# Effects of biochar application on potting media chemical properties, arbuscular mycorrhizal fungi spore density, growth and nutrient uptake of sorghum (*Sorghum vulgare* L.)

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**Abstract**. Two sets of laboratory incubation and pot experiments were conducted to characterize the chemical properties and to evaluate arbuscular mycorrhizal fungi (AMF) abundance, growth and nutrient uptake of sorghum grown in biochar–amended soil-sand potting media. Both experiments used pasteurized sand and two acid soils (Ultisol and Entisol) as the base substrate with rice hull char (RHC), coconut sawdust char (CSC), peanut hull char (PHC) and corn cob char (CCC) as potting amendment. A soil and sand without biochar served as the control. Application of PHC in Ultisol-based potting mix had shown marked increase in pH, electrical conductivity (EC), total N, total P, exchangeable K, Ca and Mg and decreased exchangeable acidity and Al. Similarly, PHC application in Entisol-based potting mix increased pH, total organic carbon (OC), total N, total P, exchangeable K and a significant decrease in exchangeable acidity and Al. On the other hand, CSC application in Ultisol- and Entisol-based potting mix resulted in higher EC and exchangeable Na values. Application of PHC and CSC in Entisol-based mix depressed plant height and biomass production. In Ultisol-based potting mixes, improved P concentration and uptake was obtained from PHC and RHC addition. Except for RHC, biochar application had reduced spore counts. Among biochar evaluated, PHC had the most superior influence on the chemical properties of potting mixes. However, increased in pH and P due to PHC and CSC application posed a detrimental effect on plant growth, biomass production and AMF abundance.
**Key Words**: biochar, arbuscular mycorrhizal fungi, Ultisol, Entisol, potting mix.

**Introduction**. Biochar is a stable and sterile solid carbon (C)-rich by-product of biomass pyrolysis, primarily intended as soil amendment to enhance soil quality and sequester C (Mukherjee et al 2011). Biochar is highly porous, nutrient-rich, usually alkaline and exhibit large specific surface area (Jindo et al 2014; Piash et al 2016). Biochar can boost soil fertility of degraded soils and reduce soil acidity by raising pH in acid soils. It also enhances water holding capacity, cation exchange capacity (CEC), adsorption of plant nutrients and creates suitable condition for soil micro-organisms (Ishii & Kadoya 1994; Lehmann et al 2003). As such, biochar can be utilized as component of potting medium for inoculum production of arbuscular mycorrhizal fungi (AMF).

The AMF play a key role in natural and agricultural ecosystems. As symbionts, AMF play an important role in plant nutrition by improving access to nutrients particularly phosphorus (P) and plant health by providing protection against soil-borne pathogens, heavy metal uptake regulation, salinity and drought tolerance and enhanced soil aggregation (Khade & Rodrigues 2009; Garg & Chandel 2010). In return, the fungi receive carbohydrates (sugars) and growth factors from the host plant (Habte 2000). With the benefits that can be provided by AMF on plant productivity and soil quality, AMF are essential to the sustainability of soil-plant systems.

Biochar can enhance mycorrhizal abundance and functioning due to the provision of suitable habitat for AMF to colonize, grow and reproduce (Thies & Rillig 2009). The surfaces and pores of biochar have the ability to adsorb soluble organic matter, gases

and inorganic nutrients which creates better environment for AMF (Thies & Rillig 2009). Biochar pores may also protect AMF against fungal grazers and can stimulate spore germination of AMF (Warnock et al 2007; Rillig et al 2010). Previous studies indicate that biochar amendments can increase mycorrhizal abundance and percent colonization in plants which resulted to better nutrient uptake thereby increasing plant growth and production (Ishii & Kadoya 1994; Yamato et al 2006; Wathira et al 2016). However, in another study of Warnock et al (2010) using non-herbaceous biochar, AMF abundance decreased with increased biochar application rates. This contrasting effect was attributed to the changes in soil properties brought about by the addition of biochar with varying properties and amount applied.

The most widely adapted technology for AMF inoculum production is pot-based culture with soil-sand mixture as common substrate (Kapoor et al 2008). The fungi are allowed to grow and multiply in conjunction with suitable host plant roots. Although the soil-and sand-based inocula are quite easy to obtain, these kinds of inocula are too heavy for extensive use in agriculture and forestry as well as environmental reclamation. Incorporation of unmodified organic materials, on the other hand, may lower stability of the inoculum since these materials are prone to microbial decomposition. In addition, these materials may contain pathogens which then lower the quality of resulting inoculant. These problems associated with the use of soil, sand or unmodified organic material as substrate may be overcome by using biochar as component of potting mix for multiplication of AMF.

Although biochar application has shown positive effects on AMF population, the magnitude of AMF response varies with the type of biochar and soil. Hence, a detailed understanding of how chemical characteristics of biochar influence AMF, as well as growth and nutrient uptake of the host plant is essential. Understanding the influence of biochar on the characteristics of potting mix is important to assess its potential as component in pot culture of AMF. Hence, the objectives of this study were: 1) to characterize the chemical properties biochar-amended acid soil-sand potting mixes; and 2) to evaluate the AMF abundance, growth and nutrient uptake of sorghum (*Sorghum vulgare* L.) grown in biochar-amended soil-sand potting media. It was hypothesized that the different biochars as potting mix component would have different effects on the chemical properties of potting media and consequently would have varied influences on AMF spore density and plant growth and nutrient uptake.

## Material and Method

***Collection, preparation, and analyses of soil and sand***. Two contrasting soils were collected from the soil surface (0-20 cm depth) of a grassland area of Biliran and PhilRootcrops experimental station of Visayas State University, Baybay City, Leyte, Philippines which will be referred to as Ultisol and Entisol respectively in succeeding sections. The soil from Biliran was a weathered soil characterized with deep red color clay texture (Soil Survey Division Bureau of Soils & Water Management 1993). The area was primarily grassland but had been cropped with *Cocos nucifera* and *Musa acuminata*. The cropped land soil of Leyte was a moderately young clay soil developed from alluvial deposits. This soil has been previously planted with *Colocasia esculenta* and *Ipomoea batatas* and had a history of inorganic fertilizer application. Both soils are clayey in texture with particle size distribution of 22.1% sand, 15% silt and 62.9% clay for Ultisol and 30.3% sand, 26.1% silt and 43.6% clay for Entisol. While sand has particle size distribution of 57.1 sand, 30% silt and 12.9% clay.

The sand was collected from Calbigaa River of Brgy. Pangasugan, Baybay City, Leyte, Philippines. Subsequently, the river sand was air-dried, sieved at 2-mm and washed 15-20 times with tap water. The sieved soils and sand were pasteurized separately at 325$^{o}$C for three consecutive days at 3 h per day. Subsamples were taken for determination of initial chemical characteristics of pasteurized soils and sand and the rest was prepared for incubation and pot experiments. The experiment was conducted under shed house condition of the Department of Soil Science, VSU, Philippines from January 2015 to June 2015.

The pH and electrical conductivity (EC) of pasteurized soils and sand in distilled water (1:5, w/v) were measured using pH and EC meters (Rayment & Higginson 1992). The exchangeable acidity was determined with 1 N KCl extraction and quantified by titrating the resulting extract with 0.1 N NaOH (Thomas 1982). The exchangeable aluminum (Al) was analyzed in the KCL extract from exchangeable acidity determination and was added with 1 N NAF and then back titrated with 0.1 N HCl solution. The total organic carbon (OC) was quantified using the Heane's method (Heanes 1984). To determine total nitrogen (N) and P, the samples were digested using Kjeldahl digestion mixture. The concentrations of N in the digest were quantified using the method of Baethgen & Alley (1989) while total P concentration was determined following the method of Murphy & Riley (1962). The potential CEC and exchangeable bases were measured using the ammonium acetate (pH = 7) method (Thomas 1982).

***Biochar production and characterization***. Rice hull, coconut sawdust, peanut hull and corn cob were air-dried and charred separately using a fabricated pyrolyzer. The pyrolyzer was from a modified metal drum (40 cm height x 15 cm diameter) with a thermometer attached to its external wall for temperature monitoring. The reactor had also ventilation tube and slit with the lid. Approximately 15 kg each of the feedstock was loaded into the reactor through the slit, covered with lid and was then heated at 400-450$^{o}$C for 3-5 h using firewood as fuel. Charring time for rice hull char (RHC) was 5 h, 3 h for coconut sawdust char (CSC), while 4 h for peanut hull char (PHC) and corn cob char (CCC). The percent recovery for RHC, CSC, PHC and CCC was 35, 26, 45 and 29% respectively. After the overnight cooling of charred material, the biochar was sifted through 2 mm-sieve. Subsequently, a subsample was taken for characterization and the rest was used for the potting medium additive.

The pH, EC, total OC, total N and P as well as potential CEC of four biochars were measured using the previously described methods for assessment of the initial characteristics of soil and sand. The total potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were extracted by wet digestion and the concentration of the cations in the digest were quantified using atomic absorption spectrophotometer.

***Incubation experiments***. The effects of different types of biochar on the chemical properties of the potting media were evaluated through two separate incubation experiments using an Ultisol and Entisol as the base substrate soil in Experiments 1 and 2 respectively. In both experiments, five potting media were formulated. In Experiment 1, the treatment mixes on volume basis (total volume of 1.7 cm$^3$) were: 1) control (50% Ultisol and 50% sand), 2) RHC 2:1:1 (50% RHC, 25% Ultisol and 25% sand), 3) CSC 2:1:1 (50% CSC, 25% Ultisol and 25% sand), 4) PHC 2:1:1 (50% PHC, 25% Ultisol and 25% sand), and 5) CCC 2:1:1 (50% CCC, 25% Ultisol and 25% sand). In Experiment 2, the five treatment mixes consisted the following: a) control (50% Entisol and 50% sand), 2) RHC 1:2:2 (20% RHC, 40% Entisol and 40% sand), 3) CSC 1:2:2 (20% CSC, 40% Entisol and 40% sand), 4) PHC 1:2:2 (20% PHC, 40% Entisol and 40% sand) and 5) CCC 1:2:2 (20% CCC, 40% Entisol and 40% sand). There were three replications per potting substrate combination. Potting media with and without biochar were thoroughly mixed, placed in plastic bags (36 cm diameter x 24 cm height cm), wetted to 75% field capacity and incubated in the laboratory at room temperature ranging from 26 to 32$^{o}$C for 20 days.

The field capacity of each substrate mixture was determined by saturating the substrate placed in the perforated tin can with water. Three replicated cans were sampled for this purpose. The cans were covered with plastic to avoid evaporation and allowed to drain for 3 days. Soil samples from each can were then taken for moisture content determination using the gravimetric method. The average moisture content was calculated and expressed as percentage moisture at field capacity.

All five treatment combinations from both experiments were arranged in randomized complete block design and every 5 days re-randomization of bags within the block was done after thorough mixing of the substrate. After incubation, the different potting media were air-dried and stored in plastic bags until used for chemical analyses.

The substrate mixes in both experiments were analyzed following the methods used for the initial characterization of soil and sand.

***Pot experiments***. Two sets of pot experiments were carried out to evaluate the effects of biochar-amended potting media on AMF spore density, growth and nutrient uptake. The composition of potting media in Pot experiments 1 and 2 was similar to that in Incubation experiments 1 and 2 respectively. For each soil type, additional control treatment without biochar was included as the uninoculated control. The six treatment combinations in Experiment 1 were: 1) uninoculated control (50% Ultisol and 50% sand without AMF), 2) inoculated control (50% Ultisol and 50% sand + AMF), 3) RHC 2:1:1 + AMF (50% RHC, 25% Ultisol and 25% sand + AMF), 3) CSC 2:1:1 + AMF (50% CSC, 25% Ultisol and 25% sand + AMF), 4) PHC 2:1:1 + AMF (50% PHC, 25% Ultisol and 25% sand + AMF ) and 5) CCC 2:1:1 + AMF (50% CCC, 25% Ultisol and 25% sand + AMF). In Experiment 2, the six treatment combinations consisted the following: 1) uninoculated control (50% Entisol and 50% sand without AMF), 2) inoculated control (50% Entisol and 50% sand + AMF), 3) RHC 1:2:2 + AMF (20% RHC, 40% Entisol and 40% sand + AMF), 4) CSC 1:2:2 + AMF (20% CSC, 40% Entisol and 40% sand + AMF), 5) PHC 1:2:2 + AMF (20% PHC, 40% Entisol and 40% sand + AMF) and 6) CCC 1:2:2 + AMF (20% CCC, 40% Entisol and 40% sand + AMF). In both experiments, the six treatment combinations were replicated four times and arranged in randomized complete block design.

Treatment mixes were prepared and incubated at the same time and in a similar manner as in the incubation experiments. After incubation, the potting mix was transferred to plastic pot (14.5 cm diameter and 27 cm in height) fitted with polyethylene bag (22 cm length x 16 cm width) inside the pot to avoid contamination and dripping of water outside the pots during watering. Pots were filled with potting mix 2.5 cm from the top. To facilitate cooling and minimize fungal contamination, the top was covered and side of the pot was wrapped with Styrofoam insulation sheet. Polyvinyl chloride (PVC) pipe (17 cm length x 4 cm diameter) with nine holes at each side was inserted into the inner side of the pot to facilitate watering. Every after watering, the top hole of the PVC pipe was covered with sterilized foam to minimize further contamination. The top cover sheet had hole (4 cm diameter) for the PVC pipe and another hole (6 cm diameter) for the growing plant. Prior to use, the pots and PVC pipes were soaked in bleach and detergent solution (15%) overnight and rinsed thoroughly with distilled water.

Immediately prior to planting, 30 g AMF soil-sand-coconut shell char based inoculum (containing 19-20 spores $g^{-1}$) was applied to each pot for treatments with AMF inoculation. Half (15 g) of the inoculum was thoroughly mixed with the potting mixture while the remaining half (15 g) was placed into a hole (approximately 2 cm diameter x 5 cm depth). Three seedlings of sorghum were planted in each pot and thinned to 1 plant per pot, 1 week after planting. In preparing the pre-geminated seedlings, seeds were surfaced sterilized in 15% sodium hypochlorite for 10 min; then rinsed with sterilized distilled water and left to germinate for five days in moist sterilized filter paper.

After transplanting, the treatment pots were arranged in a shed house with plastic roofing. Two weeks after transplanting, the positions of pots within blocks were re-randomized weekly until harvest (after 10 weeks). Plants were watered with distilled water as needed to maintain the moisture content of the medium at approximately 75% field capacity. Two weeks after transplanting, plants in all treatment pots were fertilized with 50 mL of 25% Hoagland solution with a minimal amount of phosphate (Hoagland & Arnon 1950) and biweekly thereafter until harvest.

At harvest, plant height was measured prior to cutting the shoot close to the substrate surface. The soils adhering to the roots were removed carefully and the substrate was collected. The substrate was air-dried and sieved through 2-mm sieve and a subsample was taken for spore density determination. Shoot and roots were washed with tap water, rinsed with distilled water and blot-dried using a paper towel. The different plant parts were air-dried prior to oven drying for three days in a forced draft oven set at 70$^{o}$C. The oven dried plant samples were weighed to obtain their respective

dry matter yield. Total dry matter yield was obtained by summing up the dry weight of shoot and root.

The oven dried shoot and root were ground to particle size of < 1 mm and analyzed for total N and P following the method used for quantification of total N and P content of biochar. The amount of N and P uptake was determined by taking the product of the total dry matter and their respective N or P content.

The AMF abundance as influenced by various potting media was analyzed using the substrate taken after harvest. An air-dried and sieved potting media were thoroughly mixed and a representative sample of 300 g from each potting medium was taken for AMF spore density enumeration. From each representative sample thorough mixing was done and 100 g from each sample was drawn for spore density enumeration. The spore density enumeration was done by wet sieving and decantation method following that of Habte & Osorio (2001). Quantitative estimation of AMF spores was done by placing the spores into a small plastic Petri dish with gridlines under the electric dissecting microscope. Presence of viable spores was counted by moving the Petri dish. Extraction, decantation, and quantification for each substrate were done three times and the average spore count per substrate was taken. The total number of viable spores was expressed as number 100 $g^{-1}$ of the substrate.

***Statistical analysis***. The analysis of variance and the test of means for parameters measured were done using the freeware Statistical Tool for Agricultural Research (STAR) version 2.0.1 2014. The FPLSD was selected in comparing biochar treatments.

## Results and Discussion

***Chemical properties of soil and sand***. Ultisol soil was strongly acidic clay characterized by high exchangeable Al and acidity and had low total P and exchangeable bases (Table1). Entisol soil was moderately acidic with a moderate amount of exchangeable Al and acidity, with low in total OC and total N and moderate in exchangeable bases except for a high Ca. Both soils had almost the same low EC values which are below the threshold levels for most crops.

On the other hand, the potential cation exchange capacity (CEC) value of Entisol was higher than that of the Ultisol. The regular and moderate rates of inorganic fertilizer application in Entisol could have contributed the higher amount of exchangeable bases compared to Ultisol. The river sand had almost neutral pH which could be accounted for its very low exchangeable Al and acidity. It had low total OC, total N, EC and potential CEC.

Table 1

Chemical characteristics of soil and sand

| *Property* | *Soil* | | *Sand* |
|---|---|---|---|
| | *Ultisol* | *Entisol* | |
| pH (1:5 soil to $H_2O$) | 4.87 | 5.97 | 6.33 |
| EC (ds $m^{-1}$) | 0.33 | 0.34 | 0.04 |
| Exchangeable acidity (meq 100 $g^{-1}$ soil ) | 1.85 | 0.78 | 0.14 |
| Echangeable Al (meq 100 $g^{-1}$ soil) | 1.12 | 0.37 | 0.16 |
| Total OC (%) | 2.15 | 0.97 | 0.37 |
| Total N (%) | 0.18 | 0.09 | 0.05 |
| Total P (%) | 0.25 | 0.48 | 0.38 |
| *Exchangeable bases (meq 100 $g^{-1}$ soil)* | | | |
| K | 0.03 | 0.10 | 0.08 |
| Ca | 0.06 | 3.66 | 0.45 |
| Mg | 0.14 | 0.32 | 0.21 |
| Na | 0.02 | 0.09 | 0.03 |
| CEC (meq 100 $g^{-1}$ soil ) | 17.13 | 29.30 | 4.13 |

***Biochar characteristics***. All biochars examined exhibited basic pH (> 8) with CSC the most alkaline (Table 2). Most of the biochars had low EC values (< 1 ds $m^{-1}$) except CCC (2.85 ds $m^{-1}$) and CSC (5.17ds $m^{-1}$). The total OC values varied considerably among biochars ranging from 21.07 to 58.31%, in the following order: CCC > CSC > PHC > RHC while there was no much variation with CEC values, from 18.16 to 23.32 meq 100 $g^{-1}$. The total N also varied widely with PHC having the largest amount while RHC had the lowest N content. Similarly, RHC had the lowest P concentration. For the cations, PHC was richer in total Ca and Mg compared to the rest of biochars. However, CSC had the highest total Na.

Table 2

Chemical characteristics of different types of biochar

| *Property* | *RHC* | *CSC* | *PHC* | *CCC* |
|---|---|---|---|---|
| pH (1:5 soil to $H_2O$) | 8.44 | 10.02 | 9.91 | 9.37 |
| EC (ds $m^{-1}$) | 0.98 | 5.17 | 0.97 | 2.85 |
| Total OC (%) | 21.07 | 49.38 | 32.80 | 58.31 |
| Total (%) | | | | |
| N | 0.30 | 0.60 | 1.50 | 0.60 |
| P | 0.08 | 0.38 | 0.32 | 0.24 |
| K | 0.69 | 1.32 | 1.37 | 1.46 |
| Ca | 0.35 | 0.40 | 0.44 | 0.29 |
| Mg | 0.03 | 0.06 | 0.14 | 0.04 |
| Na | 0.34 | 1.51 | 0.59 | 0.22 |
| CEC (meq 100 $g^{-1}$ char) | 23.32 | 19.40 | 18.16 | 22.08 |

***Incubation experiments: biochar-mediated changes in physico-chemical properties of acid soil-sand potting mix***

*pH.* In Experiment 1, the addition of 50% biochar materials, regardless of the type, significantly increased pH compared to the control (Table 3). The magnitude of change in soil pH varied among biochars with PHC amended potting mix having the largest difference while the RHC amended, had the least increase (Table 3). In Experiment 2, which used the Entisol-based mixture, the 20% PHC addition also resulted in substantial increase in pH (Table 3) compared to the other biochars although the increase was not similar to the Ultisol based potting mix. The difference in magnitude in the change in pH between Ultisol and Entisol based potting mixes could partly be attributed to the higher rate used in the former than in the latter. The large magnitude increase of pH upon biochar application in the Ultisol which was not observed in the Entisol suggests also that the ability of biochars to change soil pH could not be fully attributed to the rate and type of biochars amended only but also on the type of soil.

*EC.* The electrical conductivity is a measure of the concentration of soluble salts. The Ultisol and the Entisol had almost similar inherent EC properties (Table 1). But the soil-sand mixture (1:1) resulted in a higher EC value for the control of Ultisol-based than the control Entisol-based mix (Table 3). Among the Ultisol-based potting mix, the 50% added CSC to the mix raised the EC values significantly higher than the other biochar materials. Similarly, the addition of 20% CSC in the Entisol-based potting mix raised the EC values significantly higher than the other biochar materials. The trends in the influence of the different biochars were similar in both the Ultisol and Entisol soils.

Table 3

Means for the effects of biochar amendment on potting mix pH, EC, exchangeable acidity and Al, total OC, total N, total P, exchangeable K, Ca, Mg and Na in incubation Experiment 1 and 2

| *Treatment* | *pH* | *EC (ds $m^{-1}$)* | *Exchangeable (meq 100 $g^{-1}$)* | | *Total OC* | *Total N (%)* | *Total P* | *Exchangeable (meq 100 $g^{-1}$)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Acidity* | *Al* | | | | *K* | *Ca* | *Mg* | *Na* |
| Experiment 1[+] | | | | | | | | | | | |
| Control | 5.64e | 0.13e | 0.38a | 0.20a | 1.22b | 0.15c | 0.29d | 0.06c | 0.84b | 0.20e | 0.03d |
| RHC 2:1:1 | 6.23d | 0.24d | 0.15b | 0.16b | 10.29a | 0.26b | 0.47c | 1.31b | 0.56c | 0.26b | 0.08c |
| CSC 2:1:1 | 7.86b | 0.67a | 0.12bc | 0.14b | 10.56a | 0.26b | 0.74a | 1.47a | 0.85b | 0.24c | 0.39a |
| PHC 2:1:1 | 8.11a | 0.48c | 0.10c | 0.10c | 10.44a | 0.73a | 0.75a | 1.51a | 1.44a | 0.31a | 0.12b |
| CCC 2:1:1 | 6.57c | 0.52b | 0.13bc | 0.15b | 10.97a | 0.26b | 0.60b | 1.48a | 0.38d | 0.22d | 0.06c |
| LSD (%) | 0.03 | 0.01 | 0.04 | 0.03 | 0.99 | 0.06 | 0.02 | 0.08 | 0.06 | 0.01 | 0.02 |
| Experiment 2[*] | | | | | | | | | | | |
| Control | 6.04d | 0.08e | 0.15a | 0.13a | 0.52c | 0.06c | 0.44d | 0.04d | 2.64a | 0.31 | 0.09b |
| RHC 1:2:2 | 6.74b | 0.11d | 0.10b | 0.10ab | 3.28b | 0.27ab | 0.45d | 0.23c | 2.29b | 0.31 | 0.10b |
| CSC 1:2:2 | 6.79b | 0.24a | 0.08bc | 0.08bc | 3.04b | 0.19bc | 0.53b | 0.49b | 2.37b | 0.31 | 0.24a |
| PHC 1:2:2 | 6.86a | 0.16c | 0.05c | 0.05c | 5.15a | 0.40a | 0.61a | 0.82a | 2.40b | 0.31 | 0.08bc |
| CCC 1:2:2 | 6.51c | 0.19b | 0.12ab | 0.12ab | 3.42b | 0.09bc | 0.49c | 0.43b | 2.29b | 0.31 | 0.07c |
| LSD (%) | 0.05 | 0.01 | 0.04 | 0.05 | 0.77 | 0.19 | 0.02 | 0.06 | 0.12 | ns | 0.02 |

Means in a column within each experiment followed by the common letters are not significantly different at 5% level of significance;
Experiment 1[+] - Ultisol-based potting mix;
Experiment 2[*] - Entisol-based potting mix.

*Exchangeable acidity and Al*. Concomitant to the increase in pH by the biochar application was the decrease in exchangeable acidity and exchangeable Al in both the Ultisol-and Entisol-based potting mixes (Table 3). Again, PHC had the largest exchangeable acidity and Al reducing power in both soil types. The magnitude of reduction in exchangeable acidity and exchangeable Al was not as imposing as that of the rise in soil pH due to PHC and CSC. Soil-sand mixture alone (controls) had a drastic change in soil acidity during the incubation of 21 days. The varying concentrations of basic cations and alkalinity among biochars could have contributed to the varied effect in reducing exchangeable acidity and Al of the potting mix. After pyrolysis, the basic cations particularly Ca and Mg are transformed into oxides, hydroxides, and carbonates (ash) and act as liming agent when applied to soil.

Between Experiments 1 and 2, theoretically, the soil-sand mixtures could have an average of 1.00 and 0.46 meq 100 $g^{-1}$ exchangeable acidities for the Ultisol-based and the Entisol-based mix, respectively. However, the measured exchangeable acidity was only 0.38 and 0.15 meq 100 $g^{-1}$ for Ultisol-based and Entisol-based potting mix, respectively (Table 3). Furthermore, a similar trend was observed in exchangeable Al, where a reduced empirical value was obtained. The magnitude of reduction in exchangeable Al in both soil types was similar, except for CCC which accounted an 8% decrease in the Entisol and a 25% decrease in the Ultisol. In contrast, the effect of the PHC was more pronounced in the Entisol-based potting mix which had a drop of 66% in exchangeable Al, and a 50% reduction in the Ultisol-based potting mix. This trend emphasizes that liming value of biochars on soil acidity depended on biochar and soil type.

*Total OC*. Biochar amendment resulted in positive changes in total OC both in Ultisol and Entisol-based potting mixes. However, the increase in total OC was not consistent with the trend of total OC values of biochar used. In the Ultisol-based potting mix, the increase in total OC after 21 days of incubation was not proportionate to the theoretical total OC values of 11.65, 25.32, 17.03 and 29.78% for RHC, CSC, PHC, and CCC, respectively. In the Entisol-based potting mix, the measured total OC values were also different from the theoretical values of 4.75, 10.41, 7.10, and 12.20% for RHC, CSC, PHC, and CCC, respectively. It is the difference in the pattern in the changes of total OC due to biochar amendment in Ultisol-based and Entisol-based potting mixes is noteworthy. For both soil types, it was expected that CSC and CCC could contribute significantly large to the potting mixture's total OC due to their higher total OC values relative to PHC. Surprisingly, all types of biochar amendment resulted in a similar change in total OC in Ultisol-based potting mixes. On the other hand, PHC added to Entisol-based potting mixes resulted in the total OC values significantly greater than the other biochar materials. The apparent contradictory effect can be due to the difference in kind of C compounds present in the biochar and soil type.

*Total N*. Total N from the Ultisol based potting mix showed a different trend from total OC. The control had low total N but with the addition of biochar, total N increased to 0.73% with PHC. This was due to the inherently high total N in the PHC raw material. However, the other materials raised the total N similarly to 0.26%. The Entisol based potting mix had also been influenced by the PHC addition with an increase of 0.34% over the control. Yet, the different biochar materials had a different varying influence on the Entisol based potting mix compared to that in the Ultisol.

*Total P*. Amending Ultisol-sand potting mix with biochar regardless of type resulted in a positive increase in total P with RHC < CCC < CSC < PHC (Table 3). In Entisol-sand potting mixes, the pattern was different with RHC resulting in total P that was not significantly different from the value obtained from the control. The total P content of RHC is only 0.08% while the other biochars ranged from 0.24 to 0.38% (Table 2). These facts can be related to P content in the biochar and soil, as well as the biochar rate.

*Exchangeable K, Ca, Mg and Na*. In Ultisol sand–based potting mixes, adding RHC, CSC, PHC or CCC increased the exchangeable K, Ca, Mg, and Na contents compared with the control. The magnitude of increase varied with the concentration of basic cations in the biochar material. Peanut hull char, CSC, and CCC which had higher total K content than RHC had resulted in greater exchangeable K in the potting mix than RHC. Although the different pattern was observed with exchangeable Ca and Mg, the order of magnitude of increase coincides with total K concentrations in the biochar materials with PHC providing the larger amount of exchangeable Ca. Similarly, PHC resulted in highest exchangeable Mg in the potting mix while the CCC provided the least amount of exchangeable Mg. As to exchangeable Na, highest exchangeable Na was from CSC amended potting mixes which could explain the high EC value in the CSC amended potting mixes. It is worthy to note that among biochars used, CSC had the highest total Na content.

In the Entisol-sand based potting mixes, a different trend was observed. Biochar application failed to show significant influence on the exchangeable Mg in the potting. While the effects of PHC, CSC, and CCC in Ultisol-sand based potting on exchangeable K were comparable, the trend was different in Entisol-sand base potting mix with PHC resulting in highest exchangeable K. Other than K content in the biochar material could have influenced the superiority of PHC over the rest of biochar materials since CCC had the highest total K concentration among biochar materials. As to exchangeable Ca, all the biochar materials resulted in the same effect which also cannot be explained by their Ca content. Coconut sawdust char also provided the highest exchangeable Na in the Entisol sand-based potting mixes while CCC provided the lowest value. One reason is the inherent amount of total Na content in the different biochars.

The findings of the present study confirmed our hyphothesis that application of different biochars would have varied effects on soil chemical properties of potting media. The results are also consistent with the previous findings by Chintala et al (2014) who reported an increase in soil pH, EC and CEC and a decrease in soil exchangeable acidity following the addition of corn stover biochar and switchgrass biochar during 15 to 165 days of incubation period using an acid soil. Among biochars, corn stover biochar had shown marked improvement in pH, EC and CEC and a higher reduction in exchangeable acidity than switchgrass biochar. The increase in pH, EC and CEC could have been attributed to the alkalinity and high base cation concentration of the biochars. Similarly, Dume et al (2015) found that biochar application derived from coffee husk and corn cob significantly increased soil pH, EC, soil OC, total N, available P, exchangeable K, Ca and Mg levels of acidic soil. Application of coffee husk biochar showed better improvement in the soil chemical properties than corn cob biochar at all application rates (0, 5, 10 and 15 t $ha^{-1}$). Berihum et al (2017) also evaluated the effects of three types of biochar (eucalyptus biochar, corn cob biochar and lantana biochar) on the chemical properties of an acid soil. The application of biochar greatly reduced soil exchangeable acidity and increased soil pH, total N, soil OC, available P and K. From among applied biochar treatments, lantana biochar at 18 t $ha^{-1}$ application rate had a higher impact in changing soil chemical properties. These changes in soil chemical properties brought about by biochar application depends on the kind of biochar, soil types and the levels of biochar added in the soil.

***Pot experiments: effects of AMF and biochar on plant height and total biomass***. Table 4 shows that the responses of sorghum growing in the Ultisol and Entisol–sand-based potting mix varied. In the Ultisol-sand-based potting, the plants were shorter than those found in the Entisol even with the addition of AMF and biochar. The difference in plant height was not detected to be significant, even with an increasing trend with the presence of AMF and biochar. In the Entisol-based potting media, plant height was seen to be depressed by the addition of PHC and CSC. Both biochars had high total N content, even higher for PHC, and the rest of the chemical properties were sufficient for plant growth. The complex of factors affecting the expression of plant height could be attributed to the alkaline nature of the biochar. Both PHC and CSC had high alkaline pH that changed the pH to 8.11 and 7.86, respectively, in the Ultisol and 6.86 and 6.79, respectively in the Entisol (Table 3).

The root and shoot dry weight sum up the total biomass (Table 4). Biomass production, i.e., total dry weight of plants was influenced by soil type (Ultisol and Entisol), the inoculation of AMF and the biochar materials (Table 4). Both root and shoot dry matter content is significantly correlated (ranging from 0.937 to 0.998 with $p < 0.0001$) to the total biomass; hence, the discussion will be focused on the total biomass. The total biomass obtained from the Ultisol-based potting mix expectedly was improved with the addition of AMF. The biomass of the control + AMF treatment was about five times more than without AMF.

However, the presence of biochar, regardless of source, disturbed the full advantage of AMF inoculation. Instead, almost half of the potential increase of total biomass was lost with biochar. However, the addition of biochar still improved the plant's performance magnitude to at least twice without biochar application. Among the soil and plant parameters measured, only soil exchangeable Al and the plant N uptake were found correlated ($r = -0.445$ and 0.848, $p = 0.04$ and $< 0.0001$, respectively) to the total biomass of plants grown in the Ultisol-sand-based mix. The presence of high exchangeable Al in the soil is detrimental to the growth of the plants. The capacity to absorb N improved the performance of the plant. The presence of AMF countered the negative effect of exchangeable Al, which is inherently high in the Ultisol, by enabling the plant to sustain P absorption in a P-limited growing condition. Theoretically, both control and control + AMF potting mixes had total P of 0.32%, thus, the higher biomass from control + AMF compared to uninoculated control underscores that AMF strongly influenced the biomass production. This finding agrees with Watts-Williams et al (2014) who found higher P uptake in *Solanum lycopersicum* plant inoculated with AMF grown under P limited soil than non-mycorrhizal plant.

In the CSC and PHC-amended potting mixes, the theoretical total P is 0.35 and 0.32%, respectively. Although the total biomass in CSC and PHC-amended potting mixes were comparable to the AMF inoculated control, the biomass produced from the latter treatments was only less than half of the biomass from uninoculated control. The reduction in the magnitude of effect could be partly explained by the pH of the two biochar treatments. The above neutral pH in CSC and PHC-amended treatments could have resulted in deficiencies of most essential micronutrients such as iron and manganese. A meta-analysis performed by Jeffery et al (2017) showed that biochar application to soils could reduce the growth, biomass production and yield of crops especially at high biochar application rate. The reason for these decreases is that biochar may have raised the soil pH to much resulting in over-liming, thus, leading to the immobilization of micronutrients. This was confirmed also by Rondon et al (2007) who reported a reduction in biomass at biochar application rate above 60 g $kg^{-1}$.

The biomass accumulated in the Entisol could be described with a more complex relationship of factors. Inoculation of AMF did not improve the total biomass in the Entisol-sand based potting mix. However, Entisol-sand-based mix without inoculation produced greater biomass than those with biochar treated potting mix. The AMF might have a very slight effect as shown by a high biomass of plants from the control + AMF treated potting mix. Thus, the addition of AMF on biochar treated soil, particularly when the soil had already inherently high exchangeable/total P, could not warrant high biomass production.

The trend in the effects of AMF inoculation on the shoot dry weight of biochar amended soil-sand mixes was found parallel to the total dry weight. Between the two soil types, plants grown in the Ultisol-sand-based potting mixes were shorter in stature (Table 4) than those in the Entisol-sand-based potting mixes. The taller plants in the Entisol–based substrate had consequently heavier biomass than those in the Ultisol sand-based potting mixes. Heavy total biomass was then attributed to the well-developed shoot and root systems. The root systems encouraged the AMF to colonize and thus induced the increase in root surface area leading to the exploitation of nutrients (Carrenho et al 2007). Hence, inoculation of AMF improves nutrient absorption regardless of biochar applied. Increased in plant total dry weight production with AMF and biochar application has been reported also by Momayezi et al (2015). Similarly, Budi & Setyaningsih (2013) also reported an increase in neem seedling's shoot and root dry

weight by 4.547% and 6.957% in AMF and biochar amendment pots. The observed increase in plant dry weight production was attributed to the increased in P uptake of plants with AMF inoculation.

***Pot experiments: effects of AMF and biochar on N and P concentration and uptake of sorghum***. The N and P concentrations in the plant tissues differed significantly with the influence of AMF inoculation and the type of biochar added to the soil-sand-based potting mix (Table 5). In the Ultisol-sand-based potting mixes, control + AMF had lower total N in the plant tissue than the uninoculated control. The probable contributing factor to the decrease in tissue N concentration in the inoculated control treatment is the dilution of N due to the increased photosynthetic assimilation of C since the total biomass and N uptake was higher relative to uninoculated control. This result is in accordance with the study of Farzaneh et al (2011) who reported a significant drop in tissue N concentration in AMF inoculated plant compared to the control treatment. The observed reduction was attributed to the dilution effect due to higher dry matter production in the AMF treatment. While AMF inoculation was effective in increasing N plant tissue concentration in Ultisol-sand based potting mix, N concentration of plant tissue from the uninoculated control did not differ from inoculated control in the Entisol-sand-based potting mix (Table 5). With the total P, a higher concentration was obtained from inoculated control relative to the uninoculated control potting mix. Accumulated P in the plant tissue was significantly improved when biochar was added, irrespective of type. The positive effect of biochar and AMF on P uptake implies that the mentioned strategies could boost P accumulation in sorghum grown in moderately acid soil-sand based potting mixes. Comparing the values of the P accumulation in plants grown in the Entisol-sand based potting mixes from those grown in Ultisol-sand-based potting mixes, the low P content of the Ultisol soil seemed to activate the efficacy of AMF to sequester the P from the soil and provided to the plants for biomass production. In the Entisol-based plants, AMF improved P accumulation even without the biochar amelioration, *viz*. control + AMF. With the biochar addition, the significant beneficial effect of AMF inoculation in the Ultisol-sand-based potting mixes was observed only in the PHC added potting mix.

The increase in plant P uptake with AMF inoculation on biochar amended pot is in agreement with the findings of Mau & Utami (2014) who reported a higher P uptake of corn by 62.7% in pots applied with biochar and inoculated with AMF spores. Likewise, Budi & Setyaningsih (2013) observed a significant increased in P uptake of plant in the pot amended with 10% and 15% biochar inoculated with AMF. Vanek & Lehmann (2014) also found a positive biochar/AMF interaction on plant P nutrition in a low-P soil. The fungi enhance the uptake of immobile nutrient particularly P by increasing the absorptive surfaces of the root. Contrasting result was also reported by Nzanza et al (2012) where AMF inoculation and biochar application lowered leaf P by 26% when compared to the uninoculated plants.

Table 4

Means for the effects of biochar amendment and AMF inoculation on plant height at harvest, biomass of sorghum in pot Experiments 1 and 2

| *Treatment* | *Height at harvest (cm)* | *Dry weight (g plant$^{-1}$)* | | |
|---|---|---|---|---|
| | | *Root* | *Shoot* | *Total* |
| Experiment 1$^{+}$ | | | | |
| Control (uninoculated) | 81.50 | 0.71 | 2.08b | 2.79b |
| Control + AMF | 99.62 | 2.87 | 10.58a | 13.45a |
| RHC 2:1:1 + AMF | 91.75 | 1.52 | 5.24ab | 6.75ab |
| CSC 2:1:1 + AMF | 89.50 | 1.05 | 4.14ab | 5.19ab |
| PHC 2:1:1 + AMF | 101.13 | 1.27 | 6.10ab | 7.38ab |
| CCC 2:1:1 + AMF | 103.79 | 1.11 | 5.13ab | 6.24ab |
| LSD (%) | ns | ns | 7.23 | 9.24 |
| Experiment 2$^{*}$ | | | | |
| Control (uninoculated) | 132.75a | 4.48b | 18.67ab | 23.15ab |
| Control + AMF | 135.25a | 5.76a | 20.42a | 26.18a |
| RHC 1:2:2 + AMF | 136.75a | 3.67bc | 18.27ab | 21.94b |
| CSC 1:2:2 + AMF | 123.88ab | 2.54cd | 12.53c | 15.07c |
| PHC 1:2:2 + AMF | 98.75b | 1.71d | 6.43d | 8.13d |
| CCC 1:2:2 + AMF | 133.75a | 3.72bc | 16.32b | 20.05b |
| LSD (%) | 29.20 | 1.27 | 3.00 | 4.07 |

Means in a column within each experiment followed by common letters are not significantly different at 5% level of significance;
Experiment 1$^{+}$ - Ultisol-based potting mix;
Experiment 2$^{*}$ - Entisol-based potting mix.

Table 5

Means for the effects of biochar amendment and AMF inoculation on total N and P concentration and uptake and spore density in pot Experiments 1 and 2

| *Treatment* | *Concentration (%)* | | *Uptake (mg plant$^{-1}$)* | | *Spore density (spore 100 g$^{-1}$)* |
|---|---|---|---|---|---|
| | *N* | *P* | *N* | *P* | |
| Experiment 1$^{+}$ | | | | | |
| Control (uninoculated) | 2.57a | 0.07c | 72.50ab | 1.98c | 0.00c |
| Control + AMF | 1.30b | 0.07c | 154.45a | 10.02bc | 105.69a |
| RHC 2:1:1 + AMF | 0.65cd | 0.34a | 43.78b | 22.75a | 91.25a |
| CSC 2:1:1 + AMF | 0.59d | 0.20b | 30.97b | 10.28bc | 22.00bc |
| PHC 2:1:1 + AMF | 0.97bc | 0.34a | 71.83ab | 25.16a | 30.25b |
| CCC 2:1:1 + AMF | 0.58d | 0.25b | 34.43b | 15.57ab | 43.69b |
| LSD (%) | 0.34 | 0.08 | 106.83 | 11.05 | 26.00 |
| Experiment 2$^{*}$ | | | | | |
| Control (uninoculated) | 0.79ab | 0.05c | 182.62ab | 12.04c | 0.00d |
| Control + AMF | 0.88a | 0.13b | 229.65a | 35.27a | 155.00a |
| RHC 1:2:2 + AMF | 0.53b | 0.13b | 116.34bcd | 29.11ab | 106.25b |
| CSC 1:2:2 + AMF | 0.71ab | 0.19b | 105.87cd | 28.15ab | 53.50c |
| PHC 1:2:2 + AMF | 0.54b | 0.27a | 44.52d | 21.29b | 40.25c |
| CCC 1:2:2 + AMF | 0.66ab | 0.15b | 132.59bc | 29.98ab | 65.75c |
| LSD (%) | 0.31 | 0.06 | 73.32 | 9.08 | 26.24 |

Means in a column within each experiment followed by the same letters are not significantly different at 5% level of significance;
Experiment 1$^{+}$ - Ultisol-based potting mix;
Experiment 2$^{*}$ - Entisol-based potting mix.

***Spore density of AMF after planting sorghum***. Spore formation was stimulated in the inoculated controls while no single spore was formed in the uninoculated controls in both Ultisol-and Entisol-sand based potting mixes (Table 5). The absence of spores in the uninoculated controls clearly shows that there was an absence of fungal contamination in the potting mix. In the Ultisol sand based potting mix, biochar application, except for RHC, had lower spore counts; and the RHC amended potting mix had comparable spore density with the inoculated control. The reduced production of AMF spores in CSC, PHC, and CCC amended potting mixes can be attributed to their P content. Inoculated control had the least total P (Table 3) while the RHC–amended potting mix total P had slightly higher P than the control; and that total P of RHC-amended potting mix was significantly lower than that of the other biochar-amended potting mixes. Increase in soil P availability negatively affected spore formation (Ezawa et al 2000). Our study concur with the result of Warnock et al (2010) who reported a decreased in AMF abundance when significant changes in soil properties, primarily soil P availability, were observed. Adding large P containing material such as peanut shell biochar significantly increased soil available P and decreased AMF root colonization and extraradical hyphal lengths. Yusif & Dare (2017) also noticed same reduction in AMF population when soil available P and soil pH were increased following the application of large quantity (20 t $ha^{-1}$) of corn cob biochar. Our study suggests that adding large amount of P and very strongly alkaline pH biochar can depress AMF colonization and abundance in the soil by making the plant less dependent on mycorrhizal association.

In Entisol-sand based potting mix, biochar regardless of type resulted in significant reduction in a number of spores in the potting mixes. The difference in trend on spore density between Ultisol- and Entisol-sand based potting mixes emphasizes that biochar mediated effects on spore production vary with soil type. Although the addition of PHC did not result in a positive effect on spore density, its liming potential and fertilizer value cannot be discounted for acid soil-based potting mixes. Further work is needed to determine the optimum ratio of biochar, soil, and sand in the potting mix that can promote both the growth of the host plant and production of AMF propagules.

Despite the reduction in spore counts in biochar amended Entisol–sand based potting mix, the spore counts observed was correlated to the root biomass ($r = 0.3845$, $p = 0.0083$). Likewise the higher the spore count, the higher was the shoot dry matter weight ($r = 0.41$, $p = 0.0041$).

**Conclusions**. Results of this study indicate that mediated changes in potting mix chemical properties varies with biochar type, soil type and rate of biochar application. Among biochars evaluated, PHC has the most superior influence on the chemical properties of potting mixes. PHC application in Ultisol-sand based potting mixes at incubation period had shown marked increased in pH, total N, total P, exchangeable K, Ca, Mg and decreases significantly the exchangeable acidity and Al. Similarly, Entisol based potting mix amended with PHC obtained the highest increase in pH, TOC, total N, total P, exchangeable K and decreased significantly the exchangeable acidity and Al. On the other hand, CSC both in Ultisol-and Entisol based potting mix was consistent in increasing EC and exchangeable Na. Addition of PHC and CSC depressed plant height and reduced total biomass production in Entisol-based potting mix. Meanwhile, application of PHC and RHC in Ultisol-based potting mix improved tissue P concentration and uptake. Similarly, PHC application increased tissue P in Entisol-based media. Inoculation of AMF and RHC application in Ultisol-based media stimulates spore formation. However, in Entisol-based potting media, biochar application regardless of type reduced spore number.

From these results, it is clear that biochar application directly influences the chemical properties of potting media. However, these chemical changes such as increase in pH as well as P content observed in PHC and CSC have detrimental effects on the growth, biomass production of plant and AMF spore production. Moreover, the potential of biochar-amended soil-sand potting media to replace the traditional soil-sand based substrate for AMF inoculum production was not achieved in this study. However, among

biochars evaluated, RHC being inferior in promoting soil quality improvement obtained the highest AMF spore abundance.

**Acknowledgements**. We deeply thank the ACIAR-Watershed laboratory for the analysis of our soil samples, biochar and plant tissue. We also acknowledge the financial support of Department of Science and Technology-Science Education Institute Scholarsip program.

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Received: 12 September 2017. Accepted: 21 November 2017. Published online: 03 December 2017.
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How to cite this article:
Rollon R. J. C., Almendras-Ferraren A. S., Ferraren D. O., 2017 Effects of biochar application on potting media chemical properties, arbuscular mycorrhizal fungi spore density, growth and nutrient uptake of sorghum (*Sorghum vulgare* L.). AAB Bioflux 9(3):119-135.