Soil chemical properties as influenced by biochar application

4.2.3.1 Soil pH

The results of the field study show that soil pH differed substantially after utilization of different biochar doses (p<0.02, 0.001, 0.0001, and 0.0001 for 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively) as shown in Table 4.2. Generally, soil pH increased with increasing biochar application rates from 2.5 t ha^{-1} to 7.5 t ha^{-1}. At 3 MAE, there was an increase in soil pH of 8.0%, 9.8%, and 16.9% in comparison with the control when biochar was applied at 2.5 t ha $^{-1}$, 5.0 t ha$^{-1,}$ and 7.5 t ha^{-1}, respectively. An increase in pH of 9.4%, 13.7%, and 20.1% in comparison with the unamended control was observed at 6 MAE when biochar application rates of 2.5 t ha $^{-1}$, 5.0 t ha$^{-1,}$ and 7.5 t ha^{-1}, respectively were used. A similar increase in soil pH with biochar application was observed at 9 MAE and 12 MAE.

In comparison with the unamended treatment, an increase of up to 1.35 pH units was observed after the application of 7.5 t ha^{-1} biochar at 12 MAE. The intermediate rate (5.0 t ha^{-1}) increased soil pH by 0.97 pH units in comparison with the unamended treatment at 12 MAE while the biochar application rate of 2.5 t ha^{-1} enhanced soil pH by 0.54 pH units above the unamended treatment at 12 MAE. From 3 MAE to 9 MAE increasing the biochar application rate from 5.0 t ha^{-1} to 7.5 t ha^{-1} did not result in significant pH enhancement. Significant pH enhancement with increasing biochar application rates from 2.5 t ha $^{-1}$ to 7.5 t ha^{-1} was however observed at 12 MAE.

Table 4.2: Soil pH under different biochar rates

Parameter	**pH ± SE**			
Treatment	**3 MAE**	**6 MAE**	**9 MAE**	**12 MAE**
Biochar 0 tons/ha	6.50±0.21b	6.26±0.04c	6.33±0.08c	6.34±0.08d
Biochar 2.5 tons/ha	7.02±0.33ab	6.85±0.20b	6.85±0.20b	6.87±0.16c
Biochar 5.0 tons/ha	7.14±0.19a	7.12±0.20ab	7.27±0.07a	7.31±0.07b
Biochar 7.5 tons/ha	7.60±0.50a	7.52±0.32a	7.41±0.06a	7.69±0.18a
$f_{(3,8)}$	5.83	17.83	50.83	56.38
$p < 0.05$	0.02	0.0007	0.00001	0.00001

Note: *Means presented by different alphabets in each column differ substantially; Biochar 0 tons/ha=Control*

According to Pearson's correlation, a positive linear relationship was observed between biochar rates and soil pH at the different sampling periods (r =0.81, r = 0.92, r = 0.95, r = 0.97, respectively) for the four sampling periods as shown in Figure 4.5. Generally, between 65% to 90% (R^2*100) of the observations made on the pH can be attributed to the biochar rates applied (Figure 4.5). The highest biochar dose (7.5 t ha^{-1}) yielded the highest soil pH while the unamended treatment led to the lowest soil pH. In comparison with the unamended treatment, soil pH was enhanced by 16.9%, 20.1%, 17.1%, and 21.3% upon utilization of biochar doses of 7.5 t ha^{-1} at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively.

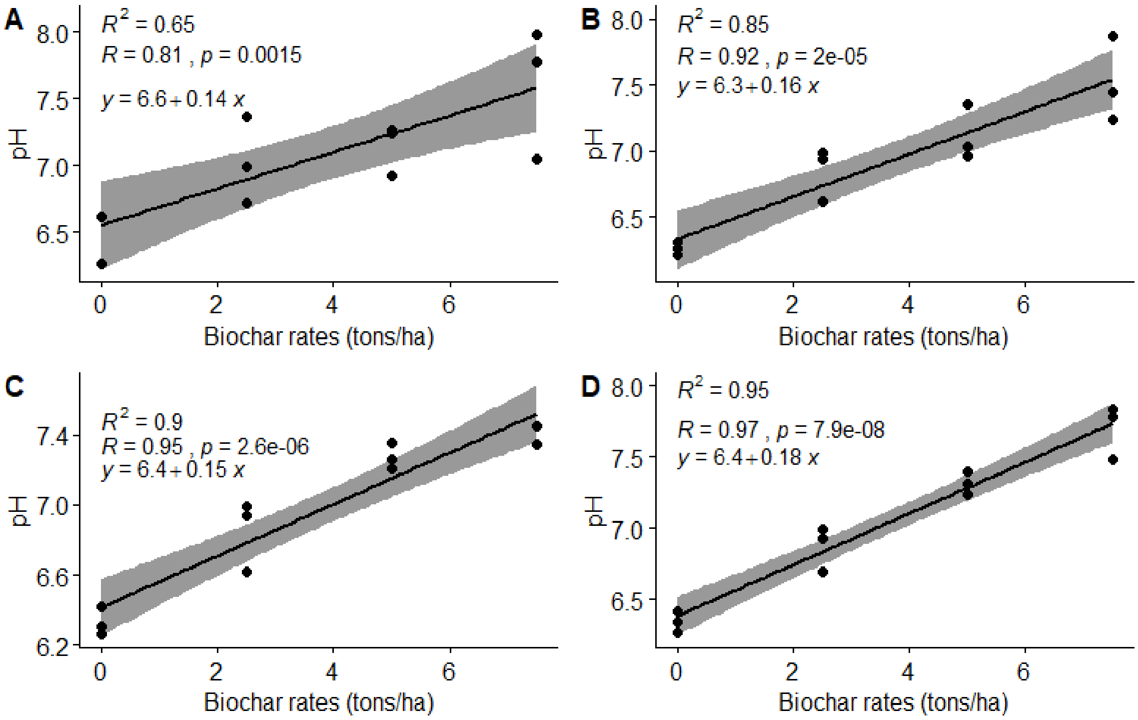


Figure 4.5: Relationship between biochar application rates and soil pH at different assessment periods

Note: *(A: 3 MAE, B: 6 MAE, C: 9 MAE, D: 12 MAE)*

The increase in soil pH with biochar application can be attributed to the inherent pH of biochar, base cation content, $CaCO_3$ content, and Calcium Carbonate Equivalent (CCE) (Shetty & Prakash, 2020). The biochar used in the present study had a pH of 8.5 which was higher than the soil pH at the study site (6.65) which could have led to the increase in soil pH. Free bases present in biochar such as Ca, Mg, and K could also have been released into the soil solution resulting in a net increase in soil pH (Shetty & Prakash, 2020). Soil pH enhancement reported after biochar utilization can also be attributed to a rise in cation exchange capacity (CEC) due to the biochar's porosity and large surface area (Trupiano et al., 2017; Mohan et al., 2018). The increase in soil pH with biochar application could also be as a result of biochar's carbonyls (COO-), phosphates (PO_4^{3-}), and other alkaline substances on the surface of biochar which neutralizes acidity and enhance soil pH (Geng et al., 2022).

A comparable study by Hailegnaw et al. (2020) observed a substantial increase in soil pH following 8% biochar utilization; the study concluded that higher biochar rates were more effective in altering soil properties than lower rates. Similarly, enhanced soil pH (1.6 and 0.8 units, respectively) was noted by Mwadalu et al. (2020, 2022) and Mohan et al. (2018) after biochar application. Geng et al. (2022) observed enhanced soil pH of up to 79.25% following the application of woody biochar. Mensah and Frimpong (2018) also observed increased soil pH after the application of Corncob biochar. Zhang et al. (2019) and Gao et al. (2017) observed enhanced soil pH by 0.5 to 1 unit, respectively. Dai et al. (2014) similarly reported enhanced soil pH of up to 2.52 units with the application of swine manure biochar at the rate of 3% (v/v).

Liming effect of biochar is dependent on the feedstock used for its preparation. Biochar has been reported to maintain soil pH within the required optimal range for plant development (Leghari et al., 2016; Yunilasari et al., 2020). The enhancement of soil pH resulting from biochar application is also influenced by the pyrolysis temperature under which the biochar was produced (Geng et al., 2022). Shetty & Prakash (2020) reported substantial improvement in soil pH after 15 days following woody biochar utilization using a rate of 5, 10, and 20 t ha^{-1}. Liming effect of biochar is important for the amelioration of acidic soils thereby enhancing land productivity (Mohan et al., 2018; Zhang et al., 2019; Geng et al., 2022).

4.2.3.2 Soil total N

Total N differed significantly after utilization of varying biochar rates ($p< 0.002$, 0.01, 0.008, and 0.02 at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively) as shown in Figure 4.6. Generally, total N was higher in plots ameliorated with biochar in comparison with the unamended treatment. Biochar application rate of 2.5 t ha^{-1} enhanced total N above the unamended control by 26.5%, 29.1%, 33.2%, and 47.9% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. The intermediate (5.0 t ha^{-1}) application rate enhanced total N above the control by 35.0%, 42.3%, 65.9%, and 73.3% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. The highest biochar application rate (7.5 t ha^{-1}) on the other hand enhanced total N above the control by 33.5%, 36.6%, 69.2%, and 97.0% at MAE, 6 MAE, 9 MAE, and 12 MAE, respectively.

The highest biochar application dosage (7.5 t ha^{-1}) yielded the highest total N across the four assessment periods. Increasing the biochar application rate from 2.5 to 7.5 t ha^{-1} did not lead to a substantial total N increase during the four assessment periods. However, the three biochar rates used had significantly higher total N than the unamended control. The unamended treatment had the lowest total N across the growth period (Figure 4.6).

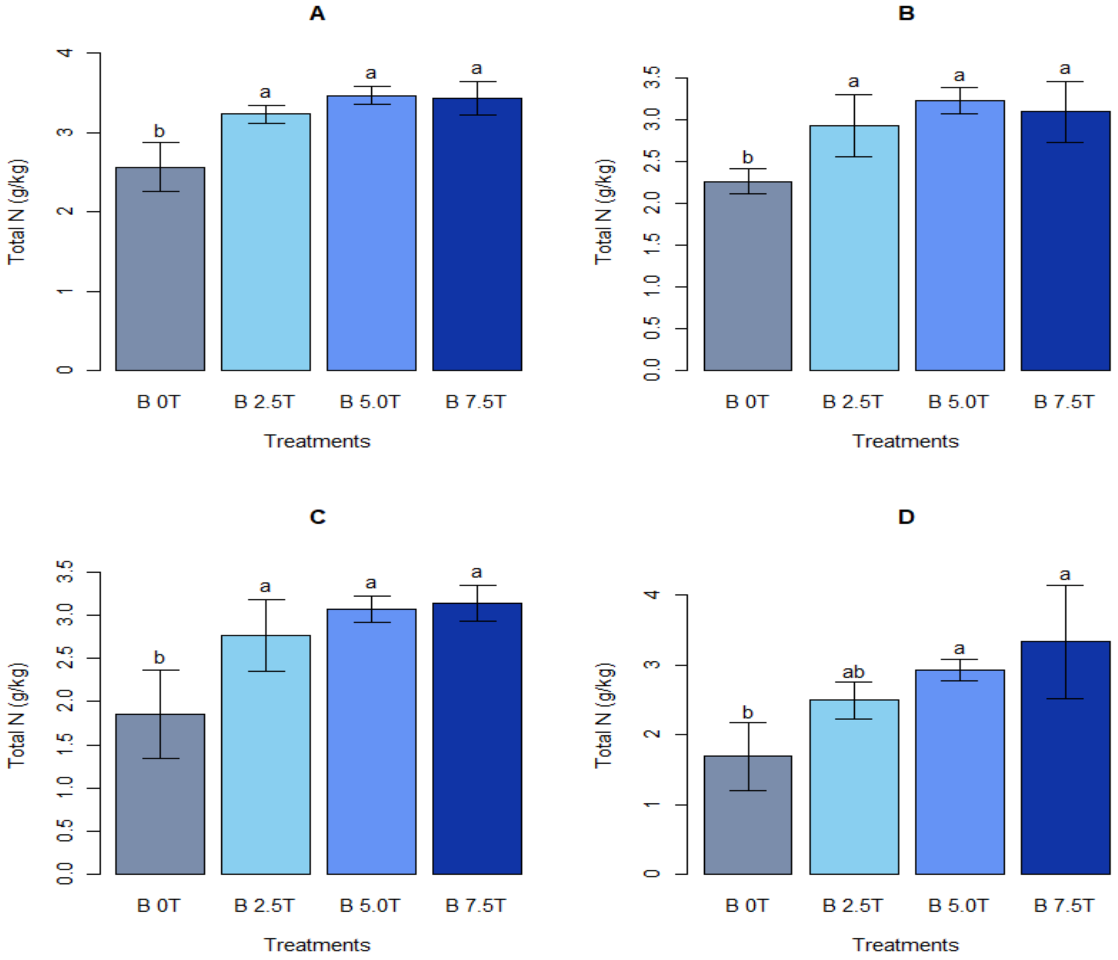


Figure 4.6: Soil Total N under different biochar rates
Note: *(i) Means presented by different alphabets in each graph differ substantially; "B" means Biochar, treatment rates are in tons/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

Figure 4.7 shows a substantial positive linear relationship between biochar rates and total N (r = 0.80, 0.72, 0.79, and 0.82) for 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. The present study also showed that there was a moderate association between biochar rates used and total N (r^2= 0.63, 0.57, 0.62, and 0.67), respectively, for the four assessment periods as highlighted in Figure 4.7.

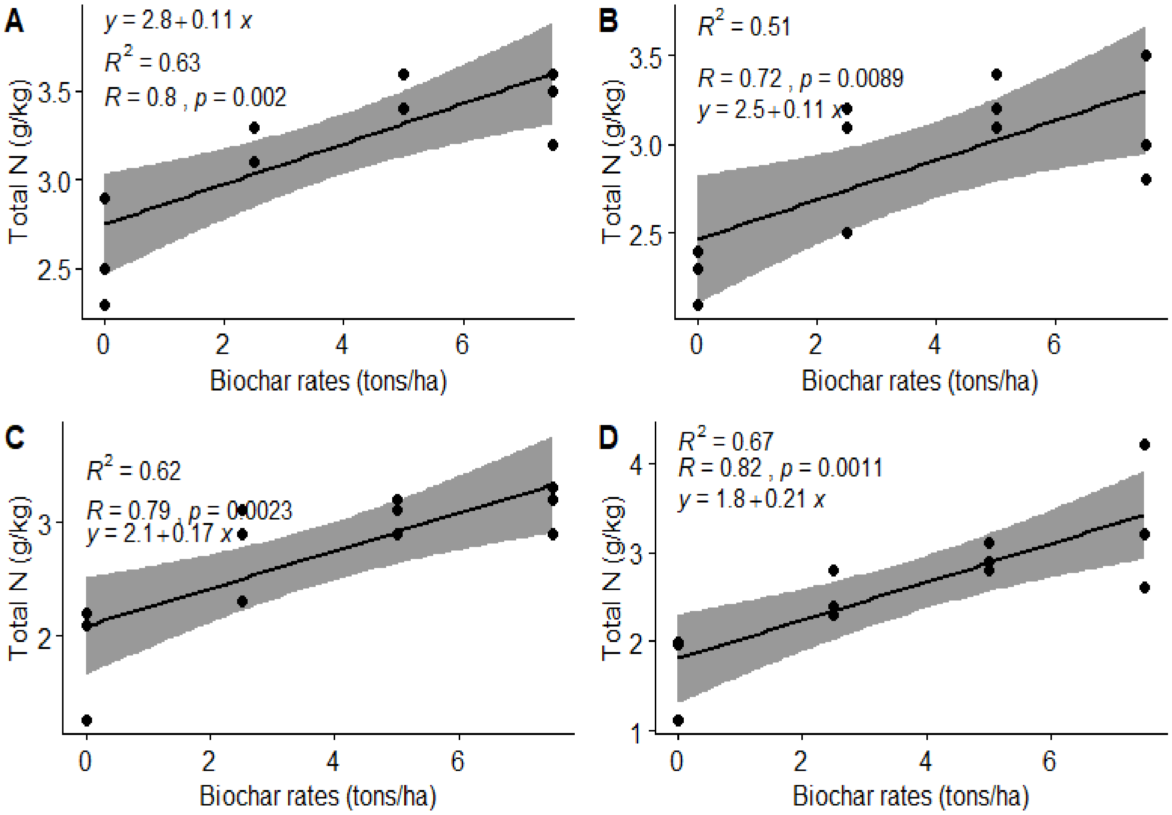


Figure 4.7: Relationship between biochar application rates and soil total N at different assessment periods

Note: *(A: 3 MAE, B: 6 MAE, C: 9 MAE, D: 12 MAE)*

The increase in total N following biochar application in the present study can be attributed to N addition from biochar itself. It can also be attributed to biochar's large surface area which enhanced the retention of NH_4^+ thus leading to improved N nutrition (Truong & Marschner, 2018). The increased total N with biochar application could be resulting from a decrease in N leaching due to biochar's high adsorption capacity (Syuhada et a. 2016; Rawat et al., 2019). Agegnehu et al.(2015) also observed a reduction in nutrient loss due to leaching after biochar use.

Mensah & Frimpong (2018), reported an enhanced N content following biochar application. Similarly, Zhang et al. (2021) reported enhanced total N after biochar application by up to 11.1% in comparison with unamended control. Ghosh et al. (2015) attributed N enhancement following biochar utilization to high N content in biochar used. Rawat et al. (2019) observed enhanced soil productivity after biochar utilization through nutrient addition into the soil such as N and retention of nutrients from other sources including the soil itself. The high N content reported in the present study could be a factor in Casuarina's nitrogen-fixing potential (Muthukumar et al., 2012). Studies have reported that Casuarina fixed up to

42.5g N_2 per tree during the first 9 months after field establishment (El-Lakany, 1986; Dommergues, 1987; Mailly & Margolis, 1992).

However, a slight decrease in total N over time was observed. This may be attributed to nutrient uptake (N) by the Casuarina trees planted as a test crop (Muthukumar et al., 2012). Nutrient uptake was however not quantified in this present study. Similar findings on the decrease in total N with time following biochar utilization were attributed to N uptake by corn plants and immobilization during the decomposition of mineralizable fractions of biochar that have low N contents (Syuhada et al., 2016).

4.2.3.3 Soil total organic carbon (TOC)

This study shows substantial differences in Total organic carbon (TOC) following utilization of varying biochar rates ($p < 0.0001$, 0.0001, 0.0001, and 0.0001 at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively). Generally, plots ameliorated with biochar recorded higher TOC than the unamended treatment across the assessment periods (Figure 4.8). The lowest biochar rate (2.5 t ha^{-1}) enhanced TOC above the control by 26.8%, 67.1%, 70.0%, and 93.8% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. On the other had the intermediate biochar application rate (5.0 t ha^{-1}) increased TOC by 82.8%, 137.4%, 165.8%, and 189.4% above the control at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. The highest biochar rate (7.5 t ha^{-1}) enhanced TOC above the control by 121.5%, 207.7%, 260.7%, and 293.4% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. Increasing biochar application rates from 2.5 to 7.5 t ha^{-1} significantly enhanced TOC as highlighted in Figure 4.8.

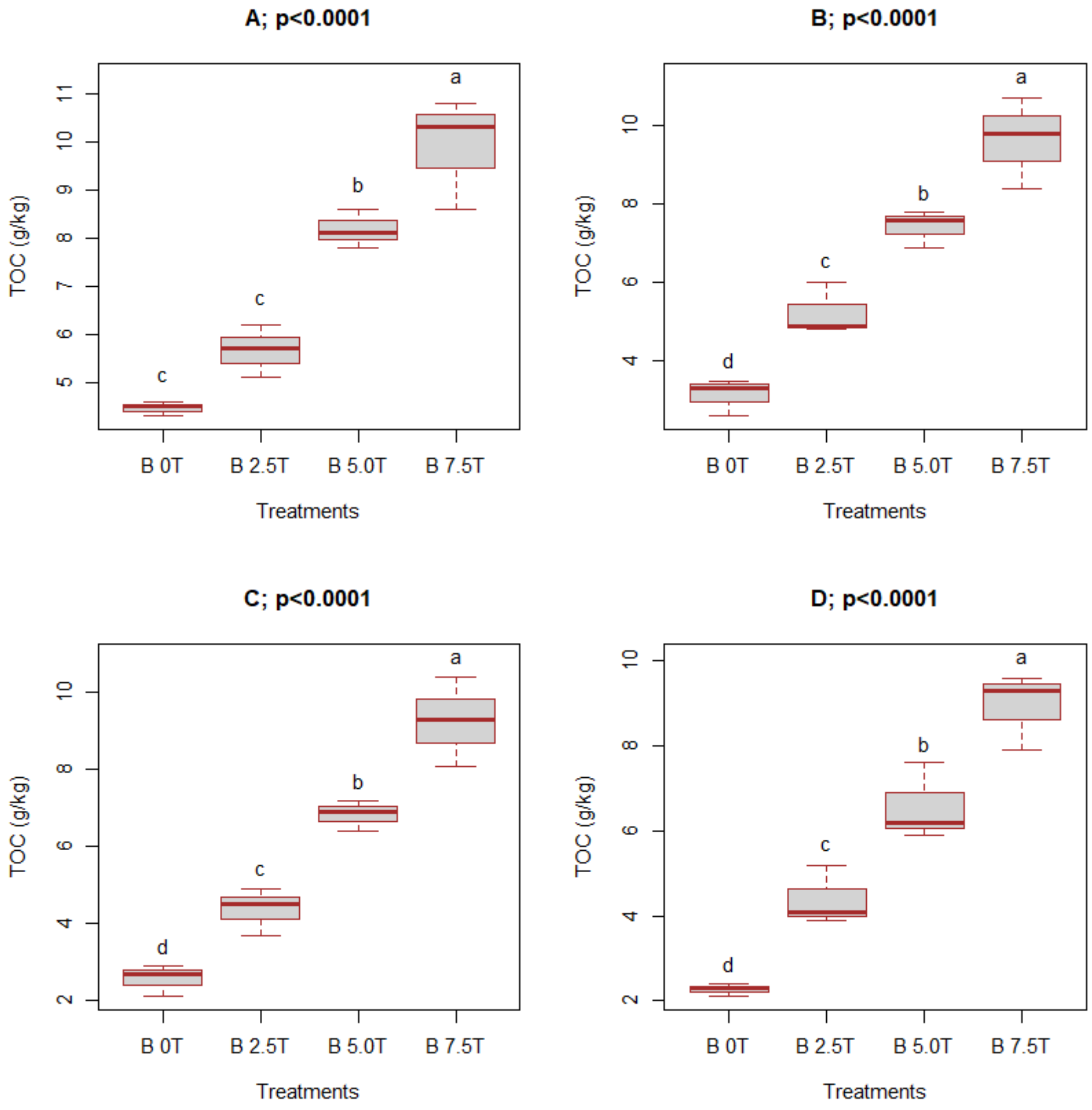


Figure 4.8: Soil TOC under different biochar rates

Note: *(i) Means presented by different alphabets in each boxplot differ substantially; "B" means Biochar, treatment rates are in tons/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

According to Pearson's correlation, a substantial positive linear relationship between biochar rates and TOC was observed as shown in Figure 4.9 (r = 0.96, 0.97, 0.97, and 0.97) for the four sampling periods respectively (Figure 4.9). The present study revealed a strong association between biochar rates applied and TOC as shown in Figure 4.9. Between 92% and 95% of observations made in soil TOC can be attributed to changes in biochar rates applied (R^2*100). Total organic carbon improvement following biochar utilization can be attributed to enhanced organic matter quantities.

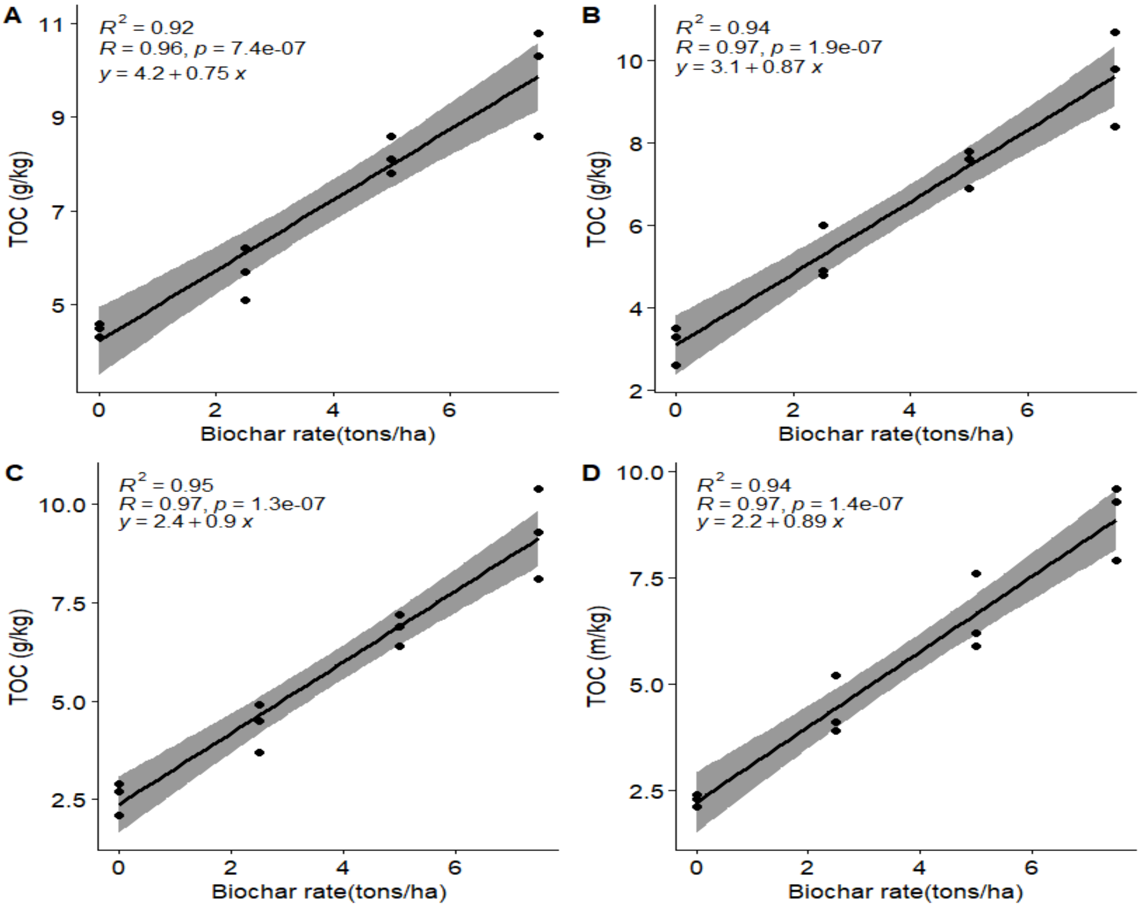


Figure 4.9: Relationship between biochar application rates and soil TOC at different assessment periods
Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Enhanced TOC resulting from biochar application can be attributed to the high C content of the biochar used in the experiment (48%). Biochar contains both labile and recalcitrant C which significantly enhances total C when mixed with soil (Syuhuda et al., 2016; Gao et al., 2017). The increase in TOC with increased biochar application rates can be attributed to the increased addition of recalcitrant C as a result of the increased application rate.

Syuhada et al. (2016) revealed that biochar rates substantially influenced TOC; this is in agreement with the present study where higher biochar application rates resulted in higher soil TOC than the lower biochar rates across the four assessment periods (Figure 4.8). Syuhada et al. (2016) attributed the increase of total C in the soil to an increasing amount of biochar added to the soil with increasing biochar rates. Similarly, Zheng et al. (2019) observed improved TOC after biochar amendment. Abdullaeva (2014) observed a substantial increase in total C following biochar utilization in comparison with the unamended treatment. Mohan et al. (2018) revealed that the application of 3% biochar enhanced organic carbon by 328% above the unamended treatment. Gao et al. (2017) and Truong & Marschner (2018) equally observed enhanced TOC following biochar utilization by < 38.19%.

The use of biochar is a suitable approach for carbon neutralization ensuing from acting as a carbon sink and reduction of greenhouse gas emissions (Xie et al. (2015). Studies have shown that biochar enables the long-term sequestration of carbon (Saletink et al., 2019). Amending one hectare with biochar sequesters approximately 13 tons of CO2eq in the soil (Lawawiec et al., 2019). During pyrolysis, 0.04 tCO2eq is emitted to the atmosphere for each ton of biomass pyrolyzed, however, 1.67 tCO2eq per ton of feedstock is stored in the soil. Biochar captures and stores atmospheric carbon in recalcitrant form thereby reducing carbon emission to the atmosphere (Tisserant & Cherubini, 2019). Biochar use in agricultural systems is a viable option for enhancing natural rates of carbon sequestration in soil and improving soil quality (Srinivasarao et al., 2013). Post-Kyoto agreements under the United Nations Framework Convention on Climate Change (UNFCCC) unilaterally accepted biochar as a viable climate change mitigation strategy (Omulo, 2020).

The soil TOC declined across the assessment periods for all the treatments with the control recording a decline of up to 49.6% while a biochar dose of 7.5 t ha^{-1} recorded a decline in TOC of up to 10.1%. Studies have shown that the decomposition of biochar ranged from 0.005% to 0.023% per day for studies conducted within one year; the decomposition reduced with increasing biochar rates (Wang et al., 2016). Biochar reduces the decomposition rate of organic matter to facilitate C sequestration in low organic C soils (Fatima et al., 2021).

4.2.3.4 Available P

Substantial available P differences were observed after utilization of varying biochar rates (p<0.001, 0.0009, 0.0001, and 0.00006 at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively). Generally, plots treated with biochar recorded higher available P than the control plots across the assessment periods (Table 4.3). Biochar dose of 7.5 t ha^{-1} led to higher available P levels across the assessment periods by 263.7%, 301.6%, 281.5%, and 263.6% above the control across the four assessment periods, respectively. The intermediate application rate (5.0 t ha^{-1}) enhanced available P above the control by 194.1%, 237.9%, 230.7%, and 175.5% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. The lowest biochar application rate on the other hand enhanced available P above the control by 152.4%, 184.4%, 151.2%, and 89.6% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. Increasing the biochar application rate from 5.0 to 7.5 t ha^{-1} did not significantly increase available P from 3 MAE to 9 MAE ($p>0.05$). However, substantial variations in available P after the use of 2.5 and 7.5 t ha^{-1} biochar were observed across the four assessments period.

Table 4.3: Available P under different biochar rates at different assessment periods

Parameter	**Available Phosphorus ± SE (mg kg^{-1})**			
Treatment	**3 MAE**	**6 MAE**	**9 MAE**	**12 MAE**
B 0 tons/ha	6.56±2.54c	5.51±1.23c	5.35±1.58c	5.30±1.10d
B 2.5 tons/ha	16.56±2.17b	15.67±2.72b	13.44±2.79b	10.05±2.08c
B 5.0 tons/ha	19.29±5.49ab	18.62±5.19ab	17.69±2.70a	14.60±2.45b
B 7.5 tons/ha	23.85±1.89a	22.13±1.42a	20.41±1.52a	19.27±0.83a
$f_{(3,8)}$	14.13	16.20	27.54	35.42
$p < 0.05$	0.001	0.0009	0.0001	0.00006

***Note** Means presented by different alphabets in each column differ substantially; Biochar 0 tons/ha=Control, B means biochar*

Results of Pearson's correlation in the current study revealed a positive linear relationship between biochar application rates and available soil P of r = 0.88, 0.88, 0.93, and 0.96, at the four assessment periods, respectively also shown in Figure 4.10. The study also revealed that there was a strong association between biochar application rates and available P across the four assessment periods (r^2 = 0.78, 0.78, 0.86, and 0.93), respectively as shown in Figure 4.10. The study further revealed that 78% to 93% of the changes observed in available P can be attributed to changes in biochar application rates applied (Figure 4.10).

The results of Pearson's correlation in the present study revealed a significant positive linear relationship between soil pH and available P of r = 0.83, 0.86, 0.97, 0.94, respectively for the four assessment periods as highlighted in Figure 4.11. The results revealed that there was a strong association between soil pH and available P of r^2 = 0.69, 0.74, 0.94, and 0.88, respectively, for the four assessment periods. The study revealed that 69% to 94% of the observation made in the available P can be attributed to changes in the soil pH.

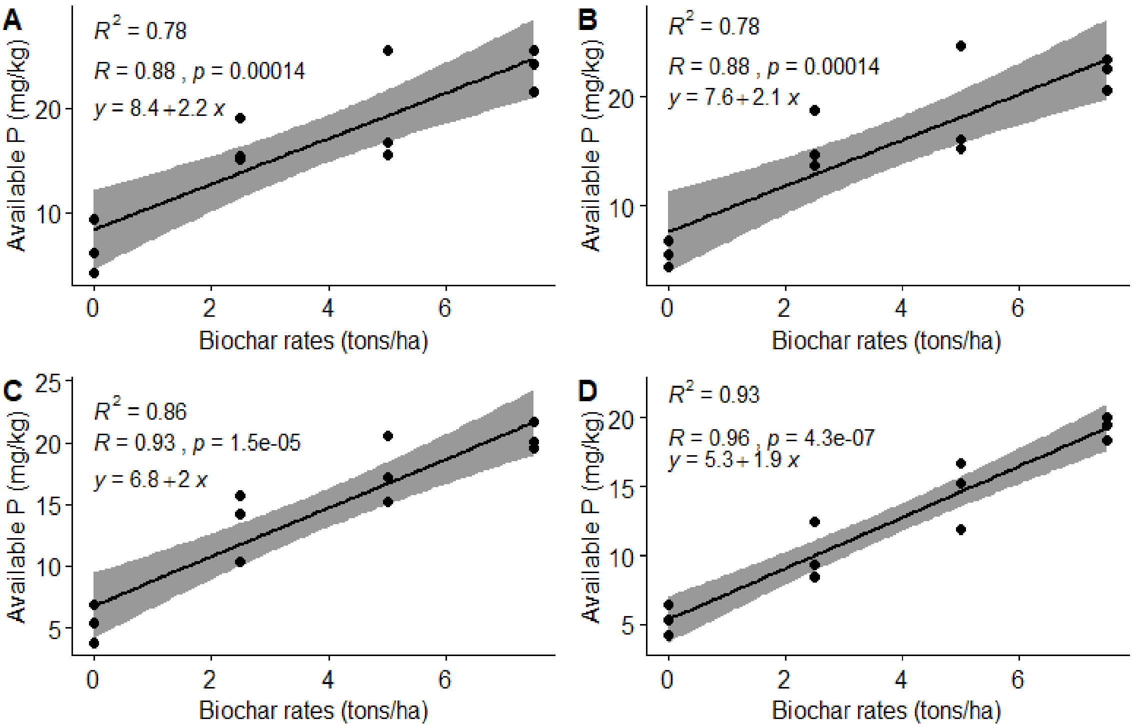


Figure 4.10: Relationship between biochar application rates and soil available P at different assessment periods

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

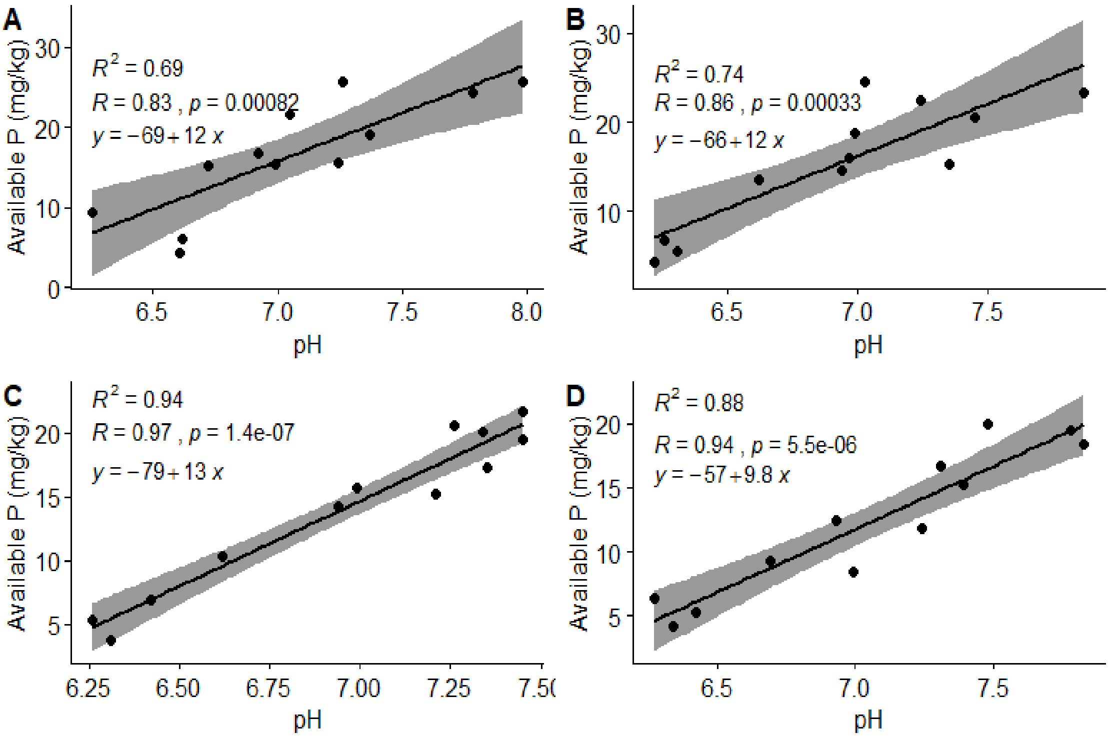


Figure 4.11: Relationship between soil pH and available P at different assessment periods

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

The increase in available P following biochar utilization as observed in this study can be attributed to P addition through biochar application. The increase in available P with increasing biochar rates could also be attributed to P addition from biochar itself. The enhanced available P could also be a result of reduced iron (Fe) and Aluminium (Al) activity due to an increase in soil pH and exchangeable bases (Mensah &Frimpong, 2018). Biochar's capacity to retain and exchange phosphate ions due to biochar's positively charged surface could also have contributed to the enhanced available P in the present study (Rawat et al., 2019).

Similar observations on enhanced available P with biochar application were made by Mensah & Frimpong (2018). The study revealed that biochar utilization substantially increased soil P with the highest P observed in the 2% sole biochar application. Ghosh et al. (2015) also observed that available P concentration increased in plots amended with biochar. Soil P has been reported to be highly pH-dependent (NRCS, 2014). Studies by NRCS (2014) and Jensen (2010) reported that available P is mainly available between pH of 6.0 and 7.5. The enhanced available P resulting from increasing biochar application rates can thus be linked to the liming effect of biochar which was enhanced when biochar rates were increased in the present study.

Glaser & Lehr (2019) reported improved available P with increased biochar application rates, a finding that is also reported in the current study. Other studies have also reported increased P concentration in the soil following biochar application (Trupiano et al., 2017; Truong & Marschner, 2018). Biochar can potentially serve as sustainable P fertilizer with its impact depending on pyrolysis temperature, feedstock, and application doses (Glaser & Lehr, 2019). Woody biochar has a higher P than biochar from manure (Li et al., 2019; Shen et al., 2016). It has also been noted that biochar prepared from high-temperature pyrolysis (>550ºC) has a larger surface area and P content (Li et al., 2019). Generally, P concentration steadily increases as pyrolysis temperature increases (Glaser & Lehr, 2019; Li et al., 2019). Li et al. (2019) observed enhanced P during the pyrolysis process by two to three folds.

Approximately 50% of P from biochar is released to the soil in form of orthophosphates and pyrophosphates at pH <9 (Li et al., 2019). Phosphorus adsorbed in biochar can be released and utilized for plant growth as part of biochar's aging process. The P increase with biochar application contributes to greater root growth thus enhancing nutrient and water uptake by plants (Li et al., 2019). There was also a slight decline in available P across the assessment periods in the present study. This can be attributed to P uptake by Casuarina trees planted as a test crop (Muthukumar et al., 2012). Nutrient uptake of Casuarina trees in the present study was however not quantified. Liao et al. (2020) reported similar findings on the decrease in soil

P with time and attributed the decline to nutrient uptake by maize and faba bean during a three-year experiment period.

4.2.3.5 Extractable Potassium

The results of this study show that extractable K was significantly different across the four assessment periods as shown in Table 4.4 (p<0.0002, 0.0006, 0.0002, 0.0007, respectively) following the application of different biochar rates. Biochar dose of 7.5 t ha^{-1} led to higher extractable K levels across the assessment periods by 101.5%, 107.2%, 125.8%, and 122.9% above the control across the four assessment periods, respectively. The intermediate application rate (5.0 t ha^{-1}) enhanced extractable K above the control by 85.3%, 77.1%, 93.5%, and 90.2% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. The lowest biochar application rate on the other hand enhanced extractable K above the control by 41.4%, 30.7%, 35.3%, and 46.5% at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. Generally, plots treated with biochar recorded higher extractable K of up than the untreated treatment. There was however lack of substantial difference in extractable K between biochar rates of 5.0 and 7.5 t ha^{-1} across the four assessment periods ($p>0.05$). The general trend in the present study was control <2.5 t ha^{-1}<5.0 t ha^{-1}=7.5 t ha^{-1} except at 9 MAE where the trend was control=2.5 t ha^{-1}<5.0 t ha^{-1}=7.5 t ha^{-1}.

Table 4.4: Extractable K under different biochar rates and at different assessment periods

Parameter	**Extractable Potassium ± SE (mg kg^{-1})**			
Treatment	**3 MAE**	**6 MAE**	**9 MAE**	**12 MAE**
Biochar 0 tons/ha	61.7±5.5c	59.7±12.9c	52.7±15.0b	51.0±6.3c
Biochar 2.5 tons/ha	87.3±12.0b	78.0±10.5b	71.3±9.7b	74.7±7.4b
Biochar 5.0 tons/ha	114.3±11.6a	105.7±9.0a	102.0±7.0a	97.0±13.8a
Biochar 7.5 tons/ha	124.3±8.6a	123.7±13.2a	117.0±5.6a	113.7±15.0a
$f_{(3,8)}$	24.93	18.24	25.71	17.50
p <0.05	0.0002	0.0006	0.0002	0.0007

Note: *Means presented by different alphabets in each column differ substantially; Biochar 0 tons/ha =control*

The results of Pearson’s correlation revealed a significant positive linear relationship between biochar rates and extractable K across the four assessment periods (r = 0.93, 0.93, 0.94, 0.93 respectively) as illustrated in Figure 4.12. The study also revealed a strong association between biochar rates and extractable K (r^2 = 0.87, 0.87, 0.89, and 0.86 respectively for the four assessment periods) whereby between 86% and 89% of the observations made in extractable K can be attributed to the response of changes made in biochar application rates as shown in Figure 4.12.

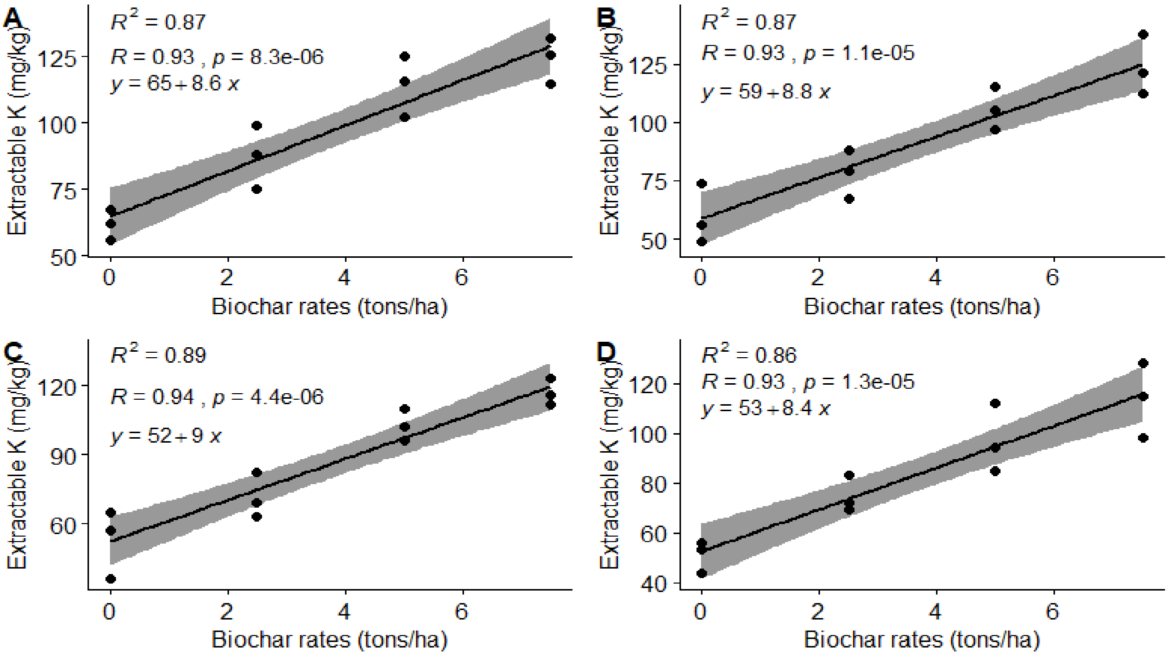


Figure 4.12: Relationship between biochar rates and extractable K at different assessment periods

***Note:** 3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Enhanced extractable K resulting from different biochar application rates in the present study can be attributed to K addition from biochar used which was high in extractable K (1166 mg kg^{-1}). The increase in extractable K with biochar application can also be attributed to increased K-solubilizing bacteria (Wang et al., 2018). The increase of extractable K in this study can be attributed to the ash content contained in the biochar used for the experiment (Mensah & Frimpong, 2018); the biochar used for the experiment had an ash content of 3.95% (w/w). The enhanced K^+ can also be attributed to the decreased K^+ leaching and release of adsorbed K^+ to the soil solution due to biochar's large surface area and high adsorption capacity. Also, K^+ availability in the soil is pH dependent with high pH enhancing K availability (Dume et al., 2015; Miller, 2016). Miller (2016) reported that K^+ availability is higher above the pH of 6.0. This could explain the higher K^+ when biochar was utilized which substantially raised soil pH.

A comparative study by Gao et al. (2017) reported enhanced extractable K of up to 22.38% following biochar application. Similar findings on improved extractable K after biochar utilization were reported by Gosh et al. (2015) and Wang et al. (2018). Syuhada et al. (2016) reported a substantial influence of biochar rates on K availability; utilization of 10 to

15 g kg^{-1} biochar recorded substantially higher K than the untreated control. Jien & Wang (2013) also reported a significant increase of K in soil with biochar application. Dume et al. (2015) observed improved exchangeable K^{+} by up to 72.9% following biochar application. The study attributed the changes in K^{+} following biochar utilization to ash content present in the biochar used for the experiment. There was also a slight decline in extractable K across the assessment periods; which can be attributed to the nutrient uptake of Casuarina trees planted as test crops (Muthukumar et al., 2012).

4.2.3.6 Extractable Calcium

The results of this study show that extractable calcium differed substantially after the use of different biochar rates at 3 MAE and 12 MAE assessment periods ($p<0.02$ and 0.03, respectively). Generally, biochar-amended soil recorded higher Ca^{2+} than the untreated control (Figure 4.13). The highest biochar dosage (7.5 t ha^{-1}) yielded higher Ca^{2+} of <412 mg kg^{-1} throughout the assessment period. An improvement in Ca^{2+} of <70% after biochar amendment (7.5 t ha^{-1}) was observed at 12 MAE (Figure 4.13). The general trend of extractable Ca in the soil in the present study was control <2.5 t ha^{-1}=5.0 t ha^{-1}<7.5 t ha^{-1}.

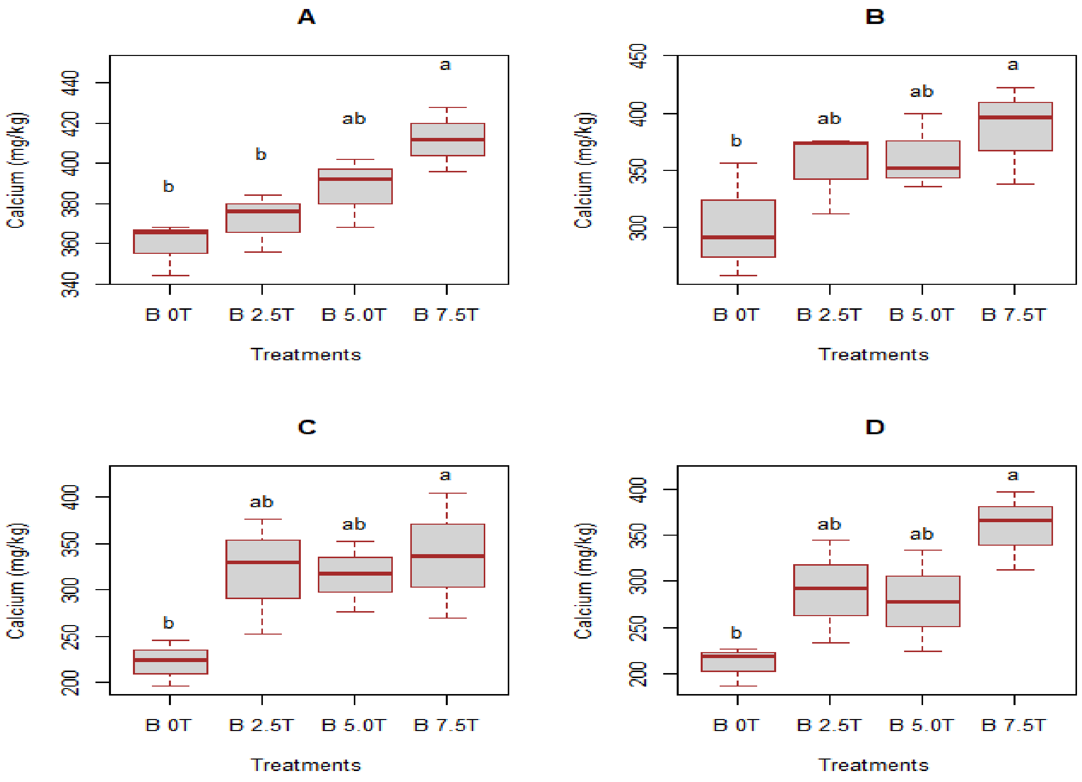


Figure 4.13: Soil Calcium under different biochar rates

Note: *(i) Means presented by different alphabets in each boxplot differ substantially; "B" means Biochar, treatment rates are in tons/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

Results of a Pearson's correlation revealed a substantial positive relationship between biochar doses and soil extractable Ca^{2+} at the four sampling periods ($r = 0.83, 0.64, 0.62$, and 0.75, respectively). These results thus show enhancing biochar rates led to improve soil Ca^{2+} across the assessment periods (Figure 4.14). The results further revealed that the association between biochar rates and soil Ca^{2+} was moderate ($r^2 = 0.69, 0.41, 0.38$, and 0.57, respectively) as shown in Figure 4.14. Only 38% to 69% of the observation made in soil Ca^{2+} can be attributed to changes in the biochar rates used.

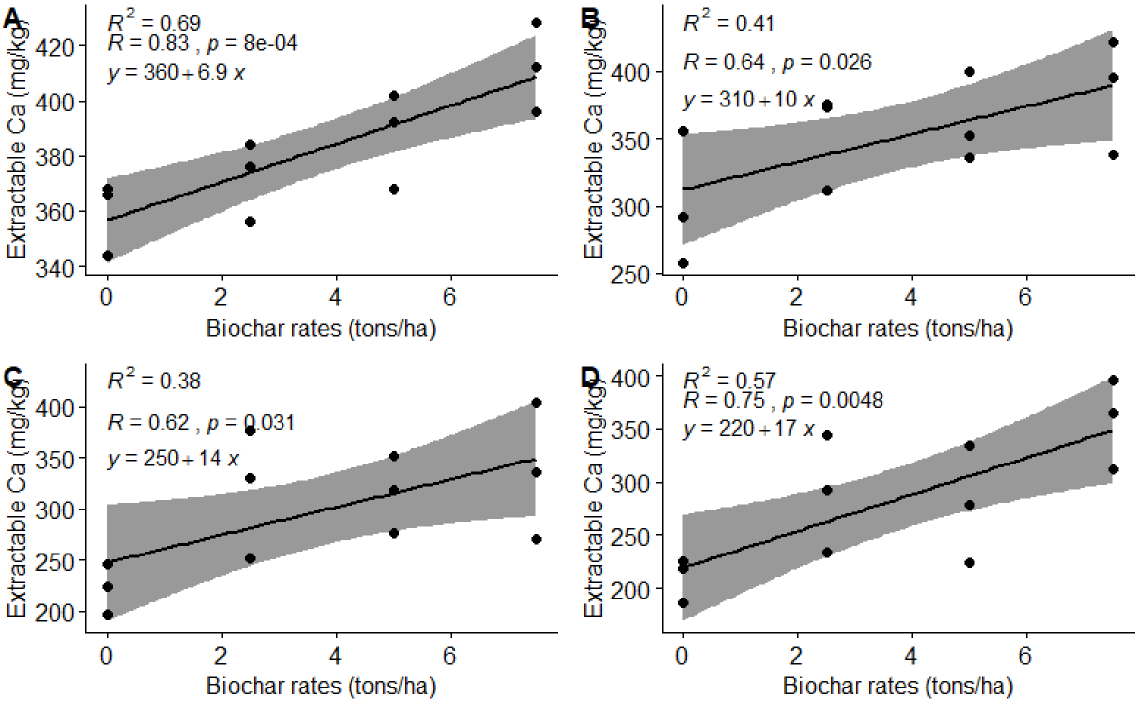


Figure 4.14: Relationship between biochar rates and extractable Ca at different assessment periods

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

The enhanced Ca^{2+} following biochar amendment can be attributed to Ca^{2+} addition from the biochar used in the experiment which was high in Ca^{2+} (746 mg kg $^{-1}$). The enhanced calcium with biochar application can also be attributed to the increase in exchangeable bases such as Ca^{2+} due to ash content (Ghosh et al., 2015); the biochar used for the present study had an ash content of 3.95% (w/w). The enhanced calcium in the present study could also be attributed to the Ca^{2+} increase in soil due to biochar's absorption capacity and its ability to release nutrients slowly for plant use (Rawat et al., 2019). The study further revealed that enhanced Ca^{2+} may be a result of reduced leaching of nutrients following biochar application.

Similar studies have also observed increased Ca^{2+} following biochar application (Jien & Wang, 2013; Ghosh et al., 2015; Mensah & Frimpong, 2018). Rawat et al. (2019) reported increased Ca^{2+} in soil following biochar application. Similarly, Silva et al. (2017) observed enhanced soil Ca^{2+} after the use of higher biochar rates; a trend observed in the present study. Shetty and Prakash (2020) similarly reported enhanced Ca^{2+} after the application of different biochar rates. Chan et al. (2008) and Hailegnaw et al. (2020) also observed increased soil Ca^{2+} following the application of biochar. Hailegnaw et al. (2020) further revealed that Ca^{2+} increased by up to 38.6% after the use of 8% biochar. This trend of enhanced Ca^{2+} was observed in plots that had low Ca^{2+} content than the biochar applied.

The moderate association between biochar rates and Ca^{2+} in the present study can be attributed to the level of Ca^{2+} which was already present in the soil before biochar utilization. There was, however, a decline in soil Ca^{2+} across the assessment periods which could be attributed to nutrient uptake by Casuarina trees used as a test crop. Nutrient uptake by Casuarina was however not quantified in the present study

4.2.3.7 Exchangeable Magnesium

The results of this study revealed that there were no significant differences in exchangeable Mg^{2+} following the application of different biochar rates as shown in Figure 4.15 ($p < 0.44$, 0.3, 0.25, and 0.12 respectively) for 3 MAE, 6 MAE, 9 MAE, and 12 MAE. In comparison with the unamended treatment, the lowest biochar rate (2.5 t ha^{-1}) did not lead to distinct trends at 3 MAE and 6 MAE assessment periods. However, there was a general increase in Mg^{2+} following the application of higher rates of biochar (5.0 and 7.5 t ha^{-1}). The highest biochar dose (7.5 t ha^{-1}) led to higher Mg^{2+} across the four sampling stages (Figure 4.15). The general trend of exchangeable Mg in the soil in the present study was control=2.5 t ha^{-1}=5.0 t ha^{-1}=7.5 t ha^{-1}.

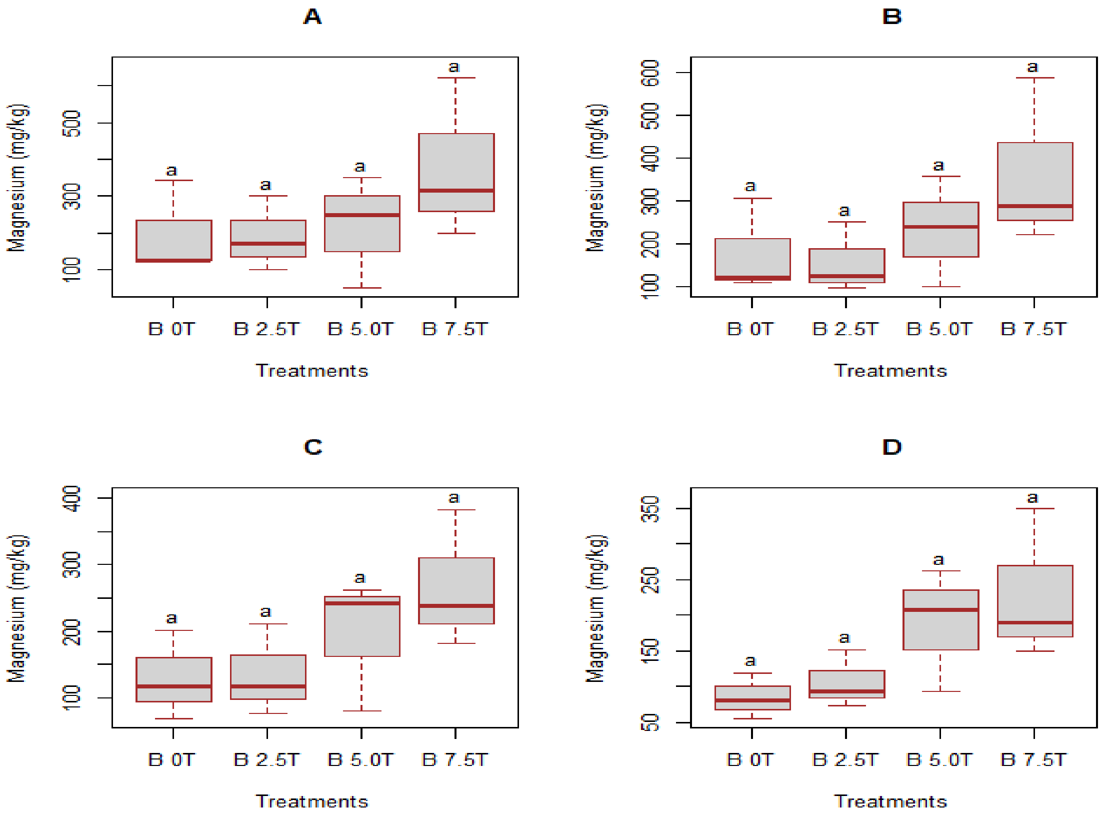


Figure 4.15 Soil exchangeable Magnesium under different biochar application rates

Note: *(i) Means presented by different alphabets in each boxplot differ substantially; "B" means Biochar, treatment rates are in tons/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

Results of Pearson's correlation conducted in this study revealed a positive linear relationship between biochar rates and Mg^{2+} (r = 0.43, 0.53, 0.59, and 0.69, respectively) for the four assessment periods (3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively) as shown in Figure 4.16. The results further revealed that only 18% to 47% of the observations made in soil Mg^{2+} can be attributed to changes in biochar rates applied.

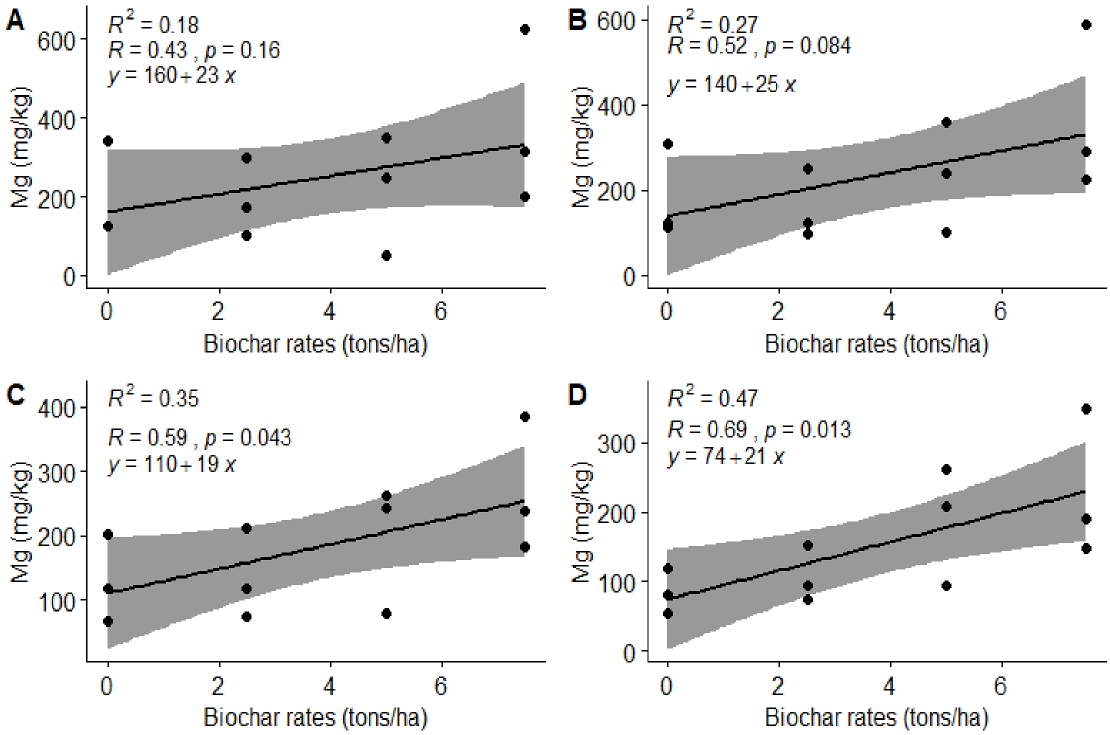


Figure 4.16: Relationship between biochar rates and extractable Mg at different assessment periods

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

The enhanced Mg^{2+} resulting from biochar utilization can be accredited to Mg^{2+} addition through biochar application. Findings of the present study are similar to those of Jien & Wang (2013), Ghosh et al. (2015), and Mensah & Frimpong (2018) who reported an increase in Mg^{2+} with biochar application. The increase in Mg^{2+} and other exchangeable cations was attributed to the ash content in the biochar used for the experiment. The Ash content in biochar used for the present experiment was 3.95% (w/w). Similar studies have observed enhanced Mg^{2+} following the application of different biochar types and rates (Rawat et al., 2019; Shetty & Prakash, 2020). The influence of biochar in the soil is highly dependent on biochar rates, baseline characteristics of the soil, feedstock used, pyrolysis temperature, and biochar type used for the trial (Aung et al., 2018; Mensah & Frimpong, 2018; Hailegnaw et al., 2020).

4.2.3.8 Cation Exchange Capacity (CEC)

The results of the present study show significant differences in soil CEC following the application of different biochar rates at 9 MAE and 12 MAE ($p < 0.05$ and 0.02, respectively) as shown in Table 4.5. The CEC was not significant at the onset of the experiment (3 MAE and 6 MAE). There was however a general increase in soil CEC after biochar utilization. A Biochar dose of 7.5 t ha^{-1} yielded the highest CEC (Table 4.5). Soil CEC was enhanced by 37.8% and 95.2% utilization of biochar dose of 7.5 t ha^{-1} at 3 MAE and 12 MAE assessment periods, respectively. The general trend of CEC in the soil in the present study at 3 MAE and 6 MAE was control=2.5 t ha^{-1}=5.0 t ha^{-1}=7.5 t ha^{-1} while at 9 MAE and 12 MAE, the trend was: control <2.5 t ha^{-1}=5.0 t ha^{-1} <7.5 t ha^{-1}.

Table 4.5: Soil CEC under different biochar application rates

Parameter	**CEC ± SE (cmol $_{(+)}$ kg $^{-1}$**			
Treatment	**3 MAE**	**6 MAE**	**9 MAE**	**12 MAE**
Biochar 0 tons/ha	5.93±0.72a	5.03±0.17a	3.65±0.32b	2.95±0.46b
Biochar 2.5 tons/ha	5.94±0.93a	5.45±0.74a	4.70±0.74ab	4.16±0.78ab
Biochar 5.0 tons/ha	6.03±1.51a	6.13±1.79a	5.33±1.40ab	4.84±1.33a
Biochar 7.5 tons/ha	8.17±2.04a	7.60±1.84a	6.15±0.85a	5.76±0.57a
$f_{(3,8)}$	1.87	2.12	3.99	5.77
p <0.05	0.21	0.17	0.05	0.02

Note: *Means presented by different alphabets in each column differ substantially; Biochar 0 tons/ha=control*

The study also revealed a significant positive linear relationship between biochar rates and soil CEC as shown in Figure 4.17. The correlation coefficient for Pearson's correlation conducted in the study for the four assessment periods (3 MAE, 6 MAE, 9 MAE, and 12 MAE) was as follows: r = 0.51, 0.64, 0.77, and 0.82, respectively. The strength of association between the two variables ranged from weak to moderate, that is 26% to 68% of the observations made in soil CEC which could be attributed to changes in biochar rates applied.

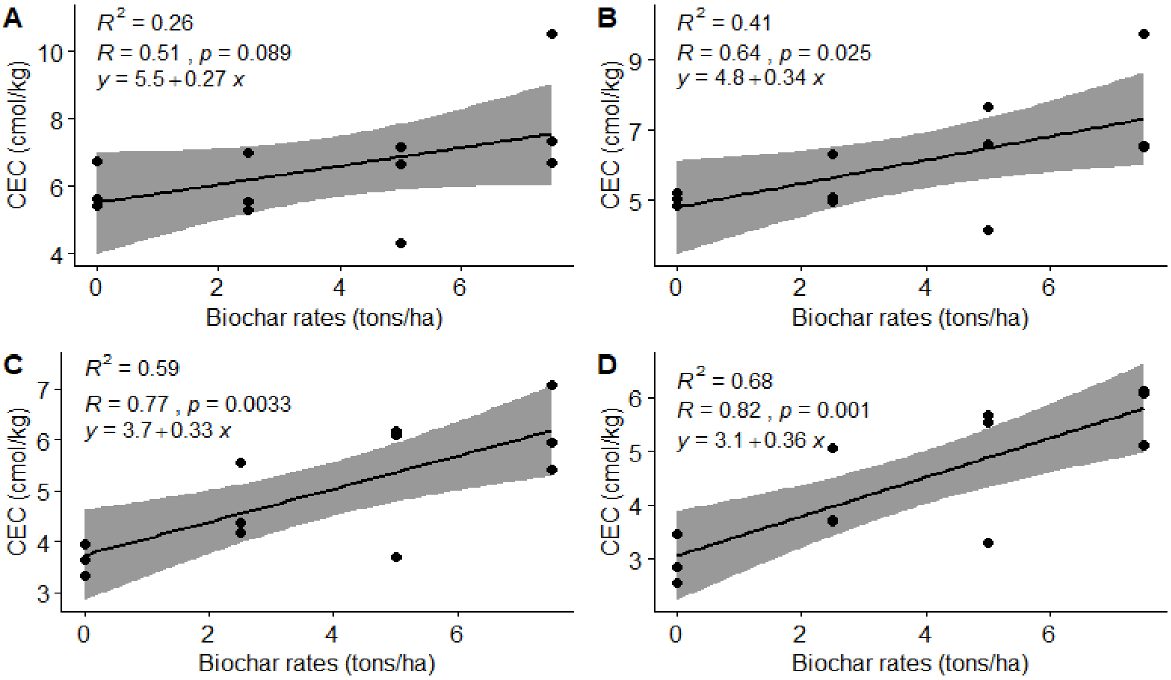


Figure 4.17: Relationship between soil biochar rates and soil CEC at different assessment periods
***Note:** 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

The high CEC after biochar amendment may be a result of the addition of exchangeable cations through biochar application (Rawat et al., 2019). The enhanced CEC resulting from biochar application can also be attributed to low biochar oxidation which increases char's carboxyl groups thus improving soil CEC (De Melo Carvalho et al., 2013; Pühringer, 2016; Mohan et al., 2018).

The CEC of soils measures the capacity of the negative charge where cationic nutrients are adsorbed by the soil which eventually makes them available for plant utilization and stops them from leaching into surface and ground waters. Studies have reported increased CEC following biochar application (Gao et al., 2017; Mensah and Frimpong, 2018; Truong & Marschner, 2018). Mohan et al. (2018) reported enhanced Na^+, K^+, $Ca^{2+,}$ and Mg^{2+} availability after biochar use. The study further reported an increase in CEC of up to 362% at higher biochar application rates in comparison with the unamended treatment.

The relatively high CEC values accrued to biochar application is an indication of its capacity to retain soil nutrients (Tomczyk et al., 2020). Studies have shown that CEC is also pH dependent whereby higher pH results in higher CEC (Weber & Quicker, 2018). This could explain the enhanced CEC after biochar utilization in the present study due to biochar's liming properties which significantly increased soil pH. Biochar's effect on CEC is dependent on the feedstock used; feedstock from animal waste has higher CEC than plant wastes (Tomczyk et al., 2020). In a study conducted in Brazilian Savannah by De melo Carvalho et al. (2013), there was a linear increase in soil pH, exchangeable Ca, exchangeable Mg and CEC while a linear decrease was observed for Al after biochar utilization. The present study's findings are contrary to the findings by Abdullaeva (2014) that reported no substantial CEC improvement as a result of biochar utilization. Soil CEC is an important soil quality index that determines the capacity of soil to supply nutrients to plants (Rawat et al., 2019).

4.2.4 Biochar's influence on soil microbial population

Findings of the present study show substantial variations in bacteria ($p<0.02$ and 0.01 at 3 MAE and 12 MAE, respectively) and fungi population ($p<0.01$ and 0.02 at 3 MAE and 12 MAE, respectively) in the soil following utilization of varying biochar rates as shown in Table 4.6. The soil had a higher bacteria population than the fungi population as shown in Table 4.6. Generally, plots ameliorated with biochar yielded a higher microbial population (bacteria and fungi) than the unamended treatment.

Table 4.6: Bacteria and fungi population under different biochar rates and assessment periods

Parameter	**CFUs g Soil^{-1}**			
	Bacteria*10^5		**Fungi *10^4**	
Treatment	**3 MAE**	**12 MAE**	**3 MAE**	**12 MAE**
Biochar 0 tons/ha (Control)	48±4.9b	49±4.0b	42±8.1c	42±8.6b
Biochar 2.5 tons/ha	54±6.6bc	57±2.6a	49±4.0bc	51±4.6ab
Biochar 5.0 tons/ha	59±4.2ab	60±5.5a	56±5.5ab	55±5.7a
Biochar 7.5 tons/ha	64±4.5a	65±6.1a	61±4.7a	62±4.0a
$f_{(3,8)}$	5.91	6.64	6.80	5.58
$p<0.05$	0.02	0.01	0.01	0.02

Note: *(i) Means presented by different alphabets in each column differ substantially*

The lowest biochar rate (2.5 t ha^{-1}) enhanced the bacteria population above the control by 12.5% and 16.3% at 3 MAE and 12 MAE, respectively. It also enhanced the fungi population by 16.7% and 21.4% during the two assessment periods, respectively. The intermediate biochar rate (5.0 t ha^{-1}) enhanced the bacteria population above the control by 22.9% and 22.4% at 3 MAE and 12 MAE, respectively. It further enhanced the fungi population by 33.3% and 45.2% during the two assessment periods, respectively. The highest biochar application rate (7.5 t ha^{-1}) recorded the highest bacteria above the control by 32.6% and 32.6% at 3 MAE and 12 MAE, respectively. It also enhanced the fungi population above the untreated control by 45.2% and 47.6% for the two assessment periods, respectively.

Generally, there was a slight rise in microbial population by <5.6% from 3 MAE to 12 MAE resulting from biochar application. However, increasing the biochar application rate from 2.5 to 7.5 t ha^{-1} did not lead to substantial improvement in microbial population as shown in Table 4.6. The general trend of the bacteria population at 12 MAE was: control< 2.5 t ha^{-1} = 5.0 t ha^{-1} = 7.5 t ha^{-1} while that of the fungi population was control = 2.5 t ha^{-1}<5.0 t ha^{-1} = 7.5 t ha^{-1}.

The increase in soil microbial population following biochar utilization may be a result of biochar's large surface area and porosity which provides a habitat for soil microorganisms. The high bacteria population in biochar treatments can also be attributed to increased organic matter which enhanced porosity and aeration providing a conducive habitat for microorganisms (Bhattarai et al., 2015). Gul et al. (2015) noted that the large surface area and porosity of biochar provide a habitat for bacteria and fungi. The study further revealed that the bacteria and fungi population was influenced by biochar's surface charge which binds microbial cells, chemical compounds, and ions and the concentration of nutrients.

Ullah et al. (2020) observed enhanced soil bacteria of up to 16% following the application of 2% biochar; the study further revealed that increasing the application rate to 4% increased the bacteria population by up to 50%. Azeem et al. (2020) revealed that biochar can promote plant development resulting from improved microbial activity in the soil through its impact on microbial abundance, bacteria/fungi ratio, and microbial community structure. Zhang et al. (2014) and Zhao et al. (2022) similarly observed enhanced soil microbial biomass of up to 763% with biochar application.

4.3 Casuarina Growth Performance under Different Biochar Application Rates

4.3.1 Effect of varying biochar rates on the height of *Casuarina equisetifolia*

The height of Casuarina plants significantly increased following utilization of varying biochar rates compared to the untreated control at 6 MAE, 9 MAE, and 12 MAE (*p* <0.05, 0.05, 0.01, respectively) as shown in Table 4.7. There were no substantial variations in Casuarina heights following the utilization of different biochar application rates at 3 MAE in comparison with the unamended treatment ($p<0.09$). The 5.0 t ha^{-1} biochar dose led to an unsubstantial increase in Casuarina height at 3 MAE and 6 MAE in comparison with the unamended treatment as shown in Table 4.7; however, it yielded higher Casuarina height than the unamended treatment. Generally, in comparison with the unamended treatment, Casuarina height was enhanced upon biochar utilization.

Table 4.7: Casuarina mean height (m) under different biochar application rates and assessment periods

Seedling height ± SE (m)	Growth period			
Treatment	**3 MAE**	**6 MAE**	**9 MAE**	**12 MAE**
Control	0.75±0.04b	1.15±0.03b	1.77±0.06b	2.77±0.07b
Biochar 2.5 tons/ha	0.89±0.09a	1.42±0.13a	2.18±0.17a	3.24±0.27a
Biochar 5.0 tons/ha	0.85±0.07ab	1.36±0.17ab	2.07±0.22a	3.18±0.14a
Biochar 7.5 tons/ha	0.89±0.06a	1.43±0.07a	2.13±0.14a	3.33±0.06a
$f_{(3,8)}$	3.02	3.95	4.19	7.21
$p < 0.05$	0.09 n.s.	0.05	0.05	0.01

Note:* *(i) *Means presented by different alphabets in each column differ substantially* ***(ii)*** *n.s. means not significant*

Amendment with the highest biochar dosage (7.5 t ha^{-1}) yielded the highest Casuarina height at two assessment periods (6 MAE and 12 MAE). In comparison with the unamended treatment, the use of biochar (7.5 t ha^{-1}) enhanced Casuarina height by 24.3% and 20.2 %, respectively, for the two assessment periods. Nevertheless, the use of the lowest and highest (2.5 and 7.5 t ha^{-1} respectively) biochar doses yielded similar mean height (0.89 m) at 3 MAE; this means height was 18.7% higher than the control (Table 4.7). At 9 MAE, biochar application at 2.5 t ha^{-1} resulted in the highest Casuarina height (2.18 m) which was 23.2% above the control treatment. The trend was 7.5 = 2.5 > 5.0 t ha^{-1} = control before 6 MAE. At 9 and 12 MAE it was 7.5 =5.0 = 2.5 t ha^{-1} > control.

Generally, Casuarina height did not differ substantially when biochar application rates increase from 2.5 to 7.5 t ha^{-1}. At 6 MAE and 12 MAE when 7.5 t ha^{-1} of biochar yielded the highest mean height, the difference in Casuarina height in comparison with 2.5 t ha^{-1} was only 0.7 and 2.7% respectively. Amongst the biochar doses used, a 5.0 t ha^{-1} rate yielded lower Casuarina mean height across the growth period compared to 2.5 and 7.5 t ha^{-1} rates. Nevertheless, the intermediate dosage (5.0 t ha^{-1}) recorded higher Casuarina mean height than the unamended treatment by 13.3%, 18.3%, 16.9%, and 14.8% across the four assessment periods, respectively.

The results of Pearson's correlation conducted revealed a substantial positive linear relationship between biochar doses and Casuarina height growth (r = + .56, +.59, +.54, and +.72 respectively) for the four sampling periods as highlighted in Table 4.8 and Figure 4.18. The results of linear regression analyses conducted to determine the strength of association between biochar rates and mean Casuarina height show that only 29%-51% (r^2*100) of the observations made can be accounted for by changes made in the biochar application rates applied.

Table 4.8: Coefficient of correlation between biochar application rates and mean Casuarina height at 4 assessment periods

Assessment period	**Coefficient of correlation (r)**	***P* value**
3 MAE	.56	0.059
6 MAE	.59	0.043
9 MAE	.54	0.072
12 MAE	.72	0.008

***NB:** MAE: Months after field establishment*

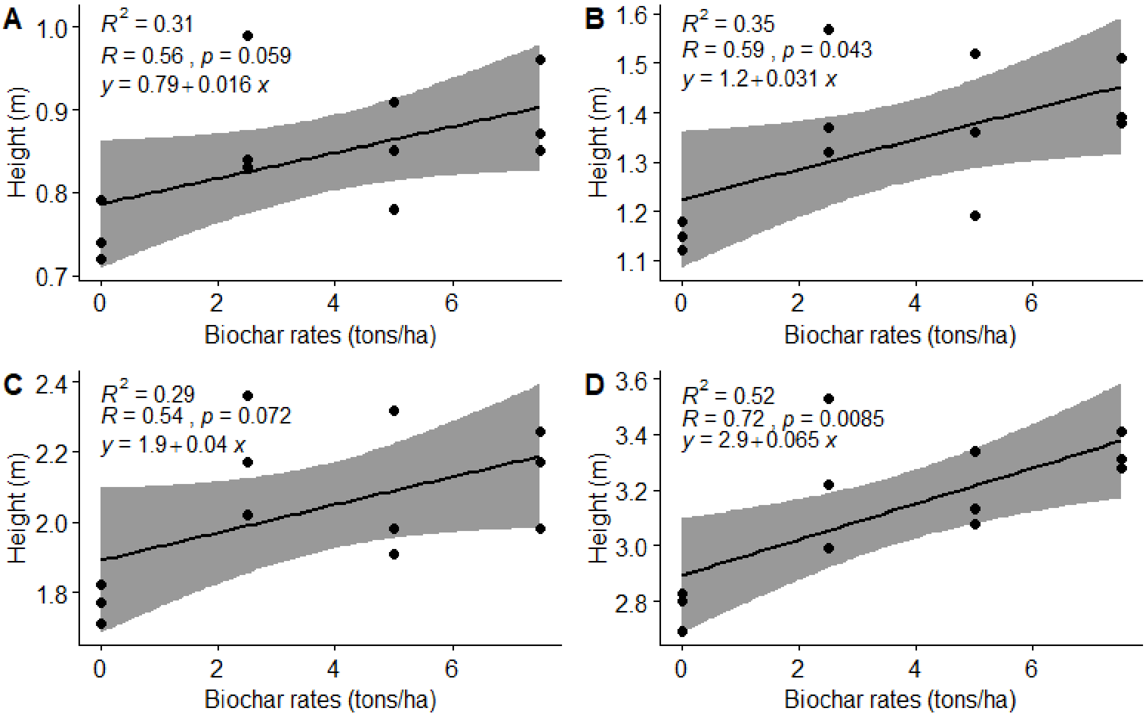


Figure 4.18: Relationship between biochar application rates and Casuarina height at different assessment periods

***Note:** 3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Enhanced Casuarina height resulting from biochar utilization can be attributed to improved availability of essential plant nutrients (N and P) and improvement of soil physical properties such as reduced bulk density and enhanced soil moisture content. The present study has shown that biochar utilization enhanced soil phosphorus, nitrogen, and potassium as shown in Table 4.3, 4.4, and Figure 4.6. Similar findings on enhanced soil nutrient availability following biochar utilization were observed by Berihun et al. (2017) and Helliwell (2015). This could also explain the trend observed in the present study where biochar utilization enhanced Casuarina mean height more than the unamended treatment throughout the assessment period. Some studies have shown that the low susceptibility of biochar to microbial processes enhances the stability of soil organic matter in the soil while improving the availability of water and nutrients to plants (Brantley et al., 2015; Tel, 2018). However, research on the effects of biochar on tree growth is limited (Wilson, 2015). Growth of crops as influenced by biochar utilization has received substantial focus in the recent past with studies being conducted to evaluate the impact of biochar on crop yields (Haider, 2017).

To understand the effect of biochar on plant growth in general, Mohan et al. (2018) reported enhanced eggplant growth due to biochar utilization in comparison with the unamended treatment. The study further revealed that soils ameliorated with 3% biochar had the highest increase in both plant height and number of leaves compared to the unamended treatment. Mohan et al. (2018) observed improved eggplant height from 8.3 cm in the first week to 20 cm (7th week) versus 6.0 cm (1st week) to 9.5 cm (7th week) in the case of unamended treatment. Comparable results to the present study on enhanced plant growth resulting from biochar utilization were also reported by Carter et al. (2013). Biochar has been reported to promote plant productivity through several mechanisms such as enhancement of physical conditions with biochar application through enhanced water holding capacity thereby providing water for plant development (Khaitov et al., 2019). In the present study, an improvement in moisture content of <108% was observed after the utilization of 7.5 t ha^{-1} of biochar at 12 MAE.

A study by Rahim (2018) equally observed substantial enhancement in height of Glycine max (L.) after biochar utilization. The study attributed this to alleviating the physicochemical stresses, particularly water and nutrient scarcity as a result of biochar utilization. The findings of the present study concur with Berek & Hue (2013) who observed substantial plant growth and biomass increase by 2 to 4 folds after biochar utilization. A study by Drabkin & Weinfuether (2014) reported that tree growth showed substantial differences on sites with biochar; the study however recommended further studies to ascertain the observed trends.

4.3.2 Effect of different biochar rates on *Casuarina equisetifolia* growth performance (Diameter at ground level and diameter at breast height)

The results of the effect of sole biochar application on Casuarina collar diameter, that is

‘Diameter at Ground Level of trees (DGL)’ over 12 months are shown in Figure 4.19 below. There was a gradual increase in DGL across the four assessment periods as illustrated in Figure 4.19. There were, however, no substantial differences in collar diameter following utilization of varying biochar application rates across the assessment periods (p < 0.91, 0.82, and 0.57, respectively) at 3 MAE, 6 MAE, and 9 MAE. Similarly, no substantial variations were observed in terms of DBH at the 12 MAE assessment period (p <0.17). The unamended treatment recorded the lowest DGL and DBH across the growth periods as highlighted in Figure 4.19. Despite the lack of substantial variations in terms of DGL and DBH after utilization of different biochar rates, generally, plots ameliorated with biochar yielded higher DGL and DBH in comparison with the unamended treatment (Figure 4.19). Biochar dose of 2.5 t ha^{-1} yielded the highest DGL and DBH across the growth period; this was 4.3%, 16.4%, 12.8%, and 30.2% above the unamended treatment for the four assessment periods, respectively.

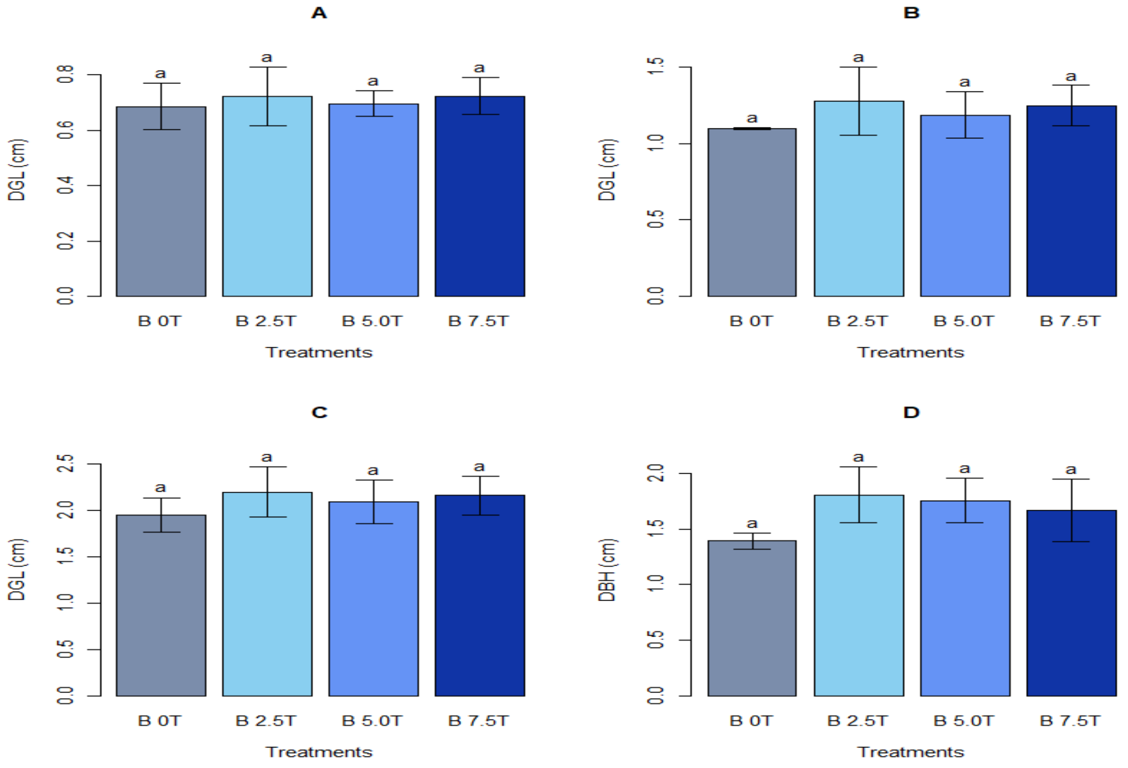


Figure 4.19.: Casuarina collar diameter under different biochar application rates

Note: *(i) Means presented by different alphabets in each graph differ substantially; “B” means Biochar, treatment rates are in tons/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

There was an unsubstantial positive linear relationship between biochar rates and Casuarina DGL/DBH (r = + .14, +.29, +.28, and +.36 for the four sampling periods) as shown in Figure 4.20. The results further revealed a very weak association between biochar application rates and Casuarina DGL/DBH. Only 2 to 13% of the observations made in Casuarina DGL and DBH can be accounted for by changes in biochar application rates. This indicates that increasing biochar application rates did not lead to substantial improvement of DGL and DBH.

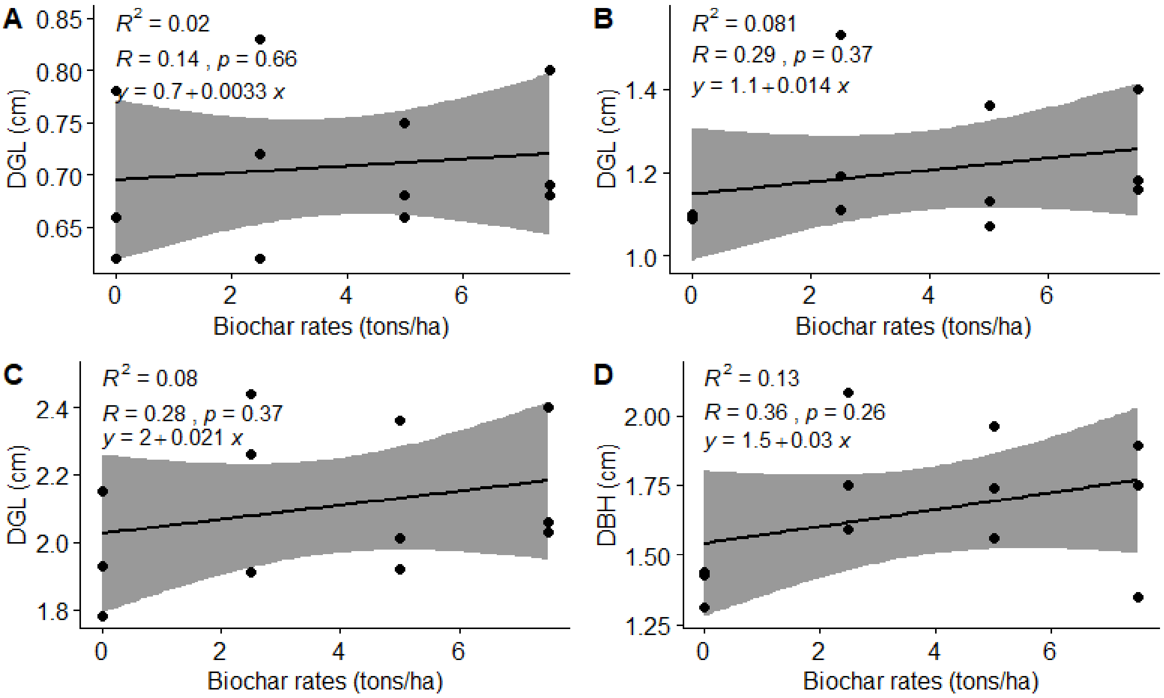


Figure 4.20: Relationship between biochar application rates and Casuarina DGL/DBH at different assessment periods

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

The slight increase in DGL and DBH with biochar utilization can be attributed to the enhancement of physico-chemical soil properties with biochar application as shown in Tables 4.3, 4.4, and 4.5. In the present study, the application of the highest biochar rate led to enhanced available P by < 301%, extractable K by up to 123%, and CEC by up to 95.2%. Biochar's impact on soil physical characteristics directly affects plant growth through enhanced root penetration as a result of reduced bulk density and soil moisture retention due to increased porosity as a result of biochar's high porosity (Rawat et al., 2019). Bulk density declined by up to 23.2% (Figure 4.1) and soil moisture improved by up to 108% (Figure 4.3) following biochar utilization in the present study.

The effects of biochar on Casuarina DGL and DBH are subject to further studies. Numerous biochar studies have focussed on agricultural soils therefore biochar's impact on tree growth is not well-studied. Further, available literature reported contradicting findings.

Palviainen et al. (2018) reported that biochar application of 10 mg ha^{-1} enhanced the diameter growth of dominant trees significantly which was 25% higher than in unamended plots. Macphail (2018) in his meta-analysis reported that biochar increased plant growth by up to 41%. The meta-analysis attributed the increase in plant growth after biochar utilization to nutrient and water retention by biochar.

4.4 Impact of Biochar Combined with Manure and Inorganic Fertilizers on Casuarina Growth

4.4.1 Casuarina seedlings’ collar diameter (DGL) growth as influenced by biochar and manure utilization

The impact of biochar and manure on seedling collar diameter was observed for 177 days and was measured periodically and found as shown in Table 4.9. There was a gradual increase in DGL across the growth period as indicated by the various treatments. Collar diameter differed substantially upon application of the various treatments at day 135 ($p < 0.05$) and day 177 ($p < 0.00005$) as indicated in Table 4.9. Seedlings ameliorated with 10% (v/v) manure had the highest DGL while seedlings treated with 10% (v/v) biochar had the lowest DGL across the growth periods. Application of 10% manure yielded Casuarina DGL which was 30.7% and 17.6% higher than the unamended treatment at days 133 and 177, respectively. Utilization of 10% biochar on the other hand yielded Casuarina DGL lower than the unamended treatment across the three growth periods (133, 147, and 177 days) by 7.7, 11.5, and 11.2%, respectively. The impact of biochar on the seedling collar is subject to further studies to understand biochar characteristics that may impact the growth of seedling collar diameter.

Table 4.9: Casuarina seedling DGL as influenced by the application of biochar and manure

Seedling DGL (cm) ± SE		**Growth period (days)**	
Treatments	**133**	**147**	**177**
Control	0.13±0.02b	0.26±0.01ab	0.357±0.03c
10% Biochar	0.12±0.15b	0.23±0.001b	0.317±0.02d
20% Biochar	0.12±0.02b	0.24±0.15b	0.407±0.01b
10% Manure	0.17±0.02a	0.27±0.03a	0.487±0.04a
10% Biochar+10% Manure	0.12±0.04b	0.26±0.03ab	0.420±0.03b
20% Biochar+10% Manure	0.16±0.03ab	0.25±0.01ab	0.420±0.02b
F (5,12)	3.161	2.432	20.378
p<0.05	0.05	0.09	0.00005

Note: *Means presented by different alphabets in each column differ substantially; n.s. means not significant.*

Seedlings ameliorated with a combination of 20% biochar and 10% manure; and those treated with 10% manure alone had higher DGL in comparison with the unamended control by 23 and 30.7%, respectively. At day 147, there were no substantial variations in DGL across treatments ($p<0.09$) but seedlings ameliorated with 10% manure yielded the highest DGL (<0.27 cm). At day 177, seedlings ameliorated with 10% manure recorded the highest DGL of 0.487 cm which was 36.4% higher than the unamended treatment and 53.6% higher than seedlings ameliorated with 10% biochar (Table 4.9).

The increase in collar diameter after manure utilization reported in the present study could be attributed to enhanced nutrient availability through mineralization of the manure as shown in Table 4.10. The high nitrates concentration after the use of 10% manure could have led to an increase in Casuarina collar diameter (Table 4.10). The high nitrates concentration in the manure treatment can be a result of N mineralization resulting from the narrow C/N ratio of the manure used for the experiment (1:7) (Ezlu et al., 2019). Ewulo et al. (2008) and Han et al. (2016) similarly observed enhanced nitrogen with manure application. Integrating biochar with manure enhances soil nitrogen (Yunilasari et al., 2020). Enhanced nitrates following manure utilization were crucial for the vegetative growth of *C. equisetifolia* seedlings (Leghari et al., 2016). Similar observations were made by Biederman & Harpole (2013) and Khaitov et al. (2019). Daldoum and Hammad (2015) equally observed enhanced collar diameter (of *Acacia senegal* seedlings) with manure application in comparison with the unamended treatment.

This study shows that seedlings ameliorated with 10% biochar yielded lower collar diameter than the unamended treatment (Table 4.9). This could be attributed to the inherent characteristics of this biochar such as the wide C/N ratio (57:1) of biochar produced from woody feedstock materials (*Prosopis juliflora*) 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 a slow 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; Gao et al., 2019).

Table 4.10: Selected chemical soil properties as influenced by different biochar and manure application rates

Parameter	Soil nutrients ± SE				
Treatments	**P (ppm)**	**K (me/100 g)**	**Na (me/100 g)**	**NO3- (ppm)**	**Mg (me/100 g)**
Control	9.02±0.81b	0.87±0.29ab	2.33±0.38a	2.18±0.89b	1.90±0.05ab
10% Biochar	12.87±3.84b	0.67±0.06ab	1.63±0.32ab	1.43±0.42b	1.78±0.20ab
20% Biochar	21.67±2.74a	0.80±0.46ab	2.23±0.29ab	2.30±1.12b	1.68±0.07b
10% Manure	12.82±3.81b	0.53±0.15b	1.50±0.60b	4.86±0.55a	1.99±0.19ab
10% B+10% M	20.77±2.62a	1.33±0.31a	2.23±0.38ab	2.11±0.88b	1.95±0.19ab
20% B+10% M	21.40±2.73a	0.57±0.12b	1.63±0.25ab	4.43±1.78a	2.06±0.27a
F (5,12)	11.3	2.1	2.8	5.5	2.1
p<0.05	0.0003	0.14	0.06	0.007	0.141

Note: *Means presented by different alphabets in each column differ substantially; B means biochar*

4.4.2 Effect of biochar and manure on the height of *Casuarina equisetifolia* seedlings

The effect of biochar and manure on the height of Casuarina seedlings is shown in Table 4.11. A gradual increase in seedling height from day 35 to day 177 for all treatments was observed. Substantial variations in seedling height were observed from day 77 across the various treatments ($p < 0.005$, 0.0004, 0.0001, 0.00005, 0.0006 and 0.0000006 for day 77, 91, 105, 119, 133, 147, and 177 respectively). The results show that seedlings ameliorated with 10% manure (v/v) yielded the highest heights of all other treatments measured across the recording periods. It was followed by the integration of 20% Biochar and 10% Manure, and the combined application of 10% Biochar and 10% Manure in that order. The unamended treatment recorded the lowest heights across the growth periods except for day 177 where seedlings treated with 10% biochar recorded the lowest (47.7 cm) height, even lower than the unamended treatment by 6.53% (Table 4.11).

Application of 10% manure, substantially enhanced Casuarina heights in comparison with the unamended treatment by 27.9, 30.8, 22, 29.5, 32.2, 27.4 and 46.8% for days 77, 91, 105, 119, 133, 147, and 177, respectively while the seedlings ameliorated with integration of 20% Biochar and 10% Manure yielded the second highest seedling heights across the growth periods (Table 4.11). Also observed was the lack of significant differences in heights among treatments from day 35 to 63.

Table 4.11: Biochar and manure's effect 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±0.71b	7.20±0.56b	9.79±0.55b	12.98±0.90d	15.77±1.12c	17.62±1.05c	19.13±0.68e	21.22±0.50c	25.50±0.71b	51.03±3.04d
10% Biochar	5.91±0.45ab	7.96±0.56ab	10.70±1.02ab	14.45±0.50c	18.29±0.42b	19.16±0.75b	20.61±1.14de	22.13±1.09c	26.04±1.48b	47.70±2.17d
20% Biochar	5.93±0.18ab	8.35±0.67a	11.51±0.59a	15.58±0.51abc	18.62±0.66b	19.52±0.53b	21.49±0.93cd	22.78±0.88c	26.86±1.58b	59.77±0.75c
10% Manure	6.15±0.29ab	8.48±0.35a	10.86±1.47ab	16.60±0.79a	20.62±0.80a	21.61±1.26a	24.77±0.82a	28.06±2.0a	32.48±2.97a	74.93±6.02a
10% B+10% M	6.05±0.26ab	8.08±0.38ab	11.08±0.44ab	15.22±0.83bc	18.60±0.89b	19.62±0.48b	22.51±0.90bc	25.21±0.40b	30.25±1.51a	65.20±1.11b
20% B+10% M	6.49±0.28a	8.42±0.70a	11.40±0.57ab	15.92±0.47ab	19.40±0.97ab	20.56±1.05ab	23.48±1.20ab	26.54±1.18ab	32.03±1.23a	68.50±0.56b
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

Note: *Means presented by different alphabets in each column differ substantially; B. Biochar; M, Manure*

Generally, biochar and manure ameliorated seedlings yielded higher Casuarina seedling heights than the unamended treatment (Table 4.11). This could be as a result of increased nutrients such as nitrogen and phosphorus from manure and biochar utilization (Table 4.10) and enhanced soil moisture holding capacity. Khaitov et al. (2019) and Biederman & Harpole (2013) equally observed enhanced soil nutrients after manure and biochar utilization. The availability of nitrogen is essential for plant vegetative growth while phosphorus plays a crucial role in root growth and development (Razaq et al., 2017). It is evident in the current study that manure application enhanced the shoot growth of *C. equisetifolia* compared to sole biochar application and the unamended treatment.

Enhanced *C. equisetifolia* seedling height following manure utilization can be attributed to increased available nitrogen with manure application as shown in Table 4.10. Towards the end of the trial, manure ameliorated plots still had nitrates concentration of 4.86 ppm which was higher than the unamended treatment by 122.9%. The application of 10% manure recorded 239.9%, 130.3%, and 9.7% higher nitrates concentrations compared to the application of 10% biochar, 10% biochar+10% manure, and 20% biochar+10% manure, respectively as shown in Table 4.10. Such an increase in plant height after manure application was also observed by Prakash et al. (2014) where seedlings of *Calendula officinalis* were used. This study also showed that biochar increased seedling height compared to the unamended treatment as indicated in Table 4.11.

Manure used had a narrow C/N ratio (1:7) which could have led to net N mineralization compared to biochar which had a wider C/N ratio as shown in Table 4.1. Previous studies have shown that application of manure with a narrow C/N ratio leads to net N mineralization thereby providing nitrogen in an available form for plant uptake (Daldoum & Hammad, 2015; Truong & Marschner, 2018). The enhanced height with the combined application of manure and biochar may be due to N, P, K and Mg addition (Table 4.10) which enhanced nutrient availability for *C. equisetifolia* seedlings growth (Berek & Hue, 2013; Drabkin & Weinfuether, 2014; Gao et al., 2019; Uddin et al., 2012). Nevertheless, there was no substantial increase in height with increased rates of biochar from 10 to 20%.

Comparable findings were conveyed by Carter et al. (2013) and (Rahim, 2018); the studies revealed that plant growth increased substantially following biochar utilization in comparison with the unamended treatment. The studies further showed that biochar amendment resulted in enhanced 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 unamended treatment. The integration of biochar and manure resulted in 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 enhancement from biochar (Gao et al., 2019) and nitrogen release from manure (Uddin et al., 2012).

4.4.3 Impact of biochar and manure on Casuarina seedling quality

Integration of 10% biochar and 10% manure yielded the highest shoot/root ratio of 4.83:1 and was followed closely by 10% manure treatment with a ratio of 4.66:1 (Figure 4.21). The shoot/root ratio of Casuarina seedlings ameliorated with a combination of 10% biochar and 10% manure was 23.2%, 40.8%, and 35.3% higher than the unamended treatment, 10% biochar, and 20% biochar, respectively. The high shoot/root ratio can be attributed to enhanced height growth with manure application as a result of enhanced nutrient availability especially nitrogen (Table 4.10).

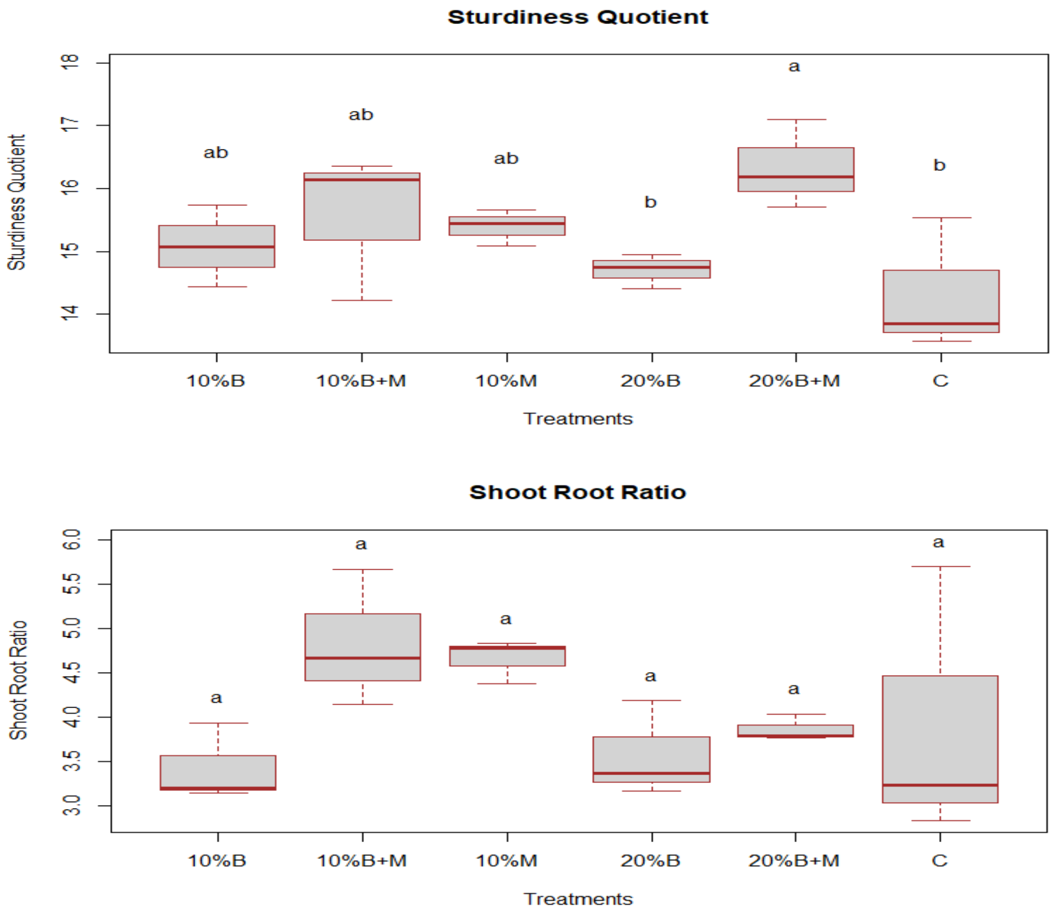


Figure 4.21: Casuarina seedlings quality indices as influenced by various treatments

Note:* *(i) *Means presented by different alphabets in each boxplot differ substantially;* ***(ii)*** *"B" refers to Biochar and "M" refers to Manure, C refers to control.*

Nitrogen is vital in the structural composition and metabolic compounds in a plant cell; it equally plays a significant role in photosynthesis thus enhancing plant growth (Nasar et al., 2023; Sharma et al., 2017). Razaq et al. (2017) observed that seedlings ameliorated with N resulted in substantially greater shoot and root growth than the unamended treatment; this could explain the trend in the present study. Seedlings ameliorated with 10% and 20% biochar yielded the lowest S: R ratio of <3.6 :1 as shown in Figure 4.21. This was within the recommended quality ratio range of 3:1 according to Jaenicke & ICRAF (1999) and Kung'u et al. (2008). The balanced seedling growth as a result of the application of 20% biochar and 10% biochar can be attributed to N and P supply through the treatment as shown in Table 4.10 which enhanced both vegetative and root growth. The use of biochar, therefore, led to the balanced growth of *C. equisetifolia* seedlings in terms of shoot and root development.

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 SQ shows a healthy seedling with low mortality, especially in dry ecosystems. A sturdiness quotient higher than 6 has been considered undesirable because it is an indication of physiological imbalance resulting in tall frail seedlings while an extremely small sturdiness quotient indicates difficulty in the seedling establishment (Jaenicke & ICRAF, 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).

4.4.4 Effect of biochar combined with manure and NPK on Casuarina growth after field establishment

4.4.4.1 Casuarina height

This study shows that Casuarina heights did not differ substantially following the application of manure, NPK, and combination of biochar, manure, and NPK ($p< 0.23$, 0.18, 0.14, and 0.48, respectively, at four assessment periods as shown in Figure 4.22.

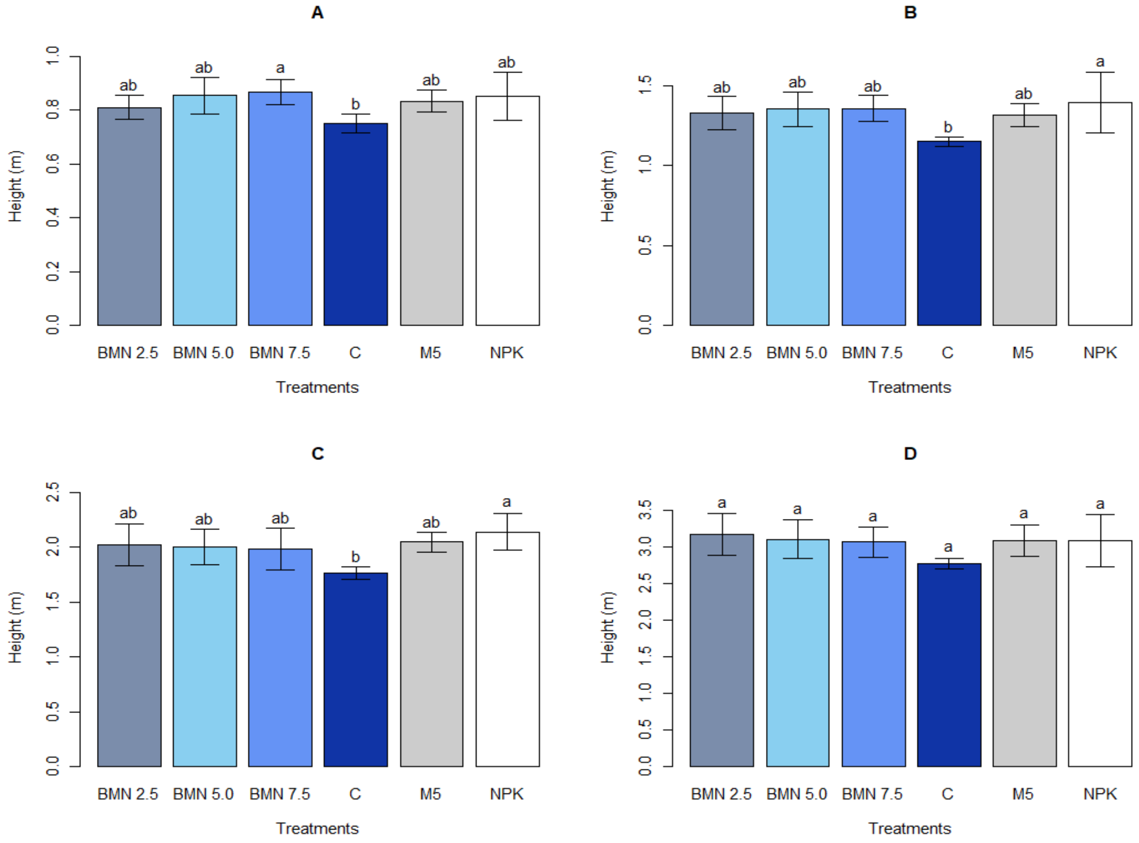


Figure 4.22: Effect of biochar, manure, and NPK on Casuarina height growth

Note: *(i) Means presented by different alphabets in each graph differ substantially (ii) "B" refers to Biochar and "M" refers to Manure, C refers to control, T refers to tons/ha, NPK 50 refers to NPK at 50kg/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

At 3 MAE, the highest Casuarina mean height was recorded in plots ameliorated with biochar (7.5 t ha^{-1}) integrated with manure and NPK as shown in Figure 4.22; this was 16% higher than the unamended treatment, 2% higher than NPK (50 kg ha^{-1}) and 4.8% higher than sole manure application (5.0 t ha^{-1}). At 6 MAE, sole NPK (50 kg ha^{-1}) yielded the highest mean height (1.39 m); which was 20.9% higher than the unamended treatment, 5.3% higher than sole manure (5 t ha^{-1}) and also higher than the integration of biochar, manure and NPK (Figure 4.22). The study further revealed that at 9 MAE, sole NPK (50 kg ha^{-1}) yielded the highest Casuarina height (2.14 m); this was 20.9% higher than the unamended treatment. Integration of biochar (7.5 t ha^{-1}), NPK, and manure unexpectedly recorded the second lowest Casuarina mean height (1.98 m) which was only 11.8% higher than the unamended treatment but lower than the sole application of NPK and manure.

At the end of the assessment period (12 MAE), the combined application of biochar (2.5 t ha^{-1}), NPK, and manure yielded the highest Casuarina mean height (3.17 m) which was 14.4% higher than the unamended treatment, 2.9% higher than sole NPK (50 kg ha^{-1}) and 2.6% higher than sole manure (5.0 t ha^{-1}). Use of intermediate and highest biochar dosage with NPK and manure recorded lower Casuarina mean heights than combined use of lowest biochar rate (2.5 t ha^{-1}) with NPK and manure (Figure 4.22). A comparison between sole biochar application at 7.5 t ha^{-1} and sole NPK application revealed that sole application of biochar yielded higher Casuarina heights than sole NPK application; however, the difference in means was not substantial ($t=0.111$, $p < 0.91$).

Enhanced plant height following the utilization of NPK fertilizer at 6 MAE and 9 MAE can be attributed to the availability of readily available N, P, and K following its application. Nitrogen is a crucial element required for the vegetative growth of plants (Razaq et al., 2017). A study by Walker et al. (2002) reported a linear relationship between plant growth rate and N application. All plants utilize N for proper growth and development and also promote shoot growth (Leghari et al., 2016). Application of P fertilizer enhances plant production and quality as well as storage and use of energy; there is a positive relationship between P level and plant development (Razaq et al., 2017). Further, the study showed that fertilizer P application promoted root growth and increased plant height. The slow growth in plots treated with NPK at 50 kg ha^{-1} at 12 MAE can be attributed to nitrates leaching in the soil (Wang et al., 2019). Zhou et al. (2006) reported that nitrate leaching in the soil varies depending on the type of soil. The study further revealed that N leaching in sandy loam ranged from 16.2 to 30.4% when nitrate fertilizer was used.

4.4.4.2 Casuarina DGL and DBH

The results of this study show unsubstantial variations in Casuarina DGL and DBH at 3 MAE and 12 MAE ($p < 0.87$ and 0.94, respectively) following the application of sole manure, NPK, and their integration with varying biochar application rates. However, substantial variations in Casuarina DGL at 6 MAE and 9 MAE ($p < 0.01$ and 0.04, respectively) as shown in Figure 4.23 were observed.

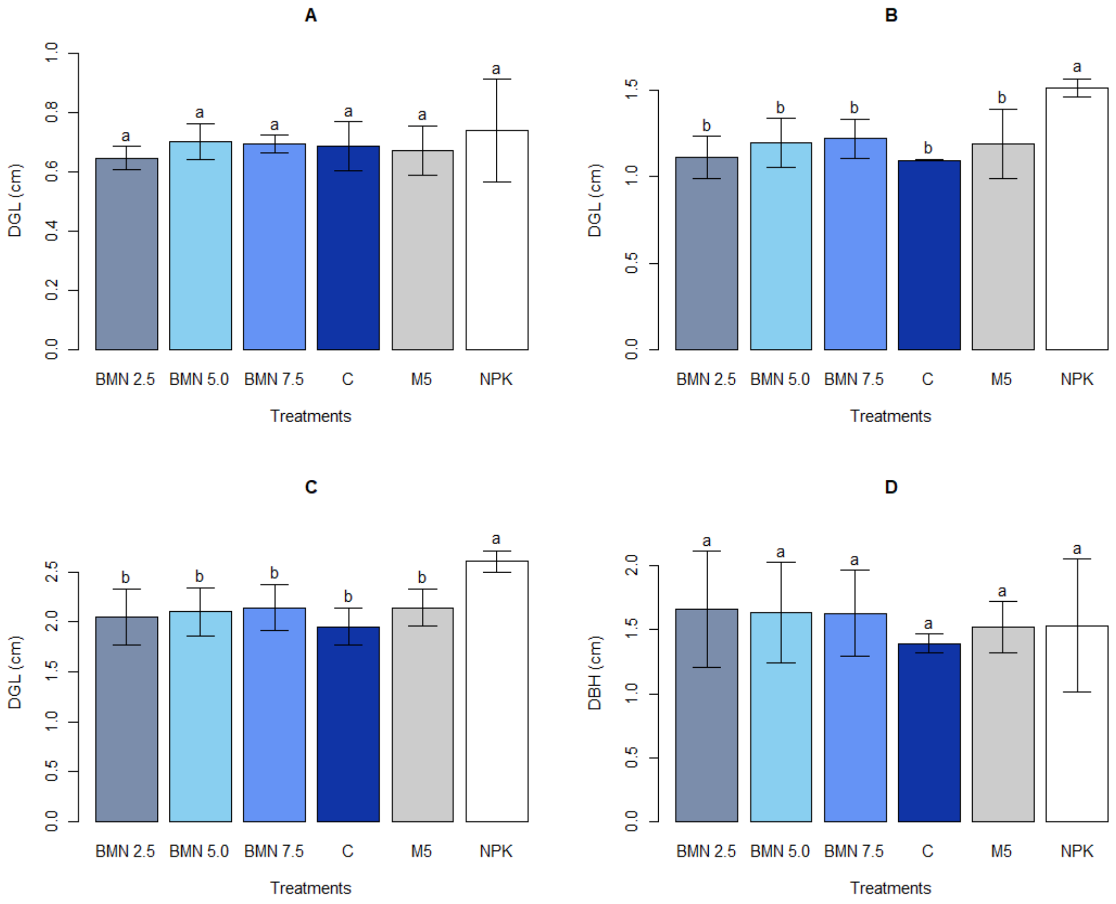


Figure 4.23: Effect of biochar, manure, and NPK on Casuarina DGL

***Note:* *(i)* Means presented by different alphabets in each graph differ substantially *(ii)* "B" refers to Biochar and "M" refers to Manure, C refers to control, T refers to tons/ha, NPK 50 refers to NPK at 50kg/ha; 3 MAE (A), 6 MAE (B), 9 MAE (C) and 12 MAE (D)*

The sole use of NPK fertilizer recorded the highest Casuarina DGL across three assessment periods which was 7.2%, 37.3%, and 33.3% above the unamended treatment for 3 MAE, 6 MAE, and 9 MAE respectively. At 12 MAE, the highest DBH of 1.66 cm was observed in the amendment with biochar (2.5 t ha^{-1}), NPK, and manure; this was 19.4% higher than the unamended control (1.39 cm). The untreated control yielded the lowest DGL and DBH across the assessment period (Figure 4.23). Sole manure (5 t ha^{-1}) recorded lower DGL and DBH than sole NPK (50 kg ha^{-1}). Integration of manure, NPK, and biochar did not result in a substantial Casuarina DGL and DBH enhancement as shown in Figure 4.23. However, the combination of biochar (7.5 t ha^{-1}), NPK, and manure resulted in higher DGL and DBH than the unamended treatment.

The substantial enhancement of Casuarina DGL and DBH following utilization of

NPK can be due to the supply of N, P, and K in available forms that were absorbed and utilized by the plants. Nitrogen is an essential element needed for plant shoot growth (Liu et al., 2014). Phosphorus on the other hand plays a crucial role in promoting healthy root growth and early shoot growth. Grant et al. (2001) observed that P stress early in the growing season can inhibit plant growth. Potassium (K) is the most abundant inorganic cation in soils, and it is important as an activator of important enzymes, improves N uptake and protein synthesis, uptake of other nutrients and water, and photosynthesis (Xu et al., 2020), and therefore ensuring optimal plant growth.

The lack of substantial increase in Casuarina DGL and DBH following manure utilization may be a result of the quality of manure used in the experiment such as low P concentration (10.5 ppm) as shown in Table 4.1. Studies have shown that the source of manure determines its effectiveness in enhancing soil fertility for crop growth (Rayne and Aula, 2020). The enhanced Casuarina DGL and DBH growth as a result of biochar utilization at 7.5 t ha^{-1} integrated with manure and NPK can be attributed to the provision of nutrients from NPK and improved soil moisture retention through manure and biochar utilization. There was a substantial improvement in soil moisture following the utilization of biochar as shown in Table 4.12. Soil moisture was enhanced by <55.3% compared to the unamended treatment at 12 MAE resulting from the integrated use of biochar (7.5 t ha^{-1}), NPK, and manure.

Table 4.12: Gravimetric soil moisture content under different treatments

Soil Moisture ± SE (%)	**Assessment period (Months)**			
Treatment	**3**	**6**	**9**	**12**
Control	5.99±0.13c	5.50±0.13b	5.10±0.12b	6.44±0.43b
NPK 50 kg/ha	6.55±0.39c	6.95±0.43ab	5.85±0.58b	5.93±1.92b
Manure 5 t/ha	8.38±0.47b	7.92±0.73ab	6.21±0.18b	8.05±1.06ab
Biochar 2.5 t/ha +M+NPK	8.39±0.29b	9.00±3.1a	7.69±0.83a	7.89±0.85ab
Biochar 5.0 t/ha +M+NPK	8.49±0.40b	8.17±0.69a	8.09±0.81a	9.25±0.68a
Biochar 7.5 t/ha +M+NPK	9.24±0.50a	8.24±0.52a	8.79±0.87a	10.00±1.88a
$f_{(5,12)}$	29.94	2.47	31.36	4.51
$p < 0.05$	0.0001	0.09	0.0001	0.02

Note: *Means presented by different alphabets in each column differ substantially; M means manure*

4.5 Effect of Soil Moisture Content and Soil nutrients on Casuarina Growth

4.5.1 Relationship between soil moisture and Casuarina growth (Height, DGL and DBH)

The present study revealed a substantial positive linear relationship between soil moisture content and Casuarina mean height across the four assessment periods as shown in Figure 4.24 below (r = 0.69, 0.85, 0.64, and 0.56) at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively. Casuarina's mean height was enhanced as soil moisture content increased following biochar utilization. The study further revealed that between 32 and 72% of observations made in Casuarina height growth can be attributed to changes in soil moisture content across the assessment periods.

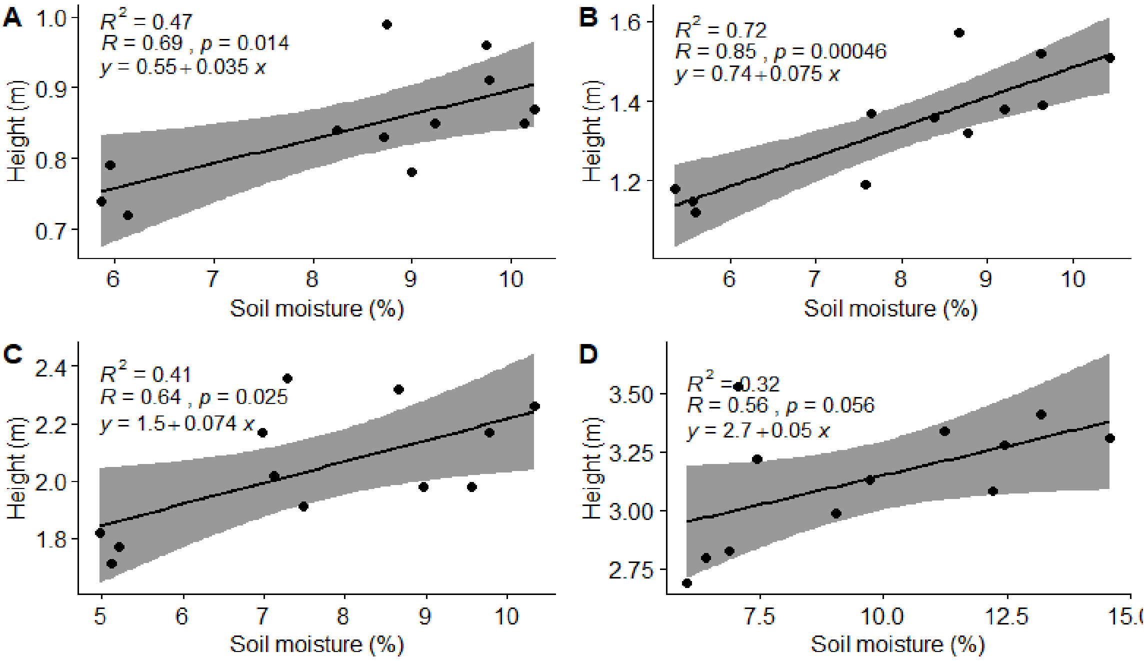


Figure 4.24**:** Relationship between soil moisture content and Casuarina height as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

However, there was no substantial association between soil moisture and Casuarina DGL and DBH across the four assessment periods as shown in Figure 4.25 (r = 0.17, 0.58, 0.36, and 0.22) at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively). Only 3 to 33% of the observations made in Casuarina DGL and DBH can be attributed to changes in soil moisture content following biochar application. The lack of a strong association between soil moisture content and Casuarina DGL and DBH is subject to further studies.

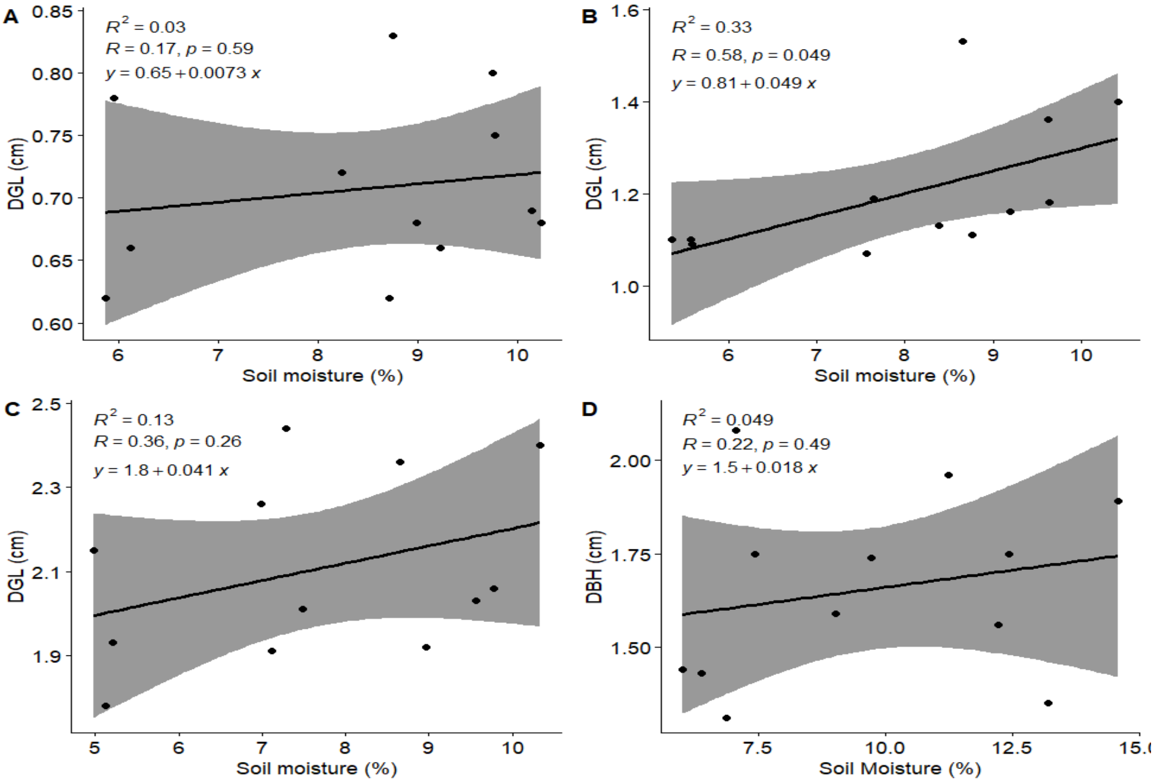


Figure 4.25**:** Relationship between soil moisture content and Casuarina DGL and DBH as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Soil moisture content substantially impacts plant growth and development (Brockley, 1981; Misra, 2003; Lee et al., 2017). Soil moisture affects plant nutrition through its influence on nutrient transport and nutrient absorption by plants (Brockley, 1981). The uptake of nutrients by plants in the soil solution depends on the rate at which nutrients may move from the surrounding soil to the root surface which is dependent on soil moisture content. Low soil moisture limits hydraulic conductivity in the soil thereby reducing the ease with which water moves to the plant roots. Enhanced soil moisture content improves plant shoot length (Lee et al., 2017).

Soil moisture impacts both the chemical and physical characteristics of soils. Soil moisture availability affects plant growth and development and particularly the ability to absorb nutrients needed (Misra, 2003). Water content in the soil equally affects nutrient supply, mechanisms of ion transport, and uptake by plants. García et al. (2008) observed that excess soil moisture reduced root growth by 36% and increased shoot growth by 13%. The same study further revealed that water deficit reduced both root and shoot growth by 26 and 32%, respectively.

4.5.2 Casuarina growth parameters (Height, DGL, and DBH)

4.5.2.1 Relationship between Total Nitrogen and Casuarina Height, DGL, and DBH

This study showed a substantial positive linear relationship between total N and Casuarina height as illustrated in Figure 4.26 (r = 0.52, 0.62, 0.68, and 0.73) at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively).

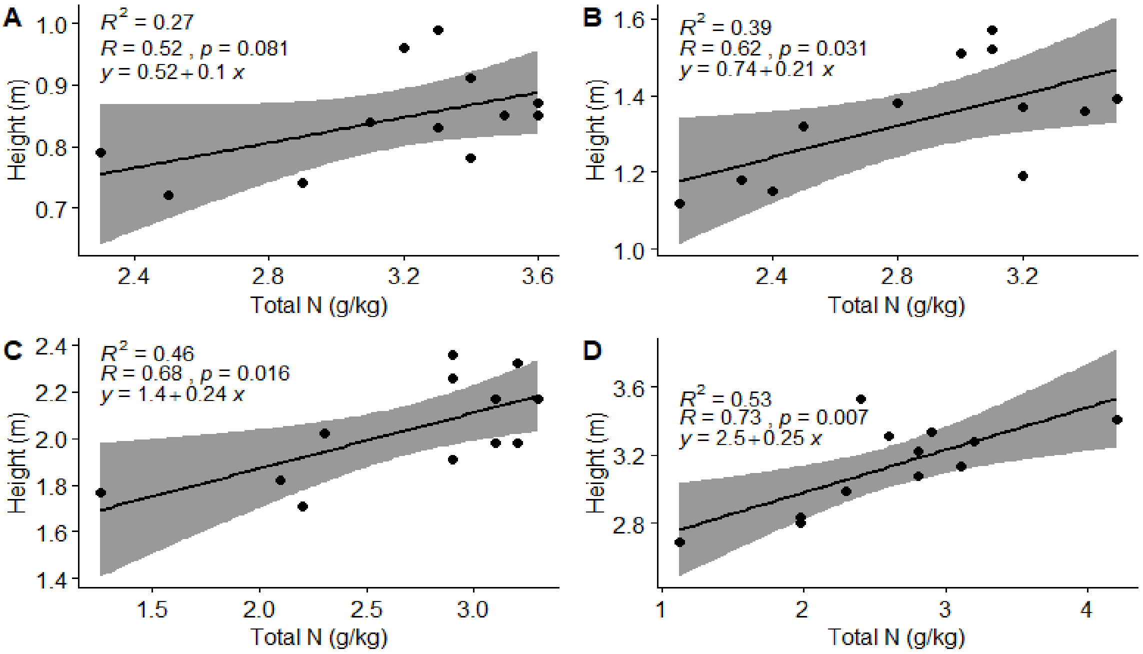


Figure 4.26**:** Relationship between Total N and Casuarina height as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

The results revealed that Casuarina height increased as the amount of total N in the soil increased. Linear regression analysis further revealed that between 27 and 53% of the observations made in Casuarina height were a result of a shift in the total N in the soil. Results of Pearson's correlation also revealed a positive linear relationship between total N and Casuarina DGL and DBH at three sampling periods (r = 0.36, 0.43, and 0.15) at 6 MAE, 9 MAE, and 12 MAE, respectively as shown in Figure 4.27. At 3 MAE there was a negative relationship between total N and Casuarina DGL (r = -0.11). However, the relationship between total N and Casuarina DGL/DBH was not substantial. Only 1 to 18% of the observation made in Casuarina DGL and DBH can be attributed to changes in total N (Figure 4.27).

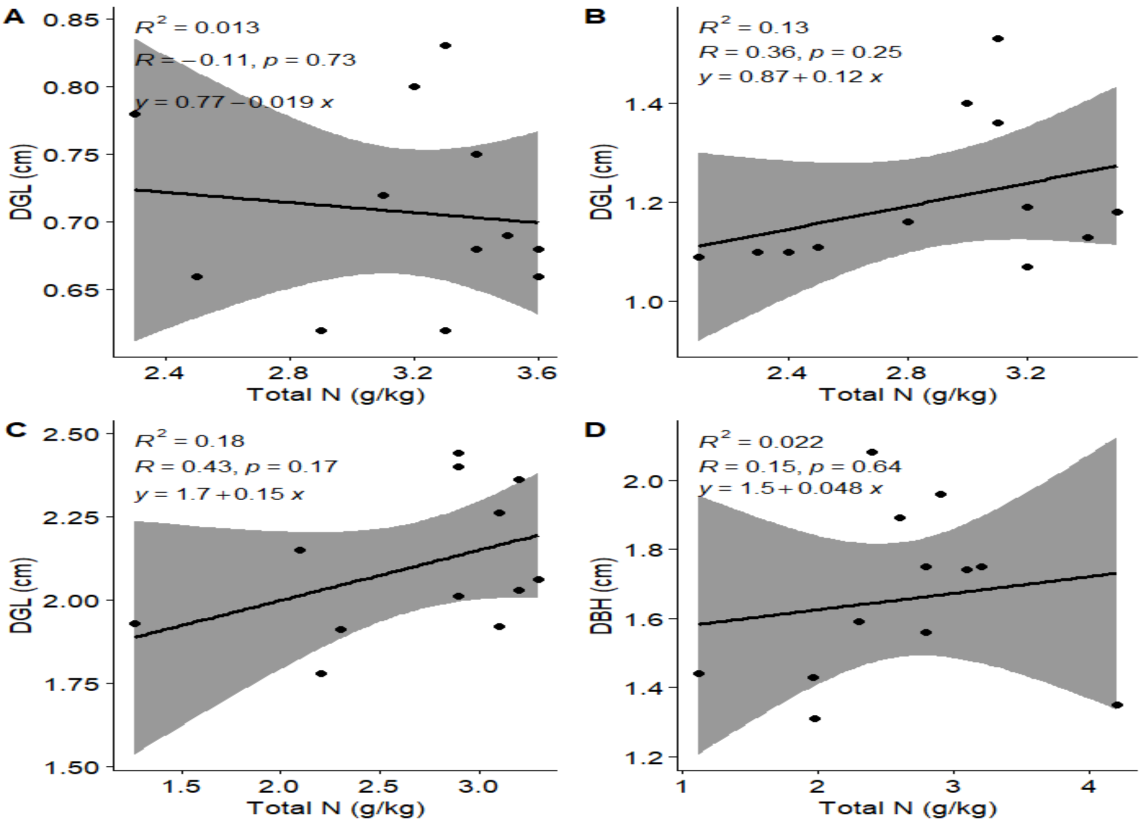


Figure 4.27**:** Relationship between Total N and Casuarina DGL and height as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

The study showed that enhanced total N following biochar utilization led to enhanced Casuarina height across the assessment periods as shown in Figure 4.26. Nitrogen plays crucial roles in various physiological processes. It promotes the vegetative growth of leaves and stems while also stimulating root growth (Leghari et al., 2016). Nitrogen is an essential element required for successful plant growth (Liu et al., 2014). This could explain the enhanced Casuarina height following enhanced total N. The study further revealed that although inorganic N accounts for less than 5% of the total N in the soil, they are the main forms absorbed by plants (Liu et al., 2014). Nitrogen contributes to structural components and metabolic compounds in a plant cell; N also plays a vital role in photosynthesis thus enhancing plant growth (Sharma et al., 2017). Seedlings ameliorated with N resulted in substantially greater height and root diameter than the unamended treatment as highlighted by Razaq et al. (2017).

4.5.2.2 Relationship between Available Phosphorus and Casuarina Height, DGL, and DBH

In terms of available phosphorus (P), a substantial positive linear relationship between P and Casuarina height was observed (r = 0.68, 0.76, 0.66, and 0.81 at 3 MAE, 6 MAE, 9 MAE, and 12 MAE, respectively). The results of this study revealed that between 44 and 66% of the observations made in Casuarina height can be attributed to changes in the soil available P (Figure 4.28). The results further showed that enhanced available P substantially improved Casuarina height.

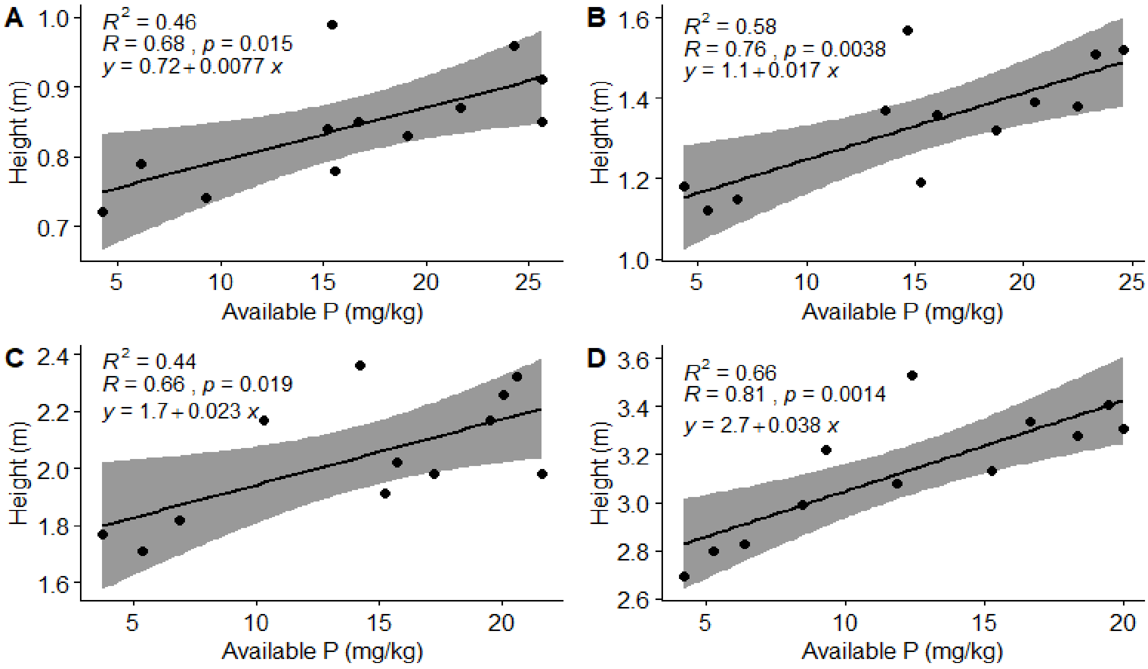


Figure 4.28: Relationship between Available P and Casuarina height as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Lack of substantial linear relationship was observed between available P and Casuarina DGL and DBH in the present study as shown in Figure 4.29. There was a weak association between available P and Casuarina DGL and DBH; only 3 to 22% of the observations made in Casuarina DGL and DBH can be attributed to changes in available P in the soil. The present study revealed that there was enhanced Casuarina height, DGL, and DBH as available P increased following biochar utilization. The enhanced Casuarina growth due to enhanced soil P can be attributed to the role played by P in plant growth and development (Sharma et al., 2017).

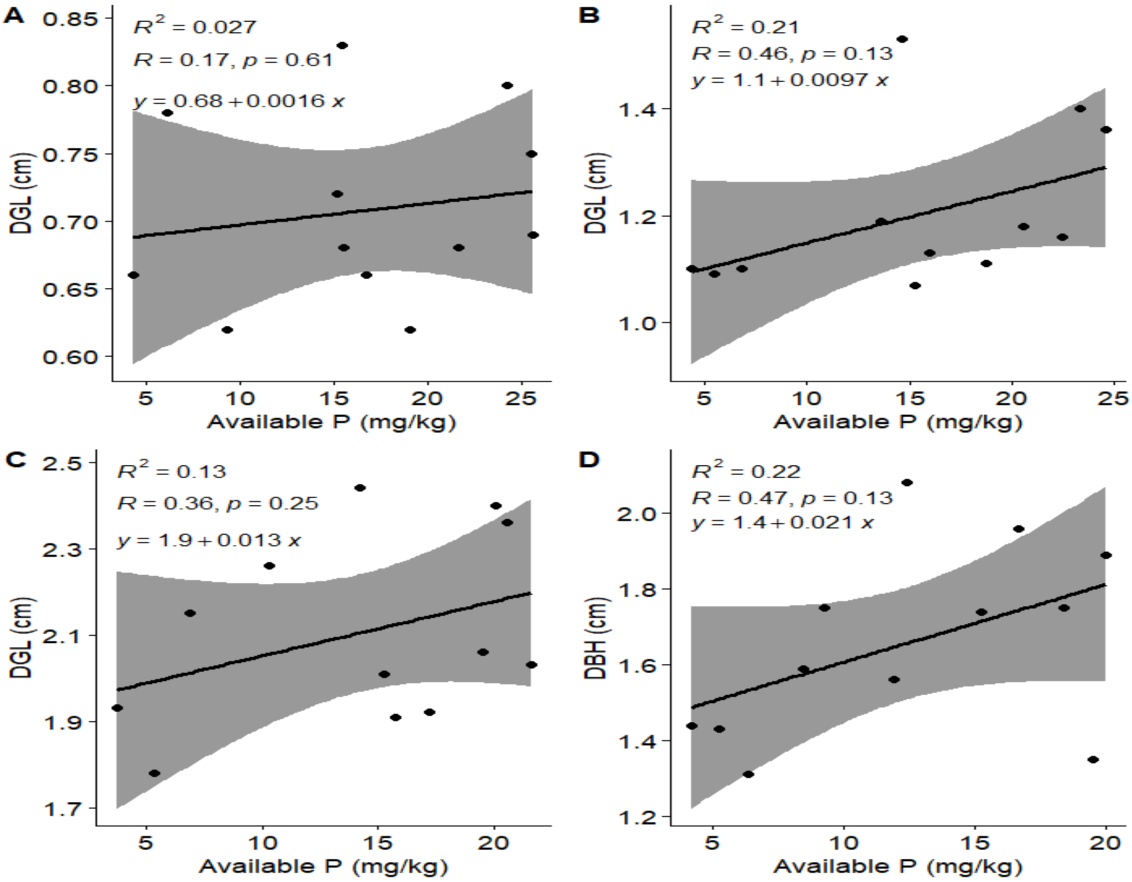


Figure 4.29**:** Relationship between Available P and Casuarina DGL and DBH as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Sharma et al. (2017) reported that phosphorus is an important nutrient for plant growth and development. It contributes in the complex of nucleic acid and structure of plants which is essential in protein synthesis regulation therefore; it also plays a vital role in cell division and the development of new plant tissue. Phosphorus is used by plants in H_2PO4^- or HPO_4^- forms which are pH dependent and increase with increasing soil pH (BC, 1999). Harman (2017) revealed that phosphorus stress early in the growing season can restrict plant growth and development thus leading to stunted growth of affected plants. Grant (2001) also reported on the importance of P nutrition for successful plant growth and development. Razaq et al. (2017) reported enhanced plant growth following P application; seedlings ameliorated with P exhibited substantially greater plant height and root diameter than the unamended treatment.

4.5.2.3 Relationship between Extractable Potassium and Casuarina Height, DGL, and DBH

A substantial linear relationship was observed between extractable potassium and Casuarina height (r = 0.57, 0.59, and 0.66 at 3 MAE, 6 MAE, and 12 MAE), respectively, except at 9

MAE (r = 0.55) as shown in Figure 4.30. There was a moderate association between available K and plant height; that is between 33 and 43% of the observations made in Casuarina height could be attributed to changes in available K in the soil following biochar application.

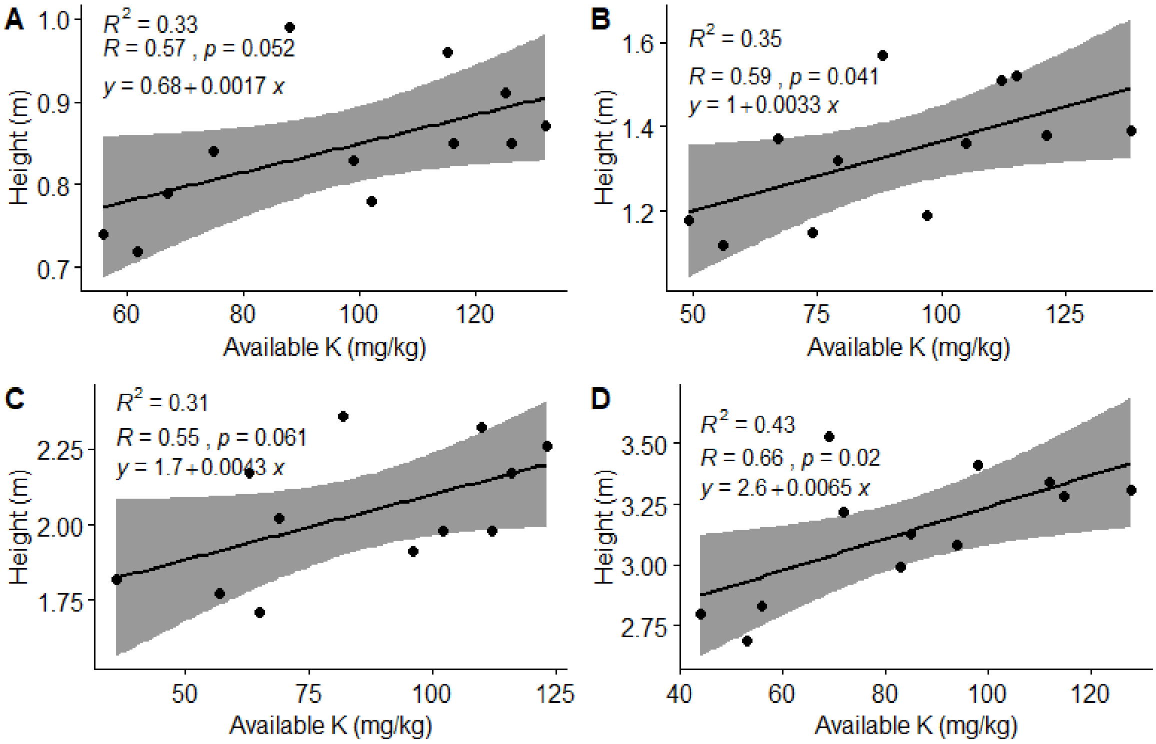


Figure 4.30: Relationship between available K and Casuarina height as influenced by biochar application at different growth stages

***Note:** 3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

In terms of DGL and DBH, there was a non-significant linear relationship between available K and Casuarina DGL and DBH (r = 0.09, 0.32, 0.27, and 0.48 at 3 MAE, 6 MAE, 9 MAE, and 12 MAE), respectively as shown in Figure 4.31. The strength of association between available K and Casuarina DGL and DBH was however very weak; only between 0.8 and 23% of the observations made in Casuarina DGL and DBH can be attributed to changes in available K in the soil as shown in Figure 4.31.

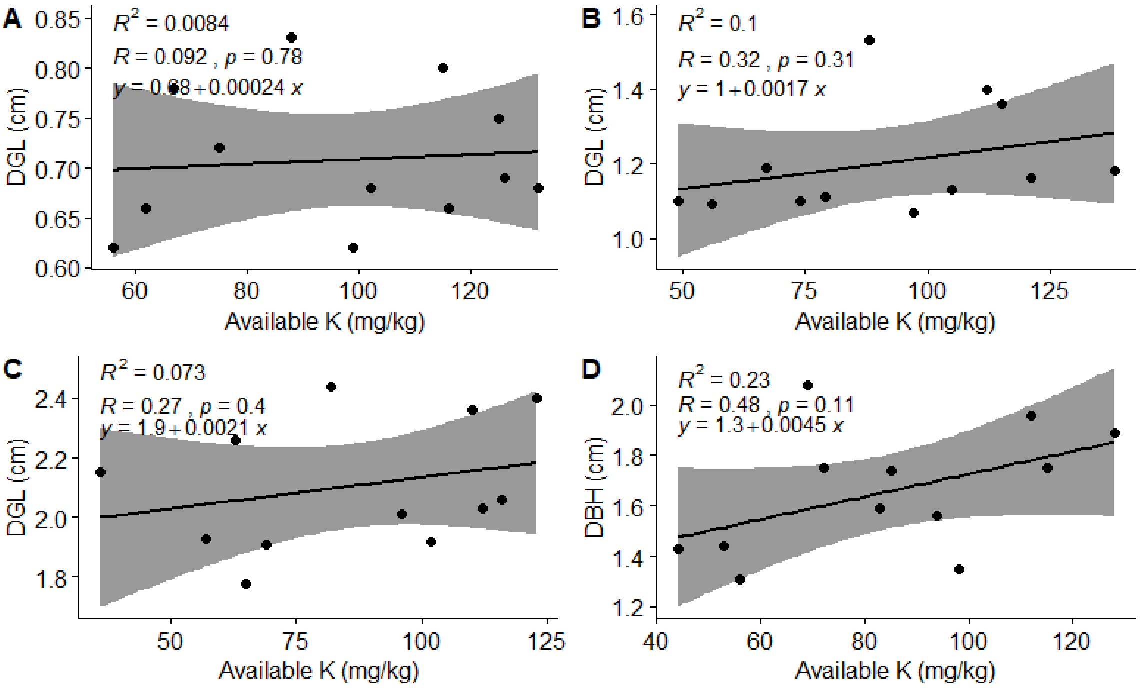


Figure 4.31**:** Relationship between Extractable K and Casuarina DGL and DBH as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D)*

Potassium is required for numerous plant growth processes such as the activation of enzymes in plant cells, regulating the opening and closing of stomata, and transport of water and nutrients throughout the plant among other processes (Prajapati & Modi, 2012; Kaiser & Rosen, 2018). Studies have shown that the supply of potassium to plants led to a substantial increase in plant growth. The studies however noted that the application of higher rates of potassium did not lead to additional benefits (Inthichack et al., 2012; Xu et al., 2020). Under drought conditions, K has also been reported to regulate the stomatal opening of plants thereby adapting to water deficits (Hasanuzzaman et al., 2018). Potassium plays various roles within the plant such as increased protein production, enhanced water-use efficiency within the plant, and the stimulation of early growth in the plant (Truong, 2017).

4.5.2.4 Relationship between soil pH and Casuarina Height

A substantial linear relationship was observed between extractable soil pH and Casuarina height (r = 0.61, 0.57, and 0.72 at 6 MAE, 9 MAE, and 12 MAE), respectively, except at 3 MAE (r = 0.54) as shown in Figure 4.32. There was a moderate association between soil pH and plant height; between 29 and 52% of the observations made in Casuarina height could be attributed to changes in soil pH following biochar application.

The influence of soil pH on plant growth can be attributed to the impact of pH on biochemical soil functions. Neina (2019) reported that pH influences a myriad of chemical, biological, and physical characteristics and processes that impact plant growth and biomass yield. It further revealed that pH controls the solubility, mobility, and bioavailability of trace elements. Soti et al. (2015) reported a substantial impact of soil pH on plant growth. The study attributed the changes in plant growth to pH's influence on nutrient availability and its direct effect on the protoplasm of plant root cells.

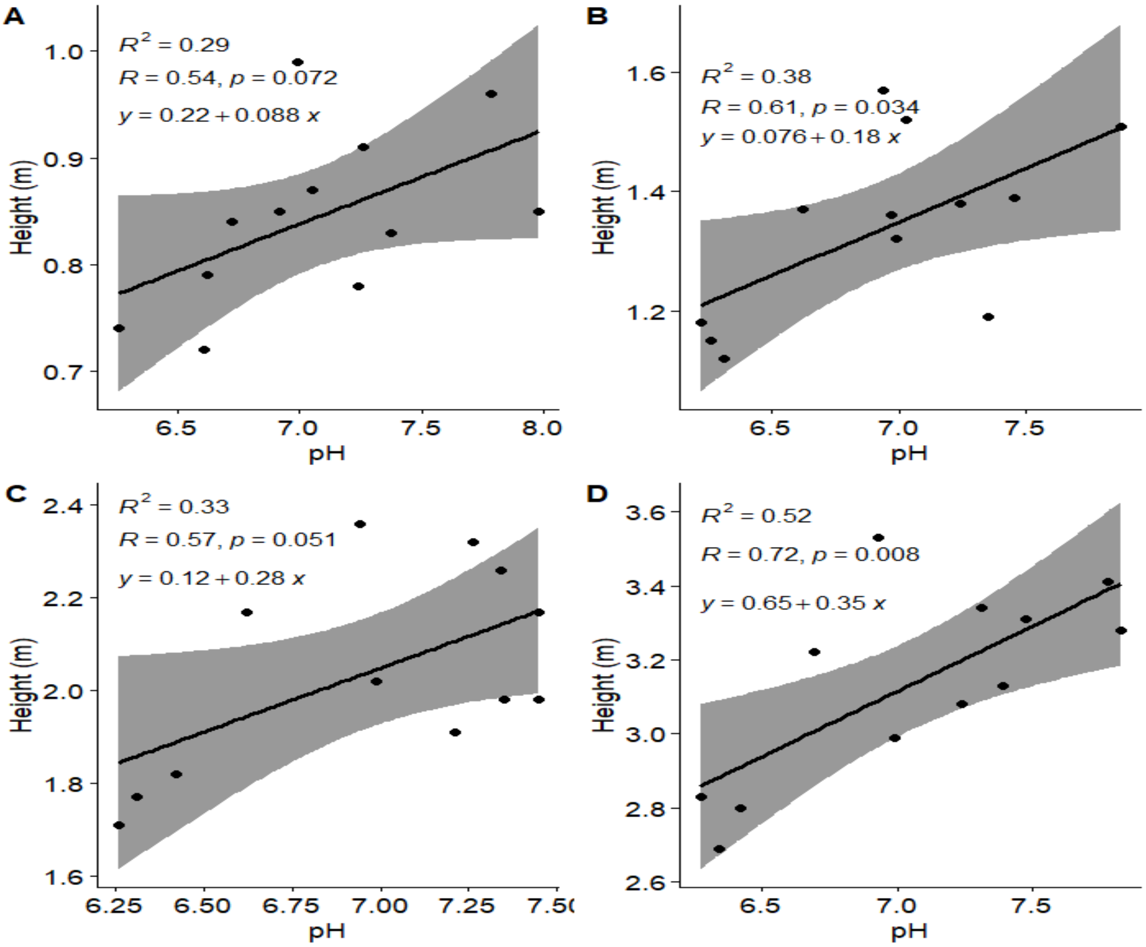


Figure 4.32**:** Relationship between soil pH and Casuarina height as influenced by biochar application at different growth stages

Note: *3 MAE (A), 6 MAE (B), 9 MAE (C), and 12 MAE (D).*

4.5.3 Combined effect of soil moisture and nutrient availability for Casuarina growth height

The current study evaluated the effect of soil moisture and different soil nutrients on Casuarina height. This study showed that soil moisture significantly affected Casuarina height growth at 3 MAE and 12 MAE assessment periods as illustrated in Table 4.13. In terms of the effect of different nutrients, soil nutrients did not substantially affect Casuarina height growth except for phosphorus which significantly affected Casuarina growth at the end of the experiment ($p<0.002$). The results further revealed a lack of substantial interaction effect between soil moisture and soil nutrients at 3 MAE and 12 MAE assessment periods.

Table 4.13: Sole and combined effects of soil moisture and soil nutrients on casuarina height

Response	**df**	**MS**	**F value**	**p value**
		3 MAE		
SM	1	0.034	7.24	0.03
pH	1	0.001	0.01	0.94
SM: pH	1	0.001	0.17	0.69
SM	1	0.034	9.36	0.02
N	1	0.003	0.84	0.39
SM: N	1	0.006	1.73	0.22
SM	1	0.034	7.57	0.02
P	1	0.001	0.18	0.68
SM: P	1	0.002	0.26	0.57
SM	1	0.034	8.09	0.02
K	1	0.004	1.07	0.33
SM: K	1	0.001	0.06	0.81
		12 MAE		
SM	1	0.242	6.09	0.04
pH	1	0.177	4.43	0.07
SM: pH	1	0.022	0.56	0.48
SM	1	0.242	6.33	0.04
N	1	0.166	4.33	0.07
SM: N	1	0.045	1.17	0.31
SM	1	0.242	14.25	0.005
P	1	0.345	20.27	0.002
SM: P	1	0.036	2.13	0.18
SM	1	0.242	5.12	0.05
K	1	0.100	2.11	0.18
SM: K	1	0.039	0.83	0.39

NB: $\alpha \leq 0.05$, MS=Mean squares, SM=Soil Moisture, N= Total nitrogen, K= Extractable potassium, P= Available phosphorus, df= degree of freedom

These results indicate that the relationship between soil moisture and Casuarina growth is not influenced by soil nutrients. Soil moisture availability affects plant roots, water absorption, and transpiration by leaves, which further affect dry matter accumulation, and ultimately affect plant growth. Plant roots are crucial for soil water absorption and their development is affected by the availability of soil moisture and aeration (Misra, 2003).

Drought stress is a vital limiting factor for plant growth and development (Seleiman et al., 2021; Chadha et al., 2019; Wang et al., 2019). This hampers plants from attaining their optimal growth potential. Plant growth is achieved through cell division and differentiation, and cell elongation and involves a complex interaction of genetic, physiological, ecological, and morphological events. Moisture stress, therefore, impacts these events as loss of turgor and impaired mitosis which results in obstructed cell elongation and limited cell division respectively (Uwimana et al., 2022). This causes diminished growth (Chadha et al., 2019). Moisture stress leads to the production of abscisic acid which triggers stomatal closure. This leads to a decline in intercellular CO_2 levels thereby limiting photosynthesis (Nasar et al., 2021; Raza et al., 2021; Yasin et al., 2022).

Chadha et al. (2019) observed that soil moisture substantially impacted plant height thereby concurring with the findings of the present study. The study observed reduced plant growth due to water stress resulting from prolonged droughts. Pardales et al. (2000) similarly observed enhanced sweet potato height where the plants were exposed to excess soil moisture. The study further revealed that plants exposed to excess soil moisture content yielded higher leaf numbers compared to plants exposed to moisture stress. Generally, plants have been reported to respond favourably to relatively high soil moisture contents with growth rate diminishing as soil moisture is depleted (Hagan, 1955).

This study revealed that phosphorus was the only plant nutrient that significantly affected Casuarina’s height. Phosphorus is crucial in most plant processes that encompass energy transfer (BC, 1999). Phosphorus equally plays a key role in the metabolism of sugars, photosynthesis, cell enlargement, energy storage and transfer, transfer of genetic information, and cell division (Gitari et al., 2020; Kamau et al., 2019; Harman, 2017). Phosphorus also promotes early shoot growth, and healthy root development and is vital in seed formation. Optimal phosphorus enhances plant water-use efficiency and improves the efficient use of other nutrients such as nitrogen. It also helps plants cope with moisture stress, contributes to disease resistance in some plants and hastens plant maturity, and protects the environment through better plant development (Harman, 2017). Grant et al. (2001) reported that P stress early in the growing season can restrict plant growth.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study evaluated the potential of biochar, manure, and NPK fertilizer in improving *Casuarina equisetifolia* growth and soil physical-biochemical properties. At the seedling stage, the results revealed that the application of 10% (v/v) manure resulted to higher Casuarina growth than the control and sole biochar application. At the field establishment stage, the results of this study showed that biochar has the potential to enhance Casuarina growth. Biochar enhanced Casuarina height by up to 24.3% after the use of the highest dose (7.5 t ha^{-1}) in comparison with the unamended treatment. Generally, all plots ameliorated with biochar yielded higher Casuarina height and DGL compared to the unamended treatment. In comparison with sole NPK at 50 kg ha^{-1} and manure at 5 t ha^{-1}, the biochar dose of 7.5 t ha^{-1} yielded higher Casuarina heights across the four assessment periods.

Biochar application equally substantially influenced soil bulk density and soil moisture content. Biochar application significantly lowered soil bulk density; bulk density declined by up to 25% after biochar utilization. This research shows that the use of biochar can assist reduce soil compaction through its effect on soil bulk density. Reduction of soil compaction will thus enhance root development thereby enhancing tree growth.

The findings of this study also show that biochar application can alleviate soil moisture stress in farmlands. Plots ameliorated with biochar yielded higher soil moisture than the unamended treatment. The highest soil moisture content was observed in plots ameliorated with biochar (7.5 t ha^{-1}) across the four sampling periods. The results also revealed the existence of a significant positive linear relationship between biochar application rates and soil moisture content and Casuarina mean height. There was, nevertheless, a substantial negative correlation between biochar rates and soil bulk density. Biochar application offers the potential for enhancing Casuarina growth (DGL, DBH, and height) through the improvement of soil physical properties which are vital in plant growth and development.

This study further showed that biochar amelioration had a positive impact on soil chemical characteristics crucial for optimal plant development. Biochar particularly enhanced soil pH thereby improving P availability. It also enhanced total N, total C, exchangeable cations (Ca^{2+}, K^{+}, Mg^{2+}), and CEC. Biochar also increased soil microbial population by up to 47.6% compared to the unamended treatment; microorganisms play a crucial role in nutrient cycling. The enhanced soil nutrients following biochar utilization are crucial for enhancing plant growth if used in agroforestry systems. The study further showed that biochar application rates should be considered when using biochar as a soil amendment to ensure optimal benefits accrued to biochar in terms of enhancing soil fertility and improving soil physical properties.

5.2. Recommendations

Based on the results,

- It is recommended that farmers in the coastal region of Kenya incorporate 5.0 t ha^{-1} of biochar during Casuarina woodlot establishment to reduce soil compaction through reduced soil bulk density and enhance soil moisture storage which will enhance Casuarina growth.
- The use of 10% (v/v) manure for Casuarina seedling production is recommended,
- Further studies are recommended to evaluate the potential of biochar produced from different feedstock on tree growth and carbon sequestration
- It is recommended that further studies are undertaken to evaluate the mechanisms under which biochar enhances the availability of soil nutrients
- There is a need to undertake a cost-benefit analysis on the use of biochar, manure and NPK for the production of Casuarina poles at the Coast.

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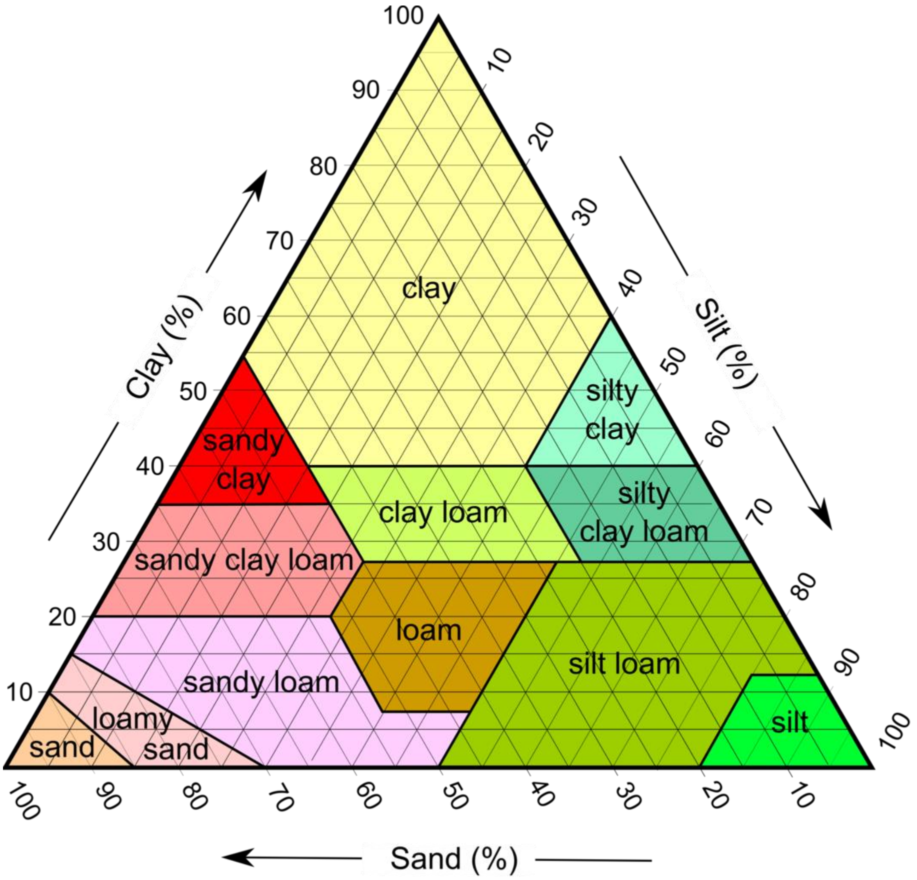
APPENDICES

Appendix I: List of Publications

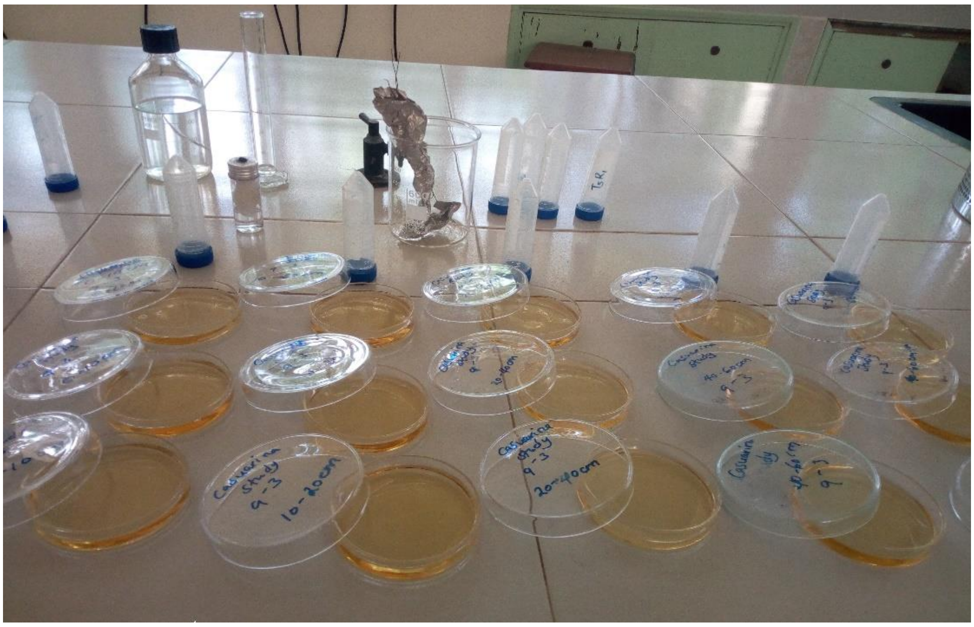
Mwadalu, R. U., Mochoge, B., and Danga, B. (2020). Effects of biochar and manure on soil properties and growth of *Casuarina equisetifolia* seedlings at the coastal region of Kenya. Scientific Research and Essays, 15(3), 52-63. https://doi.org/10.5897/SRE2020.6684

Mwadalu, R., Mochoge, B. and Danga, B. (2021). Assessing the Potential of Biochar for Improving Soil Physical Properties and Tree Growth, International Journal of Agronomy, vol. 2021, Article ID 6000184, 12 pages, 2021. https://doi.org/10.1155/2021/6000184

Appendix II: Textural Triangle



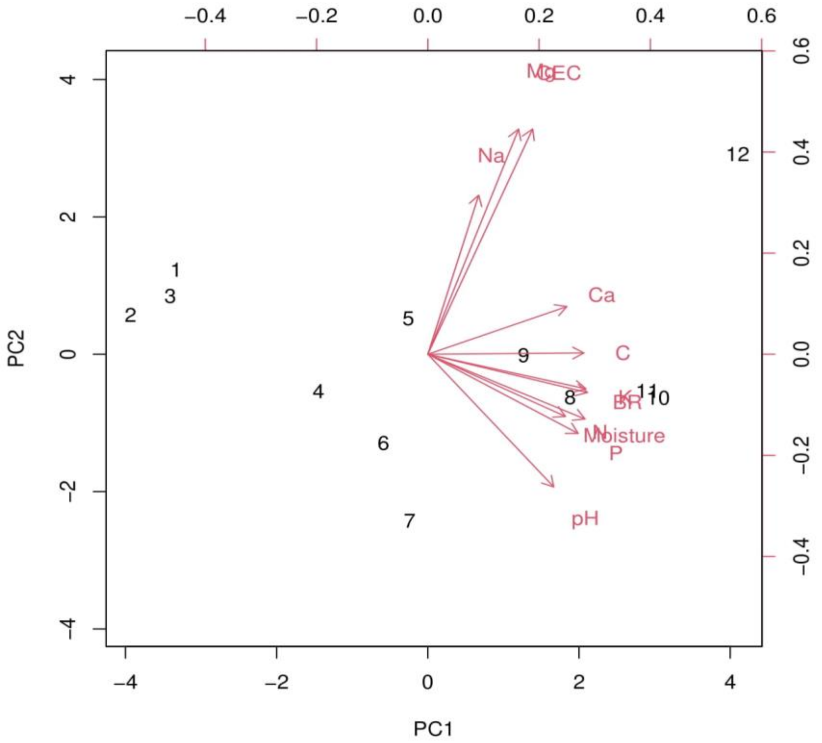
Appendix III: Determination of microbial population in the lab



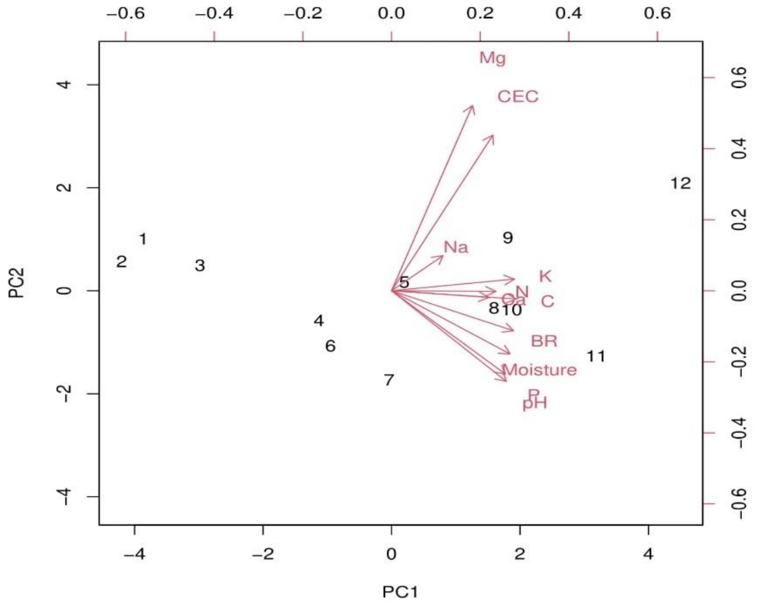
Appendix IV: Casuarina equisetifolia

Appendix V: Interaction of different parameters in the soil at different assessment periods

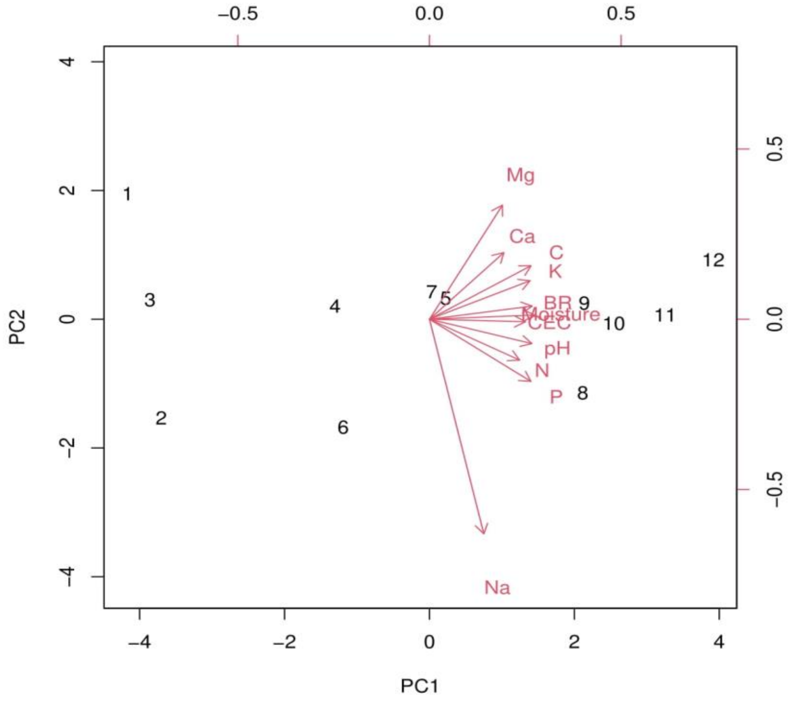
3 MAE assessment period



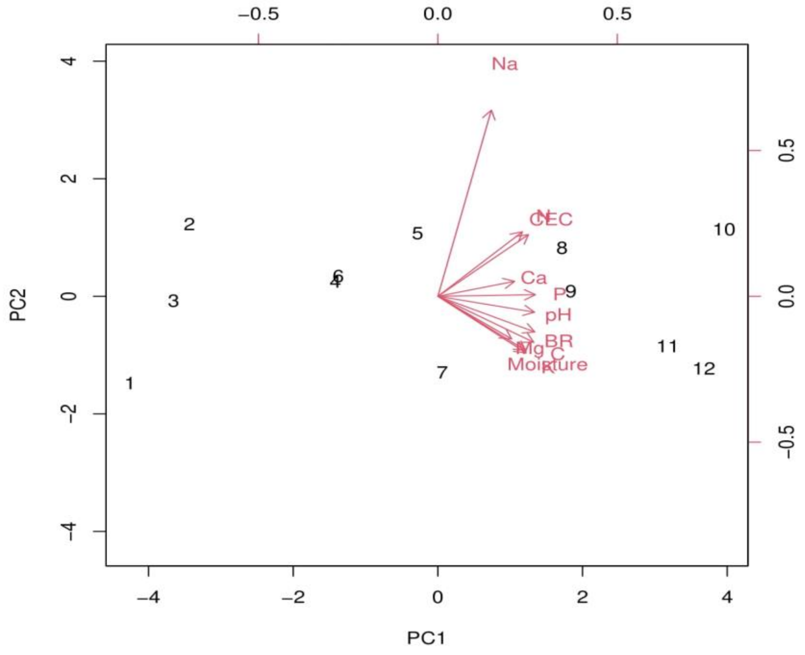
6 MAE assessment period



9 MAE assessment period



12 MAE assessment period



Appendix VI: Soil Characteristics as influenced by Biochar and Manure

Soil total C, 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**

Means presented by different alphabets in each column differ substantially

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.

Means presented by different alphabets in each column differ substantially

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

Means presented by different alphabets in each column differ substantially. CFU: Colony Forming Units.

Appendix VII: Root development and nodulation in *C. equisetifolia* seedlings

Appendix VIII: Biochar production

Appendix IX: Soil analysis procedures

Soil bulk density determination

Soil bulk density was determined at the onset of the experiment, at 3 months and at 9 months sampling period using the core ring method. A core sampler of known volume was used to obtain a sample from 0.15 m depth. Thereafter, the determination of the weight of the empty core sampler was done before obtaining the sample. The core sampler containing the soil was then oven-dried for 72 hours at 105 ºC. After 72 hours, the core sampler containing dry soil was allowed to cool and the weight of the dry soil plus the core sampler was determined. Bulk density (BD) was then determined using the following formula:

Bulk Density (g/cm^3) = $\frac{W2-W1}{V}$

Where,

W1 = Weight of empty core sampler (g)

W2 = Weight of core sampler + dried soil (g)

V = Volume of the soil (cm^3)

Determination of soil moisture content

Soil moisture content was determined gravimetrically quarterly for 1 year after the experiment's establishment. Fresh soil samples were collected from the research plots from a depth of 0-20 cm and placed in an iced-cooled box to prevent moisture loss. Three soil samples were collected per plot for soil moisture determination; the fresh soil samples were used for moisture determination. Analytical balance was then used to determine the weight of an empty can (W1). Fresh soil was then added and their weight was recorded (W2). Further, oven-drying of the samples for 72 hours at 105° C was done, then allowed to cool in a desiccator for 30 minutes. The weights of the dry soil and that of the can be then measured (W3). The gravimetric moisture content of the soil was calculated as a percentage of the dry soil mass as shown in Eq. I

$$\%\,Soil\ Moisture\ Content = \frac{\mathrm{W2-W3}}{(\mathrm{W3-W1})} * 100 \qquad (Eq. 1)$$

Where,

W1 - Weight of can

W2 - Weight of can + fresh soil

W3 - Weight of can + dry soil

Determination of soil texture/soil particle analysis

Exactly 50g of sieved air-dried soil samples was weighed using an analytical weighing balance and put into half-liter shaking bottles. Thereafter, 0.3 liters of water was added and 50 ml Calgon solution was further added and closed. A blank solution containing only 300 ml of water was included as a control. The contents were shaken overnight using a reciprocal shaker. The suspension was put into a 1-liter graduated cylinder and made up to the mark with water. The mixture was further stirred using a plunger for 2 minutes and the percent sand was measured using a hydrometer for 40 seconds. The second reading for percent clay was done 2 hours after settling down of sand suspension (Calculation Eq.2)

$$\% \, Sand\ or\ Clay = \frac{Sample\ reading - Reading\ of\ blank}{weight\ of\ sample\ taken} * 100 \qquad \text{(Eq.2)}$$

NB: This formula applies to sand or clay only. For silt, subtract sand and clay from the normal 100%.

Determination of Soil pH and Electro conductivity (EC)

Soil $pH_{(2.5:1)}$ (water) and electroconductivity (EC) were measured according to standard procedures (Anderson and Ingram, 1993; Okalebo et al., 2002). A 20g soil sample was put in a 100 ml polythene bottle followed by the addition of 50 ml deionized water. The contents were shaken for 30 minutes using a mechanical shaker. The mixture was allowed to stand for 30 minutes. The pH (water) was measured using a pH meter (Model 691) and electrical conductivity was measured with a conductivity meter (Model TOA Cm-20S).

Determination of soil total nitrogen

Total N was determined using the Kjeldahl method (Anderson and Ingram, 1993; Okalebo et al., 2002). Exactly 0.3g of ground and sieved soil were put in clean labeled digestion tubes. A digestion mixture of 4.4 ml was prepared by dissolving 0.42g of selenium (Se) powder and 14 g of lithium sulphate in 350 ml of 30% hydrogen peroxide through thorough mixing. Exactly 420 ml of concentrated sulphuric was added slowly being cooled in an ice bath. The mixture (4.4 ml) was then put in digestion tubes with samples and placed in a block digester. The digestion tubes were heated for 2 hours at 360 ° C until the solution was clear. The contents were then allowed to cool. Thereafter, 25 ml of deionized water was added and contents were transferred into a 50 ml volumetric flask and made to the 50 ml mark with deionized water.

A clear solution was then obtained for nitrogen analysis from the top of the tube after the mixture was allowed to settle. A 10 ml aliquot of the solution was then put into the distillation tubes where 10 ml of 40% sodium hydroxide was added for distillation. The extract was steamed into 5 ml of 1% boric acid and 4 drops of mixed indicator. Distillation was continued for 2 minutes from the time the indicator turned green. The distillate was removed and titrated with 0.1 M HCl until the colour changed from green through grey to a definite pink.

Calculation of Nitrogen content in the soil sample was calculated as indicated in Eq.3.

$$Nitrogen\ content\ in\ the\ soil\ (\%) = \frac{Corrected\ ml\ of N/140HCl*0.1}{weight\ of\ sample} \qquad \text{(Eq.3)}$$

Where;
Corrected ml of N/140HCl = Burette reading – The ml of N/140HCl required for the blank.

Determination of soil available phosphorus

Available P was determined using the method described by Olsen and Sommers (1982) and Okalebo et al. (2002). Exactly 5 g of air-dried soil was put into a 250 ml polyethylene shaking bottle. Thereafter, 50 ml of Olsen's extracting solution (0.5 M NaHCO3 pH 8.5) was added. The mixture was mechanically shaken for 30 minutes using a mechanical shaker. The suspension was then filtered through Whatman filter paper No. 42. The filtrate was used for the colorimetric P measurements where 5 ml of the clear filtrate was put into a 50 ml volumetric flask and 20 ml of distilled water was added. Then, the addition of 10 ml of the ascorbic acid-reducing agent to each volumetric flask was done, beginning with the standards. This was made up to the 50 ml mark with deionized water and then shaken well. The solution was left to stand for 1 hour to permit full colour development. Absorbance values of all the solutions were measured at 880 nm using a UV spectrophotometer (Model UV Spectronic 21-Milton Roy Co). Phosphorus concentrations for each sample were then calculated using the formula below.

P in sample (ppm) = C x ppm solution x df/w (Eq. 4)

Where C = the corrected concentration of P in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

Determination of extractable potassium in soil

Determination of Potassium was done spectrophotometrically (Anderson and Ingram, 1993) using a flame photometer. Five grams of air-dried soil samples were put in 250 ml clean polythene bottles where 100 ml of 1.0M neutral ammonium acetate solution was added and the contents are shaken for 30 minutes using a mechanical shaker. The contents were filtered using filter paper. Then, 5 ml of the filtrate was pipetted into a 50 ml volumetric flask where 1 ml of 26.8 % Lanthanum chloride solution was added and the contents made to the 50 ml mark with the ammonium acetate solution. Standards containing potassium at concentrations 0.0, 2.5, 5.0, 7.5, and 10 ppm potassium were prepared similarly to fall within the measurable range of the calibrated flame photometer. The flame emission intensities were measured at 766nm.

Determination of soil organic carbon

Ground air-dried soil was sieved through a 0.5 mm sieve. After, 1.0 g of the sieved sample was put in a half-liter conical flask and the addition of 10 ml of 1N $K_2Cr_2O_7$ was done, and the mixture was gently shaken. Thereafter, the addition of 20 ml concentrated H_2SO_4 was done to the mixed solution; the flask was swirled 3 times. Further, the mixture was allowed to stand for 30 minutes before the addition of 200 ml of deionized water, 1 ml of diphenylamine indicator, and 0.5 g sodium fluoride. The mixture was back titrated with $FeH_8N_2O_8S_2$ solution till the colour changed to brilliant green. The volume of the ferrous ammonium sulphate solution consumed was noted. A blank titration (without soil) was also carried out in a similar procedure and the volume of the ferrous ammonium sulphate solution consumed was noted. Due to the 77% recovery rate of organic carbon in this method, a correction factor of 100/77 (1.3) was used in calculating soil organic carbon content. The following formula was used for calculating SOC:

$$\textit{Organic carbon (\%)} = (B-S) \times NFAS \times 0.003 \times 1.3 \times 100/W \qquad \text{(Eq.5)}$$

Whereby:

B = Volume of ferrous ammonium sulfate consumed for blank titration in ml

S = Volume of ferrous ammonium sulfate consumed for sample in ml

N_{FAS} = Normality of ferrous ammonium sulfate from blank titration

W = Dry mass of soil sample (g)

Determination of Na, Ca, Mg

To prepare 1M Ammonium acetate solution of 7.0 pH, 58 ml acetic acid was put into about 600 ml of deionized water in a 2-liter beaker. Then, 70 ml of concentrated ammonium hydroxide (NH_4OH) was added under a fume chamber through a long-stemmed glass funnel so that it could be introduced into the bottom of the acid solution. The solution was then cooled for about 30 minutes and adjusted to pH 7.0 with acetic acid or NH_4OH using a pH meter. The solution was then transferred into a 1 litre volumetric flask and diluted to volume. It was then mixed in a Pyrex reagent bottle. Five grams of soil sample was then weighed and 30ml of 1M NH_4OAc was added.

The contents were shaken on a mechanical shaker for 2 hours and the mixture was then centrifuged at 2000 rpm for 5-10 minutes. Decanting the clear supernatant was carefully done into a 100ml volumetric flask. Another 30ml of NH_4OAc solution was added and shaken for 30 minutes. The mixture was further centrifuged and the supernatant was transferred into the same volumetric flask. Another 30ml of NH_4OAc solution was added and further shaken for 30 minutes. The supernatant was then transferred into the same volumetric flask and made up to the mark with NH_4OAc solution. Sodium, Calcium, and Magnesium were determined on an atomic absorption spectrophotometer (AAS).

Determination of soil cation exchange capacity (CEC)

Soil CEC was determined by the addition of exchangeable Ca^{2+}, Mg^{2+} K^{+}, and Na^{+} for pH above 6. For pH below 6, exchangeable acidity will be added to the sum of Mg^{2+}, Ca^{2+}, $K^{+,}$ and Na^{+} in meq/100g. The following formula was used:

$$\mathit{CEC}\ (\mathit{cmol}_{(+)}\ \mathit{kg}^{-1}) = \frac{Ca\ (ppm)}{200} + \frac{Mg\ (ppm)}{120} + \frac{K\ (ppm)}{390} + \frac{Na\ (ppm)}{230} \qquad \text{(Eq.6)}$$

Determination of Microbial Population (bacteria and fungi)

The measurement of the microbial population was done 3 months after the experiment's establishment and at the end of the experiment. Both bacteria and fungi populations in the soil samples were quantified using the dilution plate count method as described by Ogunmwoyi et al. (2008). For the determination of bacteria in the soil, 39 g of Nutrient Agar (NA) was dissolved in a 1000 ml conical flask of deionized water to prepare a bacteria growth medium. The mixture was then autoclaved for about one hour and dispensed into Petri dishes and 0.1 ml of the diluted soil sample was then spread on the plates containing nutrient agar (three replicates per sample). To serial dilute the soil sample, 9 ml of distilled water was measured using a measuring cylinder into test tubes and covered with cotton wool and Aluminium foil to prevent contamination. This was also autoclaved for about one hour for sterilization. One gram of the soil sample was then dissolved into the 9 ml sterilized deionized water and shaken thoroughly. Serial dilution was then done until the fifth dilution. The first dilution was spread on the nutrient agar dispensed on Petri dishes which were incubated for 12 hours in an oven at 37°C for bacteria to grow. A colon counter was then used to count the number of colony-forming units in each petri dish and the mean was determined for each sample.

The determination of the fungi population in the soil also followed the same procedure as that of determining the bacteria population in the soil except that the growth medium for fungi was Potato Dextrose Agar (PDA) instead of nutrient agar (NA). Twenty-eight grams of PDA were dissolved in 1000 ml of deionized water in a conical flask. After spreading the diluted soil sample on the Petri dishes containing dispensed PDA, it was incubated for 120 hours after which the colony-forming units (CFUs) were counted using a colony counter. The number of CFUs in the soil solution was then converted to the number of CFUs per gram of soil.

Appendix X: Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **834499**

Date of Issue: **03/January/2022**

RESEARCH LICENSE

This is to Certify that Ms.. Riziki Umazi Mwadalu of Kenyatta University, has been licensed to conduct research in Kilifi on the topic: IMPROVING SOIL PROPERTIES AND GROWTH OF CASUARINA EQUISETIFOLIA THROUGH USE OF PROSOPIS JULIFLORA BIOCHAR, MANURE AND INORGANIC FERTILIZER IN KILIFI COUNTY, KENYA for the period ending : 03/January/2023.

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