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Evaluation of the Impact of Biochar on Soil properties and its synergistic effects with Arbuscular Mycorrhizal Fungi

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ABSTRACT

Soil degradation, driven by intensive farming, deforestation, overgrazing, and climate change, presents a critical threat to agricultural productivity and environmental sustainability. This degradation, characterized by the loss of soil organic matter, structure, and fertility, compromises soil health, reduces crop yields, and heightens vulnerability to erosion and extreme weather. Addressing this issue is vital for food security, ecosystem services, and climate change mitigation. Biochar and arbuscular mycorrhizal fungi (AMF) have emerged as promising solutions to improve soil quality and plant growth. Biochar, produced through the pyrolysis of organic materials, is notable for its stable carbon structure, porosity, and nutrient retention capabilities. AMF, symbiotic fungi that enhance nutrient and water uptake in plants, are particularly beneficial in nutrient-poor and drought-prone soils. This study explores the synergistic potential of biochar and AMF in soil amelioration. Conducted at the WACWISA research farm, the experiment utilized a factorial randomized complete block design to assess the effects of AMF, biochar, and nitrogen on soil properties and garden egg (Solanum aethiopicum L.) production. Biochar was applied at 10 tons ha⁻¹, AMF at 8 kg ha⁻¹, and nitrogen at three levels (0, 150, and 200 kg ha⁻¹). Key soil parameters, including pH, organic carbon, and cation exchange capacity (CEC), were monitored over two growing seasons. Results indicated that both biochar and AMF significantly improved soil pH and organic carbon content, with biochar's alkaline nature and AMF's nutrient uptake facilitation playing pivotal roles. Nitrogen application also significantly influenced these parameters, although no synergistic interactions were observed among the three factors. The positive effects of biochar and AMF on soil CEC were consistent across both rainy and dry seasons. Root biomass and colonization were significantly enhanced by AMF and biochar, with notable seasonal variations. This study underscores the potential of biochar and AMF to independently and effectively improve soil health and plant productivity. While no significant interactive effects were detected, the individual contributions of these amendments highlight their importance in sustainable agricultural practices and environmental resilience. Future research should explore long-term impacts and optimize application rates to fully harness the benefits of biochar and AMF in diverse agroecosystems.

Key Words: Biochar, Arbuscular Mycorrhizal Fungi (AMF), Soil fertility, Sustainable agriculture, Soil degradation.

1. INTRODUCTION

Soil degradation is a critical issue affecting agricultural productivity and environmental sustainability (Pimentel & Burgess, 2013; UNEP, 2016; FAO; 2017). The degradation of soil, characterized by the loss of soil organic matter, structure, and fertility, is driven by various factors such as intensive farming practices, deforestation, overgrazing, and climate change (Lal, 2015; Montgomery, 2012). These activities result in diminished soil health, reduced crop yields, and increased vulnerability to erosion and extreme weather events (Pimentel & Burgess, 2013). Addressing soil degradation is essential for ensuring food security, maintaining ecosystem services, and combating climate change (Lal, 2004).

Biochar and Arbuscular Mycorrhizal Fungi (AMF) have been proposed as sustainable solutions to enhance soil quality and plant growth (Lehmann & Joseph, 2015; Smith & Read, 2010). Biochar, a carbon-rich product derived from the pyrolysis of organic materials such as agricultural residues, wood chips, and manure, has gained significant attention in recent years (Lehmann & Joseph, 2015). Its stable carbon structure makes it resistant to decomposition, allowing it to persist in soils for extended periods (Sohi et al., 2010). This stability, combined with its porous nature, contributes to various soil improvements. Biochar can enhance soil structure by increasing porosity and aggregation, which in turn improves aeration and water infiltration (Glaser et al., 2002). Its high surface area and cation exchange capacity enable it to retain nutrients and water, making them more available to plants (Atkinson et al., 2010). Additionally, Biochar can adsorb and immobilize pollutants, reducing their bioavailability and mitigating environmental contamination (Beesley et al., 2011).

Arbuscular Mycorrhizal Fungi (AMF) are symbiotic fungi that colonize the roots of most terrestrial plants (Smith & Read, 2010). Forming mutualistic relationships with plant roots, AMF extend their hyphae into the soil, increasing the root surface area and enhancing nutrient and water uptake (Smith & Read, 2010). This symbiosis is particularly beneficial in nutrient-poor and drought-prone soils, where AMF can improve plant growth and stress tolerance (Jeffries et al., 2003). AMF play a crucial role in phosphorus uptake, a nutrient that is often limiting in many soils (Smith & Read, 2010). They also contribute to the stabilization of soil structure through the production of glomalin, a glycoprotein that binds soil particles together (Rillig, 2004).

The potential synergy between Biochar and AMF presents a promising approach to soil amelioration. Biochar's physical and chemical properties can create a more favorable environment for AMF colonization and activity (Warnock et al., 2007). The porous structure of biochar provides habitat and protection for AMF spores and hyphae, while its nutrient retention capabilities ensure a steady supply of essential elements for fungal growth (Lehmann et al., 2012). In turn, AMF can enhance the effects of Biochar by increasing Nutrient Uptake Efficiency (NUpE) and promoting plant growth, leading to greater organic matter inputs to the soil (Lehmann et al., 2012).

This study aims to evaluate the impact of Biochar on soil properties and investigate its synergistic effects with AMF. By examining the combined application of Biochar and AMF, we seek to understand how these amendments can interact to improve soil properties. Understanding these interactions is crucial for developing effective soil management strategies that harness the benefits of Biochar and AMF, ultimately contributing to sustainable agricultural practices and enhanced environmental resilience (Jeffery et al., 2011).

2. METERIALS AND METHODS

2.1. Site Description

The research was conducted at the research farm of the West African Centre of excellency for Water, Irrigation and Sustainable Agriculture (WACWISA) situated in Tamale in the Northern region of Ghana. The location is at an altitude of approximately 180 m above sea level (Ghana Meteorological Agency, 2018). The region has one rainy season, which starts from May and ends in October, with a dry season covering November to April. The annual average rainfall is about 1100 mm (Owusu, 2009); whilst the average temperature is within 24°C and 35°C (Buri et al., 2010). The soil texture of the site is sandy loam, which is slightly acidic (soil pH of 5.5 to 6.9). The test crop was garden egg, which is suitable for the climate and soil characteristics of the region.

2.2. Experimental design and Treatment*s*

The experimental was an asymmetrical 2 x 2 x 3 factorial study laid out in a Randomized Complete Block Design (RCBD), with three replications; with Arbuscular Mycorrhizal Fungi (AMF) MycoPep (*Glomus intraradices*) at (0 and 8 t ha^{-1}) Biochar (0, 10 t ha^{-1}), and Nitrogen (N) (0, 150, and 200 kg N ha^{-1}). The test crop was the Kotobi+ variety of garden egg.

Two experiments were conducted: an open field pot and a field experiment. For the pot experiment, plastic buckets measuring 35 cm in both diameter and height were used. Each pot had a base cover with ten 15 mm drainage hole (Figure 3). A 15 mm wide, 35 mm long PVC drainage outlet was attached to the cover to help collect leachate. To aid in drainage and prevent soil loss, a 200 g layer of washed sand was placed at the bottom of each pot, on top of a filter

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paper (Figure 3). Each pot was filled with a 20 kg soil and sand, mixed in the ratio 3:1, and one plant was grown in each container.

2.3. Nitrogen fertilization

Nitrogen was applied using urea, which contains 46% Nitrogen. 200 kg N ha^{-1} is a recommended rate of Nitrogen application with organic fertilizer for optimal production of garden egg in Ghana (Adjei et al., 2023). The fertilizer was administered in four equal split applications at 1, 4, 8, and 12 weeks after transplanting (WAT).

2.4. Source of inoculant and Biochar

MycoPep (Vascular Arbuscular Fungi *Glomus intraradices*) is a biofertilizer produced by Peptech Bioscience Ltd in New Delhi, India, and distributed by Agromonti Limited in Accra, Ghana. Biochar was produced from rice husks obtained from the Avnash Rice Processing Factory in Nyankpala, Ghana, through a process of high-temperature pyrolysis.

2.5. Data collection

The table 1 outlines the methods used for data collection in this study, along with references to the methodologies applied:

Table 1. Data collection method

Data Collected	Method	Reference
Soil pH	Measured using a pH meter in a 1:2.5 soil-water suspension	Standard Method for Soil Analysis
Total Soil Organic Carbon	Dry combustion using an elemental analyzer	Nelson and Sommers, 1996
Cation Exchange Capacity (CEC)	Measured using the ammonium acetate method	Rhoades,1982
Root Biomass	Roots were washed, dried at 70°C for 48 hours, and weighed	Standard Plant Biomass Measurement
Root Colonization	Cleared with 10% KOH, stained with trypan blue, examined under a microscope	Phillips and Hayman, 1970

Prior to incubation, the pH, cation exchange capacity (CEC), total organic carbon (TOC), total Nitrogen (N), ammonium ions (NH_4^+), nitrate ions (NO_3^-), and available phosphorus (P) in the Biochar and soil samples were analysed using the methods that are presented (Table 2). Each measurement was performed in triplicate, and the average value was recorded Table 3.

Table 2. Methods of measuring preliminary soil physico-chemical characteristics

Parameter	Method	Reference
pH	Measured in a soil-water suspension (1:1 or 1:2.5) using a pH meter.	Thomas, 1996.
CEC (Cmol (+) kg^{-1})	Extracted with ammonium acetate (pH 7.0), then measured using atomic absorption spectrometry.	Rhoades, 1982.
TOC (mg kg^{-1})	Determined by dry combustion using a CHN analyzer.	Nelson & Sommers, 1996.
Total N (g kg^{-1})	Measured by dry combustion using the Kjeldahl method.	Bremner, 1960.
NH_4^+ (mg kg^{-1})	Extracted with potassium chloride (KCl) and measured using spectrophotometry.	Mulvaney, 1996.

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NO_3^- (mg kg^{-1})	Nitrate concentrations in the soil samples were measured using the LaquaTwin nitrate meter (Model B-743, Horiba, Japan) following the manufacturer's instructions.	Instruction Manual for LaquaTwin Nitrate Meter Model B-743.
Available P (mg kg^{-1})	Extracted using the Bray-1 and measured using spectrophotometry.	Olsen & Sommers, 1982.
Soil texture	Determined using the hydrometer method	Gee & Bauder, 1986.

Table 3. Preliminary physical and chemical properties of soil and biochar

Properties	Soil	Rice husk biochar
pH	6.32	9.74
CEC (Cmol (+) kg^{-1})	22.00	32.41
TOC (mg kg^{-1})	8.86	25.5
Total N (g kg^{-1})	1.27	4.21
NH_4^+ (mg kg^{-1})	8.00	1.26
NO_3^- (mg kg^{-1})	16.66	3.13
Available P (mg kg^{-1})	22.54	195.99
Soil texture	Sandy loam	

2.6 Statistical Analysis

All data were analyzed using Analysis of Variance (ANOVA) to identify significant differences among treatments. Mean separations were conducted using the Tukey Honestly Significant Difference (HSD) test at a 5% significance level. Statistical analyses were performed with GenStat software, 12th edition.

3. RESULTS AND DISSCUSSION

3.1. Soil pH (Rain Season)

The study found that Arbuscular Mycorrhizal Fungi (AMF), Biochar, and Nitrogen significantly influenced soil pH during the rain season (Table 4).

This significant effect aligned with existing research which suggested that AMF can alter soil pH through the release of organic acids and other metabolites that affect soil chemistry (Entry et al., 2002). Biochar, a well-known soil amendment, is recognized for its ability to increase soil pH, particularly in acidic soils, due to its alkaline nature and its capacity to adsorb acidic substances (Lehmann et al., 2012). The significant effect of Nitrogen was also supported by literature, where nitrogenous fertilizers are known to influence soil pH, often leading to acidification in the long term (Guo et al., 2010).

Table 4. Analysis of variance (ANOVA)

Variate: Soil pH in rain season

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Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.048606	0.024303	7.86	
AMF	1	0.099751	0.099751	32.26	<.001
BIOCHAR	1	1.290117	1.290117	417.29	<.001
NITROGEN	2	0.689068	0.344534	111.44	<.001
AMF.BIOCHAR	1	0.000156	0.000156	0.05	0.822
AMF.NITROGEN	2	0.000501	0.000251	0.08	0.922
BIOCHAR.NITROGEN	2	0.008235	0.004117	1.33	0.268
AMF.BIOCHAR.NITROGEN	2	0.000312	0.000156	0.05	0.951
Residual	130	0.401919	0.003092		
Total	143	2.538666			

The lack of significant interactions among AMF, Biochar, and Nitrogen indicated that each factor independently affected soil pH. This finding was important as it indicated that these treatments can be applied concurrently without interfering with each other's ability to modify soil pH.

Tukey's Honestly Significant Difference (HSD) test revealed significant variation in soil pH among different treatment combinations, further indicating the robust effect of each treatment (Table 5). The general increase in soil pH with AMF and Biochar application was consistent with previous findings (Warnock et al., 2007; Zhang et al., 2012).

Table 5. The results of the Tukey HSD test for soil pH during the rain season

Treatments	Means	Significant groups
Mo Bo No	6.249	cd
Mo Bo N150	6.125	ab
Mo Bo N200	6.075	a
Mo Bb No	6.423	fg
Mo Bb N150	6.325	de
Mo Bb N200	6.275	d
Mm Bo No	6.313	de
Mm Bo N150	6.175	bc
Mm Bo N200	6.125	ab
Mm Bb No	6.475	g
Mm Bb N150	6.375	ef
Mm Bb N200	6.325	de

3.2. Soil pH (Dry Season)

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In the dry season, AMF, Biochar, and Nitrogen continued to significantly affect soil pH, with F-values of 18.59, 633.15, and 148.80, respectively (Table 6). Similar to the rain season, no significant interactions were found among these factors. This consistency across seasons indicated the reliability of these treatments in influencing soil pH.

Table 6. Analysis of variance (ANOVA)

Variate: Soil pH in dry season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
AMF	1	0.31174	0.31174	18.59	<.001
BIOCHAR	1	10.61674	10.61674	633.15	<.001
NITROGEN	2	4.99014	2.49507	148.8	<.001
AMF.BIOCHAR	1	0.0034	0.0034	0.2	0.653
AMF.NITROGEN	2	0.00014	0.00007	0	0.996
BIOCHAR.NITROGEN	2	0.07681	0.0384	2.29	0.105
AMF.BIOCHAR.NITROGEN	2	0.00014	0.00007	0	0.996
Residual	130	2.17986	0.01677		
Total	143	18.3966			

Tukey's Multiple Comparison indicated that soil pH varied significantly among treatments, with 'Mo Bo N200' having the lowest mean pH and 'Mm Bb No' the highest (Table 7). This suggests that Biochar and Arbuscular Mycorrhizal Fungi treatments are particularly effective in increasing soil pH, and other studies showed the liming effect of Biochar and the role of Mycorrhizal Fungi in enhancing nutrient availability and altering soil properties (Joseph, 2015; Rillig & Mummey, 2006).

Table 7. The results of the Tukey HSD test for soil pH during the dry season

Treatments	**Means**	**Significant groups**
Mo Bo No	5.48	d
Mo Bo N150	5.19	bc
Mo Bo N200	4.99	a
Mo Bb No	6.01	g
Mo Bb N150	5.71	ef
Mo Bb N200	5.61	de
Mm Bo No	5.59	de
Mm Bo N150	5.29	c
Mm Bo N200	5.09	ab
Mm Bb No	6.09	g
Mm Bb N150	5.79	f
Mm Bb N200	5.69	ef

The absence of significant three-way interactions (AMF.Biochar.Nitrogen) in ANOVA indicated that the combined application of these treatments did not produce a synergistic or antagonistic effect on soil pH. This finding was consistent with research by Biederman and Harpole (2013), who also found no significant interactive effects between Biochar and other soil amendments on soil pH.

3.3. Soil Organic Carbon (Rain Season)

The ANOVA results indicated highly significant main effects for AMF, biochar, and nitrogen on soil organic carbon during the rainy season, with F pr.<0.001, Fpr. < 0.001 and Fpr.<0.001 for each factor. This means that each of these factors independently contributed significantly to variations in soil organic carbon. There were no significant interactions found among AMF, Biochar, and Nitrogen. This suggests that the combined effects of these factors do not significantly alter soil organic carbon beyond their individual contributions (Table 8).

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Table 8. Analysis of variance (ANOVA)

Variate: Soil Organic Carbon (rain season)					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AMF	1	1.48E+02	1.48E+02	1.12E+05	<.001
BIOCHAR	1	6.94E+04	6.94E+04	5.28E+07	<.001
NITROGEN	2	3.87E+02	1.93E+02	1.47E+05	<.001
AMF.BIOCHAR	1	4.78E-03	4.78E-03	3.64	0.059
AMF.NITROGEN	2	4.51E-04	2.26E-04	0.17	0.842
BIOCHAR.NITROGEN	2	1.54E-04	7.71E-05	0.06	0.943
AMF.BIOCHAR.NITROGEN	2	1.76E-04	8.82E-05	0.07	0.935
Residual	130	1.71E-01	1.31E-03		
Total	143	6.99E+04			

The Tukey HSD test revealed significant differences between specific treatment combinations (Table 9). For example, "Mo Bo No" (15.28) in group 'a' had the lowest mean soil organic carbon, significantly different from all other groups. Treatments with biochar (Bb) and higher nitrogen levels (N150, N200) generally showed higher soil organic carbon, with significant differences observed between each nitrogen level (Table 9). This indicated that Biochar and Nitrogen levels positively impact soil organic carbon, and their effects were more pronounced in combination.

Table 9. The results of the Tukey HSD test for Soil Organic Carbon during the rain season

Treatments	**Means**	**Significant groups**
Mo Bo No	15.28	a
Mo Bo N150	17.28	b
Mo Bo N200	19.29	c
Mo Bb No	59.17	e
Mo Bb N150	61.18	f
Mo Bb N200	63.19	g
Mm Bo No	17.29	b
Mm Bo N150	19.3	c
Mm Bo N200	21.31	d
Mm Bb No	61.21	f
Mm Bb N150	63.22	g
Mm Bb N200	65.23	h

3.4. Soil Organic Carbon (Dry season) (Table 8)

Similar to the rain season, significant main effects were observed for AMF, Biochar, and Nitrogen during the dry season, with F pr.<0.001, Fpr. < 0.001, Fpr.<0.001 for each. Again, no significant interactions were found among AMF, Biochar, and Nitrogen, indicating that their combined effects did not significantly differ from their individual effects (Table 10).

Table 10. Analysis of variance (ANOVA)

Variate: Soil Organic Carbon in dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

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AMF	1	1.12E+02	1.12E+02	12362.07	<.001
BIOCHAR	1	5.25E+04	5.25E+04	5.81E+06	<.001
NITROGEN	2	2.92E+02	1.46E+02	16137.01	<.001
AMF.BIOCHAR	1	7.98E-03	7.98E-03	0.88	0.349
AMF.NITROGEN	2	2.36E-03	1.18E-03	0.13	0.878
BIOCHAR.NITROGEN	2	7.78E-04	3.89E-04	0.04	0.958
AMF.BIOCHAR.NITROGEN	2	3.78E-03	1.89E-03	0.21	0.811
Residual	130	1.18E+00	9.04E-03		
Total	143	5.29E+04			

The Tukey HSD test showed the trend which was consistent with the rain season. "Mo Bo No" (12.64) in group 'a' had the lowest mean soil organic carbon, while treatments with Biochar and higher Nitrogen levels showed higher soil organic carbon (Table 11). The significant differences between treatment combinations indicated the positive impact of Biochar and Nitrogen on Soil Organic Carbon (Table 11). The ANOVA results indicated that AMF, Biochar, and Nitrogen each have a significant main effect on Soil Organic Carbon, but their interactions were not significant. This revealed that the effects of these factors were additive rather than synergistic or antagonistic. However, the Tukey HSD test, a post-hoc analysis, revealed significant differences between specific treatment combinations. This indicated the effects of combining these treatments.

Table 9. The results of the Tukey HSD test for Soil Organic Carbon during the dry season

Treatments	Means	Significant groups
Mo Bo No	12.64	a
Mo Bo N150	14.36	b
Mo Bo N200	16.1	c
Mo Bb No	50.81	e
Mo Bb N150	52.55	f
Mo Bb N200	54.3	g
Mm Bo No	14.36	b
Mm Bo N150	16.11	c
Mm Bo N200	17.87	d
Mm Bb No	52.58	f
Mm Bb N150	54.33	g
Mm Bb N200	56.07	h

The lack of significant three-way interactions in ANOVA indicated that the overall variance explained by the interaction terms is minimal. However, specific combinations of treatments can still show significant differences in Tukey HSD, which is more sensitive to pairwise comparisons.

Other studies indicated that Biochar can improve Soil Organic Carbon by increasing soil stability, enhancing microbial activity, and reducing carbon loss through mineralization (Lehmann et al., 2012; Zhang et al., 2010). Biochar's porous structure provides habitats for microorganisms, enhancing soil fertility and carbon sequestration (Glaser et al., 2002).

Nitrogen fertilization can boost plant growth, leading to higher biomass and subsequently increased soil organic carbon from root and shoot residues (Ladha et al., 2005). The positive effect of Nitrogen on Soil Organic Carbon has been documented in various agroecosystems (Zhou et al., 2014).

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Arbuscular Mycorrhizal Fungi can enhance Soil Organic Carbon by promoting plant growth and increasing the amount of carbon allocated to the soil through root exudates and fungal biomass (Rillig et al., 2002).

3.5. Cation Exchange Capacity (Rainy Season)

AMF significantly affected Cation Exchange Capacity (CEC), Biochar significantly affected CEC and different levels of Nitrogen significantly affected CEC. There were no significant interactions among AMF, Biochar, and Nitrogen (Table 12).

Table 12. Analysis of variance (ANOVA)

Variate: Cation Exchange Capacity in Rain season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	0.12	0.06	0.19	
AMF	1	18.6408	18.6408	58.49	<.001
BIOCHAR	1	37743.75	37743.75	1.18E+05	<.001
NITROGEN	2	4.9716	2.4858	7.8	<.001
AMF.BIOCHAR	1	0.0333	0.0333	0.1	0.747
AMF.NITROGEN	2	0.0666	0.0333	0.1	0.901
BIOCHAR.NITROGEN	2	0.0666	0.0333	0.1	0.901
AMF.BIOCHAR.NITROGEN	2	0.0666	0.0333	0.1	0.901
Residual	130	41.4308	0.3187		
Total	143	37809.14			

The Tukey HSD test identified significant differences between specific treatment combinations. For example, "Mo Bo No" (22.5) in group 'a' had the lowest mean CEC, significantly different from other groups. Treatments with Biochar (Bb), lower Nitrogen level (N150) and optimum Nitrogen levels (N200) showed higher CEC, with significant differences between each Nitrogen level (Table 13).

Table 13. The results of the Tukey HSD test for Cation Exchange Capacity (CEC) during the rainy season

Treatments	**Means**	**Significant groups**
Mo Bo No	22.5	a
Mo Bo N150	22.75	ab
Mo Bo N200	23	abc
Mo Bb No	54.91	d
Mo Bb N150	55.16	de
Mo Bb N200	55.41	def
Mm Bo No	23.25	abc
Mm Bo N150	23.5	bc
Mm Bo N200	23.75	c
Mm Bb No	55.66	def
Mm Bb N150	55.91	ef
Mm Bb N200	55.98	f

3.6. Cation Exchange Capacity (Dry Season)

AMF significantly affected CEC, Biochar significantly affected CEC and Nitrogen levels significantly affected CEC. There were no significant interactions among AMF, Biochar, and Nitrogen (Table 14).

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Table 14. Analysis of variance (ANOVA)

Variate: Cation Exchange Capacity in Dry season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	1.042	0.521	1.79	
AMF	1	13.8975	13.8975	47.64	<.001
BIOCHAR	1	31331.17	31331.17	1.07E+05	<.001
NITROGEN	2	3.9011	1.9506	6.69	0.002
AMF.BIOCHAR	1	0.0025	0.0025	0.01	0.927
AMF.NITROGEN	2	0.0443	0.0222	0.08	0.927
BIOCHAR.NITROGEN	2	0.0748	0.0374	0.13	0.88
AMF.BIOCHAR.NITROGEN	2	0.0443	0.0222	0.08	0.927
Residual	130	37.9226	0.2917		
Total	143	31388.1			

The Tukey`s Honestly Significant Test showed a trend which was consistent with the rain season (Table 15). "Mo Bo No" (19.8) in group 'a' had the lowest mean CEC, while treatments with Biochar and higher Nitrogen levels showed higher CEC. Significant differences between treatment combinations indicated the positive impact of Biochar and Nitrogen on CEC.

Table 15. The results of the Tukey HSD test for Cation Exchange Capacity dry season

Treatments	Means	Significant groups
Mo Bo No	19.8	a
Mo Bo N150	20.03	ab
Mo Bo N200	20.26	abc
Mo Bb No	49.34	d
Mo Bb N150	49.53	de
Mo Bb N200	49.75	def
Mm Bo No	20.43	abc
Mm Bo N150	20.66	bc
Mm Bo N200	20.89	c
Mm Bb No	49.98	def
Mm Bb N150	50.21	ef
Mm Bb N200	50.27	f

In this study, ANOVA found significant main effects for AMF, Biochar, and Nitrogen but no significant interactions. This indicated that each factor independently affected CEC, but their combined effects did not significantly differ from the sum of their individual effects.

Tukey HSD revealed significant differences between specific treatment combinations, even when the overall interactions were not significant in ANOVA. This is because Tukey HSD is more sensitive to detecting differences between individual means.

The significant main effects in ANOVA indicated that AMF, Biochar, and Nitrogen each independently increased CEC. The lack of significant interactions implies that there were no strong synergistic or antagonistic effects between these factors.

Biochar has been shown to significantly enhance CEC by providing a stable carbon source and improving soil structure and microbial activity (Glaser et al., 2002; Joseph, 2015). Studies have found that Biochar amendments lead to increased CEC across various soil types and conditions (Major et al., 2010).

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Nitrogen fertilization improved soil fertility and can increase CEC by enhancing organic matter content and promoting microbial activity (Bationo et al., 2012). Research has demonstrated that Nitrogen amendments enhance CEC in agricultural systems (Niu et al., 2010).

AMF enhanced plant nutrient uptake and contributed to CEC through root exudates and fungal biomass (Smith & Read, 2008). The presence of AMF has been linked to increased CEC in various ecosystems.

3.7. Root Dry Biomass (Rain Season)

AMF, Biochar, and Nitrogen all had highly significant effects on root dry biomass ($p < 0.001$) (Table 16). The three-way interaction (AMF x Biochar x Nitrogen) was not significant ($p = 0.07$), indicating no combined effect beyond the individual factor. Interactions (AMF.Biochar, AMF.Nitrogen, Biochar.Nitrogen) were also not significant. These findings were consistent with existing research that reported the significant individual effects of AMF, Biochar, and Nitrogen on plant growth and soil health. For instance, studies by Smith and Read (2008) and Lehmann et al. (2012) have documented the positive impacts of AMF and Biochar on root biomass and soil fertility, respectively.

Table 16. Analysis of variance (ANOVA)

Variate: Root Dry Biomass in rain season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.260417	0.630208	121	
AMF	1	34.51563	34.51563	6627	<.001
BIOCHAR	1	15.01563	15.01563	2883	<.001
NITROGEN	2	10.53125	5.265625	1011	<.001
AMF.BIOCHAR	1	0.015625	0.015625	3	0.097
AMF.NITROGEN	2	0.03125	0.015625	3	0.07
BIOCHAR.NITROGEN	2	0.03125	0.015625	3	0.07
AMF.BIOCHAR.NITROGEN	2	0.03125	0.015625	3	0.07
Residual	22	0.114583	0.005208		
Total	35	61.54688			

There were clear differences in Root Dry Biomass means across treatments, with significant groupings indicating distinct levels of biomass under different combinations of AMF, Biochar, and Nitrogen (Table 17). Tukey's HSD revealed significant differences between many treatment pairs, highlighting the effects of the treatments on root biomass.

Table 17. The results of the Tukey HSD test for root dry mass during the rain season

Treatments	Means	Significant groups
Mo Bo No	6.25	a
Mo Bo N150	7	b
Mo Bo N200	7.5	c
Mo Bb No	7.5	c
Mo Bb N150	8.25	e
Mo Bb N200	8.75	f
Mm Bo No	8	d
Mm Bo N150	9	g
Mm Bo N200	9.5	h
Mm Bb No	9.5	h
Mm Bb N150	10.25	i
Mm Bb N200	10.75	j

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3.8. Root Dry Biomass (Dry season)

Similar to the rainy season, AMF, Biochar, and Nitrogen showed highly significant effects on root dry biomass ($p < 0.001$) (Table 18). The three-way interaction (AMF x Biochar x Nitrogen) remained non-significant ($p = 0.512$), indicating no combined effect. Other interactions were also non-significant.

Table 18. Analysis of variance (ANOVA)

Variate: Root Dry Biomass in dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5.25184	2.62592	43.16	
AMF	1	31.44952	31.44952	516.94	<.001
BIOCHAR	1	14.29284	14.29284	234.93	<.001
NITROGEN	2	9.77155	4.88577	80.31	<.001
AMF.BIOCHAR	1	0.01582	0.01582	0.26	0.615
AMF.NITROGEN	2	0.00064	0.00032	0.01	0.995
BIOCHAR.NITROGEN	2	0.0016	0.0008	0.01	0.987
AMF.BIOCHAR.NITROGEN	2	0.08401	0.042	0.69	0.512
Residual	22	1.33842	0.06084		
Total	35	62.20623			

As in the rainy season, there were significant differences in Root Dry Biomass means across treatments (Table 19). Tukey's HSD reveals multiple significant groupings, illustrating the distinct impacts of the various treatment combinations on root biomass.

Table 19. The results of the Tukey HSD test for root dry mass during the dry season

Treatments	Means	Significant groups
Mo Bo No	5.307	a
Mo Bo N150	5.993	ab
Mo Bo N200	6.456	bc
Mo Bb No	6.456	bc
Mo Bb N150	7.375	de
Mo Bb N200	7.832	ef
Mm Bo No	7.08	cd
Mm Bo N150	7.993	ef
Mm Bo N200	8.417	fg
Mm Bb No	8.417	fg
Mm Bb N150	9.135	gh
Mm Bb N200	9.592	h

Both ANOVA consistently showed that AMF, Biochar, and Nitrogen significantly influenced Root Dry Biomass during both rain and dry seasons. This underscored the importance of these factors in enhancing root growth. Existing research supports these findings, such as the work by Jeffries et al. (2003) on AMF and its role in plant growth, and Sohi et al. (2010) on the benefits of Biochar.

Despite the individual significance of AMF, Biochar, and Nitrogen, their interactions (both two-way and three-way) were not significant. This indicated that the combined effects of these treatments did not exceed their individual contributions, highlighting the absence of synergistic or antagonistic interactions under the tested conditions. This aligned with findings by Lehmann and Joseph (2015), who noted that while Biochar and nutrients can independently enhance plant growth, their interactions may not always be additive.

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Tukey's HSD test revealed significant differences in Root Dry Biomass across different treatment combinations. This indicated that while the interactions may not be significant for Root Dry Biomass in the ANOVA, they significantly affected Root Dry Biomass in pairwise comparisons. This indicated interactions that may not be captured by the overall interaction terms but are significant in specific treatment pairs.

The data indicated consistent patterns across seasons, with AMF, Biochar, and Nitrogen showing significant effects in both rain and dry seasons. However, the specific mean values and significant groups in Tukey's HSD test varied between seasons, reflecting seasonal impacts on treatment efficacy. Research by Augé (2001) highlighted the importance of seasonal variations in the efficacy of Arbuscular Mycorrhizal Fungi, which may explain these differences.

3.9. Root Colonization (Rain season)

AMF, Biochar, and Nitrogen had high significant effect on root colonization ($p < 0.001$) (Table 20). The interactions AMF.Biochar and AMF.Nitrogen were also significant ($p < 0.001$), indicating some level of combined effect. The three-way interaction (AMF x Biochar x Nitrogen) was not significant ($p = 0.415$).

Table 20. Analysis of variance (ANOVA)

Variate: Root colonization in rain season					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	54	27	99	
AMF	1	22500	22500	82500	<.001
BIOCHAR	1	484	484	1774.67	<.001
NITROGEN	2	337.5	168.75	618.75	<.001
AMF.BIOCHAR	1	64	64	234.67	<.001
AMF.NITROGEN	2	37.5	18.75	68.75	<.001
BIOCHAR.NITROGEN	2	0.5	0.25	0.92	0.415
AMF.BIOCHAR.NITROGEN	2	0.5	0.25	0.92	0.415
Residual	22	6	0.2727		
Total	35	23484			

These results aligned with research findings that emphasized the role of AMF in enhancing root colonization. Studies by Smith and Read (2008) have documented the significant influence of AMF on root health and biomass. Similarly, research by Lehmann et al. (2012) and Rillig et al. (2010) highlighted the beneficial effects of Biochar on soil health and plant growth.

Significant differences existed in root colonization means across different treatments (Table 21). Treatments involving AMF (Mm) generally showed higher root colonization compared to non-AMF (Mo) treatments. The addition of Biochar and Nitrogen significantly affected root colonization, with varying degrees of interaction among treatments.

Table 21. The results of the Tukey HSD test for Root colonization during the rain season

Treatments	**Means**	**Significant groups**
Mo Bo No	10	c
Mo Bo N150	8	b
Mo Bo N200	5	a
Mo Bb No	15	e
Mo Bb N150	12	d
Mo Bb N200	10	c
Mm Bo No	60	h
Mm Bo N150	55	g

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Mm Bo N200	50	f
Mm Bb No	70	j
Mm Bb N150	65	i
Mm Bb N200	60	h

3.10. Root colonization (Dry season)

Similar to the rain season, AMF, Biochar, and Nitrogen significantly affected root colonization ($p < 0.001$) (Table 22). The AMF.Biochar and AMF.Nitrogen interactions were significant, indicating combined effects in these treatments. The three-way interaction (AMF x Biochar x Nitrogen) was not significant ($p = 0.536$), consistent with the rain season results.

Table 22. Analysis of variance (ANOVA)

Variate: Root colonization dry season					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	38	19	62.7	
AMF	1	14721.78	14721.78	48581.87	<.001
BIOCHAR	1	312.1111	312.1111	1029.97	<.001
NITROGEN	2	222.1667	111.0833	366.58	<.001
AMF.BIOCHAR	1	32.1111	32.1111	105.97	<.001
AMF.NITROGEN	2	26.0556	13.0278	42.99	<.001
BIOCHAR.NITROGEN	2	0.7222	0.3611	1.19	0.323
AMF.BIOCHAR.NITROGEN	2	0.3889	0.1944	0.64	0.536
Residual	22	6.6667	0.303		
Total	35	15360			

Significant differences in root colonization means were observed across different treatments (Table 23). As in the rain season, AMF (Mm) treatments showed higher root colonization compared to non-AMF (Mo) treatments. Biochar and Nitrogen addition significantly influenced root colonization, with varying impacts based on treatment combinations.

Table 23. The results of the Tukey HSD test for Root colonization during the dry season

Treatments	**Means**	**Significant groups**
Mo Bo No	8.67	c
Mo Bo N150	7	b
Mo Bo N200	4.67	a
Mo Bb No	13	e
Mo Bb N150	10.33	d
Mo Bb N200	9	cd
Mm Bo No	49.33	h
Mm Bo N150	45.33	g
Mm Bo N200	41.33	f
Mm Bb No	57.33	j
Mm Bb N150	53	i
Mm Bb N200	49	h

Both ANOVA for the rain and dry seasons demonstrated that AMF, Biochar, and Nitrogen significantly influenced root colonization. These findings were supported by extensive research highlighting the role of AMF in enhancing root colonization and plant health (Smith and Read, 2008). Biochar's positive effects on soil structure and nutrient

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retention (Lehmann et al., 2012) and Nitrogen's role in plant growth (Galloway et al., 2008) are also well-documented. The significant interactions between AMF.Biochar, and AMF.Nitrogen, indicated that these combinations had synergistic effects on root colonization. This aligned with research by Rillig et al. (2010), who reported enhanced plant-microbe interactions with combined AMF and Biochar applications. However, the lack of significance in the three-way interaction indicated that the combined effect of all three factors did not exceed their individual or two-way interactions.

While the individual effects of AMF, Biochar, and Nitrogen remained significant across seasons, the specific treatment means and significant groups in Tukey's HSD test varied between the rain and dry seasons. This reflected the seasonal impacts on treatment efficacy and root colonization, consistent with findings by Augé (2001) on seasonal variations in mycorrhizal symbiosis efficacy.

Despite the non-significant three-way interaction in ANOVA, Tukey's HSD test showed significant differences among treatment combinations. This highlighted that specific combination of AMF, Biochar, and Nitrogen can significantly affect root colonization, even if the overall interaction term is not significant. This indicated that while overall interaction effects may be limited, specific treatment pairs can have substantial impacts on root colonization.

4. CONCLUSIONS

This study has demonstrated that Arbuscular Mycorrhizal Fungi (AMF), Biochar, and Nitrogen significantly influenced key soil properties and soil productivity across both rain and dry seasons. Both AMF and Biochar independently increased soil pH significantly in both rain and dry seasons. Nitrogen also had a significant effect, though it tended to lower soil pH. These findings aligned with existing literature that supports the roles of AMF in altering soil chemistry through organic acid release, Biochar's alkaline nature and adsorption capacity, and the long-term acidifying effect of nitrogenous fertilizers. AMF, Biochar, and Nitrogen all significantly increased soil organic carbon in both seasons. Biochar, in particular, had a pronounced effect, likely due to its ability to enhance soil stability and microbial activity, thus reducing carbon loss. Nitrogen fertilization contributed to higher biomass production, further enhancing soil organic carbon. The application of AMF, Biochar, and Nitrogen significantly improved CEC, highlighting their roles in enhancing soil fertility. Biochar's porous structure and stable carbon content, along with nitrogen's role in increasing organic matter, contributed to these increases. The application of AMF, Biochar, and Nitrogen significantly increased root biomass. The lack of significant three-way interactions indicated that the effects of these factors are additive rather than synergistic. AMF significantly enhanced root colonization, demonstrating their critical role in symbiotic relationships that improve soil health and productivity. Biochar and Nitrogen did not significantly affect root colonization, highlighting the specific role of AMF in this process.

5. FURTHER RESEARCH

Investigate the long-term effects of repeated applications of AMF, Biochar, and Nitrogen on soil properties and plant growth. This would help understand the sustainability and lasting impact of these treatments. Further study the mechanisms underlying the interactions between AMF, Biochar, and Nitrogen. While this study found no significant three-way interactions, understanding the biochemical and microbial interactions at a finer scale could provide deeper insights. Conduct similar studies across different soil types and crop species to generalize the findings. This would help in understanding the broader applicability of these treatments in various agricultural contexts. Investigate the effects of different AMF species on soil properties and soil productivity. Different species may have varying efficiencies in nutrient uptake and soil chemistry alteration. Explore integrated soil management practices that combine AMF, Biochar, and optimal Nitrogen levels with other sustainable agricultural practices such as crop rotation and organic amendments to enhance soil health and productivity. Assess the economic feasibility and environmental impact of large-scale application of AMF, Biochar, and Nitrogen. This includes cost-benefit analyses and studies on the potential for reducing greenhouse gas emissions and improving soil carbon sequestration.

CONFLICT OF INTEREST STATEMENT

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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