Amending Soil With Biochar From Available Agricultural Wastes to Improve Sustainability of Cotton Production in Mali

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Research Article

Keywords: Biochar, Soil amendment, Cotton, Soil nutrients, Arbuscular mycorrhizae

Posted Date: October 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-945517/v1

Abstract

Improving the sustainability of cotton production in Mali can be achieved by returning organic matter and nutrients to degraded soils. Amendment with biochar prepared from locally available feedstocks has been suggested as a pathway to sustainability. A greenhouse study was conducted to evaluate the effect of biochar prepared from two feedstocks readily available in Mali, cotton field residue and rice hulls, on cotton plants grown to six weeks. A composted municipal biosolid was included for comparison with the more carbon-stable biochars. Four soils of contrasting properties were included in the study. Plants were measured for shoot height and mass, root length and mass, whole plant tissue nutrients, and arbuscular mycorrhizal fungi (AMF) colonization in roots. Shoot height was often improved by amendment in all soils. Root mass was improved by amendment only in the soil with the greatest clay content. Nitrogen (N) uptake was significantly depressed, and phosphorus (P) uptake was increased under biochar and compost amendment in the most coarsely textured soil. No effect on N and P uptake was observed in the soil with the greatest clay content. Ridge regression analysis showed that AMF root colonization was positively related to the P content of the amendments ($1.411*P_{amend}$), negatively related to soil P ($-0.486*P_{soil}$) and positively related to both soil pH (2.153*pH) and clay content (1.129*clay%). Results indicate that degraded soils may be restored through amendment with biochar created from locally available feedstock to improve sustainability of cotton production. Soil properties will determine the degree of benefit.

Highlights

- Biochars prepared from locally available feedstocks may be prepared on farm with low cost equipment to amend soils for improved and sustainable cotton production.
- Biochar amendments were shown to improve early shoot growth (6 weeks) in cotton grown in the greenhouse in four soils.
- Arbuscular mycorrhizal fungi (AMF) colonization of roots is beneficial to plant performance and is related to the phosphorus (P) provided by the amendment, the soil P content, soil pH, and soil clay content.

Introduction

Degradation of soil natural resources is a global crisis threatening the sustainability and security of food and fiber production (Scherr 1999; Lal 2012). Restoring and maintaining high function to soils under intensive agricultural production requires changing many currently accepted conventional practices. While the problem is global in scale, the need to better align soil management practices with the required changes is more urgently felt in less developed nations where agriculture is closely coupled to economic outcomes (Bindraban et al. 2012; Lal 2012).

Landlocked Mali in West Africa is one of the 49 least economically developed countries, according to the United Nations Food and Agriculture Organization (FAO 2014). Over 75 % of the population is rurally based, 39 % are economically active in agriculture, and 50 % of the GDP comes from the agricultural sector, suggesting a substantial dependence upon the quality of the soil for the well-being of the Malian population (Andrieu et al. 2017). Cotton is the primary cash crop in sub-Saharan Mali, often rotated with cereals and legumes (Traore et al. 2013). Cotton will therefore be a major future determinant of economic stability for Mali, and for the nearby countries of Burkina Faso, Benin, Chad, and Senegal. Sustainable management of soil resources for continued productivity will be important to the future of these countries.

Cotton is produced in the southern region of Mali where rainfall is sufficient. Ultisols, tropical ferruginous soils, predominate here (Keita 2000). These soils are low in native nutrients (particularly phosphorus), carbon (C), and water holding capacity. Increasing pressure from climate instability in the region, limited access to fertilizer, and soils low in fertility, combine to form a substantial aggregated constraint to achieving production and economic potentials (Ebi et al. 2011; Traore et al. 2013; Hall et al. 2017). Current soil management here includes slash and burn clearing of land followed with intensive tillage before planting. Slash and burn is effective at clearing weeds, raising soil pH, and increasing plant available nutrients such as phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the short term (Pypers et al. 2012). However, the practice is unsustainable and incapable of supporting a growing cotton and cereal production system. It requires long fallow periods (5-10 yrs) in between for regrowth of native vegetation and results in long-term declines in nutrient stores, soil organic matter, and overall productivity (Kotto-Same et al. 1997; Kato et al. 1999). Alternative soil management approaches are urgently required to provide for the sustainable production of all crops in Mali and the surrounding region.

Until the problem of access to fertilizers is solved, the recovery of nutrients and organic from materials closer to hand, including animal manures, composts, and biochars, remains one of the most promising options for restoring and maintaining soil fertility. Several authors have suggested that local production of biochar from available vegetative materials and organic wastes, used as a soil amendment, has the potential to provide positive benefits in sub-Saharan Africa (Mekuria and Noble 2013; Gwenzi et al. 2015; Smith et al. 2015). Kätterer et al. (2019) reported long term yield increases for corn (*Z. mays*) and soybean (*G. max*) in Kenya for 10 years following application of biochar derived from *Acacia* spp. wood. However, Kamau et al. (2019) found that corn yields in Kenya over a four-year period following biochar addition (with and without fertilizer) were not improved over fertilizer alone. The latter authors did, however, note an increase in earthworm populations. Both studies suggested further research is required.

Other studies have shown that biochar can stimulate the development of mutualistic associations between crop roots and arbuscular mycorrhizal fungi (AMF), resulting in improvements to crop performance (Warnock et al. 2010). These fungal organisms are known to benefit plants in terms of soil volume access for increased acquisition of water and nutrients, and are often credited with facilitating crop stress resistance (Watt et al. 2006). Benefits of crop/AMF association specific to cotton production include increased yield, fiber quality, and phosphorus regulation (Cely et al. 2016; Gao et al. 2020).

Despite the numerous reviews on the subject of soil improvement via biochar and direct calls for its adoption as a soil amendment in sub-Saharan Africa (Whitman and Lehmann 2009; Leach et al. 2012; Gwenzi et al. 2015), very few studies have been performed. This is especially true in the cotton producing countries of West Africa. The purpose of the current study, therefore, was to develop data on cotton response to biochar-amended soils using vegetative materials locally available to Malian farmers (cotton field residue and rice hulls) as biochar feedstock at temperatures that might be replicated on-farm. The effect of the two biochars was compared with that of compost-amended and unamended soils. The experimental hypothesis was that addition of biochar would improve soil fertility and produce agriculturally relevant benefits in cotton grown to 6 weeks in the greenhouse.

Materials And Methods

2.1. Location and Facilities

The experiment was carried out during the fall of 2017 at College Station, TX on the campus of Texas A&M University. A proxy soil representing the ultisols of southern Mali was selected, along with 3 additional soils to compare effects across textural classes and mineralogies. Biochar preparation was performed at the Department of Mechanical Engineering Combustion and Reaction Characterization Laboratory. Cotton growth experiments were performed at the greenhouse facilities within the Institute for Plant Genomics and Biotechnology.

2.2 Biochar preparation

Cotton residue (stems, leaves, and lint) collected post-harvest from a research plot at the Texas A&M University's Research Farm (30.550094, -96.435346) and rice hulls purchased from a local agricultural supplier were used as feedstocks for the two biochars used in the study. Cotton residues (CS) were dried in an oven at 65°C for 12 hours and cut into 4 cm pieces. Rice hulls (RH) were also dried at 65°C for 12 hours. Each feedstock was pyrolyzed at 350°C for 30 minutes within a quartz tube 7.5 cm x 122 cm placed in a three zone programmable Lindberg Blue M tube furnace (Thermo Fisher Scientific, Waltham MA). Argon gas was used to create the pyrolysis atmosphere, with 10 volumes of Ar gas used to flush vapors created during the process under a 0.5 L min^{-1} flow rate. Vapors were evacuated from the reactor and condensed in the waste stream. Biochars were cooled under Ar gas for 30 min. Seven batches of CS feedstock and two batches of RH feedstock biochar were prepared. Conversion efficiency as final mass percentage of starting material for the feedstocks was 35.93% for CS and 51.9% for RH. Total masses of CS and RH biochars prepared were 504 g and 577 g respectively. Prior to use in the greenhouse study, each material was forced through a 5 mm screen to reduce heterogeneity in chemical composition and particle size.

2.3 Soils

Four soils were used in the study. The following assignment of series was performed using USDA Soil Survey maps (USDA Soil Survey Staff, nd) and verified for consistency by observation. A Burleson series soil (Fine, smectitic, thermic Udic Haplustert) was collected from the A horizon (0 to 10 cm) in a field near Thrall, TX that had been planted in cotton (30.596646, -97.297634). A Hearne series soil (Fine, mixed, semiactive, thermic Typic Haplustult) was collected from the Bt1 and Bt2 horizons (25 cm to 62 cm below the surface) from a 'fill dirt' mining site in Hearne, TX (30.902194, -96.602912). A Pullman series soil (Fine, mixed, superactive, thermic Torrertic Paleustoll) was collected from the Ap horizon (0 to 6 cm) a field near Bushland, TX that had been planted in corn (35.191097, -102.098732). A Wolfpen series soil (Loamy, siliceous, semiactive, thermic Arenic Paleudalf) was collected from the A horizon in a pasture field near Centerville, TX (31.320222, -95.964693).

Soils were passed through a cone crusher before sieving to < 5 mm. Heterogeneity was minimized by mixing 40 L batches in a 50 L capacity electric cement mixer, followed by manual mixing on a plastic tarp using a combination of turning with snow shovels and inversion through pulling the outside edges of the tarp over the soil in a rotating fashion. Soils were stored in 20 L plastic containers with leak proof plastic lids until ready for use.

2.4 Greenhouse Study

All aspects of the plant growth study, including plant material collection, were performed in compliance with relevant institutional, national, and international guidelines and legislation. Seven treatments (Table 1) were evaluated in four soils for their effects on the early growth and development of cotton plants in the greenhouse. Prior to amendment with biochar, all soils received fertilizer as calcium (Ca), nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) in amounts sufficient to produce 890 kg ha^{-1} seed cotton according to the Texas A&M AgriLife Extension Service Soil, Water, and Forage Testing Laboratory recommendations (College Station, TX), regardless of soil test analysis result (Table 2). The Hearne soil received 652 mg kg^{-1} $CaCO_3$ to address soil pH conditions low enough to prohibit growth. An assumption of 2.24 Gg soil ha^{-1} (15-21 cm furrow slice) was applied to all soils to accommodate conversion of yield goal-based fertility recommendations to the mass of soil in each greenhouse pot. Both CS and RH biochars were added at low and high rates to soil. These treatments were compared with a control receiving no amendment, as well as a compost at low and high rates of addition. The compost was made from municipal biosolids, yard waste, and food waste (Synagro, Baltimore, MD). Five replications of each treatment were prepared. Experimental units were arranged randomly in 4 rows on greenhouse benches.

Table 1. Treatments and rates of amendment in greenhouse grown cotton.

#	Treatment	Abbreviation	Rate Applied (mg kg^{-1} soil)
1	Control	C	0
2	Cotton Residue Biochar Low Rate	CSBLo	554
3	Cotton Residue Biochar High Rate	CSBHi	1662
4	Rice Hull Biochar Low Rate	RHBLo	554
5	Rice Hull Biochar High Rate	RHBHi	1662
6	Compost Low Rate	CMPLo	554
7	Compost High Rate	CMPHi	1662

Table 2. Nutrients, sources, and rates added to all soils in greenhouse study.

Fertilizer Nutrient	Rate Applied (mg kg^{-1} soil)	Source
Ca	152	$CaSO_4 \cdot H_2O$
N	67	$(NH_4)_2SO_4$
P	15	$Ca(H_2PO_4)_2 \cdot H_2O$
K	60	$(NH_4)_2SO_4$
S	298	$CaSO_4 \cdot H_2O$, $(NH_4)_2SO_4$,

Deepot™ containers (D60 Steuwe and Sons, Tangent OR) 6.9 cm in diameter and 35.6 cm depth were used to hold 600 g of soil and amendment. Two untreated cotton seeds (13SO3 variety were placed 1.5 cm below the soil surface in each pot. Seeds were obtained from the Texas A&M cotton breeding program. Dr. Steven Hague (Texas A&M University Cotton Improvement Laboratory) provided identification and full permission for their use in this study. One week following germination, the smaller of the two germinated plants was removed from each pot. All Water was supplied to each pot according to an estimate of 60% water filled pore space performed by saturating each soil to measure 100 % water filled pore space. Each day prior to germination, 3 ml of reverse osmosis (RO) water was added to each pot to replace evaporated water in the germ zone. Following germination, 10 mL of RO water was added to each pot daily for the 42-day duration of the study. This practice was found to be sufficient to keep all pots near the initial weight measured prior to germination.

2.5 Chemical and physical analyses

Total C and N in soil, biochar, compost, feedstock materials, and cotton leaf tissue was analysed by the combustion method (McGeehan and Naylor 1988). Soil available nutrients P, K, Ca, S, and magnesium (Mg) were analysed by ICP-AES following Mehlich III extraction (Mehlich, 1984). Soil available micronutrients zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) were analysed by ICP-AES following extraction with DTPA (Lindsay and Norvell 1978). Nitrate N (NO_3-N) in soil was measured by Cd reduction colorimetry following extraction with 1 M KCl. Soil, biochar, compost, and feedstock pH was measured by benchtop pH meter in a 1:2 (soil:water) extract using deionized water. Electrical conductivity (EC) of soil, biochar, compost, and feedstock materials was measured using a benchtop conductivity meter in a 1:2 extract using deionized water. Total mineral nutrients P, K, Ca, Mg, Na, Zn, Fe, Cu, Mn, S, and B in biochar, compost, feedstock materials, and cotton whole plant tissue was analysed by ICP-AES following microwave assisted acid digestion (Havlin and Soltanpour 1989).

Cotton plant height was measured from the soil surface to the point where the first true leaf is joined to its stem at 42 days. Cotton shoot mass was measured on a 4-place digital balance after cutting the plant at the soil surface and oven drying at 60°C for 16 hours, or until constant weight. Cotton root mass was measured at 42 days by the following procedure. Most of the soil was removed from roots by careful and gentle rinsing with a garden hose against a 2.5 mm screen. Roots were then soaked overnight in a 3% solution of sodium hexametaphosphate and RO water and rinsed with RO water the following day to remove any remaining soil particles. Roots were then dried and weighed as with the shoots.

2.6 Statistical analysis

Analysis of variance (ANOVA) was performed using the GLM procedure in SAS software (SAS Institute; Cary, NC) applied to the model y = amendment, rate, soil, amendment*rate, amendment*soil, rate*soil, amendment*rate*soil, where y are the dependent variables measured. In the model, amendment refers to the material added to the soil (e.g. cotton biochar, rice hull biochar, or compost), rate refers to the rate of addition (Table 1), and soil refers to the soil type investigated (e.g. Burleson, Hearne, Pullman, or Wolfpen; table 4). Results of the ANOVA Type I sum of squares probability (p) values are reported herein. Post-hoc comparison of means was performed using Dunnett's test (α = 0.05), also in the GLM procedure, which compares all treatments for significant difference from the control. Regression analysis was applied to results for mean AMF association of roots using the REG procedure in SAS software. The regression model included the following independent variables: soil test P, soil pH, % clay, and amount of P, N, and C applied with each carbon amendment. The presence of multicollinearity between variables was evaluated through principal components regression (PCR) using the PRINCOMP procedure, and with the variance inflation factor (VIF) and the ridge regression adjustment to the model within the REG procedure (Schreiber-Gregory and Jackson 2017). Further post-hoc data analysis for plant response to AMF association was performed using simple regression analysis with plant nutrients, shoot height, shoot mass, root mass, and S:R ratio as dependent variables (α = 0.05).

Results And Discussion

3.1 Organic Amendment Properties

Composted municipal biosolids and biochars derived from pyrolysis of rice hulls and cotton field residues were applied as organic amendments to four soils at two rates each. The amendments varied in chemical composition (Table 3). Conversion of raw vegetative matter from rice hulls and cotton field residue during pyrolysis substantially altered their respective contents of carbon and nutrients, often resulting in greater concentrations afterwards.

Table 3. Chemical composition of organic amendments and the vegetative feedstocks used to create two biochars. Pre-pyrolysis is used to designate the vegetative feedstock source pyrolyzed to create each of the biochars.

Organic Amendment	pH	C	N	P	K	Ca	Mg
		---- g kg^{-1} ----		---------- mg kg^{-1} ----------			
Composted biosolids	6.86	508.2	15.0	11300	4630	89460	5340
Rice hulls (pre-pyrolysis)	–	347.2	4.8	970	2800	752	592
Cotton residue (pre-pyrolysis)	–	400.6	14.9	1342	8069	10606	3125
Rice hulls (pyrolyzed)	6.13	411.9	8.0	1509	5181	1486	1013
Cotton residue (pyrolyzed)	9.25	540.6	1.7	4262	20409	25979	7521
	S	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**B**
	------------------- mg kg-1 -------------------						
Composted biosolids	8840	1050	10545	438	258	172	41
Rice hulls (pre-pyrolysis)	470	<140	25	24	96	2	2
Cotton residue (pre-pyrolysis)	1422	659	225	19	26	5	26
Rice hulls (pyrolyzed)	217	<140	116	40	222	6	3
Cotton residue (pyrolyzed)	1843	2117	1737	41	79	22	51

Amendment pH ranged from 6.13 (moderately acid) for the rice hull biochar to 9.25 (alkaline) for the cotton residue biochar. This is a substantial and relevant difference for biochars intended as a soil amendment. Carbon contents ranged from 411.9 g kg^{-1} in rice hulls to 540.6 g kg^{-1} in composted biosolids. Nitrogen (N) contents were relatively low, ranging from 1.7 g kg^{-1} in cotton residue to 15 g kg^{-1} in biosolids. Other nutrients, such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and boron (B) were generally well supplied by the amendments.

The conversion of vegetative matter by pyrolysis is well known to result in changes in nutrient content in the resultant biochars. Such changes are dependent upon the conditions (e.g. temperature and duration of pyrolysis) under which the biochar is created, and the properties of the feedstock used. The final biochar products are also known to vary in their benefits for plant growth. In biochars created from wheat and rice straw, Naeem et al. (2014) reported a positive relationship between temperatures (300, 400, and 500°C) and the chemical parameters pH, C, P, K, Zn, Mn, and Fe, as was observed in this study. There was a negative relationship between temperature and N. Kloss et al. (2012) found similar results in the same temperature range for K, Ca, Mg, and P, but noted a decrease in Ca and Mg as temperatures exceeded 500°C. These authors stressed that the conditions under which biochars are created should be considered in alignment with the intended application of the product. The chemical changes observed in biochars in this study, created at 350°C, are similar to those reported by others. Although not measured in this study, it is reasonable to presume that the presence of microorganisms in the biochars is substantially lower than that of the composted biosolids, due to the exposure to sterilizing high heat.

Table 4. Select chemical and physical properties related to the fertility of the soils used to grow cotton in the study.

Soil	pH	Cond	NO_3-N	P	K	Ca	Mg	S	Na
		µmhos/cm	——— mg kg^{-1} ———						
Burleson	5.9	216	4	72	138	4026	538	30	20
Hearne	4.7	74	1	2	85	628	288	16	33
Pullman	7.7	282	19	30	514	5106	468	13	35
Wolfpen	6.8	95	1	7	28	636	27	7	1
	Fe	**Zn**	**Mn**	**Cu**	**Sand**	**Silt**	**Clay**	**Textural class**	
	——— mg kg^{-1} ———				——— % ———				
Burleson	34.1	0.91	70.8	0.67	37	30	33	Clay Loam	
Hearne	15.0	0.78	2.8	0.25	65	14	21	Sandy Clay Loam	
Pullman	6.9	0.56	14.1	0.74	38	35	27	Loam	
Wolfpen	12.9	0.11	6.3	0.17	73	20	7	Sandy Loam	

3.2 Soils Properties

Four soils used to grow cotton in the greenhouse varied in chemical, physical, and mineralogical properties (Table 4). The Burleson soil is a clay loam rich in smectites. The Hearne soil is a sandy clay loam featuring kaolinitic minerals. The Pullman soil is a loam rich in illites. The Wolfpen soil is a sandy loam with very little (7 %) clay mineral content. Soil pH values range from 4.7 (strongly acidic) in the Hearne soil to 7.7 (moderately alkaline) in the Pullman soil. All soils are deficient in plant available N, measured as NO_3-N. Only the Burleson soil sufficiently supplies P to plants without fertilization (critical level = 50 mg kg^{-1}; Texas A&M AgriLife Soil Water and Forage Testing Laboratory, College Station, TX). The two sandiest soils, Hearne and Wolfpen, are deficient in plant available K (critical level = 125). Many of the soil nutrients present ranges that straddle the critical levels for each nutrient, however, fertilizer nutrients supplied were sufficient to address plant requirements. Differences measured in the physical and chemical properties presented contribute to differences in native fertility, as well as to degree of response to the chemical properties contributed by the organic amendments.

Albuquerque et al. (2014) found biochars from different feedstocks differentially altered soil pH, EC, N, and P, and that rate of application was a significant factor. These authors found that the resultant biochar type was a significant effect on the growth of sunflower biomass. Ameloot et al. (2015) reported on biochars created from poultry litter and pine chips feedstocks at two different temperatures used to amend two acidic silt loams contrasting in organic carbon content. The poultry litter biochar contained approximately half the carbon of the pine chips biochar and approximately 16 times as much N. Supply of N was enhanced from poultry litter biochar in the soil with greater native C content and immobilization of N was exacerbated in the soil with lower native C. Immobilization, the incorporation of N into microbial biomass, prevents plant uptake of N from soil. This occurs when the supply of N is too low, relative to the supply of C, to provide both the C and N needs of microorganism cell growth and division (Hart et al. 1994). Therefore, amendment with biochars which are uniformly in excess of the C:N ratio required for N release (<20:1), will result in N immobilization and reduced plant uptake (Havlin et al. 2013). These results illustrate the complexity of interactions between biochar amendment and soil properties that will need to be accounted for as use becomes more widespread.

Table 5. ANOVA p-value results for treatments' and their interactions' (parameters) effect on shoot (top plant matter) height and mass, root mass, and shoot mass to root mass (S:R) ratio. Model tested in ANOVA was in the order presented in the table from top to bottom: y = carbon rate soil carbon*rate carbon*soil rate*soil carbon*soil*rate.

Parameter	df	Shoot height	Shoot mass	Root mass	S:R ratio
full model	**27**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
amendment	**3**	<0.0001	<0.0001	0.0005	0.0288
rate	**1**	0.3901	0.7989	0.3936	0.9088
soil	**3**	<0.0001	<0.0001	<0.0001	<0.0001
amendment*rate	**2**	0.0008	0.3007	0.4854	0.2117
amendment*soil	**9**	0.0848	0.4898	0.0066	<0.0001
rate*soil	**3**	0.9226	0.0181	0.0046	0.4848
amendment*soil*rate	**6**	0.0189	0.1973	0.1078	0.0010

3.3 Plant Shoot and Root Biomass

Cotton plants grown for six weeks in the greenhouse were evaluated for differences by treatment and treatment combination effects on shoot and root growth (Table 5). Organic amendment (carbon source) was significant ($\alpha = 0.05$) for differences in shoot height, shoot mass, root mass, and the ratio of shoot to root mass (S:R). Rate of application (554 or 1662 mg kg^{-1} soil) was not a significant affect for any plant growth parameter. Soil series was a highly significant effect for all measured properties ($p < 0.0001$). Organic amendment and soil interaction was significant for root mass and S:R only.

Comparison of means for difference from the control within each soil indicates that different growth parameters are differentially affected by the organic amendment treatments Table 6), though organic amendments were generally responsible for improvements to plant performance. Of the four growth parameters measured, shoot height for six-week-old cotton plants most frequently differed from the control. In no case were shoot heights for any organic amendment numerically lower than the control. In the Pullman soil, all treatments were significantly associated with taller plants. The Pullman soil also saw the highest frequency of significant shoot mass increases with amendment. Similar to shoot height, shoot mass was never numerically decreased with amendment. Root mass was improved in all cases, with the small exception of two treatments in the Wolfpen soil. Root mass was most frequently significantly improved in the Burleson soil.

Differences in the distribution of carbon resources between the shoot and root system are difficult to determine in terms of favorability for production systems. Generally, fewer roots are required to access water and nutrients when supplies of each are plentiful and located near the base of the plant (Johnson and Thornley 1987). However, when nutrients are deficient and/or diffuse in the soil volume, more roots are required to contact and intercept immobile species (Blum, 1996). There is a general trade-off in the plant between acquisition of soil resources and internal allocation of soil and photosynthetic resources. Brouwer (1963) described an equilibrium between shoots and roots that favored greater yield when root systems were more prolific.

In the current study, nutrients and water were supplied in sufficiency. Therefore, an interpretation could be put forth that plants with a larger S:R ratio more efficiently accessed those nutrients and water, supplying the photosynthetic activity of the plant with less energy invested below the surface. On the other hand, a larger root system with a smaller S:R ratio could indicate an investment (i.e. insurance) by the plant in buffering against future abrupt change in nutrient and/or water availability, and will provide continued carbon inputs from active exudation and post-harvest decomposition (Johnson et al. 2006). For the purposes of improving rain fed cotton production in Mali and neighboring countries, it may be presumed that both early shoot development and S:R ratio should be considered important attributes (Brown and Scott, 1984). Short-term productivity is never unimportant, yet insurance against environmental stress and natural redistribution of soil carbon will provide lasting benefits.

Table 6. Cotton plant shoot (top matter) height and mass, root mass, and shoot mass to root mass (S:R) ratio for plants grown in four soils with three organic amendments at two rates each. Bolded numbers with asterisks (*) indicate significant difference from the control treatment within each soil by Dunnett's test for comparison of means ($\alpha = 0.05$). μ = mean and σ = 1 standard deviation for the mean of 5 replicates.

Soil	Amendment	Rate	Shoot Height		Shoot Mass		Root Mass		S:R ratio	
			μ	σ	μ	σ	μ	σ	μ	σ
		mg kg^{-1}	cm		g		g			
Burleson	Control	0	26.2	1.6	2.26	0.37	0.53	0.14	4.38	0.47
Burleson	Compost	554	***32.7**	2.4	3.10	0.37	***0.81**	0.07	3.82	0.18
Burleson	Compost	1662	26.5	1.9	2.27	0.36	0.67	0.16	***3.45**	0.45
Burleson	Rice Hull Biochar	554	29.7	1.6	2.74	0.45	***0.91**	0.11	***3.05**	0.64
Burleson	Rice Hull Biochar	1662	31.1	4.3	2.80	0.36	***0.78**	0.09	***3.60**	0.34
Burleson	Cotton Biochar	554	29.8	2.3	3.03	0.67	***0.80**	0.16	3.78	0.23
Burleson	Cotton Biochar	1662	***32.6**	4.9	3.01	0.74	***0.85**	0.15	***3.52**	0.36
Hearne	Control	0	22.1	1.0	1.28	0.39	0.65	0.13	1.95	0.41
Hearne	Compost	554	***26.5**	2.0	1.66	0.22	1.02	0.39	1.83	0.71
Hearne	Compost	1662	22.7	0.3	***1.94**	0.29	1.21	0.30	1.69	0.53
Hearne	Rice Hull Biochar	554	23.5	0.7	1.53	0.26	1.18	0.13	1.29	0.14
Hearne	Rice Hull Biochar	1662	***25.2**	1.2	1.53	0.10	1.19	0.28	1.32	0.20
Hearne	Cotton Biochar	554	***27.6**	0.7	1.71	0.55	0.88	0.23	2.02	0.65
Hearne	Cotton Biochar	1662	***29.9**	0.8	***2.28**	0.54	***1.43**	0.66	1.88	0.90
Pullman	Control	0	26.2	2.4	1.43	0.21	0.51	0.06	2.77	0.15
Pullman	Compost	554	***34.0**	3.0	***2.12**	0.42	0.68	0.07	3.13	0.38
Pullman	Compost	1662	***39.9**	2.0	1.84	0.34	0.56	0.09	3.26	0.17
Pullman	Rice Hull Biochar	554	***35.7**	2.6	***2.09**	0.15	0.49	0.09	***4.35**	0.70
Pullman	Rice Hull Biochar	1662	***32.5**	2.3	1.79	0.40	0.50	0.18	***3.74**	0.98
Pullman	Cotton Biochar	554	***34.9**	2.7	***2.00**	0.22	0.67	0.14	3.04	0.38
Pullman	Cotton Biochar	1662	***35.8**	3.4	1.93	0.37	0.47	0.14	***4.23**	0.60
Wolfpen	Control	0	27.0	1.6	1.88	0.28	0.82	0.07	2.29	0.28
Wolfpen	Compost	554	30.6	2.6	2.08	0.50	0.85	0.21	2.46	0.18
Wolfpen	Compost	1662	30.1	4.1	2.33	0.30	0.90	0.13	2.60	0.24
Wolfpen	Rice Hull Biochar	554	***32.8**	4.2	2.46	0.49	0.64	0.13	***3.88**	0.39
Wolfpen	Rice Hull Biochar	1662	30.4	4.5	***2.56**	0.44	0.76	0.11	***3.38**	0.34
Wolfpen	Cotton Biochar	554	31.0	1.9	2.44	0.27	0.90	0.06	2.72	0.32
Wolfpen	Cotton Biochar	1662	***33.0**	3.1	2.48	0.24	0.89	0.12	***2.81**	0.26

3.4 Whole Plant Nutrient Content

The ANOVA results for affect of treatments on macro and micronutrients are presented in Table 7. Amendment was significant for the observed differences in all nutrients at α = 0.05 with the exception of K ($p = 0.0621$) and Mn ($p = 0.1136$). Rate of amendment application was not responsible for differences in the assimilation of any nutrient. Soil was a significant factor for all nutrients ($p < 0.0001$). Interactive effects for amendment and soil were significant determinants for differences in all nutrients.

Table 7. ANOVA p-value results for treatments’ and their interactions’ (parameters) effect on whole plant nutrient content. Model tested in ANOVA was in the order presented in the table from top to bottom: y = carbon rate soil amendment*rate amendment*soil rate*soil amendment*soil*rate.

Factor	df	N	P	K	Ca	Mg	S
full model	**27**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
amendment	3	<0.0001	0.0080	0.0621	<0.0001	0.0001	0.0181
rate	1	0.2361	0.5668	0.4644	0.1037	0.1130	0.8586
soil	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
amendment*rate	2	0.0363	0.4331	0.4619	0.1276	0.2698	0.0494
amendment*soil	9	<0.0001	0.0217	0.0005	<0.0001	<0.0001	0.0216
rate*soil	3	0.1010	0.0618	0.2064	0.1878	0.8714	0.7205
amendment*soil*rate	6	0.2287	0.1853	0.4771	0.0086	0.0742	0.3512
Factor	**df**	**S**	**Zn**	**Fe**	**Cu**	**Mn**	**B**
full model	**27**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
amendment	3	0.0181	0.0084	0.0055	<0.0001	0.1136	<0.0001
rate	1	0.8586	0.5938	0.1961	0.2020	0.4778	0.1534
soil	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
amendment*rate	2	0.0494	0.5975	0.0980	0.5356	<0.0001	0.7553
amendment*soil	9	0.0216	0.0249	0.0012	<0.0001	0.0083	0.0006
rate*soil	3	0.7205	0.1522	0.2306	0.2045	0.7905	0.0338
amendment*soil*rate	6	0.3512	0.5328	0.0306	0.0076	<0.0001	0.3441

Table 8 presents the results of Dunnett's test for comparison of means for differences in macronutrient assimilation from the control treatment. Soil series is evident as a determinant for N assimilation. The Wolfpen soil shows the strongest effect of amendment in depressing N uptake, as compared to the control. Regardless of the soil series, however, N uptake is almost uniformly depressed in the presence of organic amendments. The high rate of compost application in the clayey Burleson soil is the sole exception (35.2 mg N kg^{-1} compared to 31.6 mg N kg^{-1}). The Wolfpen soil exhibited the strongest effect of organic amendment on P uptake as well. Generally, organic amendments resulted in numeric, if not significant increases in P assimilation by the plants. Fewer differences or clear trends are visible across soil for K, Ca, Mg, and S. There are sparse examples of nutrient uptake depression for each. However, K uptake, which is important to bole development in cotton, was significantly improved in the Pullman soil in the low rates for all three amendments.

Table 8. Whole plant tissue (top matter) macronutrient analysis results for cotton plants grown in four soils with three organic amendments at two rates each. Bolded numbers with asterisks (*) indicate significant difference from the control treatment within each soil by Dunnett's test for comparison of means (α = 0.05). μ = mean and σ = one standard deviation for the mean of five replicates.

Soil	Amendment	Rate	N		P		K		Ca		Mg		S	
	(soil appl.)		μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
	mg kg^{-1}		------------ g kg^{-1} in tissue ------------											
Burleson	Control	0	31.6	5.0	2.84	0.41	22.72	2.14	18.99	2.34	6.21	0.40	6.81	1.39
Burleson	Compost	554	29.0	4.5	2.94	0.36	20.33	1.42	16.60	1.29	5.41	0.77	5.71	0.66
Burleson	Compost	1662	35.2	3.9	2.94	0.66	23.25	1.25	19.80	1.52	6.01	0.49	6.75	0.58
Burleson	Rice Hull Bioch.	554	29.6	2.8	3.03	0.14	20.71	1.41	15.61	3.18	5.24	0.87	6.39	0.90
Burleson	Rice Hull Bioch.	1662	29.4	3.0	2.82	0.53	***19.48**	0.53	15.52	2.14	5.29	0.86	5.70	0.58
Burleson	Cotton Bioch.	554	28.9	6.4	2.90	0.21	21.31	2.09	17.65	2.83	5.88	1.03	6.56	0.97
Burleson	Cotton Bioch.	1662	26.8	3.7	2.83	0.20	20.56	2.37	***14.70**	2.11	5.04	0.68	5.90	0.83
Hearne	Control	0	45.4	7.1	1.50	0.39	24.69	1.86	23.15	4.46	7.69	1.91	13.24	3.31
Hearne	Compost	554	***20.3**	10.7	1.61	0.19	28.53	5.67	***10.88**	1.91	***4.57**	0.50	***6.68**	3.27
Hearne	Compost	1662	32.4	11.7	2.00	0.19	29.75	3.16	***14.77**	2.63	***5.27**	0.96	10.11	3.55
Hearne	Rice Hull Bioch.	554	39.1	3.9	1.64	0.44	27.68	1.52	***16.03**	3.95	6.22	0.99	10.29	1.69
Hearne	Rice Hull Bioch.	1662	39.4	5.8	1.83	0.61	27.91	4.11	***13.77**	1.48	***4.96**	0.33	9.85	3.48
Hearne	Cotton Bioch.	554	32.4	9.8	1.42	0.58	26.07	5.01	19.28	5.38	6.46	1.29	9.84	3.43
Hearne	Cotton Bioch.	1662	34.9	5.0	1.23	0.29	25.62	2.94	17.76	2.12	6.40	0.70	8.95	1.07
Pullman	Control	0	23.6	4.3	1.82	0.27	26.56	3.15	20.70	1.75	5.96	0.53	4.17	0.42
Pullman	Compost	554	***18.8**	1.2	2.26	0.45	***30.57**	1.58	20.01	1.43	6.36	0.59	3.98	0.18
Pullman	Compost	1662	20.8	2.5	2.07	0.24	29.40	1.57	20.51	1.87	5.86	0.23	4.59	0.54
Pullman	Rice Hull Bioch.	554	23.5	2.2	***2.50**	0.36	***32.60**	2.07	23.36	1.55	6.48	0.38	4.77	0.53
Pullman	Rice Hull Bioch.	1662	***18.9**	1.0	1.79	0.21	29.57	2.99	20.29	2.85	5.86	0.70	4.04	0.36
Pullman	Cotton Bioch.	554	21.1	2.7	2.36	0.30	***31.50**	2.05	22.23	2.55	6.36	0.56	4.63	0.83
Pullman	Cotton Bioch.	1662	21.8	1.8	2.30	0.44	29.95	1.84	22.25	3.30	6.34	0.87	4.91	0.65
Wolfpen	Control	0	30.1	3.2	1.16	0.22	11.53	0.97	17.33	1.81	2.63	0.29	5.62	1.49
Wolfpen	Compost	554	***24.2**	2.2	***1.70**	0.15	11.64	1.42	18.98	3.11	2.52	0.23	5.28	1.73
Wolfpen	Compost	1662	***21.9**	3.0	1.59	0.10	10.53	0.44	13.69	2.50	***2.04**	0.20	4.96	1.95
Wolfpen	Rice Hull Bioch.	554	***25.4**	3.1	***1.69**	0.24	13.59	1.72	***12.48**	1.98	2.57	0.36	4.60	0.77
Wolfpen	Rice Hull Bioch.	1662	26.9	2.9	***1.79**	0.21	***14.17**	1.22	***11.80**	1.48	2.44	0.41	4.94	1.01
Wolfpen	Cotton Bioch.	554	***23.7**	2.8	***1.72**	0.33	12.58	1.51	14.88	2.08	2.64	0.43	5.44	0.88
Wolfpen	Cotton Bioch.	1662	***22.5**	1.8	***2.06**	0.43	13.94	2.15	***13.36**	1.83	2.65	0.31	4.85	0.78

Table 9 presents the results of Dunnett's test for comparison of means for differences in micronutrient assimilation. There was no effect on Zn assimilation. Plant uptake of Fe and Cu however, was differentially affected by soil. Amendments improved Fe uptake in the Pullman soil and depressed uptake in the Wolfpen soil. Amendments depressed Cu uptake in the Burleson soil and the Hearne soil, while cotton grown in the Pullman and Wolfpen soils were almost uniformly unaffected. No effect was present for Mn. A small number of examples indicated depression of B uptake in the sandier soils, Hearne and Wolfpen.

Table 9. Whole plant tissue (top matter) micronutrient analysis results for cotton plants grown in four soils with three organic amendments at two rates each. Bolded numbers with asterisks (*) indicate significant difference from the control treatment within each soil by Dunnett's test for comparison of means (α = 0.05). μ = mean and σ = one standard deviation for the mean of five replicates.

Soil	Amendment	Rate	Zn		Fe		Cu		Mn		B	
		(soil appl.)	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
		mg kg^{-1}	---------- mg kg^{-1} in tissue ----------									
Burleson	Control	0	25.7	5.5	37	17	6.4	1.2	76	15	62	5
Burleson	Compost	554	22.1	2.4	26	1	*5.2	0.8	66	15	63	6
Burleson	Compost	1662	26.9	2.8	34	4	*4.9	0.8	88	20	67	8
Burleson	Rice Hull Bioch.	554	23.1	2.2	*25	3	5.8	0.4	71	11	64	4
Burleson	Rice Hull Bioch.	1662	22.5	2.7	*21	1	6.5	0.8	67	12	58	7
Burleson	Cotton Bioch.	554	23.5	4.3	30	5	*5.2	0.7	86	44	64	11
Burleson	Cotton Bioch.	1662	20.8	3.2	32	2	*5.3	0.2	62	11	55	2
Hearne	Control	0	40.4	11.0	176	53	11.4	2.1	66	16	112	28
Hearne	Compost	554	27.2	6.3	88	28	*6.7	1.2	87	25	*60	13
Hearne	Compost	1662	33.7	6.7	78	10	*8.4	1.0	81	7	*73	17
Hearne	Rice Hull Bioch.	554	37.8	6.4	87	25	9.4	1.5	65	12	88	23
Hearne	Rice Hull Bioch.	1662	39.5	9.2	125	35	10.2	1.5	96	46	78	20
Hearne	Cotton Bioch.	554	29.7	9.1	189	93	9.5	2.3	51	19	80	17
Hearne	Cotton Bioch.	1662	33.8	7.2	140	46	*8.1	0.9	43	10	92	18
Pullman	Control	0	11.4	2.1	26	7	5.9	0.7	44	8	67	5
Pullman	Compost	554	11.9	2.8	31	3	5.8	0.7	35	4	62	8
Pullman	Compost	1662	10.9	1.5	*32	3	5.5	0.6	41	6	59	11
Pullman	Rice Hull Bioch.	554	13.0	2.4	*36	3	*6.2	0.4	43	5	59	6
Pullman	Rice Hull Bioch.	1662	9.4	1.2	*38	2	4.8	0.5	36	3	63	6
Pullman	Cotton Bioch.	554	12.1	1.4	*37	3	5.7	0.5	42	8	67	7
Pullman	Cotton Bioch.	1662	12.0	1.9	*39	3	5.7	0.6	38	6	65	14
Wolfpen	Control	0	20.1	3.3	49	11	5.6	0.8	293	83	84	13
Wolfpen	Compost	554	19.0	2.1	*36	5	5.7	0.8	186	81	74	15
Wolfpen	Compost	1662	16.4	3.5	*28	5	5.0	0.7	216	71	*55	9
Wolfpen	Rice Hull Bioch.	554	21.9	4.7	*29	4	6.0	1.0	203	82	63	12
Wolfpen	Rice Hull Bioch.	1662	24.0	4.1	*28	2	5.3	1.0	347	54	*54	15
Wolfpen	Cotton Bioch.	554	18.6	5.6	*33	5	5.2	1.1	308	42	71	12
Wolfpen	Cotton Bioch.	1662	17.6	3.9	*31	6	5.2	1.0	184	91	*58	10

Whole plant N in this study (20.8 - 45.4 mg N kg^{-1}) was in the range of 1.6 to 4.5 mg N kg^{-1} reported by others for young cotton plants (Joham 1950; Harris 1960). The general depression of uptake of N in amendment treatments raises the concern that additional N fertilizer may be required in the field to ensure early nutritional needs for cotton crops are met. Whole plant P was generally increased by amendment. However, the values obtained in this study (1.2 - 3.0 mg P kg^{-1}) were lower than those from the same studies (3.15 - 5.1 mg P kg^{-1}). Other macronutrients reported by Joham (1950) and Harris (1960) for young whole plants including K (20.8 - 46.1 mg kg^{-1}), Ca (15.1 - 34.6 mg kg^{-1}), and Mg (4.0 - 5.36 mg kg^{-1}). However, these nutrients were well under the range for many treatments in the Wolfpen soil.

When relating the nutrient uptake results to shoot matter, the depression of N in every case may be at least partially explained as a dilution effect arising from the increase in shoot height and mass (Table 6 and Table 8). This statement does not imply a prediction on sufficiency or deficiency beyond the six-week period of this study. However, with P uptake there was a general positive coincidence between P increase and shoot increases. Because growth (as shoot height, shoot mass, and root mass) was generally stimulated by amendment, depressions and increases in the uptake of all other nutrients may be considered through the same lens.

There is a lack of data from recent studies on whole plant nutrients at early stages of growth. There were no identifiable sources of information on whole plant S, or the micronutrients Zn, Fe, Cu, Mn, and B. Early growth of cotton biomass and nutrient uptake can substantially influence later development stages, this study provides important data in that regard that is currently in short supply.

3.5 AMF/Root Association

Mutualism between cotton roots and AMF occurs only when there is an unsatisfied need that AMF can provide to that plant (Hale et al. 2011). Therefore, harnessing the benefits of this relationship towards cotton production following biochar amendment in sub-Saharan Africa will depend upon better understandings the complex nature of the system. For example, if biochar amendment were to suppress the association between AMF and roots, then our understanding of the benefits of biochar would perhaps need to be tempered. On the other hand, if biochar were to uniformly stimulate AMF association with cotton roots, the attendant benefits would be expected, including increased P acquisition, drought resistance, and pest tolerance (Watt et al. 2006).

Multicollinearity evaluation resulted in different choices for the model depending on whether PCR, VIF, or ridge regression were employed. For example, P supplied via carbon amendment, and not soil test P, was identified as highly related by PCR. On the other hand, ridge regression identified only soil-associated properties as significant at $\alpha = 0.05$. Ultimately, carbon amendment P content was included ($p = 0.0547$) in the final regression model as the data appear to support the inclusion of factors other than those exclusively associated with the four soils (Figures 1 and 2). Note that an intercept term was not significant for the model. Regression parameter estimates from the SAS software REG procedure are presented in Table 10.

Table 10. Ridge regression parameter estimates, standard errors, and probabilities (p-values) for relating measured AMF % density in cotton roots to soil and amendment properties.

Variable	**Parameter Estimate**	**Standard Error**	**p-value**
Carbon amendment P	+1.411	0.698	0.0547
Soil test P	-0.486	0.137	0.0017
Soil pH	+2.153	0.976	0.0372
% Clay	+1.129	0.354	0.0040
ANOVA	**Sum of Squares**	r^2	**p-value**
Model (n = 28)	26634	0.8614	< 0.0001

Regression results indicate that total P in the carbon amendments were positively related with AMF root colonization, while soil test P was negatively related. Soil pH and % clay content of the soils were both positively related to AMF root colonization in the range of values included in this study. Other factors evaluated (amendment C and N) were not significant. Because AMF are frequently associated with biotic and abiotic stress resistance in major crop plants, a better understanding of early association patterns as a function of soil property and amendments will be important to managing them for crop production benefits.

In our study, AMF inoculants were prepared from fresh soils taken from the same sites as the processed soils used to grow cotton. It was our intention to work only with native populations, as exotic species have occasionally been shown to be less beneficial to plant growth and less inter-functional with other native macro and microflora (Klironomos 2003; Koziol and Bever 2017; Kouadio et al. 2017; Rezacova et al. 2020). Therefore, soil specific effects are to be expected, by design. However, the current study supports positive correlation between AMF/root association and clay content, plant available soil P, and pH reported in previous studies.

Previous research has indicated that AMF colonization of plant roots is negatively related to plant available P in soils, whether native to the soil or supplied as fertilizer (Douds and Schenck 1990; Konvalinkova et al. 2017). However, P supplied to soil in organic amendments was demonstrated to stimulate AMF association with roots. The chemical forms in plant residues, manures, and composts are primarily organic P compounds (Sadras 2006; Azuara et al. 2013). The chemical forms in biochar are primarily insoluble inorganic P complexes with aluminium (Al), Ca, Mg, and Fe that are more stable than organic P (Uchimiya et al. 2015). Neither are immediately plant available, and therefore require some energy to mineralize or solubilize. Hence, the assistance to plants from AMF towards increasing available P favors increased mutualistic interaction. This mechanism explains the contrasting negative effect of native soil P with the positive effect of amendment P on AMF / root association.

There is very little evidence directly comparing soil pH as a factor in AMF association with plant roots. Graw (1979) reported that AMF efficiency in increasing P uptake from Ca^{2+} and Al^{3+} complexed phosphate (PO_4^{3-}) applied to soil in Mexican marigold (*T. minuta* L.) and nigerseed (*G. abyssinica*) over the range of 4.4 to 6.6 pH. The range of soil pH values in this study was wider (4.7 to 7.7), extending the evidence for this effect. Carrenho et al. (2007), however, reported that liming to increase soil pH (4.5-7.0) did not result in significant effect for AMF colonization of peanut (*A. hypogaea* L. variety Tatú), sorghum (*S. bicolor* (L.) Moench variety AG 1017), or corn (*Z. mays* L. variety IAC Taiúba) in the field. This may have been an artifact of ecosystem perturbations caused by introduction of the calcareous dolomite liming agent ($CaMg(CO_3)_2$), rather than a response to soil pH. Porter et al. (1987) found that different species of AMF associated with cereal crops predominated in acidic soils (*A. laevis*) and alkaline soils (*Glomus sp.* WUM 3) in western Australian, supporting the concept that external adjustments to soil pH may not favour the activity of native adapted AMF species.

The body of knowledge on AMF association affected by soil clay content is not well developed. Our study found that soil clay was positively related to AMF association with cotton roots. Porter et al. (1987) found a weak positive relationship with clay content. Carrenho et al. (2007) reported a negative effect for soil clay on AMF colonization, stating that sandy soils stimulated development of AMF association while clayey soils inhibited it. There is a need to continue filling the knowledge gap in this specific area of inquiry.

Post-hoc simple regression analysis for direct relationships between AMF as an independent variable and the plant performance parameters nutrient assimilation, shoot height, shoot mass, root mass, and S:R ration indicated that there were relationships only for N (17.19 + 0.19*AMF; model p = 0.0189) and shoot mass (2.61 - 0.02; model p = 0.0065). Although a relationship between AMF association and P uptake was expected, it was not supported by the data in this study. Increased N uptake associated with AMF has been reported for celery (Ames et al. 1983) and lettuce (Tobar et al. 1994; Azcon et al. 2008). Hodge et al. (2001) attributed improved N uptake to improved organic matter degradation. Contrary to the negative effects regarding shoot mass, Konvalinková et al. (2017) reported increased shoot mass following inoculation with AMF in leeks (*A.* porrum) and ryegrass (*L. perenne*), but not in medic (*M. truncatula*). Their study was performed in the glass house in pots filled with sand and zeolite mix. Plants were harvested at 63 days, as opposed to 48 in our study. The effects in very young plants, where AMF associations are only recently established, may reflect larger initial percentage investments of plant photosynthetic product than those in more mature stages of growth development.

Brundrett et al. (1985) reported that AMF hyphae penetrated leek root cells within 2 days, that arbuscules (structures for transfer of products between plant and host) were formed within 4 days, and that vesicles (structures for storage of product) were formed within 5 days of inoculation. It is clear that mutualistic associations between AMF and host plant can establish very rapidly. The observations in this study were that hyphae, arbuscules, and vesicles were present all present in cotton roots harvested at 6 weeks. However, it was beyond the scope of this study to further evaluate allocation of resources between host and AMF symbiont as a function of plant developmental stage.

Conclusion

Cotton grown in the greenhouse to six weeks in four contrasting soils was generally improved in terms of shoot height, shoot mass and root mass following amendment with composted municipal biosolids, rice hull biochar, and cotton biochar. Beneficial or deleterious effects of organic amendments in terms of plant nutrient assimilation were both soil and nutrient specific. One notable and consistent effect was the depression of N uptake following amendment, which suggests that early application to ensure sufficient mineralization occurs or supplemental fertilizer N will be required. Plant root colonization rate by AMF was found to be positively related to the P supplied by carbon amendments, soil pH, and soil clay content. Colonization was negatively affected by native plant available (soil test) P level. Uptake of N was positively affected, while shoot mass was depressed by AMF colonization. Plant uptake of P was not affected by AMF colonization. The most important results from this study provide cautions that the response of early growth in cotton to organic amendments and AMF colonization will be substantially affected by soil properties. This is not a new idea, but one that is too frequently ignored. This study indicates that there is a substantial positive potential for early growth stimulation of cotton by amending Malian soils with biochars made from readily available feedstocks.

Declarations

Funding: This project was funded through the United States Department of Agriculture Foreign Agriculture Service USDA FAS

Conflicts of Interest: Not applicable

Availability of data and material: Requests should be directed to the *corresponding author

Code availability: Not applicable

Authors' contributions: Not applicable

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

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Figures

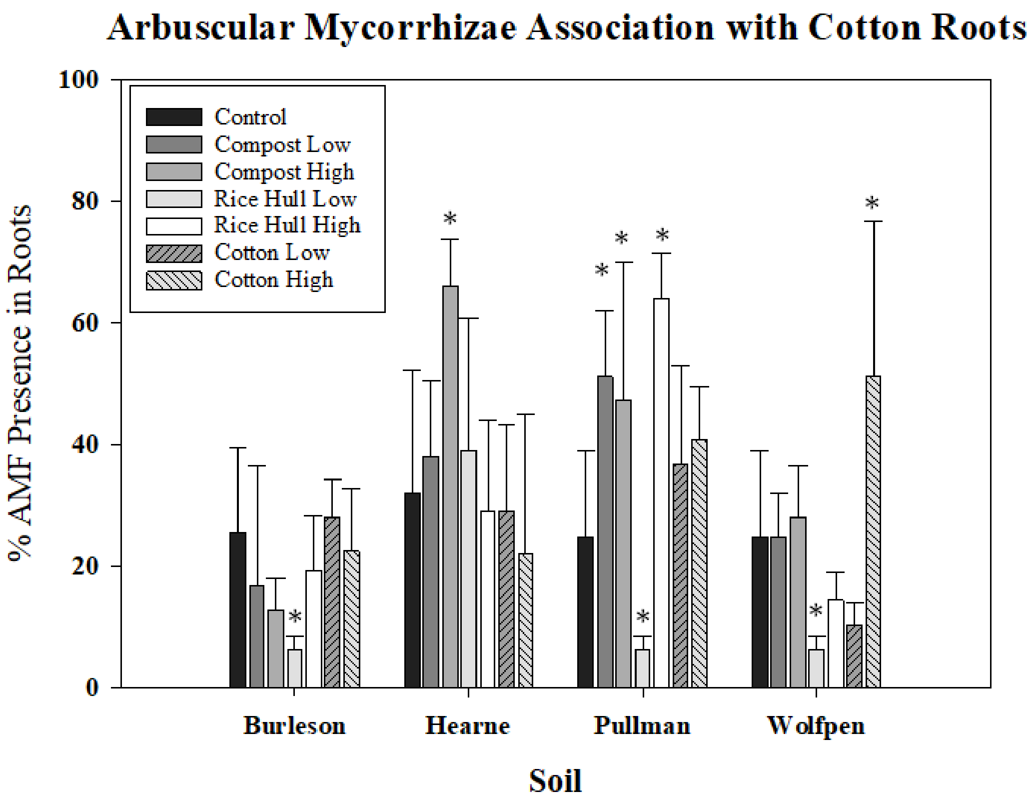

Figure 1

Bar chart for means of AMF % density in cotton roots grown in four soils receiving three organic amendments applied at two rates each, grouped by soil. Low rate of soil application = 554 mg kg-1. High rate of soil application = 1662 mg kg-1. Asterisks (*) indicate significant difference from the control treatment within each soil by Dunnett's test for comparison of means (α = 0.05).

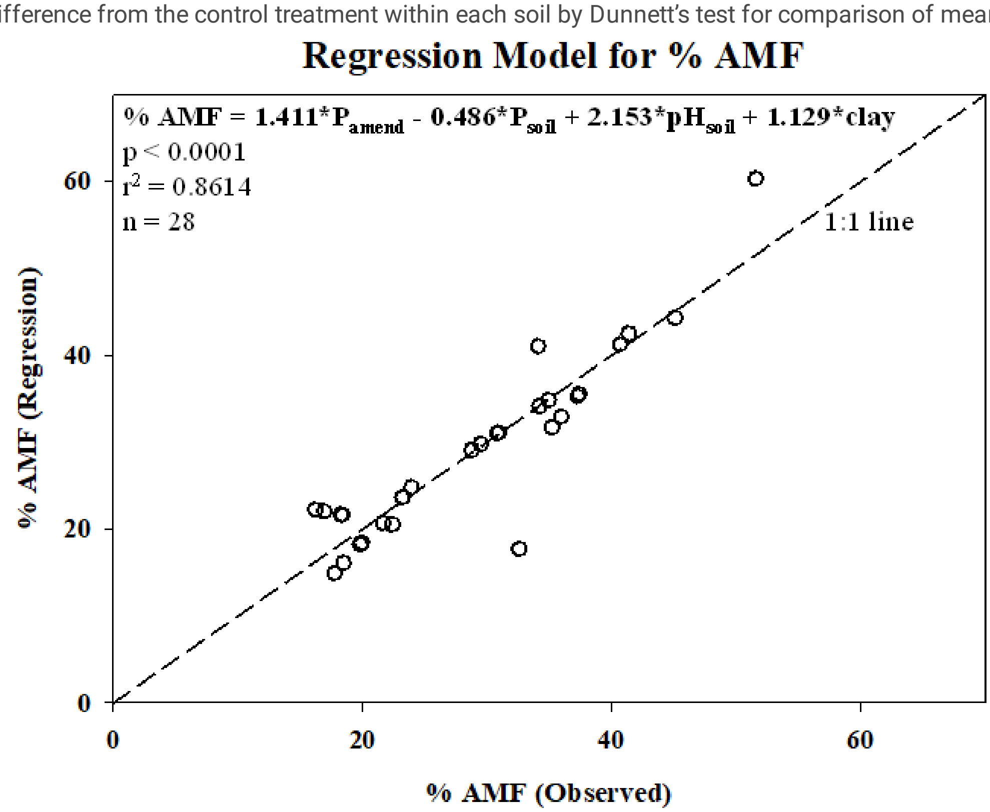

Figure 2

Results for ridge regression analysis of soil and organic amendment parameters' effect on AMF % density in cotton roots grown in four soils receiving three organic amendments applied at two rates each. Regression model with parameter estimates for the effect of each parameter in top of plot box. p = p-value, r2 = correlation coefficient, and n = number of treatment means included for regression model.