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Effects of biochar and gypsum soil amendments on groundnut (*Arachis hypogaea* L.) dry matter yield and selected soil properties under water stress

Munsanda Ngulube[1], Alice Mutiti Mweetwa[1*], Elijah Phiri[1], Samuel Christopher Muriu Njoroge[2], Hendrix Chalwe[1], Victor Shitumbanuma [1] and Rick Lynn Brandenburg[3]

[1]Department of Soil Sciences, School of Agricultural Sciences, University of Zambia, Box 32379, Lusaka, Zambia.
[2]ICRISAT/CGIAR, Chitedze Agricultural Research Station, P. O. Box 1096, Lilongwe, Malawi.
[3]Department of Entomology and Plant Pathology, North Carolina State University 3305 Garden Hall, Campus Box 7613, Raleigh, NC 27695-7613, USA.

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The effects of amending soil with gypsum and biochar on groundnut chlorophyll concentration, water use efficiency (WUE), biomass yield and selected soil properties were investigated under water stress. Gypsum ($CaSO_4.2H_2O$) was applied at 0 and 200 kg/ha, groundnut shell biochar at 1, 2 and 4% *w/w* of soil, and water at 100, 70 and 40% of daily plant water requirement (PWR) as main, sub and sub-sub plots, respectively, in a split-split-plot design. Biochar neutralized the acid soil, significantly raising soil pH from 5 to 7.15 and increasing cation exchange capacity by 75%. Biochar amended at 1 and 2%, increased groundnut dry matter yield by 28%. The optimum biochar application rate for dry matter yield was 1.4% w/w. Biochar application at 4% and irrigation at 40% of PWR reduced the WUE by 45 and 50%, respectively. Chlorophyll concentration index was highest at 40% of PWR. The results suggest that biochar has potential to raise soil pH, increase moisture retention and improve crop performance. Applying water at 100% PWR can increase groundnut dry matter yields, while higher gypsum application rates may be required to affect crop performance.

Key words: Biochar, dry matter, crop evapotranspiration, groundnut gypsum, water use efficiency.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is nutritious, rich in protein, carbohydrate, fibre, unsaturated fats, and minerals such as phosphorus (P), calcium (Ca), magnesium (Mg), vitamins E, B complex and vitamin K (Settaluri et al., 2012). It is the second most cultivated crop after maize in Zambia, consumed as a major source of protein, used as animal feed, and an important fertiliser crop because it fixes nitrogen, making it a very lucrative cash crop (Mukuka and Shipekesa, 2013). However, production and yields are low (690 kg/ha) and approximately only 50 to 70% of the potential yield under rainfed conditions (FAOSTAT, 2016). This is mainly due to climatic and soil constrains such as; poor rainfall distribution, soil acidity, low soil Ca and low soil moisture

*Corresponding author. E-mail: alicemweetwa@yahoo.com. Tel: 260-971-803-918.

retention. In addition, pests, disease, poor agronomic practices such as late planting, weeding, and planting uncertified poor quality seeds also reduce crop yields (Chabala et al., 2014: Tunwari et al., 2018). Traditionally, it is a rain-fed crop, with most groundnut farmers being small-holder; they have limited resources to and seldom use inputs (fertilisers, pesticides, lime, and supplemental irrigation systems) to address these production constraints (Mukuka and Shipekesa, 2013).

Groundnut is sensitive to drought stress at flowering and pegging which reduces fruit set. The availability of Ca in the soil for plant uptake is largely dependent on the soil moisture levels in the geocarposphere; so during periods of drought, groundnuts show deficiencies of Ca and cracks in pod shells tend to develop resulting in reduced phytoalexin production, increased number of pops, cracked pods, and increased susceptibility of kernels to aflatoxin contamination by *Aspergillus* spp. infection (Njoroge et al., 2013). Ca can be supplied in several forms, for example, as a sole lime application which has been shown to reduce aflatoxin contamination by 72% (Waliyar et al., 2013). Gypsum application rates for groundnuts range from 200 to 1000 kg/ha when less than 0.25 cmol/kg Ca is present in the soil (Nyambok, 2011).

Biochar is the product after any organic material is charred in the presence of limited O_2, by a process called pyrolysis (Abel et al., 2013), and can be used as a soil amendment. Studies have shown that biochar as a soil amendment has unique properties which allow it to offset some climatic and soil constraints brought about by changing climate. When amended to soil, biochar can improve the fertility of soil by buffering against temperature fluctuations, neutralizing acidity, increasing cation exchange capacity (CEC), increasing base saturation, increasing organic matter content, sequestering carbon, improving nutrient retention and increasing moisture retention (Cornelissen et al., 2013). Martinsen et al. (2014) and Xu et al. (2015) found that incorporation of biochar to soils planted with maize and groundnuts significantly increased yields.

Constraints affecting groundnut yield such as low soil Ca, soil acidity and exposure to prolonged dry spells have generally been investigated independently. However, these soil constraints rarely occur independently. This paper reports the sole and combined effects of gypsum and biochar on leaf chlorophyll concentration index (CCI), biomass dry matter (DM), water use efficiency (WUE), crop evapotranspiration (ETc) of the groundnut crop under water stress, and on the effects of biochar on soil pH and CEC.

MATERIALS AND METHODS

Site description and soil sampling

Soil was collected from the experimental site at the Agricultural Technology Development Centre (ATDC) of the University of Zambia Agricultural Demonstration Centre, located in Chongwe, Zambia (latitude 15° 21' 25" South and longitude 28° 27' 25" East, 1,260 m above sea level). The field was used to grow maize in the previous cropping season (2015/2016). This site falls in Agro-ecological region IIa of Zambia (receives an average rainfall of 800 to 1,000 mm/y during the cropping season that runs from November to March). The soil is sandy loam, belongs to the Chromic Luvisol taxonomy based on the World Reference Base (WRB) Classification System (IUSS Working Group WRB, 2015). For the greenhouse pot experiment and soil characterization, subsamples were collected randomly across the field at a depth of 0 to 20 cm to form a composite soil sample.

Soil characterization

The fine earth fraction of the composite soil sample was analysed for selected chemical and physical properties. The soil reaction (pH) was measured in 0.01 M $CaCl_2$ with a 1:2.5 soil: solution ratio (Van Reeuwijk, 1992) read on a pH meter (Hanna, HI2210-01 Benchtop pH/mV Meter). The EC was measured in a 1:5 soil: solution ratio (Richards, 1954) using a conductivity meter (Hanna, HI98312 DiST® 6 EC/TDS/temperature Tester). Available P was determined by the Bray 1 Method (Bray and Kurtz, 1945) read on a spectrophotometer (UV/Visible, Jenway 6305). The total N and organic matter were determined by Kjeldahl method (Bremner and Mulvaney, 1982), and Walkley and Black (1934) chromate reduction method, respectively. Exchangeable acidity (Al^{+3} and H^+) was determined by the titration method (McLean 1965), while exchangeable bases (K^+, Mg^{+2} and Ca^{+2}) and CEC were determined by the leaching method (Rowell, 1994). The bases were read on a Flame Atomic Absorption Spectrophotometer (AAS Perkin Elmer Analyst 400). The hydrometer method (Day, 1965) was used to determine the soil texture, and bulk density was determined according to the Blake (1965) Core Ring Method.

Biochar production, characterization and gypsum characterization

Groundnut shells underwent pyrolysis in a homemade kiln to produce biochar. The biochar was pounded with a mortar and pestle and passed through a 1 mm sieve for characterization. The CEC was determined by the leaching method (Rowell, 1994), total C (Walkley and Black, 1934), total N was determined using the Kjeldahl method (Bremner and Mulvaney, 1982) and neutralizing value (NV) using the titrimetric method (Faithfull, 2002). Dry ashing (Campbell and Plank, 1998) and 30 ml of 1 M HNO_3 was used for the extraction of total P and total bases Ca, Mg, K, and Na. The P was read on a Jenway 6305 UV/Visible spectrophotometer and the bases were read on a Perkin Elmer Analyst 400 flame AAS. The ash content was determined according to the American Society for Testing and Materials (ASTM) D1752-84 (2007) at 750°C. Gypsum was characterized for Ca% and S% according to the American Society for Testing and Materials (ASTM) C 471M – 01(2002), where Ca% was read on the AAS and S% was determined by weight using barium chloride.

Greenhouse experiment

At the University of Zambia (UNZA) in the school of Agricultural Sciences (at 15° 23' 24" S and 28° 19'48" E, at an altitude of 1260 m) a greenhouse pot experiment was set up in a spilt-split plot experimental design. The treatments comprised a combination of three factors; gypsum ($CaSO_4.2H_2O$ which contained 28% Ca and 11% S) at 0 and 200 kg/ha, groundnut shell biochar at 0, 1, 2 and

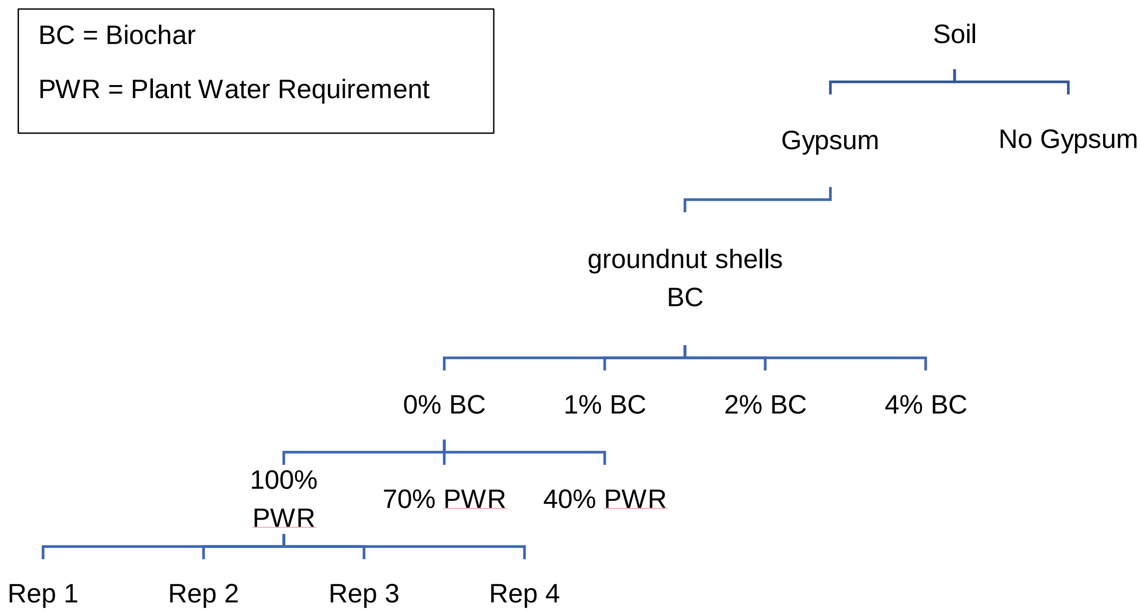

Figure 1. Treatment combinations in greenhouse pot experiment.

Table 1. Selected soil characteristics.

USDA textural class	Soil reaction (pH) in 0.01 $CaCl_2$	Exchangeable acidity (cmol/kg)	EC (mS/cm)	Total N (%)	Available N (mg/kg)		Plant available P	Organic matter	C: N ratio
					NH_4^+	NO_3^-	(mg/kg)	(%)	
Sandy loam	4.02	0.26	0.13	0.05	13.58	17.66	12.26	0.98	10:01
Critical levels	6.5		≤ 3.2	0.2	-	-	10.0	2.0	20:1

4% (*w/w* in 5 kg soil), and daily plant water requirement (PWR) at 100, 70 and 40%, giving a total of 24 treatments (Figure 1). Each treatment combination had 4 replications giving a total sample size of 96 plants. Biochar was homogeneously mixed into the soil at planting and all treatments received the optimum PWR (100%) calculated using the Food and Agriculture Organization (FAO) irrigation scheduling program CROPWAT version 8.0. The MGV 5 groundnut variety was pre-germinated by incubating the seed at 25°C for 7 days on moist Petri dishes. Four germinated seeds were planted per pot, and then thinned to 1 plant per pot after 10 days. Gypsum was applied when the first flowers appeared (40 days after planting (DAP), while plant water stress treatments were introduced at full bloom (59 DAP) until maturity.

The drainage, change in water storage (by weight), evaporation (evaporation-pan), maximum and minimum daily ambient temperatures (maximum-minimum thermometer) were measured throughout the growing season. The soil pH readings were taken at 35 DAP, by inserting a direct pH meter electrode (SCT-pH-PEN-5, Boston, USA) into the soil. The chlorophyll meter readings (SCMR) were taken at the vegetative stage (V3), first reproductive stage (R1) and third reproductive stages (R3) which were at 41, 54, 99 DAP, respectively; each recorded between 10 and 11 AM using a portable SPAD Chlorophyll Meter (CCM-2000 plus). Fungal diseases and insect pests were controlled by periodical spraying of insecticides and fungicides. At maturity (182 DAP), the biomass and pods were harvested. For each plant, the roots, shoots and pods were separated, and sun dried for 4 days and then weighed.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the effects of biochar on the soil pH and CEC. A 3-way ANOVA was used to determine the effects of biochar, gypsum and water on the CCI, DM, water balance components and WUE of the crop under water stress. Fisher's least significant difference (LSD) was used to separate the treatment means. Simple correlation was also done where the ANOVA showed significant differences. The data was analysed using R Statistical Package (Version 3.3.2) as a split-split plot design.

RESULTS

The soil used in the study was a sandy loam with a low soil pH of 4.02. Some selected soil properties are presented in Table 1. The biochar had a high pH of 10.34 and high ash content (24.48%). The total N and organic carbon content were 1.24 and 18.7%, respectively, giving a C:N ratio of 15:1. Important biochar properties are

Table 2. Selected biochar characteristics.

Total N (%)	Total P (mg/kg)	Total cations (g/kg)				CEC (cmol(+)/kg)	Organic carbon (%)	C: N ratio	Ash (%)
		Na^+	K^+	Ca^{2+}	Mg^{2+}				
1.24	5.32	6.30	10.99	11.92	10.00	11.25	18.7	15 :1	24.48

Table 3. Effect of biochar on soil bulk density, exchangeable bases, cation exchange capacity (CEC) and soil reaction (pH).

Treatment	Bulk density (g/cm^3)	Exchangeable bases (cmol(+)/kg)				CEC (cmol(+)/kg)	Soil reaction (pH) in water
		Na^+	K^+	Ca^{2+}	Mg^{2+}		
Soil	1.31	0.22	0.04	0.51	0.24	2.00^c	5.00^d
Soil + 1 % Biochar	1.40	0.24	0.08	0.45	0.26	3.20^{bc}	6.03^c
Soil + 2 % Biochar	1.36	0.27	0.10	0.48	0.30	3.25^{ab}	6.38^b
Soil + 4 % Biochar	1.35	0.28	0.16	0.48	0.32	3.50^a	7.15^a
CV (%)	2.26	2.16	2.16	2.16	2.16	2.18	1.99
P- value	0.84	0.43	<0.001***	0.71	<0.001***	0.04*	<0.001***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 Means followed by the same letter in a column are not significantly different at α=0.05.

presented in Table 2.

Incorporation of biochar into the soil at 1, 2 and 4% biochar, had very little effect on the bulk density, Na^+ and Ca^{+2} but significantly raised the pH (*P* <0.001), K^+(*P* <0.001), Mg^{+2}(*P* <0.001) and CEC (*P*=0.04) (Table 3). The exchangeable bases in the biochar were found to be at 0.69, 5.89, 0.5, 0.38 cmol (+)/kg for Na^+, K^+, Ca^{+2} and Mg^{+2}, respectively. The biochar contained a higher level of K^+ as compared to Na^+, Ca^{+2} and Mg^{+2}, which raised the soil K^+ from 0.04 cmol (+)/kg to 0.16 cmol (+)/kg at 4% biochar. The soil CEC was also raised from 2 to 3.5 cmol (+)/kg at 4% biochar.

The results show that applying water at 100, 70 and 40% PWR only had a significant effect on the chlorophyll concentration at the third reproductive stage (R3) (99 DAP)(Figure 2). Water applied at 40% PWR increased CCI by 22% at R3 as compared to water applied at 100% PWR.

Biochar application had a significant effect on the chlorophyll concentration at all three stages of crop growth. The chlorophyll concentration readings ranged from 23.3 to 32.99, 22.9 to 33.47 and 20.4 to 33.7 CCI at V3, R1 and R3, respectively with CCI differences of 42, 46 and 40% at V3, R1 and R3, respectively among biochar treatments. Gypsum had no significant effect on the CCI at all stages of growth.

The groundnut dry biomass yield increased with the addition of biochar and was highest at 1% (97.25 kg/ha) and lowest at 4% (43.58 kg/ha) (Figure 3). Applying biochar at 1% increased DM by 28%, while 4% reduced the DM by 43% and the optimum application rate of biochar was at 1.42% w/w. Applying 100% PWR gave DM of 2 and 3-fold greater than at 70 and 40% PWR, while gypsum had no significant effect. The pooled effect of biochar, gypsum and water on dry matter yield (DM) of the groundnuts at maturity (182 days) is presented in Figure 4.

Gypsum application did not directly or indirectly affect the change in soil water storage (dS), drainage (D), crop evapotranspiration (ETc) as displayed in Figure 5. Application of biochar also had no significant effect on dS and ETc (P = 0.896 and 0.563, respectively) but significantly affected the D (P = 0.0076) (Figure 6). The water application rates had no significant effect on dS (P = 0.394) but significantly affected the D and ETc components (P < 0.001 and P < 0.001, respectively (Figure 7). The D and ETc components were highest at 100% PWR while at 40% PWR resulted in a 35% decrease in ETc.

The effect of biochar, gypsum and water on water use efficiency of root (WUE_R), shoot (WUE_S) and total (WUE_T) biomass is displayed in Figure 9. Biochar at 1 and 2% had no effect on WUE_T, while 4% biochar significantly reduced the WUE_T by 45%. Water applied at 40, 70 and 100% had a significant effect (P<0.001) on the WUE as it ranged from 0.018 to 0.036 g/mm, representing a 50% reduction in WUE at 40% PWR.

DISCUSSION

The soil used in this study belongs to the Chromic Luvisol taxonomy (IUSS Working Group WRB, 2015). The pH of the soil was 4.02 which is extremely acidic (Hazelton and Murphy, 2007) and below the optimal pH range of pH 5.5 (slightly acidic) to 7.0 (neutral) suitable for growing groundnuts (Nyambok, 2011). This might have resulted in deficiencies in exchangeable bases (K, Ca, and Mg), P and molybdenum. Even at this low pH, P (12.3 mg/kg) was adequate for cop production. This could be attributed

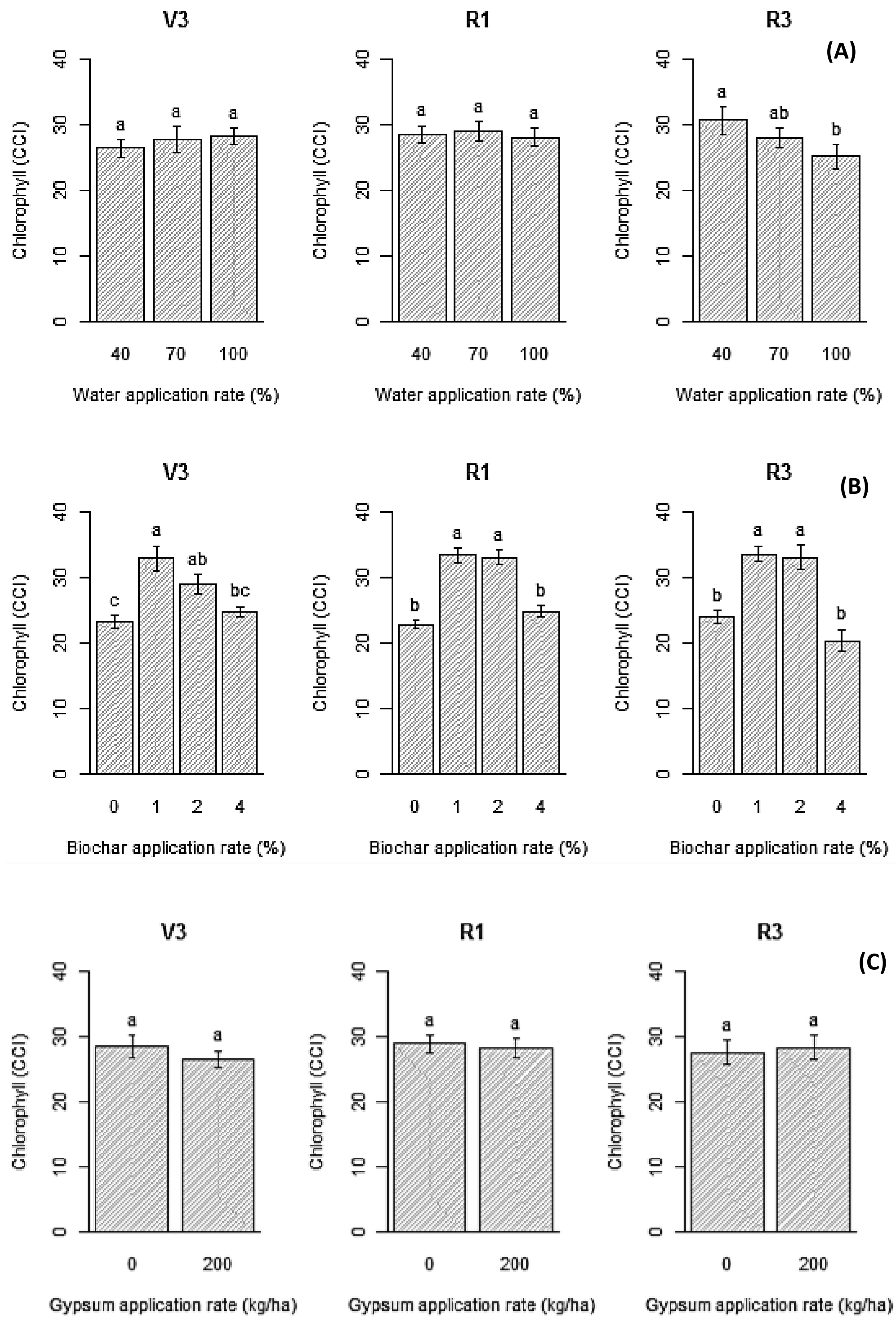

Figure 2. (A) Effect of water application rates on chlorophyll concentration at V3, R1 and R3. (B) Effect of biochar application rates on chlorophyll concentration at V3, R1 and R3. (C) Effect of gypsum on chlorophyll concentration at V3, R1 and R3.

to residual P from fertilizer application in the previous growing season. Sub-optimal levels of P and molybdenum inhibit early root development in legumes (Muhati et al., 2011).

The biochar was notably higher in K, Ca and Mg as compared to Na. High levels of Na are not desirable as this leads to the destruction of soil structure and additionally, this is not a plant nutrient. The ash content

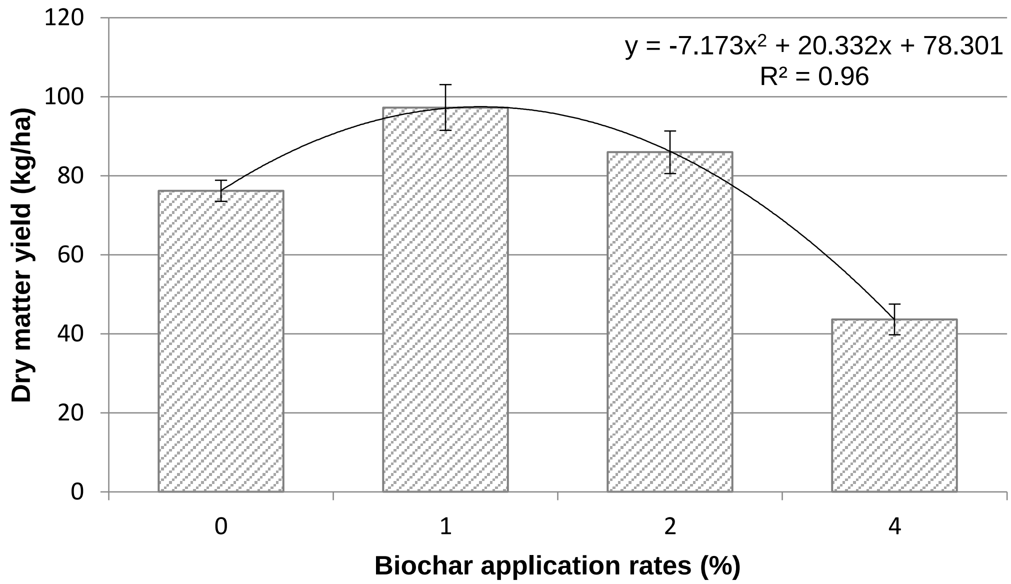

Figure 3. Effect of biochar application rate on the groundnut dry matter yield at maturity (182 days).

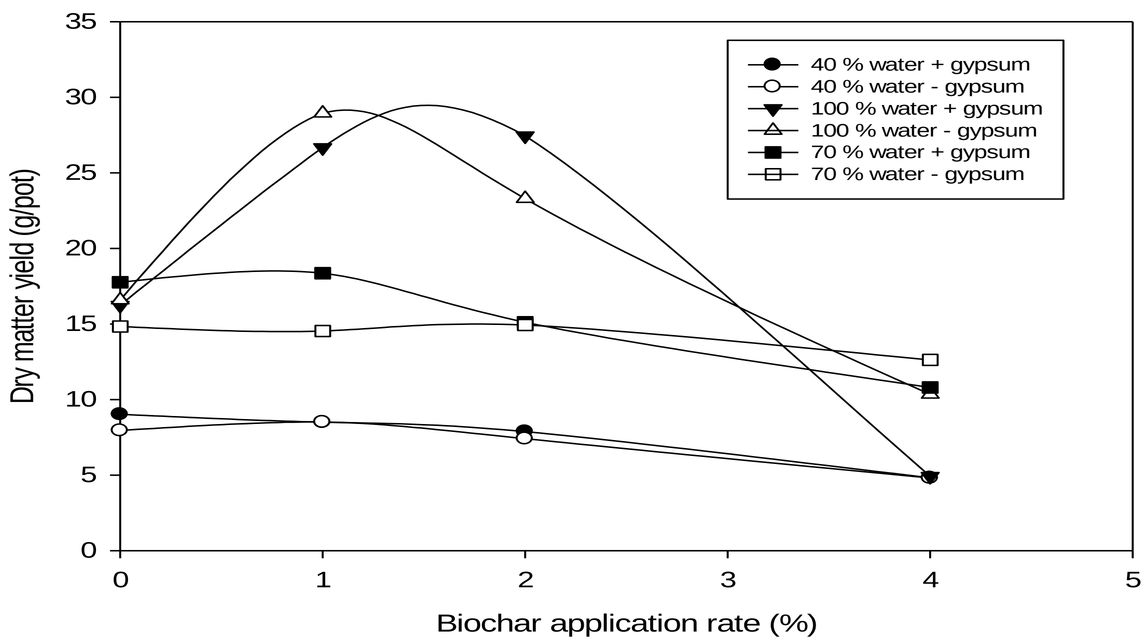

Figure 4. Effect of biochar, gypsum and water application rates on groundnut dry matter yield at maturity (182 days).

was rather high at 24.5%, indicating a high mineral content. The C/N ratio was 15 which is within the general range of 7 and 500 for biochar C/N ratios (Herbert et al., 2012). This narrow C/N ratio indicates a potential for this biochar to supply N to the groundnut crop (Singer and Munns, 1987). The neutralizing value was too low to be measured. The biochar neutralized the acidic soil by raising the soil pH from 5 to 7.15 at 4% biochar. The characteristic large specific surface area of the biochar (Lehmann et al., 2011) and high concentration of total bases (Table 2) readily displace the H^+ ions on the soil colloids by adsorption, thereby raising the low pH of the soil. There was a positive linear relationship that gave R^2 = 0.8698 between CEC and soil pH at different rates of biochar applied (Martinsen et al., 2014).

Water and gypsum had no effect on the chlorophyll

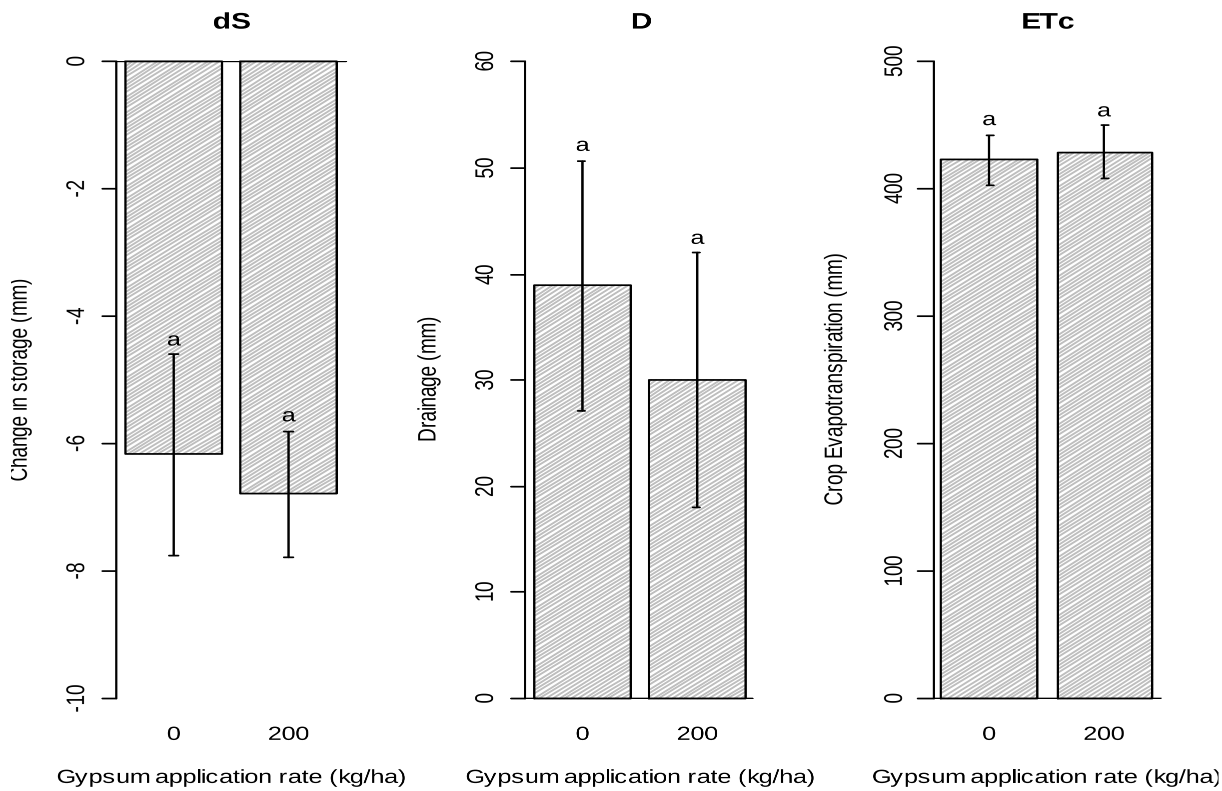

Figure 5. Effect of applying gypsum on change in storage (dS), drainage and crop evapotranspiration (ETc).

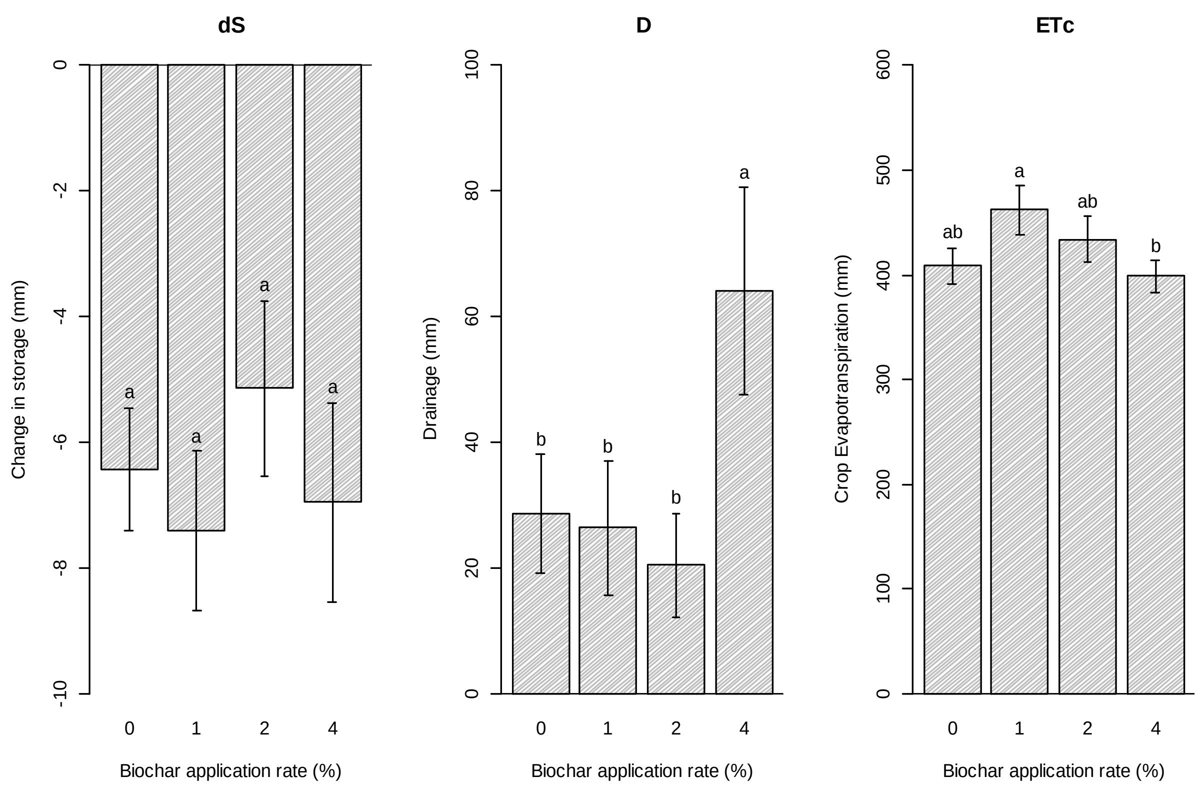

Figure 6. Effect of biochar application on soil water balance components; change in storage (dS), drainage (D) and crop evapotranspiration (ETc).

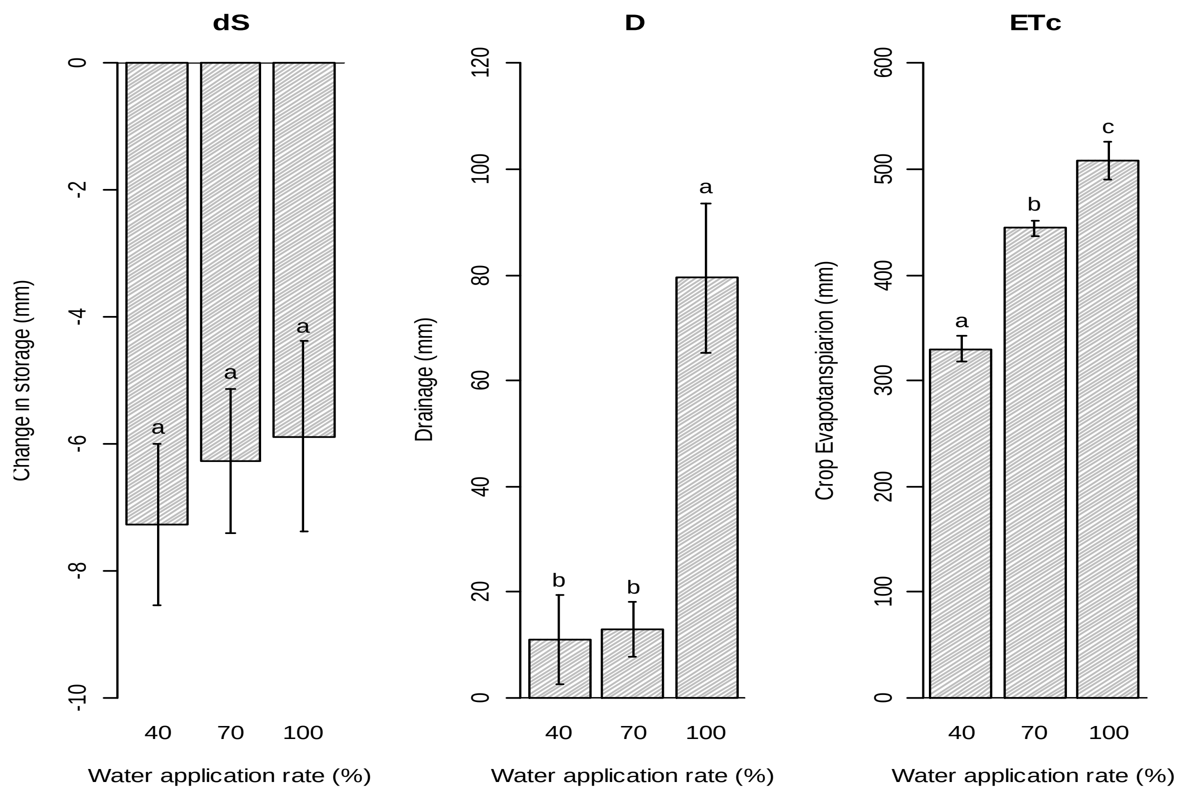

Figure 7. Effect of water application on soil water balance components; change in storage (dS), drainage (D) and crop evapotranspiration (ETc).

concentration at V3 and R1 because the treatments had not yet been initiated at these two stages. At R3, increase in water application rate resulted in a reduction (P=0.02) in chlorophyll concentration, where the chlorophyll readings were 30.71, 28.10 and 25.22 CCI for 40, 70 and 100% PWR, respectively. The highest leaf chlorophyll reading was in the 40% PWR treatments. This observation contradicts research findings by Akhkha et al. (2011) in wheat under deficit water conditions, where the chlorophyll concentration reduced with an increase in water stress. The drop-in chlorophyll production as the water application rate increased may have been due to leaching of ions essential for chlorophyll formation such as N, Mg and S (Marschner, 2012; Mathowa et al., 2012) associated with high drainage rate. Gypsum had no effect on the CCI at R3 possibly due to the low rate of application (200 kg/ha). Some studies have shown that trial application of gypsum at 50 mg/kg results in the maximal chlorophyll content in lettuce (Prasit et al., 2009)

Application of biochar had significant effect on the chlorophyll concentration at all three stages of crop growth (V3, R1 and R3). The CCI readings were lowest at 0 and 4% biochar and highest at 1 and 2%. The low CCI at 0% biochar was because of the low soil pH which affected nutrient availability, thus inhibiting plant growth and chlorophyll production. Nutrients such as N, P and Mg are directly involved in chlorophyll production, these nutrients tends to be low in acidic soil. At 4% biochar, the low CCI may have been because of 4% biochar having had the highest drainage across the biochar treatments; therefore, nutrient leaching was also highly likely. This may have resulted in nutrient deficiency which retarded crop growth and reduced chlorophyll production (Mathowa et al., 2012).

At 40% PWR, the application of gypsum to the soil had no effect on the DM regardless of the level of biochar incorporated in the soil (Figure 4). Moisture is a key factor for crop development and also affects availability of nutrients in the soil solution such as Ca (Gascho and Davis, 1994). The lower CCI observed at 4% biochar and 100% PWR could either be attributed to high moisture levels in the root environment leading to anaerobic conditions or to leaching of nutrients through drainage. The excess water resulted in retardation of crop development and growth at 4% biochar.

Application of biochar at 1 and 2 % was beneficial to the plant as it increased the dry matter yield. At 100% PWR and biochar application rate of 1.42% w/w the highest DM yield for groundnuts was achieved which agrees with a study by Xu et al. (2015), where groundnut biomass and pod yields increased by 2- and 3- times on the red ferrosol and redoxi-hydrosol when biochar was

Figure 8. The effect of biochar application at 0, 1, 2 and 4% (left to right) on groundnut plant growth at 100 PWR.

incorporated at 0.375 to 6.00% w/w, respectively. Martinsen et al. (2014) also detected a similar effect with addition of biochar resulting in an increase in maize yield though not of groundnuts because the presence of biochar increased plant available water (PAW), CEC, available K^+, pH, in the acid tropical soils. Overall only biochar and water influenced the crop development.

Application of biochar had no significant effect on dS, ETc and D. Although gypsum supplies the soil with Ca and P, its application did not enhance crop growth or affect the dS, D and ETc components. Typically Ca and P are known to be involved in plant cell elongation and protein synthesis (Jain et al., 2011; Kumar and Sharma, 2013), but gypsum did not enhance crop growth, therefore had no effect on the dS, D or ETc. The higher the biochar application rate, the higher the soil moisture retention between irrigation intervals was observed, leaving less space in the soil pores to hold more water at each irrigation; allowing more drainage at higher biochar application rates (4%). At the highest biochar application rate, smaller groundnut plants were observed. This diminished stature resulted in reduced water uptake and subsequent larger volumes of excess water loss as drainage. On the other hand, 0, 1 and 2% biochar drained the least amount of water as the soil was not saturated and plants were larger which took up more water (Figure 8).

The drainage was highest where 100% PWR was applied because more water was applied to the soil each day. The ETc was highest where 100% PWR was applied, followed by 70%. The trend of decrease in ETc with a decrease in water applied to the soil was expected. As aC3 crop species, water stress leads to an increase in photorespiration; in order to prevent more water loss through transpiration stomata begin to close (Akhkha et al., 2011). Application of gypsum did not affect the WUE_R, WUE_S and WUE_T. This could be because the gypsum applied to the low Ca soil was too low to enhance crop growth as earlier alluded to concerning the DM. On the other hand, application of biochar had a substantial effect on the WUE_R, WUE_S and WUE_T. Biochar application at 0, 1 and 2% resulted in higher WUE, while application at 4% had a negative effect. Excess water at 4% could have inhibited plant growth due to nutrient leaching and anaerobic conditions, as previously mentioned. Water, like biochar had a notable effect on the WUE_R, WUE_S and WUE_T. The general trend was that the less water applied, the lower the WUE_R, WUE_S and WUE_T, because the more moisture stress the crop experienced, the less it was able to use water efficiently (Songsri et al., 2013).

Conclusion

Incorporation of biochar in the soil significantly increased the pH of the strongly acidic soil to neutral and also significantly increased the CEC of the soil. Applying biochar to the soil at rates of 1 and 2% w/w biochar significantly increased leaf CCI (at V3, R1 and R3 growth stages) and DM yield. The results suggest that biochar application at 1.42% could give the best response for groundnut production. Applying biochar to the soil had no significant effect on the ETc. Gypsum applied at 200 kg/ha did not affect the leaf chlorophyll concentration,

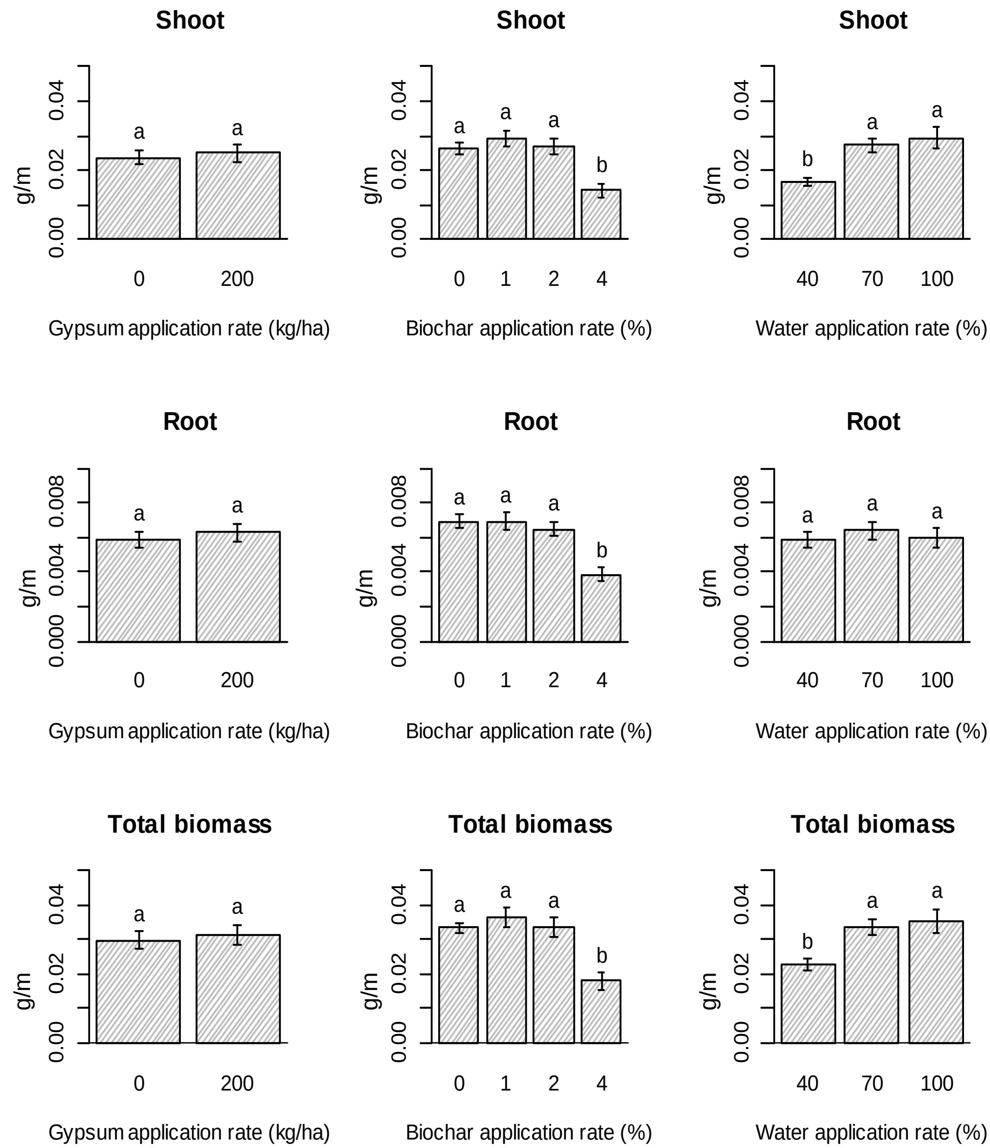

Figure 9. Effect of biochar, gypsum and water on water use efficiency (WUE) of shoot, root and total biomass.

DM, ETc and WUE as it was not sufficient to notably contribute to the crop growth in this low calcium soil. There was no significant interactive effect of applying gypsum and biochar on the CCI, DM, ETc and crop WUE under water stress conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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