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RESEARCH ARTICLE

Effect of Biochar Application and Arbuscular Mycorrhizal Inoculation on Root Colonization and Soil Chemical Properties

Sunusi Abdu Yusif*[a] and Michael Olajire Dare[b]

[a] Department of Soil Science and Agricultural Engineering, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto. P.M.B. 2346, Sokoto, Nigeria.

[b] Department of Soil Science and Land Management, College of Plant Science and Crop Production, Federal University of Agriculture Abeokuta, P.M.B. 2240, Ogun State, Nigeria.

* Corresponding author email: yusif.sunusi@udusok.edu.ng , yusifsunusiabdu@gmail.com

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ABSTRACT

Field experiment was carried out in 2013 and 2014 to investigate the effects of biochar application and arbuscular mycorrhizal (AM) inoculation on AM root colonization and selected soil chemical properties of two tomato genotypes. The experiment was laid out in a split-split plot arrangement with two tomato genotypes in the main plots, five rates of biochar applied in the sub plots and two levels of AMF inoculation in the sub-sub plots. Data were subjected to analysis of variance and significant means separated using Duncan's Multiple Range Test ($P<0.05$). The results indicated that mycorrhizal inoculation had little or no influence on AM root colonization, soil organic carbon, soil pH and available phosphorus. Application of 20 t ha^{-1} of biochar significantly increased ($P< 0.05$) soil pH and available P compared with the control while 10 t ha^{-1} and 15 t ha^{-1} of biochar produced higher organic carbon than other rates. In conclusion, biochar increased soil pH but was not high enough as to have detrimental effect on soil properties. It is, therefore, recommended that biochar with very strongly alkaline pH should only be used in soil with low pH for improvement of soil chemical and AM root colonization.

Keywords: biochar; AM root colonization; inoculation; chemical properties

1 Introduction

Application of biochar to soil have been shown to enhance soil quality [1] and to increase crop yields by improving soil properties such as water and nutrient retention [2], and also increased pH and C levels [3]. According kolb *et al* [4], biochar application to soil can change soil nutrients availability by improving soil properties, enhancement of host plant performance and higher colonization rates of the host plant roots by AMF. However, Wallstedt *et al* [5] reported a contrasting observation that biochar applications did not always benefit soil. For example, biochar application into the soil may decrease the nutrient availability or create unfavourable nutrient ratios in the soils. Gaur *et al* [6] reported that this adverse effect could be more pronounced when biochar has a very high C/N ratio and a portion of the biochar is decomposable or high rate of biochar application leading to N immobilization. Some reports emphasize that biochar amendments can increase % AM root colonization in plant roots [7] while others show decrease in AMF abundance [8]. Inhibited colonization after char amendment might be due to improved availability of P [9]. Moreover, Abbott *et al* [10] reported that mycorrhizal colonization in peanut plants was significantly depressed by adding P. Mycorrhizal fungi are frequently included in soil management activities in crop production, and widely used as soil inoculums additives [11]. Given the above possibilities for negative responses by soil to biochar amendments [12], knowledge of the effects of biochar on AM root colonization and selected soil chemical properties is paramount for environment-friendly functions of AMF in biochar amended soil. This study aims to determine the effect of biochar and AMF inoculation on AM root colonization and selected soil chemical properties.

2 Materials and Methods

2.1 Experimental Site

The experiment was carried out at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, in 2013 / 2014. The area is located between Latitude 7°12' N and Longitude 3° 20' E and lies in the south-western Nigeria in the transitional zone. Daily temperature ranges between 24 °C and 30° C and annual rainfall in the area ranges between 1100 mm and 1300 mm.

2.2 Experimental Materials and Design

The total plot size was 315 m^2 with the sub-sub plot size of 2 m × 1 m, a spacing of 1 m between and within sub-sub plots. The plant spacing was 30 cm between and within the plant stands consisting of eighteen plants per sub-sub plot. The field layout was split- split plots design with two tomato genotypes in the main plots, five biochar rates of application (0, 5, 10, 15, 20 t h^{-1}) in the sub plots and two levels of AMF inoculation (with and without) in the sub-sub plots. The AMF (*Glomus mosseae*) inoculation was done during the nursery planting at the rate of 80 g of AMF inoculants per 5 kg of sterilized top soil while the biochar was applied two weeks before transplanting (WAT). Weeding was done using hoe at 4 and 8 WAT while cypermethrin (insecticide) was applied at 2, 6, and 9 WAT at the rate of 450 ml of active ingredients to 100 liters of water per hectare of land using knapsack sprayer.

2.3 Soil and Root Sampling

Soil samples for analyses were taken before planting and after harvest. The rhizosphere soils from three plants in the middle row of each sub-sub plot along with the roots of tomato plants were dug out at a depth of about 0-20 cm for AM root colonization studies. Another set of soil samples from six different points from each sub-sub plot were collected to evaluate for soil pH, organic carbon and available P. The root samples were stored in 50% ethanol until processing.

2.4 Quantification of AM Root Colonization

Approximately 20 root bits of 1cm size were chosen randomly from each sub-sub plot for AM colonization studies following Phillip *et al* [13] method. The root samples from 50 % ethanol were washed thoroughly and then placed in 10 % KOH and heated in water bath for 15 minutes and rinsed. The root samples were then stained with a mixture of 1:1:1 of glycerol, lactic acid and distilled water and 0.05% methyl blue solution and heated for 5 minutes and then rinsed again. Glycerol (50 %) was added to preserve the root samples and mounted on compound microscope slides for visualizing the fungal structure. Quantification of AM colonization was done based on presence/absence of arbuscules, hyphae or vesicles [14] and percentage of root colonization quantified as follows:

$$\text{AM root colonization (\%)} = \frac{\text{Number of roots colonised}}{\text{Total number of roots examined}} \times 100$$

2.5 Laboratory Analysis of the Soils

Soil pH was determined in 1:1 soil-water suspension [15], organic carbon by Walkley-Black oxidation method [16], total nitrogen (N) by micro- Kjeldahl distillation method [17], available P by Bray 1 method [18], exchangeable K and Na by the flame photometer method, Ca and Mg by EDTA titration method [19]. Particle size analysis was done using hydrometer method [20]. The analyses were carried out at Soil Science and Land Management Laboratory, FUNAAB.

2.6 Data Analysis

Data obtained from this study were subjected to separate ANOVA using PROC GLM in SAS to compute mean and then separated using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

3 Results and Discussions

3.1 Physicochemical Characteristics of Soil and Biochar Chemical Characteristics

The field soil recorded 6.8 pH in H_2O (1:1), 778 g kg^{-1} sand, 134 g kg^{-1} clay, 88 g kg^{-1} silt, 1.48 % OC, 0.21 % N, 10.13 mg kg^{-1} available P, 0.62 cmol kg^{-1} K, 0.5 cmol kg^{-1} Ca, 2.9 cmol kg^{-1} Mg, 0.86 cmol kg^{-1} Na (Table 1). The biochar used for this study recorded 10.2 pH in H_2O (1:1), 14.4 % OC, 1.94 % N, 31.00 mg kg^{-1} total P, 2.29 % K, 0.022 % Mg, and 0.13 % Fe (Table 2).

Table 1: *Physicochemical properties of the soil used for this study*

Properties	Soil
pH H_2O (1:1)	6.8
Sand g kg^{-1}	778
Clay g kg^{-1}	134
Silt g kg^{-1}	88
Textural class	Sandy loam
O C %	1.48
N %	0.21
Available P (mg kg^{-1})	10.13
Exchangeable Bases (cmol kg^{-1})	
K	0.62
Ca	0.5
Mg	2.9
Na	0.86

Table 2: *Chemical composition of biochar used for this study.*

PARAMETERS	BIOCHAR
pH H_2O (1:1)	10.12
O C%	14.4
N%	1.94
Total P (mgkg^{-1})	31.00
K %	2.29
Mg %	0.022
Fe %	0.13

3.2 Mycorrhizal Effect on AM Root Colonization, Soil pH, Organic Carbon and Available P

The results from this study showed that mycorrhizal inoculation in biochar amended soil had little or no contribution to AM root colonization (Table 3), soil pH, organic carbon and available P as shown in Table 4.

Table 3: *Effects of genotype, biochar and AMF inoculation on AM root colonization of tomato plants.*

	% AM root colonization
Genotype (G)	
Ex-Lafia	34.00^a
Ex-Lokoja	33.33^a
SE±	2.35
Biochar rate(B) t ha^{-1}	
0	32.08^a
5	33.75^a
10	40.00^a
15	31.67^a
20	30.83^a
SE±	3.71
AMF (A)	
+	36.00^a
-	31.33^a
SE±	2.35
Interaction	
G*B	ns
G*A	ns
B*A	ns
G*B*A	ns

Means within the same column with the same letters are not significantly different according to Duncan's New Multiple Range Test at ($P<0.05$).

ns: not significant at $P<0.05$; AM arbuscular mycorrhizae, (-) uninoculated, (+) uninoculated.

However, non-mycorrhizal plots had higher values of organic carbon. Non-significant effect of mycorrhiza on root colonization was reported by Osonubi *et al* [21] who found no increase in AM colonization on the field since even uninoculated plot have some indigenous mycorrhiza. Abbott *et al* [10] also reported that mycorrhizal colonization in peanut plants was significantly depressed by adding P. Sanchez *et al* [22] related the reduction in the AM root colonization, and functionality of AMF towards nutrient availability probably to high soil fertility level, which reduced the dependence of the plants on mycorrhizae and therefore restricted the development of these fungi to the root cortex. Since non-mycorrhizal plots were assumed to have less number of mycorrhizal organisms, then, low utilization of organic carbon is expected compared to mycorrhizal plots. This suggests that there was biologically active C in the biochars, which was utilized by soil microorganisms as an energy source [23].

3.3 Biochar Effect on AM Root Colonization, Soil pH, Organic Carbon and Available P

Biochar application didn't influence any increased in AM root colonization as given in Table 3. This agrees with reports by Warnock *et al* [8] who found decrease in AMF abundance due to biochar amendment. Inhibited colonization after biochar amendment might be due to improved availability of P [9]. Moreover, Abbott *et al* [10] reported that mycorrhizal colonization in peanut plants was significantly depressed by adding P. Soil pH was significantly ($P<0.05$) higher with 15 t ha^{-1} and 20 t ha^{-1} followed by 10 t ha^{-1} and then 5 t ha^{-1} and lastly by 0 t ha^{-1}of biochar rates as given in Table 4. The 20 t ha^{-1} of biochar produced significantly ($P<0.05$) higher available P while 15 t ha^{-1} produced higher soil organic carbon when compared with control as given in Table 4. Increase in soil pH, organic carbon and available P in soils treated with increasingly larger quantities of biochar were observed [8; 24; 25]. Soil pH as given in Table 4 and shown in Figure 1 and available P (Table 4) were increased with increase in biochar application rates with little or no influence by mycorrhizal inoculation. This could be attributed to the very strongly alkaline pH of the maize cob biochar and the tendency to raise soil pH was certain. Meanwhile, high organic carbon obtained could be attributed to high organic carbon in the maize-cob biochar [26; 27]. Available P was also higher with biochar application rates and attributed to higher amounts of total P in the maize cob and high pH that could help in breaking the bond of Al and Fe complexes with P in the soil thereby releasing more P into soil solution [28; 29]. No significant interaction was observed among genotype, biochar and AMF in AM root colonization (Table 3). Significant interaction was observed between biochar and AMF and between genotype and AMF in soil pH and available P respectively (Table 4).

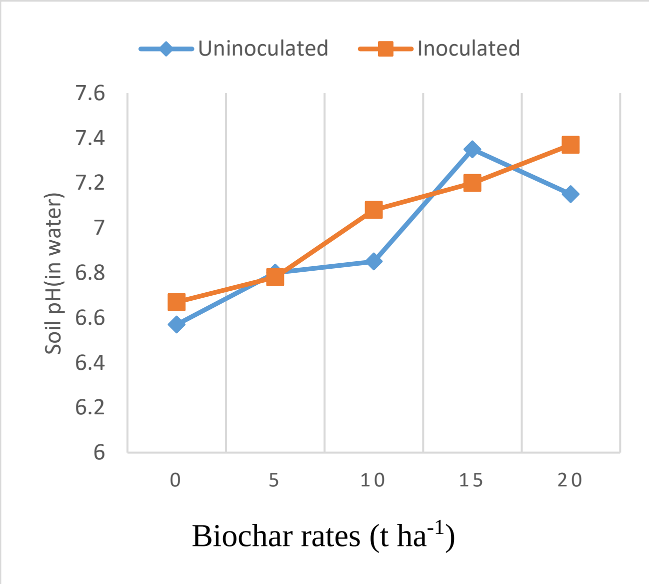

Figure 1: *Soil pH as affected by the interaction of biochar rates and AMF inoculation in the field*

Table 4: *Effects of genotype, biochar and AMF inoculation on soil chemical properties.*

	Soil pH H_2O	% OC	Available P (mg kg^{1})
Genotype (G)			
Ex-Lafia	7.02^{a}	1.26^{b}	11.82^{a}
Ex-Lokoja	6.95^{a}	1.61^{a}	12.52^{a}
SE±	0.03	0.04	0.46
Biochar rate(B) t ha^{-1}			
0	6.62^{d}	1.29^{b}	10.93^{b}
5	6.79^{c}	1.34^{ab}	12.19^{ab}
10	6.97^{b}	1.54^{a}	10.87^{b}
15	7.28^{a}	1.53^{a}	13.05^{ab}
20	7.26^{a}	1.45^{ab}	13.83^{a}
SE±	0.04	0.06	0.72
AMF (A)			
+	7.02^{a}	1.33^{b}	12.08^{a}
-	6.94^{a}	1.53^{a}	12.27^{a}
SE±	0.03	0.04	0.46
Interaction			
G*B	ns	ns	ns
G*A	ns	ns	*
B*A	*	ns	ns
G*B*A	ns	ns	ns

Means within the same column with the same letters are not significantly different according to Duncan's New Multiple Range Test at ($P<0.05$).

ns: not significant at $P<0.05$. (-)uninoculated,(+) uninoculated, OC = Organic carbon, P = Phosphorous.

4 Conclusions

Biochar application remarkably improved soil organic carbon, soil pH and available P. However, the increase in soil pH was not high enough as to have detrimental effect on soil properties. Mycorrhizal inoculation showed little or no effect on AM root colonization, soil organic carbon, soil pH, and available P. It is, therefore, recommended that biochar with very strongly alkaline pH should be used only in soil with low pH for improvement of soil chemical and AM root colonization.

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