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Influence of alternative soil amendments on mycorrhizal fungi and cowpea production

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Abstract

Alternative soil amendments (worm compost, pyrolyzed carbon [biochar]) and crop symbioses with arbuscular mycorrhizal (AM) fungi have the potential to reduce food production costs while promoting sustainable agriculture by improving soil quality and reducing commercial (N and P) fertilizer use. Our greenhouse studies investigated the influence of alternative soil amendments on AM fungi associated with cowpea (*Vigna unguiculata* [L.] Walp.) and common bean (*Phaseolus vulgaris* L.) by examining productivity and plant nutrition. We conducted an experiment to select a cowpea or common bean genotype based on AM fungal colonization, seed production, and seed nutritional content. We then grew the selected cowpea genotype (Resina) in low-fertility soil with 10 different soil amendments (combinations of biochar, worm compost, and/or commercial fertilizers) plus a non-amended control. There were no significant differences in AM fungal colonization of cowpea plants grow with different soil amendments. However, an amendment blend containing worm compost, biochar, and 50% of the typically recommended commercial fertilizer rate produced plants with similar aboveground biomass, protein concentration, and total protein production, with increased tissue K, P, and Zn concentration and total content, compared to plants receiving only the recommended (100%) rate of commercial fertilizer. As previous research links uptake of P and Zn with plant-mycorrhizal symbioses, our

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results indicate cowpea nutritional benefits may be derived from AM partnership and alternative soil amendments. These synergies between alternative soil amendments and AM fungi may help reduce farm costs while maintaining or improving crop yield and nutrition, thus increasing global food and nutrition security.

Keywords: Microbiology, Agriculture

1. Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important legume crop in many countries, with notable drought tolerance (Rivas et al., 2016). Both the seeds and leaves of cowpea can reduce malnutrition in food insecure populations, and pulse crop cultivation can improve soil fertility (Anyango et al., 2011; Vilakati et al., 2016; Lal, 2017). However, cowpeas are often grown on marginal soil without fertilization, leading to subsistence level yields (Kyei-Boahen et al., 2017).

One key to successful cowpea cultivation may be enhancing mutualistic partnerships with beneficial soil microbes, such as arbuscular mycorrhizal (AM) fungi. Field research from sub-Saharan Africa reports diverse AM fungal genera associating with cowpea roots (Diop et al., 2015; Johnson et al., 2016). Mycorrhizal fungi form beneficial associations with up to 80% of terrestrial plants, primarily increasing water and phosphorus (P) uptake in exchange for plant-derived carbon (Smith and Read, 2008). In natural ecosystems, especially in the case of locally adapted plant-soil-mycorrhizal partnerships, AM fungi can significantly improve plant nutrition (Rúa et al., 2016). Though there are numerous challenges in harnessing AM fungi to increase crop yield, they present a great opportunity to improve agricultural sustainability in multiple ways, such as reducing crop fertilizer and water requirements (Bender et al., 2016; Thirkell et al., 2017).

Crop genotypes vary considerably in their association with AM fungi, and it is important to determine the mycorrhizal responsiveness of genotypes utilized in research, indicated as a response ratio of plant biomass production of a genotype grown with AM fungi compared to the same genotype grown without AM fungi, with all other nutrient conditions held constant. For example, we found substantial differences between AM benefits to grain yield and nutritional quality of six *Sorghum bicolor* genotypes (Cobb et al., 2016). Previous research indicates positive mycorrhizal responsiveness for cowpea and common beans (*Phaseolus vulgaris* L.) (Nanjareddy et al., 2017; Oruru et al., 2018). Therefore, we focused on the influence of conventional and alternative nutrient management on AM fungi and the production/nutrition of common bean and cowpea. In our study, we initially screened genotypes of cowpea and common bean to select a productive genotype—selecting a cowpea cultivar, Risina del Trasiorfino, based on its AM fungal root colonization, seed yield, and seed nutritional quality for our follow-up greenhouse experiment.

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In addition to crop genetics, many conventional agricultural practices can negatively affect AM fungi, potentially disrupting the establishment and function of plant-fungal symbiosis. Previous research suggests AM fungal abundances, diversity, and benefits are reduced following high fertilizer rates, monoculture, fallow periods, and heavy tillage (Richardson et al., 2011; Bowles et al., 2016; Manoharan et al., 2017). Increasing AM fungal abundance in association with agricultural crops is of global importance. Dwindling P reserves threaten future food security (Herrera-Estrella and Lopez-Arredondo, 2016; Chen and Graedel, 2016), and in many countries, soil erosion far outpaces pedogenesis (Pimentel, 2006; Oliver and Gregory, 2015; Tully et al., 2015). However, if mycorrhizas can be managed as "natural bio-fertilizers" in farm systems, they could improve crop nutrition (particularly P uptake) and production efficiency (Berruti et al., 2015; Cobb et al., 2016; Faucon et al., 2017). In addition, AM fungi have been shown to enhance soil aggregation, stability, and/or water-holding capacity (Wilson et al., 2009; Willis et al., 2013; Mardhiah et al., 2016). Therefore, it is critical for researchers to develop mycorrhiza "smart" agricultural practices that do not inhibit plant-fungal associations, ultimately regenerating and sustaining AM abundance and functions in agroecosystems.

Commercial fertilizers are critical to improving yields in developing countries; however, they represent a substantial cost to farmers (Brunelle et al., 2015). Due to this cost, community projects worldwide are utilizing various types of compost to reduce local reliance on fertility amendments from external sources (Misra et al., 2003). Chaoui et al. (2003) reported that high rates of earthworm-based composts (vermicomposts) improved crop-nutrition similarly to the application of commercial fertilizers, with the additional benefits of slow nutrient release, reduced leaching, and protection from salinity stress. Maji et al. (2017) found vermicompost amendments significantly increased AM root colonization of *Pisum sativum*, compared to corresponding *Pisum* plants grown without vermicompost. A recent review suggests research in a wide array of conditions, is needed to further examine the potential benefits of vermicompost for crop production (Abbott et al., 2018). Compost can maintain or increase the AM fungal root colonization of several agricultural crops, though effects may vary due to the chemical and biological qualities of different types of compost (Duong et al., 2012; Cavagnaro, 2015).

Previous research suggests there may also be multiple beneficial outcomes from applying pyrolyzed carbon (biochar) in agroecosystems, such as enhanced N fixation by legumes and improved yields in cereal-legume intercropping systems (Liu et al., 2017a). A meta-analysis of biochar reported amendments typically improved recycling of organic waste and aboveground plant productivity, but not belowground productivity or AM abundance compared to non-amended plants (Biederman and Harpole, 2013). However, Vanek and Lehmann (2015) found additions of biochar improved AM fungal root colonization for bean plants, compared to non-amended plants. Mickan et al. (2016) reported similar results of increased colonization for

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subterranean clover (*Trifolium subterraneum*) grown in water-limited agricultural soils. A recent meta-analysis concluded biochar, as a nutrient source, increases yields in tropical rather than temperate soils; however, this analysis did not include assessments of nutrient use efficiency (Jeffery et al., 2017). More research is needed to determine the diverse and complex impacts of biochar on agroecosystems (Abbott et al., 2018).

Our greenhouse study assessed the influence of alternative soil amendments (worm compost, biochar, reduced commercial fertilizers) on AM fungi and the productivity and plant nutrition of cowpea. It is critical to determine potential links between belowground plant-mycorrhizal interactions and aboveground biomass production and plant nutrition (protein, Ca, Fe, K, Mg, P, and Zn). Synergies between AM fungi and alternative soil amendments may help reduce commercial fertilizer use while maintaining quality agricultural yields, thus reducing farm costs while providing human dietary needs.

2. Materials and methods

2.1. Experimental setup

Our greenhouse studies included: 1) genotype screening of two cowpea (Purple-hull, Resina del Trasiorfino [Resina]) and two common bean (Dicta 105, Masaai Red) genotypes to determine a model genotype, based on AM fungal colonization, seed yield, and seed nutrition and 2) assessment of the influences of AM fungi and alternative soil amendments on aboveground productivity and nutrition of the selected genotype. Cowpeas were sourced from Baker Creek Heirloom Seeds and Dr. Beebe at the International Center for Tropical Agriculture (CIAT) provided common bean genotypes. Genotypes were selected in consultation with seed providers based on hardiness and improved nutrition.

For genotype screening, cowpea and common bean genotypes were grown in pots (26.5 cm diameter × 45 cm height) filled with 22 liters of Renfrow/Grainola (eroded silty clay Mollisol/Alfisol) native grassland soil collected from the Oklahoma State University Range Research Station. Baseline soil contained 20 mg kg^{-1} plant-available N, 6 mg kg^{-1} plant-available P, 154 mg kg^{-1} plant available K, 0.88% OM, and pH of 6.8, as determined by the Oklahoma State University Soil, Water, and Forage Analytical Laboratory. Soil samples were dried at 65 °C overnight and ground to pass through a 2 mm sieve. Soil pH was measured by glass electrode in a 1:1 soil:water suspension (Thomas, 1996). Soil NO3-N and NH4-N were extracted with 1 M KCl solution and quantified by a Lachat Quickchem 8000 Flow Injection Autoanalyzer (LACHAT, 1994). Plant-available P and K were extracted using Mehlich 3 solution (Mehlich, 1984) and quantified by a Spectro Blue inductively coupled plasma (ICP) spectrometer (Soltanpour et al., 1996). Soil organic C was determined using a LECO Truspec dry combustion C analyzer (Nelson and Sommers, 1996). A randomized complete block (RCB) design was used with four replications of each genotype × amendment combination. Soil was not inoculated with additional AM fungal propagules. Plants were either: 1) non-amended, 2) amended with diammonium phosphate (18% N, 46% P, 0% K) and urea (46% N, 0% P, 0% K) to target soil N and P concentrations of 50 mg kg^{-1} plant-available N and 30 mg kg^{-1} plant-available P, or 3) amended with 480 g of worm compost (pH = 6.2; plant-available N = 0.07%; plant-available P = 0.1%; plant-available K = 0.2%; Total C = 7.32%; moisture = 29.8%).

Once we selected the cowpea cultivar Resina as our model genotype (see Table 1), we conducted a follow-up study as an RCB design with six replications of each soil amendment. Cowpea plants were grown in 22 cm diameter × 22 cm height pots filled with 7.5 L of course sand mixed in a 2:3 ratio with Renfrow/Grainola (eroded silty clay Mollisol/Alfisol) native grassland soil collected from the Oklahoma State University Range Research Station. This soil was selected due to low fertility: baseline soil:sand mix contained 13 mg kg^{-1} plant-available N, 5.6 mg kg^{-1} plant-available P, 118 mg kg^{-1} plant available K, 0.40% OM, and pH of 7.4, as determined by the Oklahoma State University Soil, Water, and Forage Analytical Laboratory. Sand was included in this experiment to improve water infiltration of the heavy clay grassland soil we collected. Sand was autoclaved prior to mixing with soil, and the mix was not inoculated with additional AM fungal propagules. For this sand:soil mix, we determined the abundance of AM phospholipids (hyphal biomass) to be 3.55 nmol g^{-1} of soil and the abundance of AM neutral lipids (storage biomass) to be 23.55 nmol g^{-1} of soil (see White and Ringelberg, 1998; Allison and Miller, 2005; Sharma and Buyer, 2015). These values are significantly different (paired t-test, $p < 0.001$, n = 8) compared to abundances found in both Oklahoma native rangelands (PLFA = 5.76 nmol g^{-1} and NLFA = 37.12 nmol g^{-1}) and in Oklahoma agricultural soils (PLFA = 1.35 nmol g^{-1} and NLFA = 4.72 nmol g^{-1}), suggesting AM fungal biomass was neither extremely high nor extremely low in our sand:soil mix.

Table 1. Mycorrhizal root colonization (MRC; %), seed yield (g), and total seed protein (g), K, Mg, P, Zn content (mg) per cowpea or common bean plant.

Genotype	MRC	Yield	Protein	K	Mg	P	Zn
Purple-hull	48^{a}	13.8^{b}	3.3^{b}	194^{b}	30^{ab}	73^{b}	0.9^{ab}
Resina	48^{a}	18.6^{ab}	4.2^{a}	210^{b}	37^{a}	97^{a}	1.0^{a}
Dicta 105	37^{ab}	15.7^{b}	3.3^{b}	217^{b}	28^{b}	70^{b}	0.7^{b}
Masaai Red	30^{b}	19.8^{a}	4.2^{a}	295^{a}	37^{a}	92^{ab}	0.9^{ab}

Cowpea (Purple-hull, Resina) and common bean (Dicta 105, Masaai Red) genotypes. Results were pooled across treatments when there were no significant cultivar by treatment interactions. Data presented as LS means (n = 12). Within a column, values labeled with the same letter do not differ significantly ($p < 0.05$).

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2.2. Soil treatments and greenhouse conditions

Commercial fertilizer (N and P) recommendations for cowpea producers are based on target soil concentrations (mg kg^{-1}) and yield goals. For this study, we determined the standard (recommended) soil N and P concentrations as 50 mg kg^{-1} plant-available N and 30 mg kg^{-1} plant-available P (Zhang and Raun, 2006). After genotype screening, we utilized Resina as our model genotype. To compare potential interactions between commercial fertilizers and alternative soil amendments (e.g. release of N and P, absorption of N and P), experimental treatments with reduced or increased commercial fertilizer targets were also included. Soil was either non-amended (control) or amended with: 1) worm compost, 2) biochar, 3) both worm compost and biochar, 4) 100% of the recommended fertilizer rate (N and P), 5) biochar and 150% of the recommended fertilizer rate, 6) biochar and 100% of the recommended fertilizer rate, 7) biochar and 50% of the recommended fertilizer rate, 8) worm compost and biochar and 150% of the recommended fertilizer rate, 9) worm compost and biochar and 100% of the recommended fertilizer rate, or 10) worm compost and biochar and 50% of the recommended fertilizer rate (Table 2).

Soil treated with commercial fertilizers (N and P) received diammonium phosphate (18% N, 46% P, 0% K) and urea (46% N, 0% P, 0% K). All biochar amendments consisted of 30 grams added to each pot (0.0044% by weight). Biochar was produced at

Table 2. Plant-available N and P added (mg), total soil concentrations (mg kg^{-1}), and properties for experimental soil amendments during setup.

Soil amendments	N added	P added	N total	P total
Control	-	-	13.0	5.6
WC	59.2	84.3	21.7	18
B	10.2	29.9	14.5	10
WC + B	69.4	114.2	23.2	22.4
NP 100	251.6	165.9	50	30
B + NP 150	431.8	297.8	76.5	49.4
B + NP 100	261.8	195.8	51.5	34.4
B + NP 50	91.8	93.8	26.5	19.4
WC + B + NP 150	491.0	382.2	85.2	61.8
WC + B + NP 100	321.0	280.2	60.2	46.8
WC + B + NP 50	151.0	178.2	35.2	31.8

Properties	Added	pH	Carbon	Moisture
WC	120g	6.2	7.32%	29.8%
B	30g	9.49	85.5%	56.4%

Soil Amendments: Control = Non-amended, WC = Worm Compost, B = Biochar, NP = Commercial Fertilizers (% of Recommended Rate).

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500–700 °C from a pinewood-based wood product (plant-available N = 0.08%; plant-available P = 0.23%; plant-available K = 0.65%; see Table 2). Worm compost amendments were established as 120 grams per pot (0.0176% by weight) (plant-available N = 0.07%; plant-available P = 0.1%; plant-available K = 0.2%; see Table 2). Commercial fertilizers (suspended in 500 mL of water), biochar, and worm compost were applied 24 hours before seeding and incorporated in proximity to anticipated seed depth (~2 cm). This procedure simulates a trench and fill amendment application method (Filiberto and Gaunt, 2013) that can be utilized with hand tools in the field.

In both studies, six seeds were sown directly into soil (~2 cm depth) moistened with 500 mL of water per pot. During genotype screening, plants were thinned to one per pot. In the follow-up study, plants were thinned to two per pot. Plants were maintained under well-watered conditions (watering every 2–4 days with 500 mL per pot) for both experiments. Greenhouse temperatures were maintained between 20 and 32 (daily mean 25) °C and photosynthetically active radiation (PAR) in ambient light ranged 918–1027 mmol m^{-2} s^{-1}. During genotype screening, plants were grown until senescence (~100 days) and multiple seed harvests occurred as pods became dry (combined for final seed yield). In the follow-up study, plants were harvested 45 days after emergence and aboveground biomass (dry weight) was determined.

2.3. Seed and tissue nutrients

The USDA-ARS Children's Nutrition Research Center in Houston Texas assessed seed K, Mg, P, and Zn concentrations, using the methods of Farnham et al. (2011). Oklahoma State University Soil, Water, and Forage Analytical Laboratory determined percent protein and mineral concentrations for plant tissue samples (combined leaf and stem biomass). Samples were dried at 85 °C overnight and ground to pass through a 1 mm screen. The moisture content was determined by drying each ground sample at 105 °C overnight. Total nitrogen (TN) and C were determined using a dry combustion C/N Analyzer (LECO Truspec) and crude protein was calculated by multiply TN by 6.25 (NFTA, 1993). Plant tissue mineral concentrations (Ca, Fe, K, Mg, P, and Zn) were analyzed by a Spectro Blue ICP following acid digestion with a block digester in nitric acid (NFTA, 1993) and calculated in parts per million (mg kg^{-1}). Plants with minimal biomass can appear to have similar nutritional quality to plants with greater biomass on a per gram basis (Ellouze et al., 2016); therefore, to reduce this potential complication while comparing the relative total nutrient accumulation of plants across each of our studies, total production and protein/mineral concentration data were combined [(production in grams) × (concentration %)]/100 to calculate total protein and mineral contents for seeds or aboveground plant biomass.

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2.4. Intra-radical mycorrhizal abundance

At harvest, root systems were washed free of soil. Nine randomly selected subsamples were collected from roots 5−10 cm below plant stems, cleared (KOH 10% for 24 hours), stained with trypan blue (0.05% for 24 hours), and scored for percentage root length colonized by AM fungi using the magnified gridline intersect method (McGonigle et al., 1990). We used a digital microscope (Hirox KH 7700) to estimate the total root colonization.

2.5. Statistical analysis

Intra-radical AM abundance, seed, aboveground plant biomass, protein and mineral concentration/content were analyzed using generalized linear mixed models (RCB design). Residuals were checked for normality and Ca, Mg, and K concentrations were square root transformed. For genotype screening, we pooled results across treatments and reported responses that were significantly different by cultivar as long as these responses did not have a significant treatment by cultivar interaction. Results are reported as least square (LS) means, and the Tukey multiple comparison method was utilized to separate means. All tests of significance were performed at $p < 0.05$. The data analysis was generated using SAS® version 9.4.

3. Results

3.1. Genotype screening

When comparing seed protein and mineral concentrations (mg kg^{-1}) between cowpea and common beans, common beans had significantly greater K while cowpeas had significantly greater protein, Mg, P, and Zn (data not shown, n = 12). The cowpea genotype Resina produced seeds with significantly greater total protein and P content compared to Purple-hull cowpea and common bean genotype Dicta 105 (Table 1). While total seed yield and nutritional content were similar for Resina and common bean genotype Masaai Red, the roots of Resina plants were significantly more colonized by AM fungi, compared to Masaai Red roots (Table 1). Resina was selected as the model genotype for our follow-up study based on similar nutritional quality and greater root colonization.

3.2. Aboveground plant production

In our second experiment, plants amended with commercial fertilizers, regardless of rate, produced significantly more vegetative biomass following 45 days of growth compared to non-amended plants or plants treated with only compost and/or biochar

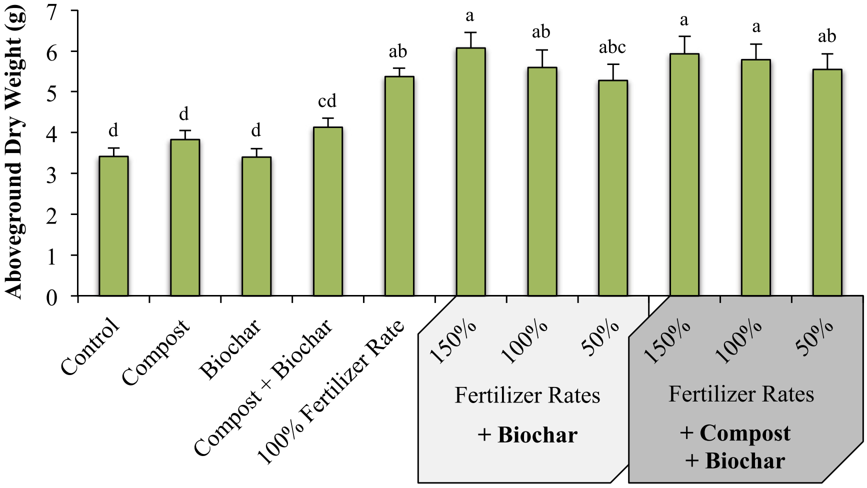

Fig. 1. Aboveground cowpea biomass production (dry weight in grams, two plants per pot) cultivar Risina, grown for 45 days in non-amended soil (control) or soil amended with worm compost (compost), biochar, and/or commercial (urea and diammonium phosphate) fertilizers. The recommended (100%) N and P fertilizer rate was 50 mg kg^{-1} plant-available N and 30 mg kg^{-1} plant-available P (150% = 75/45 mg kg^{-1}; 50% = 25/15 mg kg^{-1}). Bars represent LS means, +SE (n = 6). Bars labeled with the same letter do not differ significantly ($p < 0.05$).

(Fig. 1). The average biomass of plants amended with worm compost, biochar, and 50% of the recommend commercial fertilizer rate were similar to plants amended with the recommended (100%) commercial fertilizer rate.

3.3. Protein production

There were no significant differences in plant tissue protein concentration (%) between treatments (means ranged from 11.13 ± 1.02 to 15.82 ± 1.02) (data not shown, n = 6). However, total protein production of plants grown with some of the combinations of commercial fertilizers, worm compost, and/or biochar, was significantly greater compared to non-amended plants (Fig. 2). The average protein production of plants amended with worm compost, biochar, and 50% of the recommend commercial fertilizer rate were similar to plants amended with the recommended (100%) commercial fertilizer rate.

3.4. Tissue mineral concentrations and content

Total aboveground plant tissue concentrations (mg kg^{-1}) of Fe were not significantly different across treatments, and non-amended plants typically had similar or reduced mineral concentrations compared with amended plants (Table 3). Differences in total plant tissue mineral contents (mg) followed similar trends across all analyzed minerals. The greatest values were found for plants amended with

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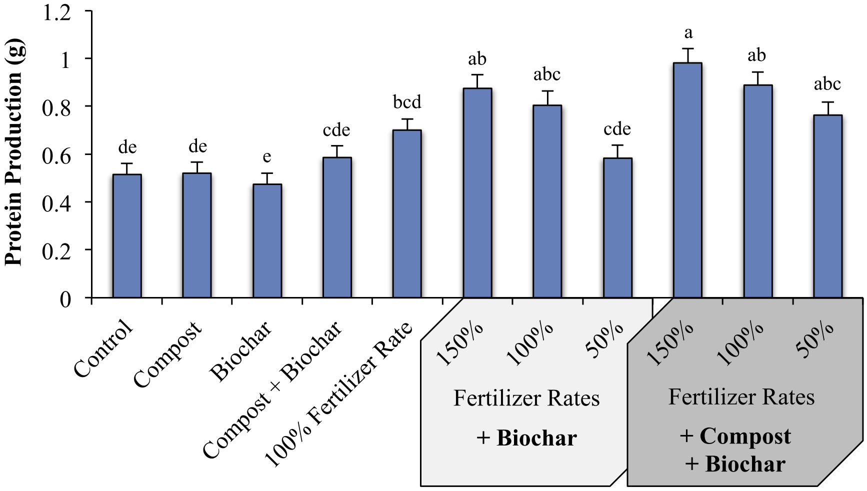

Fig. 2. Cowpea protein production (grams, two plants per pot) of cultivar Risina, grown for 45 days in non-amended soil (control) or soil amended with worm compost (compost), biochar, and/or commercial (urea and diammonium phosphate) fertilizers. The recommended (100%) N and P fertilizer rate was 50 mg kg^{-1} plant-available N and 30 mg kg^{-1} plant-available P (150% = 75/45 mg kg^{-1}; 50% = 25/15 mg kg^{-1}). Bars represent LS means, +SE (n = 6). Bars labeled with the same letter do not differ significantly ($p < 0.05$).

fertilizers combined with compost and/or biochar (Table 4). Plants amended with worm compost, biochar, and 50% of the recommend commercial fertilizer rate had greater total K, P, and Zn compared to plants amended with the recommended (100%) commercial fertilizer rate (Table 4).

Table 3. Aboveground cowpea tissue mineral concentrations (mg kg^{-1}) of plants (two per pot) grown in soil amended with each of the experimental soil amendments.

Soil amendment	Ca	Fe	K	Mg	P	Zn
Control	13030^{b}	85.3^{a}	18714^{ab}	3211^{c}	1870^{b}	28.6^{bc}
WC	16973^{a}	93.6^{a}	21261^{a}	3881^{bc}	2438^{ab}	37.0^{ab}
B	16809^{a}	132^{a}	22313^{a}	4226^{abc}	2670^{ab}	39.3^{ab}
WC + B	18382^{a}	126^{a}	21823^{a}	3950^{bc}	2928^{a}	39.5^{a}
NP 100	19874^{a}	101^{a}	18714^{b}	5207^{ab}	1866^{b}	24.0^{c}
B + NP 150	19332^{a}	116^{a}	16814^{ab}	5615^{a}	2724^{ab}	28.0^{bc}
B + NP 100	20179^{a}	129^{a}	17449^{ab}	5703^{a}	2483^{ab}	26.5^{bc}
B + NP 50	17192^{a}	101^{a}	17360^{ab}	4701^{ab}	2912^{a}	30.4^{abc}
WC + B + NP 150	19262^{a}	117^{a}	20002^{a}	5411^{a}	2676^{ab}	28.6^{bc}
WC + B + NP 100	18340^{a}	108^{a}	19975^{a}	4943^{ab}	2894^{a}	34.7^{ab}
WC + B + NP 50	19559^{a}	117^{a}	21385^{a}	4652^{ab}	2864^{a}	35.9^{ab}

Soil Amendments: Control = Non-amended, WC = Worm Compost (120 g), B = Biochar (30 g), NP = Commercial Fertilizers (% of Recommended Rate). Data presented as LS means (n = 6). Within a column, values labeled with the same letter do not differ significantly ($p < 0.05$).

Table 4. Total aboveground cowpea tissue mineral contents (mg) of plants (two per pot) grown in soil amended with each of the experimental soil amendments.

Soil amendment	Ca	Fe	K	Mg	P	Zn
Control	44.3^{d}	0.29^{b}	64.4^{d}	10.9^{d}	6.3^{e}	0.10^{d}
WC	66.5^{bcd}	0.37^{b}	83.0^{cd}	15.2^{cd}	9.5^{cde}	0.14^{cd}
B	55.4^{cd}	0.43^{ab}	74.5^{cd}	13.9^{cd}	8.7^{de}	0.13^{cd}
WC + B	77.5^{c}	0.53^{ab}	92.5^{bcd}	16.7^{bcd}	12.3^{bcd}	0.17^{abc}
NP 100	105.2^{a}	0.54^{ab}	76.7^{cd}	28.0^{a}	9.5^{cde}	0.12^{cd}
B + NP 150	119.5^{a}	0.72^{a}	103.7^{abc}	35.0^{a}	16.9^{a}	0.17^{abc}
B + NP 100	112.1^{a}	0.72^{a}	97.3^{abcd}	31.4^{a}	13.8^{abc}	0.15^{bcd}
B + NP 50	91.8^{ab}	0.53^{ab}	92.4^{bcd}	25.0^{abc}	15.2^{ab}	0.16^{abc}
WC + B + NP 150	113.7^{a}	0.69^{a}	117.9^{ab}	32.2^{a}	15.6^{ab}	0.17^{abc}
WC + B + NP 100	113.5^{a}	0.67^{ab}	123.8^{a}	30.7^{a}	18.0^{a}	0.21^{a}
WC + B + NP 50	107.3^{a}	0.63^{ab}	117.8^{ab}	25.8^{ab}	15.6^{ab}	0.20^{ab}

Soil Amendments: Control = Non-amended, WC = Worm Compost (120 g), B = Biochar (30 g), NP = Commercial Fertilizers (% of Recommended Rate). Data presented as LS means (n = 6). Within a column, values labeled with the same letter do not differ significantly ($p < 0.05$).

3.5. Intra-radical mycorrhizal abundance

Plants amended with only biochar had the greatest average AM fungal root colonization. However, none of the soil amendments resulted in root colonization that was significantly different from non-amended cowpeas (Fig. 3).

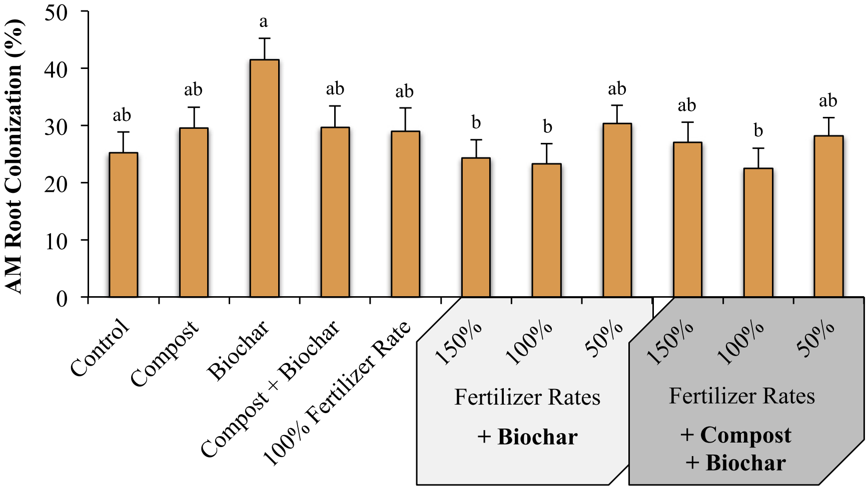

Fig. 3. Mycorrhizal root colonization (% root length colonized) of cultivar Risina, grown for 45 days in non-amended soil (control) or soil amended with worm compost (compost), biochar, and/or commercial (urea and diammonium phosphate) fertilizers. The recommended (100%) N and P fertilizer rate was 50 mg kg^{-1} plant-available N and 30 mg kg^{-1} plant-available P (150% = 75/45 mg kg^{-1}; 50% = 25/15 mg kg^{-1}). Bars represent LS means, +SE (n = 6). Bars labeled with the same letter do not differ significantly ($p < 0.05$).

4. Discussion

Our results indicate there are potential benefits to utilizing worm compost and biochar as alternative soil amendments for cowpea cultivation, as long as total plant-available nutrients are provided at recommended concentrations to support target crop yields. Commercial fertilizers generally improved both production and plant nutrition, as compared to plants receiving no fertilizers, thus indicating there were soil nutrient limitations in our studies. Amendment with only biochar provided ∼33% of recommended plant-available P, amendment with only worm compost provided ∼60%, and amendment with both provided ∼75%. However, aboveground cowpea production was not significantly increased in soil amended with compost and/or biochar, compared to non-amended plants. This suggests commercial fertilizers will continue to play a key role in agriculture, even as alternatives are incorporated into production systems.

Potassium, P, and Zn are critical for plant-growth and performance, and there is strong evidence AM fungi help acquire and transfer these nutrients to host plants (Smith and Read, 2008; Lehmann et al., 2014; Zhang et al., 2017). Tissue concentrations of K, P, and Zn were similar for non-amended plants as compared to plants amended with the recommended (100%) rate of N and P fertilizers. However, those non-amended plants also produced significantly less aboveground biomass compared to plants amended with any rate of commercial fertilizers. Amendment with worm compost, biochar, and 50% of the recommended rate of fertilizers (WC + B + NP 50) resulted in similar aboveground biomass production (with greater K, P, and Zn concentrations) compared to plants amended with the 100% recommended fertilizer rate. In addition, WC + B + NP 50 amended plants had greater total K, P, and Zn plant tissue content compared to plants amended with the 100% recommended fertilizer rate.

Although AM fungal root colonization was not different between cowpeas grown with additions of WC + B + NP 50 and the 100% recommended fertilizer rate, inclusion of biochar and worm compost likely altered soil carbon pools and soil structure (Du et al., 2017) with potential influences on nutrient dynamics and mycorrhizal functioning. We suggest these alterations may have resulted in increased total aboveground cowpea tissue K, P, and Zn content. In particular, biochar additions can affect N mineralization, though outcomes are difficult to predict across experimental conditions (Nguyen et al., 2017). Because we did not observe improved plant growth in treatments containing only biochar and/or compost, we propose inclusion of commercial fertilizers, even at reduced rates, may still be necessary to meet the nutrient requirements of cowpea. Further research is needed to elucidate potential nutrient use efficiency improvements following amendments of biochar, compost, and reduced commercial fertilizers.

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Our previous research with sorghum (*Sorghum bicolor*) showed similar improvements in plant tissue mineral contents, particularly P, for plants amended with reduced commercial fertilizer rates + biochar + worm compost; however, root colonization was also ∼60% greater for those plants (Cobb et al., 2018). While root colonization does not always indicate improved nutrient uptake (Klironomos, 2003), it is often correlated, such that greater colonization tends to improve plant nutrient uptake (Treseder, 2013; Cobb et al., 2016). In our current study, the slow-release of N and P from worm compost, combined with N and P absorption by biochar and reduced commercial fertilizers (WC + B + NP 50), may have provided sufficient nutrients for cowpea production without altering AM fungal abundance.

Previous studies, using ^{33}P radiotracers, have tracked P movement from biochar into plant tissue via AM symbiosis, indicating AM fungi can "mine" biochar for nutrients (Hammer et al., 2014). In addition, biochar can increase AM root colonization and P uptake in some crop species (Vanek and Lehmann, 2015), although this may not be observed under all conditions (Biederman and Harpole, 2013). It is difficult to make general conclusions due to variability of biochar characteristics and qualities (Keiluweit et al., 2010). Biochar can be made under different production conditions (e.g., temperature, materials). Some conditions result in biochar that provides substantial nutrient additions to soil systems while other types of biochar are primarily composed of carbon and will absorb soil nutrients (Singh et al., 2010). The biochar selected for our experiment was produced from pinewood at 500−700 °C, composed of >85% organic C; Zhao et al. (2017) reported similar biochars have significant P absorption capacity compared to biochar produced from manure and/or at lower temperatures. In our study, colonization of cowpeas amended with only biochar tended to be greater than several other treatments containing commercial fertilizers, and may be due to the relatively low concentration of plant-available P provided by biochar alone. Indeed biochar might intensify plant P limitation due to its P absorption capacity in soil (Li et al., 2016).

There are several key opportunities for future research related to the influence of alternative soil amendments on AM fungi and cowpea production. The scope of our current study included a limited number of common bean and cowpea genotypes, and it is important to expand the assessment of diverse cultivars. For example, extensive genotype screening may enable effective artificial selection for improved crop mycorrhizal responsiveness. Additionally, plant-available N may have been underestimated in our study. Future research could include more comprehensive assessments indicating impacts of alternative amendments on soil N dynamics, for example, by estimating N loss to the atmosphere or utilizing N radiotracers to track plant tissue acquisition from this various nutrient sources. Finally, soil type may have had an influence on our results; although soil used in our genotype screening was from the same site as that used in our alternative amendment experiment, soils were not identical. Edaphic factors such as the composition of sand, silt, and clay

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may substantially influence AM symbiosis and other rhizosphere processes. It is critical for future research to assess how alternative amendments impact plant-soil-microbial ecology and plant growth across soil types.

Previous research supports the idea that there is an *optimal* zone of soil fertility where neither AM fungal abundance nor crop yields are compromised (Deng et al., 2017). Because legumes use a C_3 photosynthetic pathway, and C_3 plants are generally less mycorrhizal responsive compared to C_4 species (Wilson and Hartnett, 1998), this range of ideal soil fertility could differ greatly by crop species, crop genotype, soil type, and several other factors. However, we suggest alternative fertility inputs may help achieve and maintain soil fertility for both high crop yields and AM productivity. Wang et al. (2012) found the P content in biochar can take several growing seasons to become fully plant-available. Therefore, biochar applications may increase the plant-available nutrient pool for several seasons in a farm field setting. Combining this effect with the slow release of nutrients from worm compost (Chaoui et al., 2003) may provide more agronomic efficiency over time, compared to only utilizing commercial fertilizers.

Certainly, alternative soil amendments should only be considered if crop biomass and nutrient uptake are not compromised. In our study, additions of WC + B + NP 50 caused no significant reduction in cowpea biomass or protein, while significantly increasing plant tissue K, P, and Zn contents, as compared to plants receiving twice as much commercial fertilizer (100% of the recommended rate). These findings may help reduce the costs of soil fertility management through the incorporation of locally produced worm compost and biochar. In addition, alternative fertilizers may provide small business opportunities though products such as worm compost or biochar in developing countries (Hoornweg et al., 1999; Scholz et al., 2014). For sustainable food production, it will be critical to further assess biochar amendment rates that optimize outcomes for specific crops and soils, as results of application can vary (Liu et al., 2017b). Large quantities of biochar have been shown to significantly decrease AM fungal root colonization in some systems (Warnock et al., 2010), but smaller annual additions may avoid this outcome. Further research should also include assessments of alternative and commercial fertilizer amendments on cowpea seed production and quality. However, our results are promising; indicating alternative amendments have the potential to improve agricultural sustainability.

Declarations

Author contribution statement

Adam Cobb, Gail Wilson: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Carla Goad: Analyzed and interpreted the data; Wrote the paper.

Michael Grusak: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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