FROM SOIL ECOLOGY TO HUMAN NUTRTITION:

CROP SYMBIOSIS WITH ARBUSCULAR

MYCORRHIZAL FUNGI IN AGROECOSYSTEMS

By

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Abstract: The Green Revolution helped us reduce global poverty, hunger, and malnutrition over the past 30 years. My research is part of an emerging *Brown Revolution* that is unlocking the power of living soil to sustainability provide human needs. We are losing soil more quickly than it is being replenished – worldwide, an area of farmland the size of half an Oklahoma erodes away every year; however, arbuscular mycorrhizal (AM) fungi are microorganisms that can stabilize and enhance soil, while benefitting most of our crops with increased water and nutrients. A teacup of healthy soil contains enough AM fungi to stretch across 30 football fields from end to end, but some agricultural practices reduce the abundance of AM fungi on farms. These practices degrade soil stability and fertility over time, resulting in a waste of water, a waste of fertilizers, and environmental damage. My research seeks to harness the benefits of AM fungi for sustainable food production and nutrition. We focused on sorghum and cowpea, because they are important in many developing countries, but their efficiency and drought-tolerance also make them ideal for places like Oklahoma. In the greenhouse and field, we assessed differences in plant response to AM fungi to discover which crop genotypes are the most effective partners. Then we examined how that partnership affected agricultural efficiency and seed (grain) nutritional contents, such as protein, zinc, and iron. We also assessed the impact of alternative fertility amendments (biochar, worm compost, reduced commercial fertilizers) and farm management practices (intercropping) on AM abundance, crop yield, and nutrition. More AM responsive genotypes and alternative amendments were shown to increase yield and/or key nutritional contents. Nearly 1 out of every 3 people on this planet are malnourished, and they often need more dietary protein, zinc, and iron; therefore, our results indicate that AM fungi improve soil health and human health as well. Crop genetics, alternative soil amendments, and farm management can enhance the benefits of AM fungi, and we can utilize them to help us regenerate our soils and nourish our growing population.

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CHAPTER I

The effects of arbuscular mycorrhizal fungi on grain production and nutrition of sorghum genotypes: Enhancing yields and quality through ecological partnership

ABSTRACT: The responsiveness of 3 hybrid sorghum genotypes and 3 open-pollinated sorghum genotypes to arbuscular mycorrhizal (AM) fungi and commercial fertilizers was assessed. This comparison was conducted to link grain production and quality with crop nutrition strategies (AM symbiosis versus fertility amendments). The open-pollinated genotypes produced an average of 206% more vegetative biomass and 285% more grain per plant, compared to hybrid genotypes when grown with AM fungi and no fertilization. Furthermore, the average protein production of open-pollinated genotypes was increased 320%, compared to hybrid genotypes grown under the same low-fertility conditions. Percent AM root colonization was 149% greater in open-pollinated genotypes compared to hybrid genotypes, and across all genotypes, AM colonization was significantly correlated with total grain mineral content. African and Latin sorghum genotypes were significantly more responsive to mycorrhizal symbiosis than US hybrid genotypes for nutrient uptake and subsequent grain production and quality, while hybrid genotypes were significantly more responsive to fertilization.

INTRODUCTION

Grain sorghum (*Sorghum bicolor*) is of growing interest as a food crop in the context of global climate change and mounting fertilizer costs because of superior production under dry and low-fertility conditions, as compared to corn (*Zea mays*) (Assefa et al. 2013). Sorghum (also called milo) has been, by some accounts, cultivated in sub-Saharan Africa and South Asia for over 5000 years (De Wet and Harlan 1971). It is common in US agriculture, with annual production estimated at nearly 11 million Mt in 2014 and over 15 million Mt in 2015 according to USDA Annual Crop Reports (USDA 2015; 2016).

One key to successful sorghum cultivation may be enhancing mutualistic partnerships with beneficial soil microbes, such as arbuscular mycorrhizal (AM) fungi. Mycorrhizas are structural relationships consisting of both the fungus and its host root system; they are considered nutritional symbioses, as they are primary providers of phosphorous and other trace minerals for the majority of land plants. Arbuscular mycorrhizal fungal hypha associated with plant roots can extend the reach of root systems and increase access to growth-limiting resources. The amount of hyphal biomass produced by AM fungi can be substantial (Miller et al. 1995); therefore, these fungi play a critical role as carbon sinks and in structuring soils (Wilson et al. 2009; Zhu and Miller 2003). A review by Gosling et al. (2006) reported AM fungi not only play a role in improving plant nutrition but also disease resistance, water use efficiency, soil structure and beneficial microbial activity in natural ecosystems. However, AM benefits have been depleted in many agroecosystems because farm management practices such as soil fertilizer applications (particularly phosphorus [P]) reduce fungal abundance by reducing plant dependence on the symbiosis (Richardson et al. 2011). Furthermore, agricultural

fertilizers are widely recognized as off-farm pollutants, with water quality and natural ecosystem function negatively impacted by nutrient runoff (Daigle 2003). Increased energy, mining, and transportation costs have increased farmers' cost burden from fertilizer inputs. Lower input agriculture is essential for environmental preservation. Under sustainable conditions, the multiple benefits of AM symbiosis can play a pivotal role in maintaining soil fertility and stabilization of soil structure while enhancing plant water uptake and food quality (Ellouze et al. 2014). In many of the tropical regions where low P soils are common, farmers lack access to commercial fertilizers (Ngwene et al. 2010). Phosphorus limitations are also expected to increase in agriculturally developed countries, as food production must scale with human population growth (Cordell et al. 2009).

Potentially, enhancing symbiotic activity with AM fungi provides a path to maintain or improve food production and nutrition with fewer economically and environmentally costly fertilizer inputs (Elbon and Whalen 2015). Oruru and Njeru (2016) highlighted farming practices that could be utilized to replenish the AM fungal symbiosis and particularly benefit smallholder farmers; however, there are many variables, such as crop genetics, involved in harnessing the benefits of AM fungi in agriculture. Indeed, numerous studies indicate plant genotypes vary in their responsiveness to mycorrhizas (Liu et al. 2000; Smith and Read 1996). Determining the mycorrhizal responsiveness on agricultural genotypes is a critical first step in designing farm systems where AM fungi can improve water and nutrient-use efficiency, enhance soil structure, and sustain quality yields.

Symbiotic partnerships between agronomic crops and soil microbes have recently become a focus of research and discussion of 21st Century food systems (Denison 2012). Research linking AM fungi to crop productivity, as well as nutritional value of the food products is essential to understand the practical value of these soil microbial allies in agroecosystems. This research should be considered a step toward breeding sorghum that is more resource efficient and appropriate for subsistence farmers and sustainable agriculture.

Our research seeks to understand the role of AM fungi on sorghum genotype grain production and nutritional value. Mycorrhizal colonization has previously been assessed for some sorghum genotypes (Mehraban et al. 2009), but the belowground assessment still needs to be linked to crop productivity and grain quality. Therefore, the primary objectives of our greenhouse study were (1) to assess responsiveness of African and Latin American open-pollinated (OP) genotypes, and hybrid sorghum genotypes to AM symbiosis in low-fertility and N and P fertilized soil and (2) to assess the role of AM fungal symbiosis on plant production, grain quality (starch characteristics, protein and mineral concentrations), and the total grain nutrient content produced by sorghum genotypes under two soil fertility levels.

Because sorghum utilizes a C_4 photosynthetic pathway and C_4 grasses have been shown to be obligate symbionts with AM fungi (Wilson and Hartnett 1998), we hypothesized sorghum plants are highly dependent on the symbiosis for growth and grain production in low fertility soils. Although grain quality and nutrient content were not examined in previous studies, mycorrhizal responsiveness, determined by differences in mycorrhizal and mycorrhizal-suppressed total plant dry weight, have been assessed for

many native prairie plants (Wilson and Hartnett 1998) and for crops such as sugarcane, corn, and soybean (Kelly et al. 2001). Hetrick et al. (1993; 1995) reported early land races of wheat (*Triticum* spp.) were highly responsive to AM fungi, yet modern wheat genotypes were less responsive and total plant dry weight was reduced, compared to their early ancestors, when grown in low fertility soil. Therefore, we hypothesized that OP sorghum genotypes are more responsive to AM fungi compared to hybrid genotypes, but that hybrid genotypes are more dependent on fertilizer applications than AM symbiosis for yield and grain quality. We further hypothesized that, in low fertility soils, the greater AM responsiveness of OP genotypes will result in increased grain protein and mineral content, compared to the less AM responsive hybrids that are more responsive to fertilizer.

MATERIALS AND METHODS

Experimental Setup. Two greenhouse studies were conducted – one in 2012 and a replicate study in 2013. A randomized complete block design was used with four replications in each trial, and a complete factorial design of six sorghum genotypes subjected to four soil treatments. The sorghum breeding and genetics program at Kansas State University provided open-pollinated African sorghum genotypes: Ajabsido (MNO9-7018) and Macia (PRO9110-4319); open-pollinated Latin American genotype Sureno (PRO9110-4317); and US hybrid genotypes Dekalb (54-00), Pioneer (84G62), and Seneca. The soil treatments were: 1) non-amended native soil (control); 2) native soil with fertilization (N and P); 3) native soil with fungicide (to suppress AM fungal activity); and 4) native soil with both fungicide and fertilizer amendments.

Ninety-six pots (26.5 cm diameter x 48cm height) were filled with 22 liters of Renfrow/Grainola (eroded silty clay Mollisol/Alfisol) soil collected from the Oklahoma State University Range Research Station (pH = 7.25, N = 16 ppm, P = 4 ppm, K = 137 ppm, OM= 0.59%). The Soil, Water, and Forage Analytical Laboratory at Oklahoma State University analyzed the baseline soil samples. Soil NO_3-N and NH_4 were extracted by 1M KCl solution and analyzed using the Lachat Quickchem 8000 Flow Injection Autoanalyzer (Kachurina et al. 2000). Two grams of soil were extracted with 20 ml Mehlich 3 solution (Mehlich 1984) for plant available P and K, and the concentrations of P and K in the extract were measured by an inductively coupled plasma emission spectroscopy (ICP)(Pittman et al. 2005). Soil pH was measured using a pH electrode in a 1:1 soil to water suspension. Soil organic matter (SOM) was determined by dry combustion using the LECO Truspec CN analyzer (Nelson et al. 1996). This field-collected soil was not inoculated with additional soil microbes and contained only ambient AM fungi.

For each N&P fertilized pot, 1.0g of monopotassium phosphate (0-52-34) was applied (rate equivalent to 100 kg per ha total phosphate and 292 kg per ha total potassium), and 1.4g of ammonium nitrate (34-0-0) was applied (rate equivalent to 120 kg per ha total nitrogen). For each non-fertilized pot, 0.55g potassium chloride (0-0-60) was applied to non-fertilized pots (at rate of 292 kg per ha total potassium) to ensure uniform potassium across the study. Fertilizers were dissolved in 1 L of water and applied at the beginning of the study and at sorghum bloom. Fungicide was applied at planting and every 3 weeks thereafter as a solution of 7.8 grams Topsin® (Thiophanate-Methyl) dissolved in 1 L of water per pot.

Sorghum seedlings were germinated in vermiculite and transferred to pots at the second-leaf stage. Replanting was allowed for failed transplants for the week following transplant. One liter of water was provided to all plants (including control) when fertilizer or fungicide was applied to ensure uniform soil moisture across the study. Plants were maintained under well-watered conditions, watering every 2-4 days. Greenhouse temperatures were maintained between 21-32 °C for both trials. Plants were harvested at 16 weeks, following grain maturation. Total grain production (dry weight) and aboveground plant biomass (dry weight) were determined at harvest.

Quantification of grain and starch characteristics. The USDA-ARS Center for Grain and Animal Health Research in Manhattan, Kansas, USA determined grain hardness, moisture, diameter, weight, protein, protein digestibility, starch granule size distribution, and amylose/amylopectin ratios. Hardness was determined (Single Kernel Characterization System) by crushing the grain, which was then recovered and utilized in the other evaluations (as per Kaufman et al. 2013). Subsamples of the crushed material were used to determine total protein using N combustion (Leco N combustion analyzer) and total starch (Megazyme total starch analysis). Sorghum protein digestibility was evaluated using the methods of Wong et al. (2009). Starch was isolated from the crushed grain using the method of Park et al. (2006). Starch granule size distribution (laser diffraction analysis) (Wilson et al. 2006) and amylose/amylopectin ratios (dual wavelength iodine binding) (Kaufman et al. 2015) were assessed. Correlations of all protein and starch analyses were evaluated against variations in the total digestibility of each sample. Grain production and protein concentration data were combined to calculate total protein production per plant. Grain and starch characteristics were unable to be

assessed for the mycorrhizal-suppressed non-fertilized treatment because production of grain was insufficient for analysis.

Quantification of mineral concentrations. The USDA-ARS Children's Nutrition Research Center in Houston, Texas assessed grain mineral concentrations for some of the minerals that are critical for human and animal health (Ca, Cu, Fe, K, Mg, P, and Zn). Using methods of Farnham et al. (2011), two subsamples (~0.25 g dry weight) of each ground sample were digested and processed for elemental analysis. Elemental analysis was performed using inductively coupled plasma–optical emission spectroscopy (CIROS ICP Model FCE12). Tissue mineral concentrations were determined on a dry mass basis (μg g–1 or mg g–1), and an average value was derived from the two sub-samples of each replicate. Grain production and mineral concentration data were combined to calculate total grain mineral contents per plant. Mineral concentrations were unable to be assessed for the mycorrhizal-suppressed non-fertilized treatment because production of grain was insufficient for analysis.

Quantification of AM Colonization. A subsample of live roots were removed from the soil, washed, stained with trypan blue and scored for percent AM colonization using the magnified gridline intersect method (Mcgonigle et al. 1990). This method uses a digital microscope (Hirox KH 7700) to measure the percentage root length colonized by hyphae, vesicles, coils, and arbuscules, which were combined to determine total percent colonization.

Statistical Analysis. AM root colonization, productivity, grain protein content, protein digestibility, and mineral content response variables were analyzed using mixed models

methods. Grain characterization and starch characteristics were analyzed using generalized linear mixed models methods. The Tukey multiple comparison method was utilized for significant effects and results reported as least square means. Spearman correlation method was performed for grain mineral to AM root colonization correlations. Mycorrhizal and fertilizer responsiveness were calculated for each genotype origin (OP and hybrid) as follows. Percentage mycorrhizal responsiveness = [(dry mass of mycorrhizal plant) – (dry mass of mycorrhizal-suppressed plant) / (dry mass of mycorrhizal plant)] * 100 (Hetrick et al. 1996; Wilson and Hartnett 1998). Percentage fertilizer responsiveness = [(dry mass of fertilized mycorrhizal plant) – (dry mass of non-fertilized mycorrhizal plant) / (dry mass of fertilized mycorrhizal plant)] * 100. Differences between mycorrhizal and mycorrhizal-suppressed plants, and differences between fertilized and non-fertilized plants were determined using mixed models methods. Responsiveness was considered to be significant when the total dry masses of the compared treatments were assessed as significantly different as determined by Tukey multiple comparison. All tests of significance were performed at the nominal 0.05 level. The data analyses were generated using SAS® version 9.4. Copyright © 2013 SAS Institute Inc. SAS and all other SAS Inc. product of service names are registered trademarks of trademarks of SAS Institute Inc., Cary, NC, USA.

RESULTS

Vegetative production. Open-pollinated genotypes produced significantly greater vegetative biomass compared to the hybrid genotypes when AM fungi were not

suppressed (hereafter referred to as mycorrhizal soil) and fertilizer was not added. However, fertilization of mycorrhizal soil resulted in similar growth for all genotypes (Figure 1). In non-fertilized soil, with fungicide application (hereafter referred to as mycorrhizal-suppressed soil), vegetative production was significantly reduced as compared to mycorrhizal soil that was non-fertilized. However, all genotypes responded to fertilization in mycorrhizal-suppressed soils with equivalent or increased growth compared to corresponding non-fertilized mycorrhizal soil, with the exception of Ajabsido (African) genotype. Ajabsido produced significantly greater biomass in mycorrhizal soil without fertilizers compared to fertilized mycorrhizal soil (Figure 1).

Grain Production. African genotypes produced significantly more grain than the genotype Sureno or any of the hybrid genotypes in non-fertilized mycorrhizal soil; however, fertilization narrowed these differences in productivity, with all genotypes producing similar total grain in fertilized mycorrhizal soil expect for the hybrid Seneca (Figure 2). Mycorrhizal fungal suppression without additions of fertilizers resulted in a lack of grain production across all genotypes. African genotypes had similar grain yield regardless of fertilizer or fungicide applications. However, fertilization of mycorrhizal-suppressed soil reduced grain yield of Sureno OP genotype compared to when it was grown in fertilized mycorrhizal soil (Figure 2). Fertilizing mycorrhizal-suppressed soil significantly increased grain production for hybrid genotypes compared to other treatments, indicating hybrids are highly responsive to fertilizer applications.

Grain Protein Concentration and Production. Grain protein concentrations were significantly different due to the interaction between genotype and treatments. Hybrid genotypes produced grain with lower protein concentration than OP genotypes in non-

fertilized mycorrhizal soil, but fertilization reduced these differences in protein concentration (Figure 3). The highest grain protein concentration was in Sureno OP genotype in non-fertilized mycorrhizal soil (17.35% ± 0.58) and the lowest concentration was in Dekalb hybrid genotype in non-fertilized mycorrhizal soil (7.37% ± 0.82) (data not shown). Additionally, there were differences in total grain protein production (grain production x grain protein concentration), with African genotypes producing significantly greater total amounts of grain protein per plant than the other genotypes (Figure 3). The addition of fertilizers to mycorrhizal soil increased grain protein production in Sureno and the hybrid genotypes, but reduced protein production in African genotypes. When fertilizers were applied to mycorrhizal-suppressed soil, grain protein production was significantly increased in the hybrid genotypes compared to other treatments, with variable effects on the OP genotypes (Figure 3).

Root Colonization Percentage. When grown in non-fertilized mycorrhizal soil, all hybrid genotypes were significantly less colonized, compared to the OP genotypes (Figure 4). This significant difference in colonization between genotypes was less pronounced following additions of fertilizer or fungicide, as both these applications significantly reduced the percentage root length colonization in all genotypes. Mycorrhizal suppression by fungicide consistently reduced AM colonization to ~10% or less for all sorghum genotypes.

Mycorrhizal Responsiveness. There was a significant difference (P-value <0.0001) in the interaction of genotype origin (OP and hybrid) and soil (mycorrhizal and mycorrhizal-suppressed). Total plant dry weight between OP genotypes (59.87g ± 4.24) and hybrid genotypes (18.99g ± 4.28) (data not shown) in mycorrhizal soil were

significantly different, but there was not a significant difference in total plant dry weight between OP genotypes (7.78 ± 4.52) and hybrid genotypes (12.66g ± 4.34) (data not shown) in mycorrhizal-suppressed soil. Therefore, a significant difference in mycorrhizal responsiveness was calculated (Table 1).

Fertilizer Responsiveness. There was a significant difference by the interaction of genotype origin (OP and hybrid) and fertilizer amendment (fertilized and non-fertilized). Total plant dry weight between OP genotypes (60.09g ± 7.41) and hybrid genotypes (18.88g ± 7.18) when they were not fertilized were significantly different, but there was not a significant difference in total plant dry weight between OP genotypes (50.69 ± 7.43) and hybrid genotypes (43.27g ± 7.18) when they were fertilized. Therefore, a significant difference in fertilizer responsiveness was calculated (Table 1).

Grain Mineral Concentrations and Total Content. There were significant calcium, and potassium, and zinc grain concentration differences between OP and hybrid genotypes with higher concentrations in OP genotypes compared to hybrid genotypes; this was pronounced in non-fertilized mycorrhizal soil. Grain phosphorus, magnesium, and iron concentrations were significantly different by genotype with higher concentrations present in Ajabsido and Sureno (OP genotypes) than Dekalb and Seneca (hybrid genotypes), and Pioneer and Macia characterized by intermediate concentrations. There were no significant differences in copper concentrations by genotype or treatment. Total grain mineral content (combination of grain production and grain mineral concentration) followed similar trends across all analyzed minerals by genotype and treatment, with few significant differences (Table 2). Fertilization resulted in minor changes for all genotypes, with few significant differences. Application of fertilizer to mycorrhizal-

suppressed soil resulted in a significant increase in mineral contents, as compared to non-fertilized mycorrhizal soil, for only the hybrid genotypes. In non-fertilized mycorrhizal soil, correlations between AM root colonization and per plant grain mineral contents were significant for every mineral analyzed (Figure 5).

Grain Protein Digestibility. For each genotype, fertilizer and/or fungicide did not significantly influence protein digestibility for that genotype. However, the interactions between genotype and treatments (fertilizer and/or fungicide) were significantly different for different genotypes (Figure 6). These differences did not significantly contrast between OP and hybrid genotype origins.

Grain Physical Characterization. There were few significant differences in grain physical characteristics from the interaction of genotype and treatment (Table 3), and those differences are primarily the result of differences in genotype rather than treatment.

Grain Starch Analysis. There were no significant differences in grain starch granule distribution between the OP genotypes (A-granule = 51.16%, ± 1.152; B-granule = 38.59%, ± 0.95; C-granule = 10.01%, ± 0.23) and hybrid genotypes (A-granule = 55.70%, ± 1.50; B-granule = 34.64%, ± 1.21; C-granule = 9.34%, ± 0.29) (data not shown, n = 12). There was a significant difference in percentage of grain amylose between genotypes but not by genotype origin (Ajabsido = 25.40a ± 0.30, Macia = 22.99b ± 0.27, Sureno = 23.52b ± 0.27, Dekalb = 26.34a ± 0.40, Pioneer = 25.99a ± 0.20, Seneca = 22.92b ± 0.35) (data not shown, n = 4); however, treatments did not have a significant effect. Because of this, only one season of the study was analyzed for starch characteristics.

DISCUSSION

In our study, open-pollinated OP genotypes produced 206% more vegetative biomass and 285% greater grain, compared to hybrid genotypes in non-fertilized mycorrhizal soil. This increase in biomass production was directly related to a 149% increase in percent AM root colonization. Additionally, grain protein concentrations were linked to mycorrhizal colonization, with OP genotypes outperforming hybrids. Most mineral concentrations differed by genotype, or genotype origin (OP and hybrid), and in non-fertilized mycorrhizal soil were often similar or significantly greater for OP genotypes as compared to hybrid genotypes. Similarly, total grain protein production of OP genotypes was 320% greater, on average, and total grain mineral contents were significantly correlated to AM root colonization of non-fertilized plants grown with mycorrhizal fungi. However, protein digestibility, starch granule distributions, amylose content, and grain size, weight, moisture, and hardness did not appear to be influenced by mycorrhizal root colonization of any sorghum genotypes, but rather by plant genetic traits.

Hybrid genotypes receiving both fungicide (mycorrhizal-suppressed) and fertilization generally produced greater amounts of grain, total protein, and had greater mineral content, as compared to production in non-fertilized soils. Fertilization did not increase production or grain quality in Sureno or the African genotypes, with the exception of protein production of Ajabsido. These results indicate AM fungi provided similar benefits for Sureno and the African genotypes as high rates of N&P fertilization, in regards to plant growth and grain production and quality. Importantly, this was not observed for hybrid genotypes, presumably because mycorrhizal responsiveness has been reduced in these genotypes.

The major goals of crop breeding and genetics programs are to develop superior seed-lines, improved in biomass production capability and high grain quality and quantity. However, genetic enhancement and plant breeding efforts to improve genotypes for grain production may alter the strong positive plant-fungal relationship and leave the resulting genotypes less appropriate for low-input agriculture or where there is little access to fertilizer inputs. For example, the high mycorrhizal responsiveness of ancestral land races of wheat was *suppressed* in modern genotypes – even in low-P soil (Hetrick et al. 1993; Hetrick et al. 1995), presumably because they were bred and selected under high P input. We suggest breeding and selection of sorghum can produce more efficient genotypes if maintaining or enhancing this mutualistic association is included as a focus. It is critical to understand the interactions of plant hosts and fungal partners if breeders are to enhance the symbiotic relationship rather than inadvertently lose it through selective breeding.

Mycorrhizal fungi were essential for the growth and reproduction of all genotypes, as fungicide reduced AM colonization to an average range of 4-11%, with significant reduction in plant biomass and little grain production for each genotype. Additions of N and P fertilization to fungicide-treated plants compensated for the loss of AM fungi and all sorghum genotypes produced equivalent or greater biomass and grain, compared to corresponding mycorrhizal plants. However, hybrid genotypes were generally more responsive to fertilization, resulting in increased vegetative biomass and grain production compared to control, while OP genotypes had a negative fertilizer response. Our results found African sorghum genotypes were more dependent on mycorrhizal symbiosis than hybrid genotypes for nutrient uptake and subsequent grain

production, particularly in low-fertility soils. These outcomes support the research hypotheses: that in low fertility soils, the greater AM responsiveness of OP genotypes would result in increased grain production, total protein, and mineral content, compared to the less AM responsive hybrids that are more dependent on fertilizer applications for yield and grain quality.

It may be suggested that plant-microbe responsiveness be enhanced through genetic selection of the fungal strains in farm soils. However, breeding agricultural crops to manage the relationship with AM fungi may be more effective than developing agricultural strains of AM fungal inoculum. As Sanders and Croll (2010) explain, knowledge of AM fungal genetics is currently constrained by the enigmatic nature of the organism's genome and reproduction. Because there are numerous nuclei in each fungal spore, mycorrhizal offspring may rapidly exhibit drastically different phenotypes and degrees of benefit for partner plants (Angelard et al. 2014). However, in the past 10 years, new tools for isolating and analyzing chemical exudates of plants and soil biota have propelled understanding of the rhizosphere environment, with conceptual frameworks and models continuing to become more precise and complex, and these tools may allow for efficient selection of genotypes that are both highly responsive to AM symbiosis and produce high grain quality/quantity. The initiation and formation of AM symbiosis is dependent on the production and regulation of an array of chemicals both within the host plant and rhizosphere (Abdel-Lateif et al. 2012; Bonfante and Genre 2015; Gutjahr 2014; Pozo et al. 2015; Takeda et al. 2015). Modern crop breeding may inadvertently disrupt this intricate chemical dialog between mutualist plants and AM fungal partners. If so, this uncoupling may help explain the loss of mycorrhizal

responsiveness in modern hybrids compared to responsiveness observed in OP genotypes. Even though our results suggest a loss of symbiotic potential occurred following modern sorghum breeding, we suggest our results also indicate an opportunity to harness the benefits of AM fungi for sustainable sorghum production through selective breeding, possibly under low fertility, may create a selective pressure for sorghum to invest more resources in its chemical dialog with AM fungi, enhancing root-fungal interactions and increasing grain quantity and quality. For example, the same phytohormones may be responsible for suppressing the activity of parasitic fungi while enhancing AM symbiosis (Dor et al. 2011).

Our study indicates host plant and AM fungal ecological partnership translates to improved grain production and grain quality in low-nutrient soils. A hypothetical case based on our results, using a smallholder farmer with little access to fertilizers, demonstrates the value of sorghum genotypes that rely on AM symbiosis. By planting 20,000 sorghum seeds of a highly mycorrhizal responsive genotype (Ajabsido or Macia), production would be 298.6 kg more grain with approximately 36.2 kg more total grain protein, compared to the average production of fertilizer-responsive hybrid genotypes (Dekalb or Pioneer). Grain harvested from those 20,000 plants would also contain approximately 1060 mg more iron and 900 mg more zinc compared to the total content that would be contained in the less mycorrhizal responsive genotypes. This hypothetical case illustrates context-appropriate genetics for small-scale subsistence farmers. However, as costs escalate, large-scale agricultural systems are under increasing pressure to optimize every farm input. Our results indicate it is imperative for crop breeders to

include plant-microbial partnerships as an additional focus for breeding programs to deliver efficient genotypes for sustainable agricultural systems.

CONCLUSION

Natural ecosystems display stability because of symbiotic interactions. We must understand these ecological dynamics and operationalize systems that can add to the health of agricultural soils and the quality of the food produced. Sustainable agriculture may be improved if breeders select for traits, like AM responsiveness, that reduce fertilizer inputs and enhance soil quality, while maintaining, or possibly increasing, global nutrition security. These goals may be reached through a better understanding of the mechanisms and genetics underlying plant-soil-microbial interactions, such as arbuscular mycorrhizal fungi and agricultural crops.

Beneficial soil fungi present a great opportunity to make global agriculture more efficient, more sustainable, and more productive (Ellouze et al. 2014; Rodriguez and Sanders 2015). In light of the multi-layered processes of the rhizosphere, it is critical that crop genotypes are assessed for symbiotic potential, that crop genomes are mapped to uncover the traits associated with mycorrhizal partnership, and that these traits are linked to productivity and food nutrition. We encourage best management practices for AM fungi in combination with crops genotypes selected for high AM responsiveness as a path to sustainability, through fertilizer cost reduction and reduction of their negative environmental impacts, while still providing human nutritional needs.

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TABLES AND FIGURES

Table 1. Mycorrhizal and fertilizer responsiveness (%) for open-pollinated and hybrid sorghum genotype origins.

Genotype Origin	Mycorrhizal Responsiveness (%)	Fertilizer Responsiveness (%)
Open-pollinated	+87.0a	-18.5b
Hybrid	+33.3b	+56.4a

Dry weight of mycorrhizal and mycorrhizal-suppressed plants and mycorrhizal responsiveness (%) of sorghum genotypes by origin. Mycorrhizal responsiveness calculated Percentage mycorrhizal responsiveness = [(dry mass of mycorrhizal plant) – (dry mass of mycorrhizal-suppressed plant) / (dry mass of mycorrhizal plant)] * 100 (Hetrick et al. 1996; Wilson and Hartnett 1998). Dry weight of fertilized and non-fertilized plants (mycorrhizal) and fertilizer responsiveness (%) of sorghum genotypes by origin. Percentage fertilizer responsiveness = [(dry mass of fertilized mycorrhizal plant) – (dry mass of non-fertilized mycorrhizal plant) / (dry mass of fertilized mycorrhizal plant)] * 100. Within a column, means that do not share a letter are significantly different ($P < 0.05$).

Table 2. Total grain mineral content (mg) for all sorghum genotypes by treatment.

Treatments: M = mycorrhizal non-fertilized, M+F = mycorrhizal fertilized, MS+F = Mycorrhizal-suppressed fertilized

Treatment	Genotype	Ca	Cu	Fe	K	Mg	P	Zn
M	Ajabsido	2.91a	0.11abc	0.76bcd	83.2abc	28.6abc	54.7abc	0.54bc
	Macia	2.14ab	0.10abc	0.80bcd	75.4abc	24.8bc	50.3abc	0.64abc
	Sureno	1.16bc	0.05d	0.35d	36.1bc	16.1c	34.0c	0.29cd
	Dekalb	0.69bc	0.03d	0.27d	33.7bc	12.2c	23.2c	0.19cd
	Pioneer	0.46c	0.02d	0.22d	16.8bc	6.6c	13.6c	0.11d
	Seneca	0.71bc	0.03d	0.29d	25.5bc	9.6c	20.8c	0.14cd
M+F	Ajabsido	1.03bc	0.05cd	0.58cd	62.3bc	24.0bc	52.4abc	0.31cd
	Macia	2.05ab	0.07bcd	0.48cd	77.4abc	22.5bc	49.6abc	0.34bcd
	Sureno	1.56bc	0.06bcd	0.51cd	66.8bc	25.6bc	56.1abc	0.37bcd
	Dekalb	1.74bc	0.07bcd	0.55cd	56.4bc	23.0bc	49.2bc	0.35bcd
	Pioneer	1.60bc	0.07bcd	0.56cd	57.8bc	23.0bc	49.3bc	0.38bcd
	Seneca	0.88bc	0.04d	0.38d	38.3bc	13.1c	29.5c	0.18cd
MS+F	Ajabsido	3.69a	0.13ab	1.10abc	118.5a	39.6ab	83.8ab	0.74ab
	Macia	2.38ab	0.13abc	0.92abcd	90.3abc	34.1abc	73.9abc	0.79ab
	Sureno	1.21bc	0.07bcd	0.51cd	44.2bc	18.2c	41.5bc	0.37bcd
	Dekalb	3.70a	0.14ab	1.39ab	133.8a	47.8a	93.8a	0.82a
	Pioneer	3.77a	0.15a	1.51a	136.2a	48.9a	98.2a	1.12a
	Seneca	2.73a	0.10abc	0.99abc	97.0ab	32.3abc	67.6abc	0.48bcd

Within a column, means that do not share a letter are significantly different ($P < 0.05$).

Table 3. Grain physical characteristics* for all sorghum genotypes by treatment.

Treatments: M = mycorrhizal non-fertilized, M+F = mycorrhizal fertilize
MS+F = Mycorrhizal-suppressed fertilized

Treatment	Genotype	Hardness	Moisture (%)	Diameter (mm)	Weight (mg)
M	Ajabsido	58.60c	8.94ab	2.29a	28.91a
	Macia	76.15abc	8.73ab	2.25a	31.43a
	Sureno	89.09a	8.28b	2.26a	29.81a
	Dekalb	67.87bc	9.12ab	2.48a	35.05a
	Pioneer	72.48abc	8.78ab	2.49a	34.28a
	Seneca	82.75ab	8.59ab	2.43a	33.88a
M+F	Ajabsido	60.49c	8.84ab	2.65a	34.43a
	Macia	79.15abc	8.86ab	2.11a	28.17a
	Sureno	87.86ab	8.54ab	2.27a	29.93a
	Dekalb	69.14ab	8.95ab	2.23a	31.84a
	Pioneer	77.15abc	8.82ab	2.41a	30.79a
	Seneca	88.82a	8.71ab	2.06a	25.34a
MS+F	Ajabsido	59.43c	9.44a	2.29a	28.55a
	Macia	79.15abc	8.55ab	2.21a	31.28a
	Sureno	85.34ab	8.36b	2.11a	28.64a
	Dekalb	77.73abc	9.59a	2.32a	34.62a
	Pioneer	78.77abc	8.88ab	2.51a	32.90a
	Seneca	88.49a	9.22a	2.21a	29.06a

Within a column, means that do not share a letter are significantly different ($P < 0.05$).

*Single-kernel characterization system

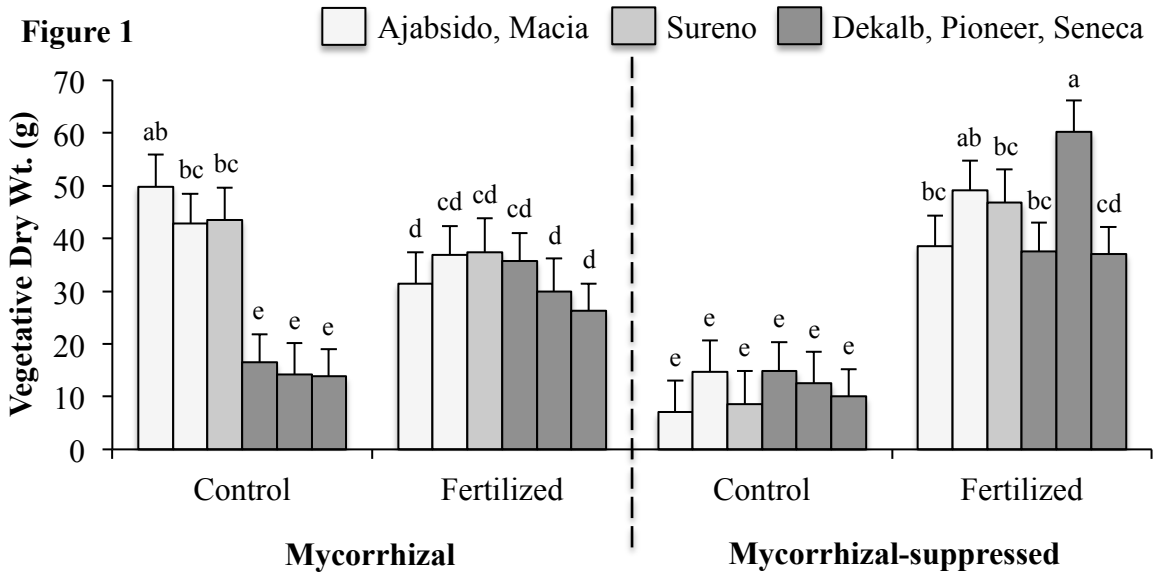

Figure 1. Vegetative dry weight for sorghum genotypes in mycorrhizal and mycorrhizal-suppressed soils, with no amendment (control), or fertilized with N&P. From left to right, genotypes are open-pollinated African (Ajabsido and Macia), Latin American (Sureno), and hybrid (Dekalb, Pioneer, and Seneca). Bars represent means, + SE (n = 8). Bars that do not share a letter are significantly different ($P < 0.05$).

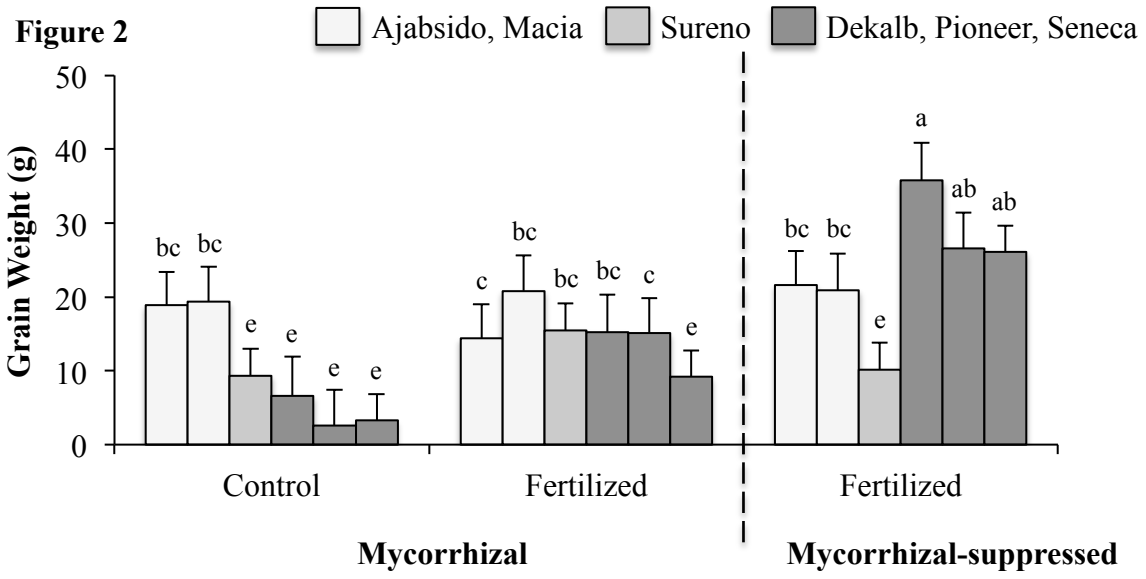

Figure 2. Grain production for sorghum genotypes in mycorrhizal and mycorrhizal-suppressed soils, with no amendment (control), or fertilized with N&P. From left to right, genotypes are open-pollinated African (Ajabsido and Macia), Latin American (Sureno), and hybrid (Dekalb, Pioneer, and Seneca). Bars represent means, + SE (n = 8). Bars that do not share a letter are significantly different ($P < 0.05$).

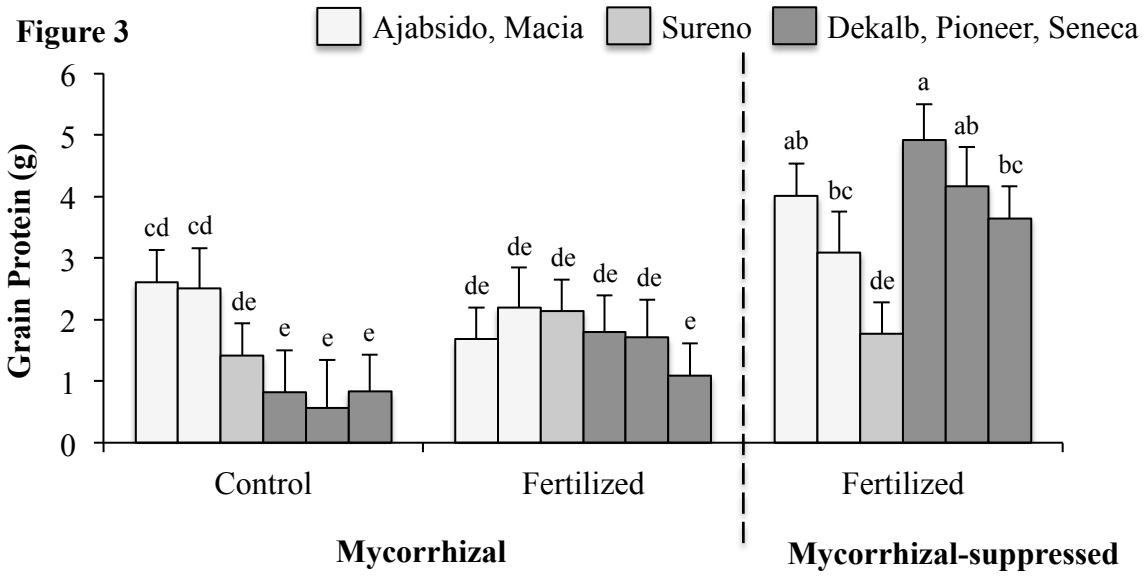

Figure 3. Total grain protein production for sorghum genotypes in mycorrhizal and mycorrhizal-suppressed soils, with no amendment (control), or fertilized with N&P. From left to right, genotypes are open-pollinated African (Ajabsido and Macia), Latin American (Sureno), and hybrid (Dekalb, Pioneer, and Seneca). Bars represent means, + SE (n = 8). Bars that do not share a letter are significantly different ($P < 0.05$).

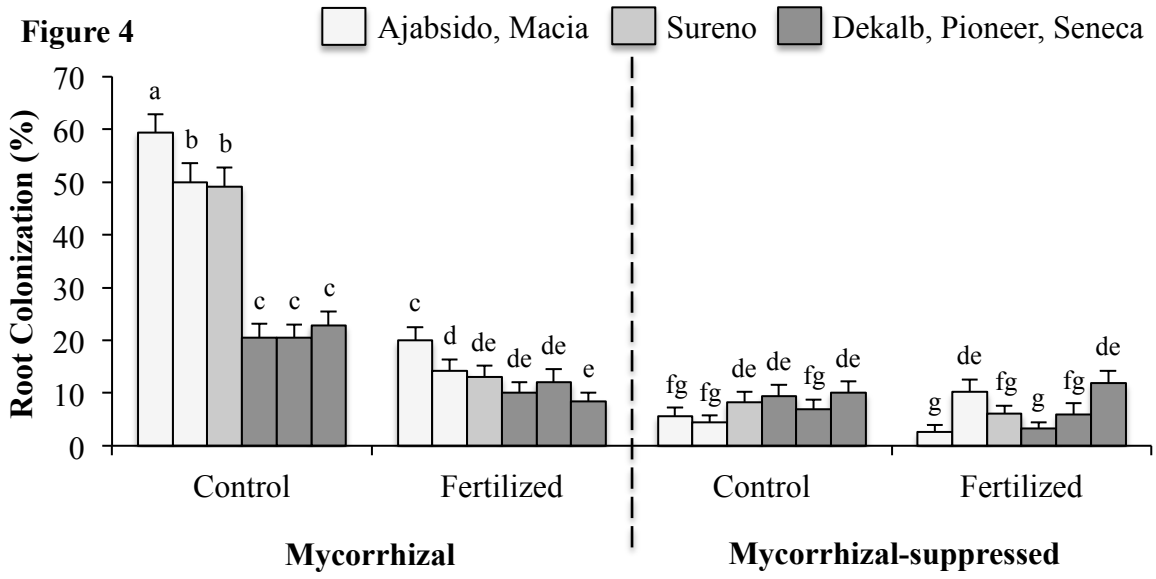

Figure 4. Percent root colonization by AM fungi for sorghum genotypes in mycorrhizal and mycorrhizal-suppressed soils, with no amendment (control), or fertilized with N&P. From left to right, genotypes are open-pollinated African (Ajabsido and Macia), Latin American (Sureno), and hybrid (Dekalb, Pioneer, and Seneca). Bars represent means, + SE (n = 8). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 5

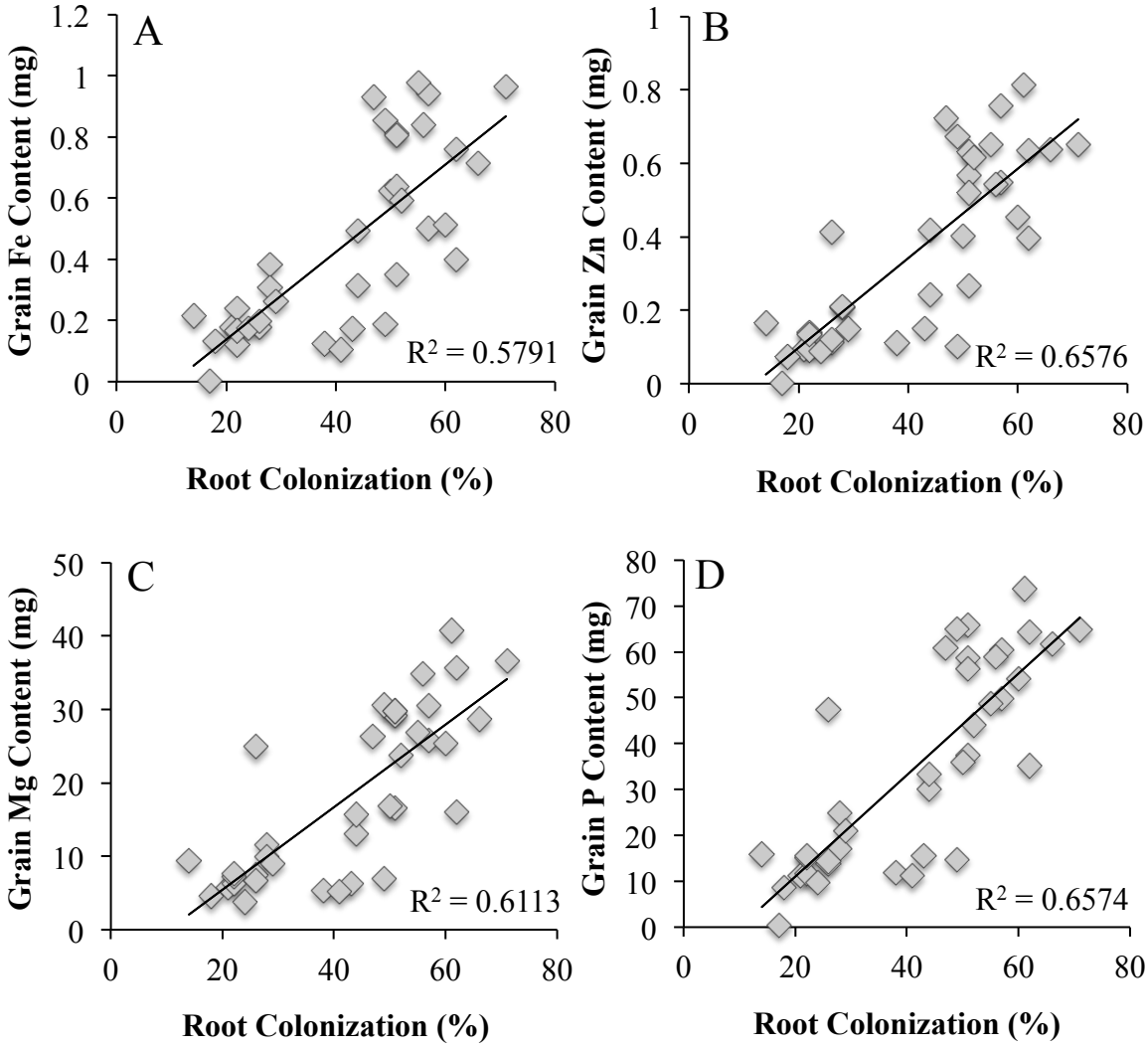

Figure 5. Relationship between mycorrhizal root colonization and total grain mineral content of **A)** iron, **B)** zinc, **C)** magnesium, and **D)** phosphorous. These minerals are of particular importance to human, animal, and plant nutrition. All mycorrhizal sorghum genotypes grown in non-fertilized soil are included. Spearman correlations were significant for all minerals.

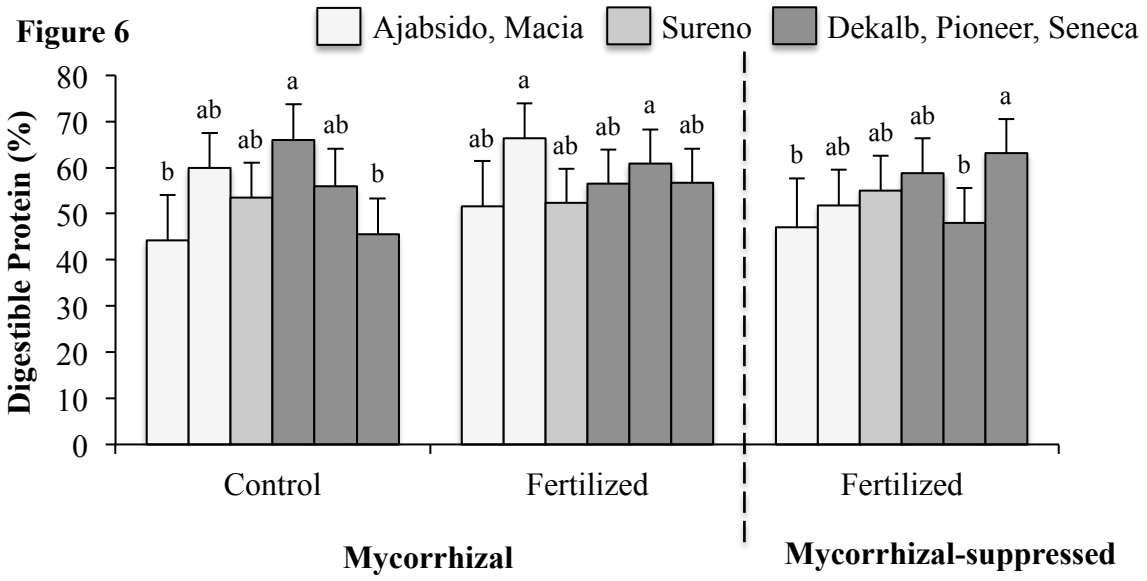

Figure 6. Grain protein digestibility for sorghum genotypes in mycorrhizal and mycorrhizal-suppressed soils, with no amendment (control), or fertilized with N&P. From left to right, genotypes are open-pollinated African (Ajabsido and Macia), Latin American (Sureno), and hybrid (Dekalb, Pioneer, and Seneca). Bars represent means, + SE (n = 8). Bars that do not share a letter are significantly different ($P < 0.05$).

CHAPTER II

Soil ecology and the sustainable production of common bean and cowpea: Arbuscular mycorrhizal fungi, alternative farm inputs, and human nutrition

ABSTRACT: The association of arbuscular mycorrhizal (AM) fungi with two genotypes of common bean and two genotypes of cowpea were assessed in two greenhouse studies, to investigate the role of AM symbiosis in agricultural production with alternative fertility amendments (compost and biochar). All genotypes were grown in local low-nutrient soil. In the first study, plants were not fertilized (controls), amended with commercial fertilizers (N&P), or amended with worm compost. Measurements included AM root colonization, seed production, and total seed protein and mineral content. Productivity and total seed protein were similar between fertilizer and compost treatments, which were significantly improved compared to control. The genotypes Masaai Red (common bean) and Risina del Trasiorfino (cowpea) produced greater total seed (dry weight) and greater total protein than the other genotypes. Compared to common beans, cowpea genotypes had significantly increased seed zinc content and their roots were more highly colonized by AM fungi regardless of treatment, with the cowpea genotype Risina del Trasiorfino being the most highly colonized overall. We selected Risina as the model genotype of our second experiment, and we assessed 10 different soil

amendments (combinations of commercial fertilizers, biochar, and worm compost). For each soil amendment, we assessed AM root colonization, vegetative biomass at 45 days, and plant tissue total protein and mineral content. Commercial fertilizers (N&P) significantly decreased AM fungal colonization and increased vegetative production when included in any amendment. The combination of worm compost with biochar and/or commercial fertilizers, significantly improved total protein and plant tissue potassium and zinc compared to corresponding plants grown with soil amendments not containing worm compost. Our results indicate alternative fertility inputs have the potential to maintain belowground symbiosis and replace a portion of commercial fertilizer use without reducing agricultural productivity

INTRODUCTION

Cereal grains are important components of worldwide diets, yet research shows some of the essential amino acids (AAs) required for human and animal protein synthesis are not highly available from some cereals – particularly grain sorghum and maize (Cervantes-Pahm et al. 2014). There is also a growing body of evidence on the impact of antinutritional factors present in many widely consumed staples (Gilani et al. 2012), and diet diversity is a universally recognized strategy in reducing risk of malnutrition (Ruel 2003). Many subsistence farmers rely on diets of mostly maize and sorghum, but the inclusion of pulses, like cowpea (*Vigna unguiculata*) and common bean (*Phaseolus vulgaris*), can help protect populations from AA deficiencies, such as lysine malnutrition. For example, traditional sorghum foods in sub-Saharan Africa (SSA) prepared with

added cowpea flour showed significantly improved protein/AA and protein digestibility compared to food prepared without added cowpea (Anyango et al. 2011).

These strategies to improve human nutrition using plant-proteins – as opposed to animal source proteins – are essential to meeting the needs of a growing human population efficiently and sustainably (Day 2013). Chronic human malnutrition has led to an increased focus on biofortification of food crops through genetic improvement and farm soil health management (Carvalho and Vasconcelos 2013). We must improve the sustainable cultivation of pulse crops in addition to cereal crops because they are widely consumed, farmers often grow them during different seasons than cereals, and many people depend on them for their caloric and nutritional needs.

A review by Gosling et al. (2006) reported arbuscular mycorrhizal (AM) fungi play a role in improving plant nutrition, disease resistance, water use efficiency, soil structure and beneficial microorganism activity in natural ecosystems, but they have been depleted in many agroecosystems because of farm management practices. Oruru and Njeru (2016) highlighted some of the farming practices that could replenish AM fungi and be particularly beneficial to smallholder farmers. There are many variables, such as crop genetics, involved with harnessing the benefits of AM fungi, and simply adding more AM fungi to farm soils has not consistently resulted in improved yield or sustainability (Berruti et al. 2015). However, the increased soil stability associated with AM fungal activity can enhance global food production systems if farmers select genotypes and agricultural practices that support the symbiosis (Ellouze et al. 2014). Attention to the management of AM fungi in agroecosystems may also reduce the need

for fertilizer applications through improved plant nitrogen and phosphorus uptake (Hodge and Fitter 2010) and reduced nutrient leaching (Asghari and Cavagnaro 2011).

Worldwide, humans consume common bean, by volume, more than any other pulse crop, and tropical bean farmers need improved seeds that are optimized for their specific issues (Beebe et al. 2014). Cowpea is also an important legume crop in many developing countries, but is often grown on poor soil without fertilization – leading to subsistence level yields (Ayodele and Oso 2014). However, field research reported as many as eight genera of AM fungi associating with cowpeas in SSA (Diop et al. 2015). This indicates AM fungi might provide some of the nutrient requirements of cowpeas and potentially boost seed nutritional quality.

Our greenhouse studies assess belowground AM symbiosis with pulse crops under commercial and alternative fertility inputs (worm compost, pyrolyzed carbon [biochar]), and whether there is a link between the AM association of different genotypes and resulting vegetative and seed production and nutrition. This link may provide direction for international agricultural development and sustainable cropping systems, and improve our understanding of how to incorporate alternative amendments while still maintaining high pulse crop yields.

There are several exciting synergies between AM fungal activity and the use of alternative soil fertility amendments. Commercial fertilizers have been used as a means of ensuring higher yield in farming systems for decades, however, ecological issues, such as eutrophication, have been associated with these inputs (Daigle, 2003). Additionally, increased energy, mining, and transportation costs have added external cost burdens to

farmers. Fertilizers are critical to improving yields in SSA; however, commercial fertilizers represent a substantial cost to farmers (Thurow 2013). Alternatives such as worm compost or biochar might improve yields at reduced expense, and need to be assessed in soil agroecology experiments.

Community projects around the world are using worm compost to reduce local reliance on external fertility sources (Misra et al. 2003). Chaoui et al. (2003) reported that earthworm-based composts (vermicomposts) improved crop-nutrition on par with commercial fertilizers, with the additional benefits of slow nutrient release, reduced leaching, and protection from salinity stress as compared to commercial fertilizers. A review by Cayuela et al. (2013) concluded compost also typically maintains or enhances AM fungal root colonization.

The feasibility of biochar production in developing countries will need to be assessed on a case-by-case basis that takes local natural resources into account. For example, Duku et al. (2011) concluded that incorporating locally produced biochar into agricultural fields in Ghana showed great potential for increasing the sustainability of local farming systems. Additional research suggests multiple beneficial outcomes from applying biochar in agroecosystems, including improved N-use efficiency in rice (Qian et al. 2014), greater AM root colonization in common beans (Vanek and Lehmann 2015), and improved water uptake and drought tolerance in wheat (Blackwell et al. 2010).

For sustainable food production, it is critical to find biochar amendment rates that optimize outcomes for particular crops and soil types, as large quantities of biochar have been shown to significantly decrease AM fungal root colonization in some systems

(Warnock et al. 2010). Smaller, yearly additions may avoid this consequence, as soil biota and soil chemistry would be affected more gradually. In developing countries, it will be important to utilize farm management that relies both on research-based methods and local technological capacity (Chambers 1983). Our studies emulate a 'trench and fill' application method, which applies amendments in proximity to seeds under a shallow layer of soil (Filiberto and Gaunt 2013). This method can be utilized with simple hand tools in the field.

With growing interest in incorporating alternative fertility amendments in sustainable agriculture and economic development, it is critical to assess the interactions of those amendments with different crop genotypes and AM fungi. Our current studies evaluate these with an overall goal of linking belowground characteristics with plant production and seed nutrition.

Assessing AM root colonization of crop genotypes and the effect of fertility amendments on colonization and seed production are key steps in determining if AM symbiosis is critical to sustainable agriculture. The primary objectives of Study 1 were (1) to assess AM root colonization for common bean and cowpea genotypes grown in low nutrient soil and amended with commercial fertilizers (N&P) or worm compost, (2) to assess seed production and nutritional quality (protein and mineral content) of the cowpea and common bean genotypes, and (3) to select the genotype with the highest percentage of AM root colonization, greatest productivity, and high seed nutritional quality for use in Study 2.

Study 1: We hypothesize cowpeas will be more colonized by AM fungi than common beans. Additionally, we hypothesize worm compost amendments will support similar production and seed nutrient content, but increased AM fungal root colonization, as compared to commercial fertilizers regardless of genotype. Furthermore, we hypothesize that genotypes with greater AM fungal root colonization will produce greater quantity of seeds with higher total nutrition (protein, minerals) compared to genotypes with less colonization in non-amended control.

If the mycorrhizal association of common bean and cowpea genotypes varies significantly, as was observed for grain sorghum genotypes (Chapter 1), AM fungal root colonization, productivity, and seed nutritional content will likely also vary across soil treatment and genotype. Therefore, we will select a model genotype, which has considerable mycorrhizal association and positive response to worm compost, for our second experiment. The primary objectives of Study 2 were (1) to assess AM root colonization for a genotype selected from Study 1 grown in low nutrient soil with ten different fertility amendments (combinations of commercial fertilizers, worm compost, and/or biochar), (2) to assess the role of AM fungal symbiosis on vegetative production and total tissue nutrition (protein and mineral content) at 45 days post plant emergence, and (3) to link productivity and nutrition to the three fertility amendment components (commercial fertilizers, compost, biochar) to discover a combination or combinations that have similar or improved results compared to typical farm applications.

Study 2: We hypothesize the selected cowpea genotype (Risina del Trasiorfino) will have increased AM fungal root colonization in treatments with biochar and/or worm compost compared to corresponding treatments not containing biochar and/or compost,

and the inclusion of worm compost in any of the treatments will improve vegetative production and tissue quality. We also hypothesize that additions of commercial fertilizers will result in the greatest growth and tissue quality, however, combining biochar and worm compost with 50% of the typical commercial fertilizer rate will result in similar or improved plant production and root colonization, as compared to the treatment with a 100% rate of commercial fertilizers. Furthermore, we hypothesize that increased AM root colonization will improve productivity and tissue quality as compared to non-amended control.

Linking AM fungi, crop genotype, and alternative fertility inputs will expand our abilities to improve soil health, food production, and nutritional quality outcomes in sustainable farm systems. The practical application of best crop genotypes and management practices may increase the benefits of AM fungi in agroecosystems and reduce the need for commercial fertilizers – mitigating negative environmental impacts while providing human dietary needs.

MATERIALS AND METHODS

Study 1 experimental setup. A greenhouse study was conducted to assess seed production, seed nutrition, and AM root colonization of common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) genotypes. A randomized complete block design was used with four replications. A factorial design included two common bean genotypes; two cowpea genotypes; and soil that was not-amended (control), amended with commercial fertilizers (N&P), or amended with worm compost. The International

Center for Tropical Agriculture in Cali, Columbia provided the common bean genotypes (Dicta 105 and Masaai Red), and Baker Creek Heirloom Seeds provided the cowpea genotypes (Six-week Purple-hull and Risina del Trasiorfino). Four total replications of each genotype were grown in: 1) non-amended native soil (control), 2) native soil with fertilization (N and P), and 3) native soil amended with worm compost. The soil was not inoculated with additional soil microbes and contained ambient AM fungal propagules.

Sixty-four Pots with a 26.5 cm diameter and 45cm height were filled with 22 liters of Renfrow/Grainola (eroded silty clay Mollisol/Alfisol) soil collected from the Oklahoma State University Range Research Station (average: pH = 7.4, N = 20 ppm, P = 3 ppm, K = 154 ppm, OM = 0.88%, Fe = 15.5 ppm, Zn = 2.1 ppm). The Soil, Water, and Forage Analytical Laboratory at Oklahoma State University analyzed all soil samples. Soil NO_3-N and NH_4 were extracted by 1M KCl solution and analyzed using the Lachat Quickchem 8000 Flow Injection Autoanalyzer (Kachurina et al. 2000). Two grams of soil were extracted with 20 ml Mehlich 3 solution for plant available P and K, and the concentrations of P and K in the extract were measured by an inductively coupled plasma emission spectroscopy (ICP)(Pittman et al. 2005). Soil pH was measured using a pH electrode in a 1:1 soil to water suspension. Soil organic matter (SOM) was determined by dry combustion using the LECO Truspec CN analyzer (Nelson et al. 1996).

Study 1 treatments. Monopotassium phosphate (0-52-34) fertilizer was applied at a rate equivalent to 100 kg per ha total phosphate and 132 kg per ha total potassium, and ammonium nitrate (34-0-0) fertilizer was applied at a rate of 60 kg per ha total nitrogen. Potassium chloride (0-0-60) was applied to all non-fertilized pots (at 132 kg per ha rate) to ensure uniform potassium across the study. Fertilizers were dissolved in 1 L of water

prior to application. Worm compost was applied at a rate of 90,000 kg per ha (sample average: pH = 6.8, total N = 1.1% [of mass], total P = 0.5%, total K = 0.5%, Total C = 12.9%, Fe = 6154 ppm, Zn = 610 ppm, moisture = 27.85%) was applied, adding an equivalent of 714 kg per ha total N, 357 kg per ha total P, and 357 kg per ha total K. The Oklahoma State SWAFL analyzed compost samples to determine composition using the methods of Peters et al. (2003). Half of the compost was incorporated under the top 2 liters of soil in proximity to anticipated seed depth (~2cm), which simulates a field application method where soil is pulled back along the planting row to accommodate compost inputs before being replaced over the applied compost (Filiberto and Gaunt 2013). One-half of the fertilizers and compost were applied at the beginning of the study and half at first plant bloom to simulate a 'side-dressing' field application method (Cavigelli et al. 2013).

Study 1 conditions. Common bean and cowpea seeds were sown directly into moistened soil. Upon sprouting, plants were thinned to one plant per pot. Normal watering occurred every 2-4 days, as needed. Greenhouse temperature ranged from 19-30 °C. Seeds were harvested after maturation and pod drying (multiple pod production cycles were allowed for each plant), and seed production (dry weight) was determined. Root samples were collected at plant senescence to assess AM root colonization.

Study 2 experimental setup. A follow-up greenhouse study was conducted to assess mycorrhizal response of the selected cowpea genotype (Risina del Trasiorfino) grown with alternative agricultural amendments. A randomized complete block design was used with six replications. Two cowpea plants were grown in each pot treated with: 1) non-amended soil (control), 2) soil with 100% typical fertilizer rate (N&P), 3) soil amended

with worm compost, 4) soil amended with biochar, 5) soil amended with both worm compost & biochar, 6) soil amended with biochar and 150% fertilizer rate, 7) soil amended with biochar and 100% fertilizer rate, 8) soil amended with biochar and 50% fertilizer rate, 9) soil amended with worm compost & biochar and 150% fertilizer rate, 10) soil amended with worm compost & biochar and 100% fertilizer rate, and 11) soil amended with worm compost & biochar and 50% fertilizer rate.

Sixty-six pots with a 22 cm diameter and 22 cm height were filled with 7.5 liters of sand mixed with Renfrow/Grainola (eroded silty clay Mollisol/Alfisol) soil collected from the Oklahoma State University Range Research Station Station in a 2:3 ratio (final mix: pH = 7.4, N = 13 ppm, P = 5.6 ppm, K = 118 ppm, OM = 0.40%, Fe = 8.19 ppm, Zn = 0.33 ppm). Soil/sand mix was selected to simulate soil texture of on-going field trials. The soil was not inoculated with additional soil microbes and contained ambient AM fungal propagules.

Study 2 treatments. In treatments containing commercial fertilizer (N&P), diammonium phosphate (18-46-0) and urea (46-0-0) were applied. The 100% typical fertilizer rate was established as equivalent to 100 kg per ha total nitrogen and 60 kg per ha total phosphate (150% = 150/90 kg per ha, 50% = 50/30 kg per ha). Each treatment containing biochar applied a rate of 2290 kg per ha (produced at 500-700 C, pinewood-based, average pH = 9.49, Total N = 0.66%, Total Ash = 9.2%, Total Organic Carbon = 85.5%, Fe = 3839 ppm, Zn = 9.2 ppm, moisture = 56.4%). Each treatment containing worm compost applied a rate of 4580 kg per ha (average: pH = 7.4, N = 0.53%, P = 0.1%, K = 0.2%, Total C = 7.32%, Fe = 5987 ppm, Zn = 51 ppm, moisture = 29.8%) adding an equivalent of 17 kg per ha total N, 3.2 kg per ha total P, and 6.4 kg per ha total K. Commercial

fertilizers, biochar, and worm compost were applied 24 hours before seeding and incorporated below the top 1 liter of soil in proximity to anticipated seed depth (~2cm). This procedure simulates a trench and fill field application.

Study 2 conditions. Cowpea seeds were sown directly into moistened soil. Upon sprouting, plants were thinned to two per pot. Normal watering occurred every 2-4 days, as needed. Greenhouse temperature ranged from 20-32 ºC. Plants were harvested 45 days after emergence and plant production (dry weight) was determined. Root samples were collected at plant senescence to assess AM root colonization.

Quantification of seed mineral concentrations. The USDA-ARS Children's Nutrition Research Center in Houston Texas assessed seed Ca, Cu, Fe, K, Mg, P, S, and Zn concentrations for each sample. Using methods of Farnham et al. (2011), two subsamples (~0.25 g dry weight) of each ground sample were digested and processed for elemental analysis. Elemental analysis was performed using inductively coupled plasma–optical emission spectroscopy (CIROS ICP Model FCE12). Tissue mineral concentrations were determined on a dry mass basis (μg g–1 or mg g–1), and an average value was derived from the two sub-samples of each replicate.

Quantification of protein and tissue quality. The Soil, Water, and Forage Analytical Laboratory at Oklahoma State University determined percent protein for seed samples and protein and mineral concentrations for tissue samples. Samples were dried at 85ºC over night and ground to pass through a 1 mm screen. The moisture content was determined gravimetrically following drying each ground sample at 105ºC overnight. Total nitrogen (TN) and carbon were determined using a LECO Truspec dry combustion

Carbon/Nitrogen Analyzer (Undersander et al. 1993) and crude protein was calculated by multiply TN by 6.25. Mineral concentrations (Ca, Fe, K, Mg, P, and Zn) of the forage were analyzed by a Spectro Blue ICP following acid digestion (Undersander et al. 1993) and calculated in micrograms per gram (ppm).

Quantification of AM Colonization. Live roots were subsampled, washed, stained with trypan blue, and scored for AM colonization using the magnified gridline intersect method (Mcgonigle et al. 1990). This method uses a digital microscope (Hirox KH 7700) to measure the percentage root length colonized by hyphae, vesicles, coils, and arbuscules, which were combined to determine total percent colonization.

Statistical Analysis. The response variables of AM root colonization, seed production, plant biomass production, and protein and mineral concentrations and content were analyzed using mixed models methods. The Tukey multiple comparison method was utilized for significant effects. All tests of significance were performed at the nominal 0.05 level. All data analyses were generated using SAS® version 9.4. Copyright © 2013 SAS Institute Inc. SAS and all other SAS Inc. product of service names are registered trademarks of trademarks of SAS Institute Inc., Cary, NC, USA.

RESULTS

Study 1

Seed Production. There were not significant differences in seed production among genotypes grown in non-amended soil (control), however, commercial fertilizers or

compost significantly improved productivity of every genotype compared to the control (Figure 2). There was a significant effect of genotype on seed production (Table 1). Masaai Red (common bean) and Risina (cowpea) were more productive than the other genotypes regardless of treatments, and Risina (which was selected as the genotype for use in Study 2) averaged 18.58g (data not shown).

Seed Protein Concentration and Production. Seed protein concentrations were significantly different by treatment and genotype but not by the interaction of treatment with genotype (Table 1). Common beans had significantly lower protein concentration than cowpeas (Table 1), with the highest concentration in the Purple-hull genotype (25.20% ± 0.37) and the lowest concentration in Masaai Red genotype (21.87% ± 0.39)(data not shown). By combining seed production and seed protein concentration data, total seed protein production was calculated in grams. Results followed a similar trend to total seed production, with significantly improved protein production for all genotypes growth in commercial fertilizer or compost compared to non-amended control (Figure 3). Genotype also had a significant effect on protein production (Table 1). Masaai Red (common bean) and Risina (cowpea) produced more total protein regardless of treatments, and Risina averaged the highest (4.17g)(data not shown).

AM Root Colonization Percentage. There were no significant differences in AM root colonization by the interaction of treatment and genotype (Figure 1). However, there was an overall significant contrast (Table 1) between common beans and cowpeas. Roots of cowpea genotypes were more highly colonized by AM fungi regardless of treatments, and Risina was the most highly colonized (47.8%)(data not shown).

Seed Mineral Concentrations and Content. There were significant differences in seed mineral concentrations by genotype origin (Table 2) with common beans having a higher concentration of calcium and potassium than cowpeas, but cowpeas having a higher concentration of copper, magnesium, phosphorus, and zinc compared to common beans. Seed mineral concentrations were also significantly different by treatment (Table 2) with compost or commercial fertilizers increasing iron, phosphorus, and magnesium concentrations compared to non-amended control but decreasing copper, while commercial fertilizers decreased zinc and compost decreased calcium concentration compared to other treatments. There were no significant differences in sulfur concentrations by genotype or treatment, but there were significant differences by the interaction of those factors (Table 2) with the highest concentrations in Masaai Red and Risina when they were grown in soil treated with commercial fertilizers or compost.

By combining seed production and mineral concentration data, total seed content for each mineral was calculated in grams. Differences in mineral contents followed similar trends across most of the analyzed minerals, with some significant differences (Table 2). Common bean genotypes had higher total seed calcium and potassium content compared to cowpeas, while cowpea genotypes had significantly higher total seed zinc content. There was also a significant genotype effect for some minerals (Table 2) with Masaai Red and Risina having more copper, iron, and sulfur content as compared to other genotypes and Masaai Red, Risina, and Purple-hull having more magnesium and phosphorus content as compared with Dicta 105.

Study 2

Vegetative Production at 45 Days. Plants treated with commercial fertilizers, regardless of rate, produced significantly more vegetative biomass following 45 days of growth compared to non-amended control plants or plants treated with only compost and/or biochar (Figure 5). Orthogonal contrasts for the eight treatments containing biochar determined that, when included, worm compost did not have a significant effect on production within those treatments and the significant differences within those treatments were the result of including commercial fertilizers.

Plant Tissue Protein Concentration and Production. Tissue protein concentrations were significantly different by treatment, with the combination of biochar, compost, and a 150% rate of commercial fertilizers resulting in the highest tissue protein concentrations (16.77% ± 0.93) and the combination of biochar with a 50% rate of commercial fertilizers resulting in the lowest concentration (11.13% ± 0.85)(data not shown); however, no treatments resulted in protein concentrations that were significantly different from plants grown in non-amended soil. Orthogonal contrasts for the eight treatments containing biochar determined that, both worm compost and/or commercial fertilizers significant increased protein concentration compared to treatments without compost and/or commercial fertilizers. By combining vegetative production and plant tissue protein concentration data, total protein production was calculated in grams. There was a significant difference between treatments, with several of the biochar blends producing significantly more total protein compared to the control (Figure 6). Orthogonal contrasts for the eight treatments containing biochar determined that inclusion of commercial fertilizers significantly increased total protein production. For those eight treatments, the

inclusion of worm compost also significant increased total protein production by an average of 0.12g.

AM Root Colonization. Compared to control, there were not significant treatment effects on cowpea root colonization by AM fungi; however, the biochar treatment had the highest average colonization (Figure 4). Orthogonal contrasts for the eight treatments containing biochar determined that, when included, commercial fertilizers significantly decreased overall AM root colonization, while worm compost did not have a significant effect on root colonization for these treatments.

Plant Tissue Mineral Concentrations and Total Content. Tissue mineral concentrations were significantly different by treatment for calcium, potassium, magnesium, phosphorus, and zinc but not iron. Various combinations of biochar, worm compost, and commercial fertilizers resulted in the highest tissue concentrations of calcium, potassium, and phosphorus compared to the lowest concentrations for the non-amended control, except with zinc where use of fertilizer by itself resulted in the lowest tissue concentration and magnesium where amendment with only worm compost resulted in the lowest concentration. By combining vegetative production and tissue mineral concentration data, total tissue content of each mineral was calculated in grams. The total plant mineral content followed similar trends across all analyzed minerals, with significant differences resulting from the addition of fertilizers (Table 3). Orthogonal contrasts for the eight treatments containing biochar determined that treatments containing worm compost increased total potassium and zinc content compared to treatments without worm compost.

DISCUSSION

In Study 1, the cowpea genotypes hosted a higher percentage of AM fungal root colonization as compared to common beans, as we hypothesized. The difference in root colonization did not appear to have an effect on crop performance as the common bean genotype Masaai Red and the cowpea genotype Risina del Trasiorfino had similar total seed production and nutritional content, regardless of treatment, and outperformed the other genotypes overall. This did not support our hypothesis, as we hypothesized greater AM root colonization would improve seed production and nutritional content (particularly in non-amended soil). Treatment with worm compost did not increase AM root colonization as we hypothesized. Cavagnaro (2015) proposed that because plants differ in their dependence on AM symbiosis, application of composts on different plant species often results in a range of production outcomes. Because legumes use a C_3 photosynthetic pathway, and C_3 plants are generally less mycorrhizal responsive compared to C_4 species, it is possible these results would differ for C_4 crops such as corn and sorghum (Wilson and Hartnett 1998). Previous research found significant correlations between AM fungal root colonization of grain sorghum genotypes in low fertility soil and total grain mineral content for key elements like iron, phosphorus, magnesium, and zinc (Chapter 1); however, there were no significant correlations between root colonization and these minerals for common bean and cowpea genotypes.

Worm compost supported similar productivity and seed nutritional content as compared to commercial fertilizers, as we hypothesized, and both fertility amendments significantly improved these responses as compared to control. These results concur with Chaoui et al. (2003) and Cayuela et al. (2013) and partially support our hypothesis. The

general lack of significant differences in seed micronutrient concentration or content between worm compost and commercial fertilizer treatments is noteworthy because worm compost contains additional micronutrient content that is not supplied by commercial N&P fertilizers. For example, previous research assessing compost with high levels of zinc, as compared to conventional fertilizers not containing zinc, did not detect significant productivity or grain zinc differences in corn (Hirzel and Walter 2015). This suggests that some crops or genotypes have a limited need or uptake capacity for zinc, and additions via compost will not always result in more zinc content in the seeds.

Although the common bean genotype Masaai Red and cowpea genotype Risina produced similar seed quantity and nutritional quality, Risina was characterized by greater AM colonization and seed zinc content. Therefore, we selected Risina as the model genotype for Study 2. The additional seed zinc content we assessed may link with the greater AM root colonization of the cowpea genotypes. There is growing evidence for improved plant zinc uptake via the mycorrhizal pathway (Lehmann et al. 2014; Smith and Read 2010). Because it is relatively immobile in soil, the additional AM fungi hosted in the cowpea roots may have improved plant acquisition of zinc.

In Study 2, we assessed fewer significant differences in AM root colonization between different treatments than we hypothesized. While treatments containing higher rates of commercial fertilizer had significantly reduced colonization compared to the biochar only treatment, neither was significantly different from non-amended control. Treseder and Allen (2002) and Johnson et al. (2006) discuss that AM fungi have substantial N&P requirements for their own physiological function; therefore low soil fertility can constrain their abundance and, alternatively, high soil fertility often causes

the plants to reduce carbon allocation to the fungal partner. This suggests an optimal zone of soil fertility exists in which AM abundance can be enhanced through careful farm soil nutrient management (Figure 7). This may also indicate that less mycorrhizal responsive C_3 species have a narrow optimal zone of mycorrhizal benefit. We proposed that some of our study's treatments (with commercial fertilizers) resulted in excessive soil N&P with a resultant loss of AM fungi, while some treatments (non-amended, compost only) resulted in insufficient soil N&P and AM fungi could not be adequately supported.

The relatively high AM root colonization following biochar amendment may suggest biochar intensified plant nutrient limitation through nutrient absorption from the surrounding soil. Observations of AM fungi mining biochar, and ^{33}P radiotracers have traced P movement from biochar into plant tissue via AM symbiosis (Hammer et al. 2014). These results led researchers to advocate the potential of combining biochar and AM fungal management – utilizing the symbiont to access nutrients from the biochar reservoir. Our results may also indicate that biochar can boost host plant root colonization by AM fungi and concurs with Vanek and Lehmann (2015).

When looking at biochar research, it is difficult to make general conclusions about how a particular plant-soil-microbial system will be impacted because of variable biochar characteristics (Keiluweit et al. 2010). A meta-analysis of biochar use in agriculture reported amendments typically resulted in significantly increased aboveground plant productivity, crop yield, rhizobia nodulation, and key nutrient concentrations, however, belowground productivity and percent AM root colonization were typically not significantly different from non-amended plants (Biederman and Harpole 2013). Lehmann et al. (2011) explained that cases where AM fungi abundance is

decreased by biochar are likely related to soil enrichment with plant-available nutrients. The range of biochar physical and chemical properties are affected by production temperature, time, and biomass inputs and can result in biochar that provides substantial nutrient additions to soil systems or is relatively resistant to microbial breakdown (Singh et al. 2010; Zimmerman 2010). The biochar we selected for our experiment was produced at high temperature and was therefore composed primarily of carbon and insoluble elements that would not be expected to significantly enrich soil N&P or reduce AM fungal root colonization.

Our results also indicate that worm compost can improve plant protein production and zinc content when combined with biochar and fertilizers. Compost can add zinc directly to zinc deficient soil. In comparison, we did not observe increases in zinc uptake following compost amendments in Study 1. However, soil zinc was 6.36 times more abundant, in Study 1, compared to the soil used in our second study. Meta-analysis of the influence of AM fungi on zinc mobilization and crop plant nutrition concluded fungal mediation was affected by soil zinc concentration (Lehmann et al. 2014) and that AM fungi generally improved crop plant zinc nutrition.

Alternative fertility inputs (biochar, compost) improved fertilizer efficiency in Study 2. Compared to the 100% rate of commercial fertilizers, the combination containing biochar or biochar & worm compost with 50% rate of commercial fertilizers produced similar vegetative biomass, protein, and tissue mineral content, as we hypothesized. This indicates that some of the cost of soil fertility management with commercial fertilizers may be replaced by locally produced worm compost and biochar.

This would also provide small business opportunities for the manufacturing of these products in developing countries (Hoornweg et al. 1999; Scholz et al. 2014).

There is potential for biochar to mitigate GHG production by reducing N_2O emissions in agroecosystems (Thomazini et al. 2015). Across a range of soil types, biochar addition significantly reduced N_2O emission (from 10% to 90% reduction) in a denitrification incubation study (Cayuela et al. 2013). The authors hypothesized the physical and chemical characteristics of the biochar facilitate the conversion of N_2O to N_2 gas. Lehmann et al. (2011) proposed that freshly produced biochars might release ethylene compounds that are partially responsible for observed alterations of soil microbial processes such as denitrification; potentially reducing human-induced climate change in addition to boosting agroecosystem efficiency.

CONCLUSION

Cavagnaro et al. (2015) reported that AM fungi can promote wide-scale sustainable nutrient cycling and use-efficiency directly through plant uptake and soil nutrient stabilization. In both our studies percent AM root colonization did not drop below 20%, regardless of treatment or genotype. Hosting AM fungi represents a substantial carbon cost to the plant, and indicates that there are enough benefits to compensate for the price. In Study 1, differences in seed production and nutritional quality were not observed between the more highly colonized cowpea genotypes and the less colonized common bean genotypes. In Study 2, AM root colonization was not highly variable between treatments; therefore we did not detect significant differences in production/nutrition that

could be attributed to the symbiosis. However, in previous experiments, where suppression of AM fungi was utilized to assess the role and strength of the symbiosis, bean plants were typically dependent on AM fungi except in high fertility soils (Ortas and Akpinar 2006; Yaseen et al. 2013). We propose that AM fungi were benefitting the pulse crops in our experiments and more research is needed to assess the interaction of AM fungi with plants grown in soil amended with compost and biochar.

For pulse crops, there are clear benefits to utilizing these alternative fertility amendments, to efficiently improve production and nutritional quality. Commercial fertilizers improved almost every production/nutrition factor we assessed, however, the rate of fertilization could be reduced to half the typical application without significant reductions in production/nutrition when the commercial fertilizers were combined with compost and/or biochar. This may provide a global benefit, in addition to reducing fertilizer costs and improving soil health, if farmers begin to apply yearly additions of biochar and compost along with commercial fertilizers. Government agencies, development organizations, and farmer interest groups should invest in training local producers to utilize these alternative inputs as a poverty reduction strategy.

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TABLES AND FIGURES

Table 1. P-values of the fixed effects and contrasts of Study 1.

Fixed Effects & Contrasts: Genotype = G, Treatment = T, Genotype Origin* = GO

Fixed Effects	Seed Production	Seed Protein Concentration	Total Seed Protein	Root Colonization
G	0.0022	<0.0001	0.0072	0.0402
T	<0.0001	<0.0001	<0.0001	0.7096
G x T	0.0921	0.1437	0.1909	0.6554
Contrasts				
GO	0.1570	<0.0001	0.9038	0.0071
GO x T	0.2737	0.7293	0.5241	0.5216

*Genotypes were categorized by origin: common beans (Dicta 105 & Masaai Red) and cowpeas (Purple-hull & Risina del Trasiorfino).

Table 2. P-values of the seed mineral concentrations and total seed mineral contents of Study 1.

Fixed Effects & Contrasts: Genotype = G, Treatment = T, Genotype Origin* = GO

	Ca	Cu	Fe	K	Mg	P	S	Zn
Fixed Effects	**Seed Mineral Concentrations**							
G	<0.0001	<0.0001	0.0793	<0.0001	<0.0001	0.0003	0.1683	<0.0001
T	0.0001	<0.0001	0.0003	0.0803	0.0035	<0.0001	0.5944	0.0056
G x T	0.3080	0.0081	0.0005	0.1636	0.0180	0.0215	0.0112	0.0864
Contrasts								
GO	<0.0001	0.0002	0.8756	<0.0001	<0.0001	<0.0001	0.7182	<0.0001
GO x T	0.0973	0.3592	<0.0001	0.1488	0.0146	0.0015	0.0065	0.4100
	Total Seed Mineral Contents							
G	0.0006	0.0102	0.0002	0.0002	0.0027	0.0115	0.0050	0.0106
T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G x T	0.4512	0.6470	0.0078	0.0396	0.0812	0.1448	0.0915	0.2287
Contrasts								
GO	<0.0001	0.2224	0.0215	0.0008	0.5155	0.5675	0.0875	0.0157
GO x T	0.2290	0.6478	0.0052	0.0808	0.8690	0.1566	0.1228	0.7976

*Genotypes were categorized by origin: common beans (Dicta 105 & Masaai Red) and cowpeas (Purple-hull & Risina del Trasiorfino).

Table 3. Total cowpea (Risina) plant tissue mineral content (mg) by treatment.

Treatments: CON = Control, WC = Worm compost, B = Biochar, NP = Nitrogen & Phosphorus fertilizers (% Rate)

Treatment	Ca	Fe	K	Mg	P	Zn
CON	14.8d	0.09c	21.8c	3.6d	2.1d	0.03d
WC	24.5cd	0.14c	31.6bc	5.6bcd	3.5cd	0.05bcd
B	18.8d	0.14c	25.9c	4.7cd	2.9d	0.04cd
WC + B	30.9cd	0.20bc	37.9bc	6.7bcd	5.0bcd	0.07bc
NP 100%	59.5ab	0.29ab	42.6abc	16.2a	5.5bcd	0.07abc
B + NP 150%	71.0a	0.41a	62.7ab	20.3a	10.1a	0.10a
B + NP 100%	60.1ab	0.40a	55.3ab	16.2a	7.4abc	0.08abc
B + NP 50%	48.1abc	0.27abc	48.0abc	12.9abc	7.8ab	0.08ab
WC + B + NP 150%	67.8a	0.40a	70.5a	18.9a	9.1a	0.10a
WC + B + NP 100%	63.5a	0.37ab	70.5a	17.1a	10.2a	0.12a
WC + B + NP 50%	57.6ab	0.32ab	65.6a	13.9ab	8.3ab	0.11a

Within a column, means that do not share a letter are significantly different ($P < 0.05$).

Figure 1

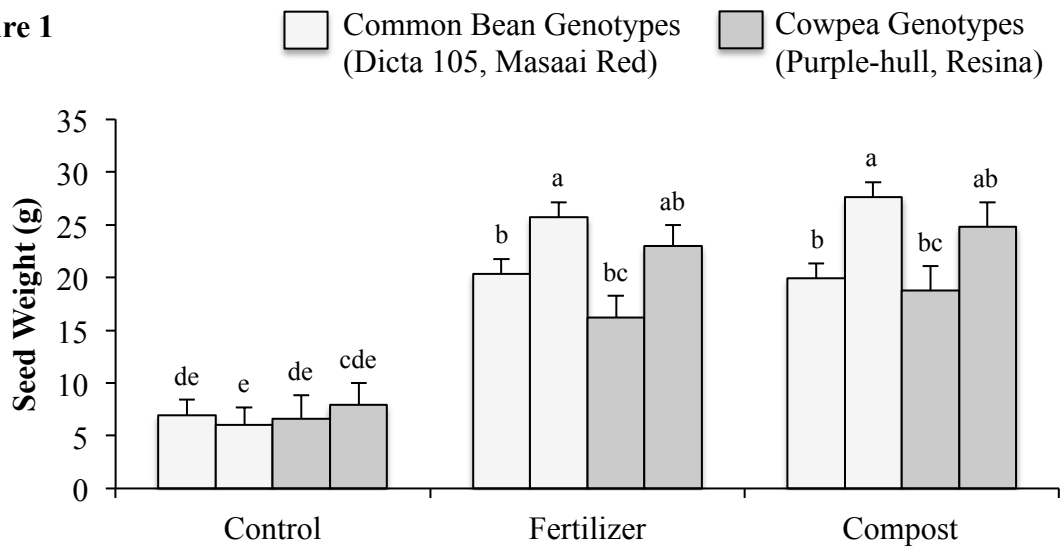

Figure 1. Seed production in grams of common bean and cowpea genotypes grown in soil that was non-amended (control) or amended with commercial fertilizers or worm compost. From left to right, genotypes are common beans (Dicta 105 and Masaai Red), cowpeas (Purple-hull and Risina). Bars represent means, + SE (n = 4). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 2

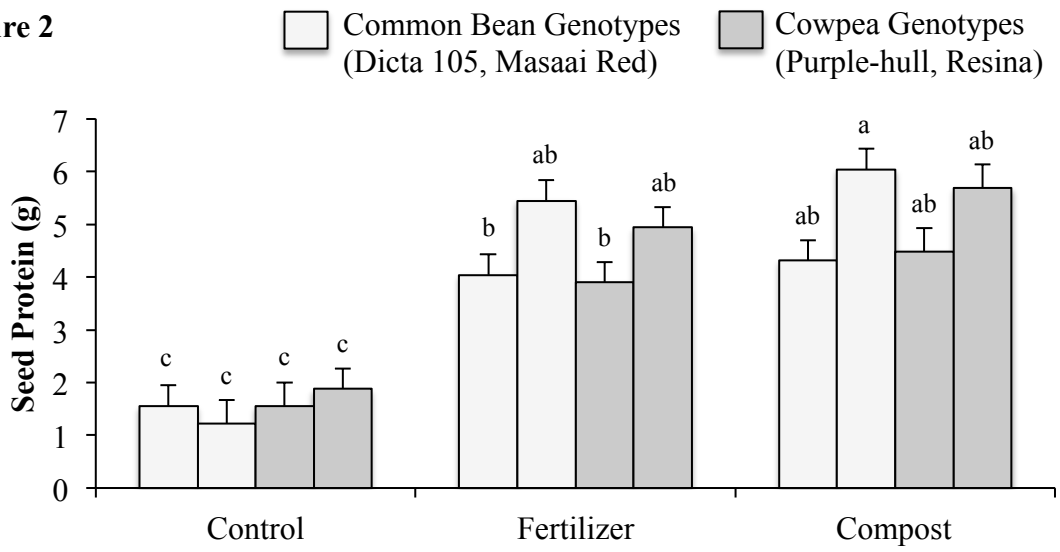

Figure 2. Seed protein production of common bean and cowpea genotypes grown in soil that was non-amended (control) or amended with commercial fertilizers or worm compost. From left to right, genotypes are common beans (Dicta 105 and Masaai Red), cowpeas (Purple-hull and Risina). Bars represent means, + SE (n = 4). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 3

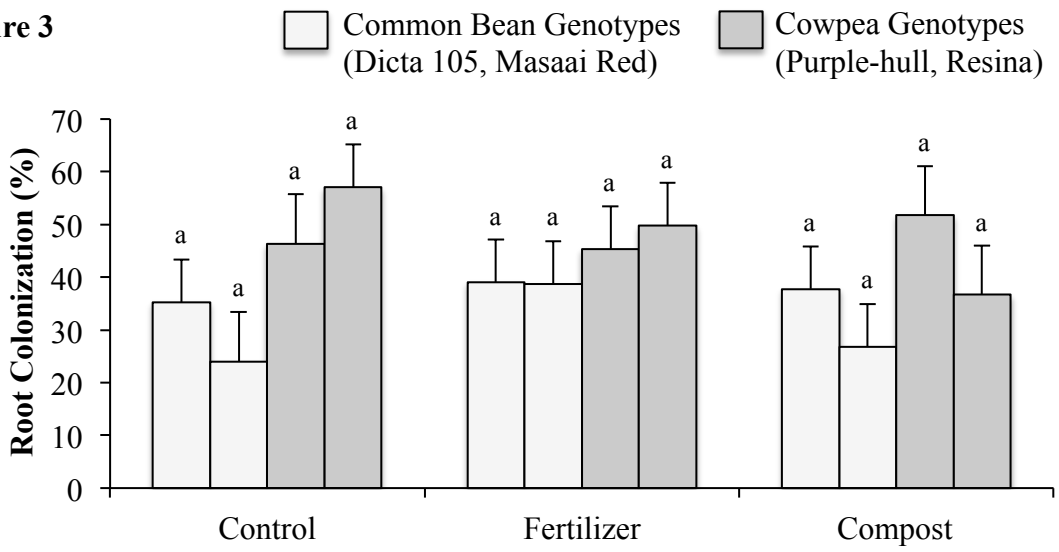

Figure 3. Percent AM root colonization of common bean and cowpea genotypes grown in soil that was non-amended (control) or amended with commercial fertilizers or worm compost. From left to right, genotypes are common beans (Dicta 105 and Masaai Red), cowpeas (Purple-hull and Risina). Bars represent means, + SE (n = 4). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 4

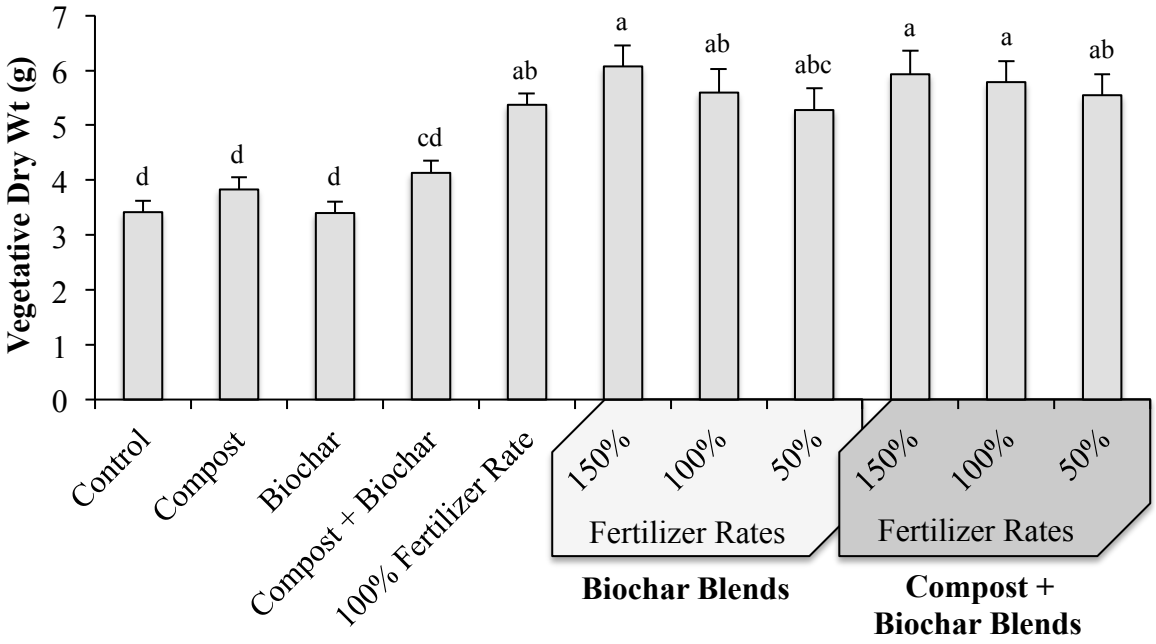

Figure 4. Total vegetative production (dry weight) of Risina (cowpea) grown in non-amended (control) or amended soils. Bars represent means, + SE (n = 6). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 5

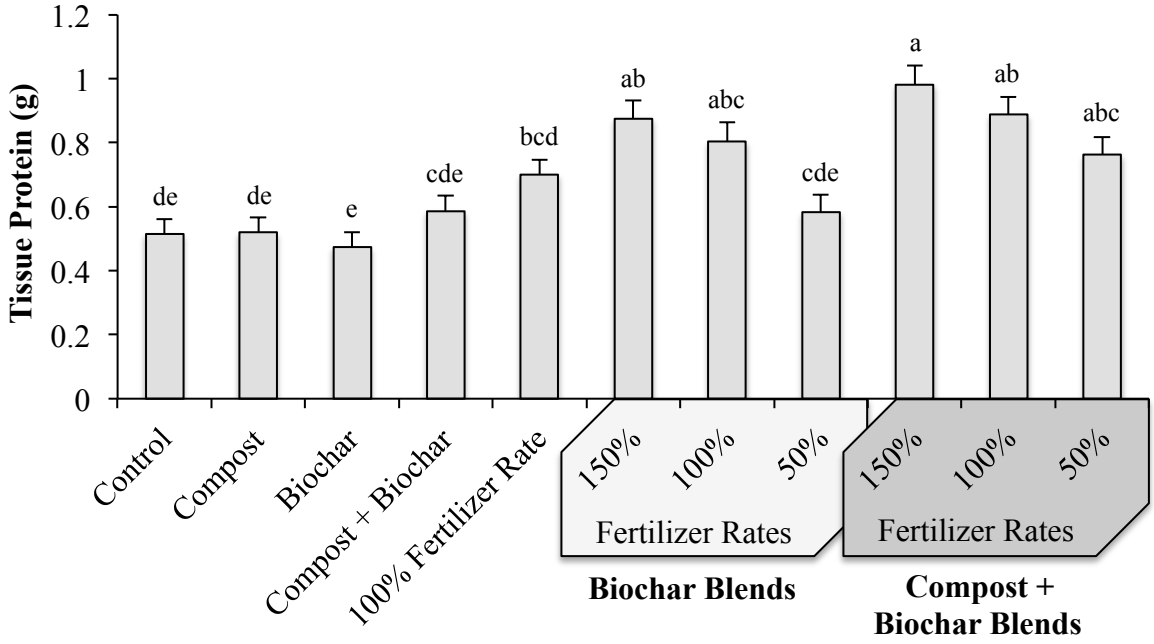

Figure 5. Total plant tissue protein of Risina (cowpea) grown in non-amended (control) or amended soils. Bars represent means, + SE (n = 6). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 6

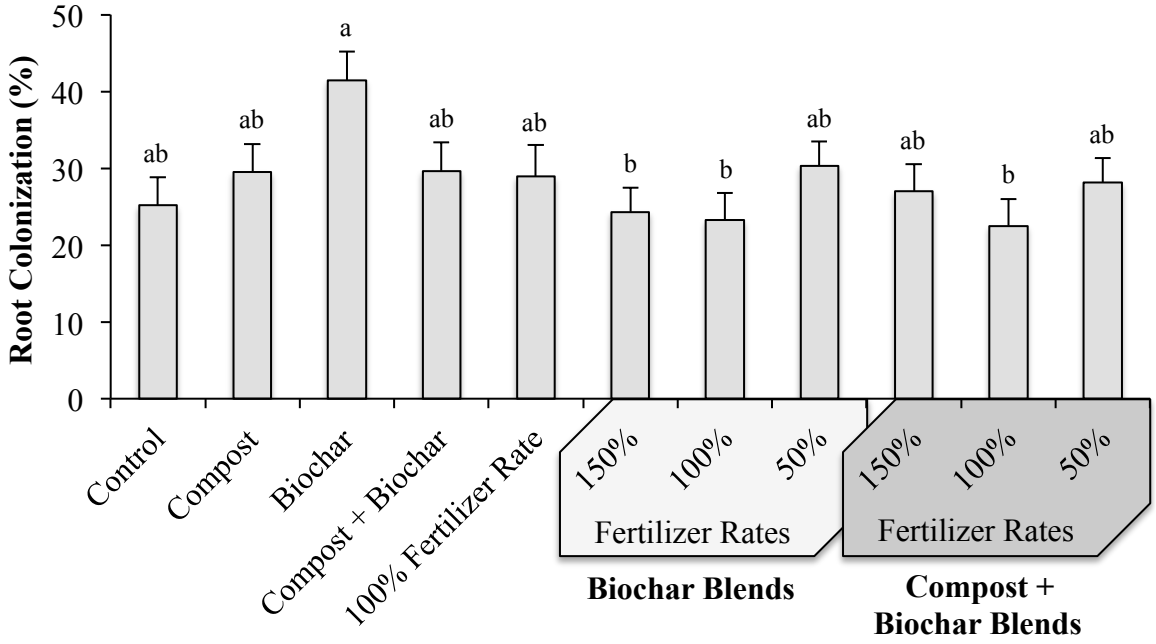

Figure 6. Percent AM root colonization of Risina (cowpea) grown in non-amended (control) or amended soils. Bars represent means, + SE (n = 6). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 7. Recreated from Treseder & Allen (2002) and Johnston et. al (2010).

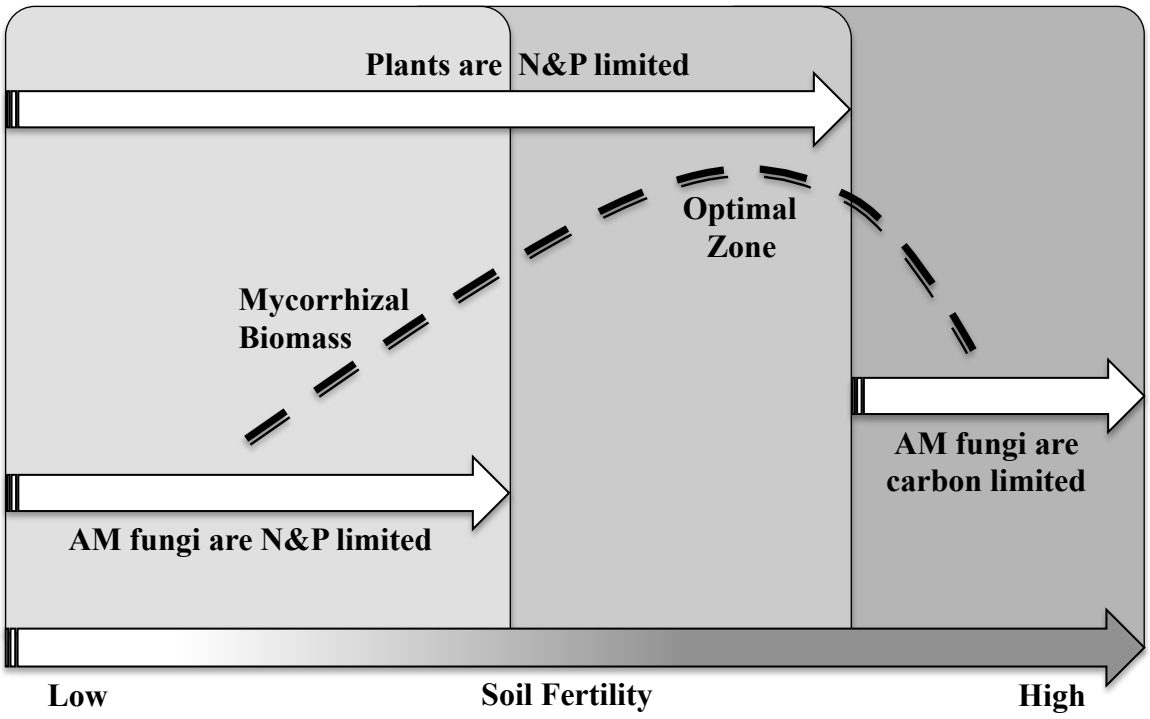

Figure 7. Arbuscular mycorrhizal fungi have substantial N&P requirements for their own physiological function, and low soil fertility can constrain their abundance while high soil fertility can result in reduced plant carbon allocation to the fungal partner. This suggests an optimal zone of soil fertility exists in which mycorrhizal fungal abundance can be enhanced through careful farm soil nutrient management.

CHAPTER III

Grain quality and soil microbial assessments of hybrid and open-pollinated sorghums: Linking farm fertility inputs, cropping systems, and genotype

ABSTRACT: Four genotypes of grain sorghum (two hybrids and two open-pollenated genotypes) were assessed for grain production, protein and mineral concentrations, and grain physical and starch quality characteristics, as well as their influence on soil microbial communities, particularly inter- and extra-radical arbuscular mycorrhizal (AM) fungi, in a field trial at two sites. All genotypes were grown as sole crops, while one hybrid and one open-pollinated genotype were also intercropped with cowpea to compare results between the two farm management systems. All genotypes were grown in low-fertility soils at Oklahoma State University Wes Watkins Research and Extension Center (Lane, Oklahoma, USA) and the Samuel R. Noble Foundation (Ardmore, Oklahoma). We established three treatments: a non-amended control, commercial fertilizers (N&P), and worm compost. Genotype had a significant effect on grain nutritional quality, and grain physical characteristics, with the open-pollinated genotypes characterized by higher Ca, Mg, P, S, and protein concentrations, compared to hybrid genotypes, and hybrid genotypes characterized by a higher proportion of grain amylose and completely hydrolysable starch, regardless of fertilizer or management treatment. Intercropping

and/or amendment with worm compost generally resulted in similar or improved grain quality as compared to sole cropping and/or amendment with commercial fertilizers. Differences were detected in the microbial communities associated with different genotypes, and there a higher fungal to bacterial biomass ratio present following amendment with worm compost. Grain production was not significantly different between genotypes or treatments, but percentage AM root colonization was significantly greater for plants grown in soil amended with worm compost, as compared to commercial fertilizers.

INTRODUCTION

Sorghum bicolor (also called milo) has been, by some accounts, cultivated in sub-Saharan Africa and South Asia for over 5000 years (De Wet and Harlan 1971). Annual production in the US was estimated at nearly 11 million Mt in 2014 (USDA 2015) and over 15 million Mt in 2015 (USDA 2016). While the high tannin content of many grain sorghum genotypes generally reduces protein digestibility for humans and animals, high grain antioxidant content may also provide health benefits for people with adequate dietary protein (Wong et al. 2010). Enhancing grain sorghum nutritional quality is an important breeding consideration (Taylor et al. 2014), as this would benefit farming communities around the globe. Additionally, understanding the variation in starch characteristics between different genotypes is key to improving sorghum use in various food products (Kaufman et al. 2013a). Interest in reducing or eliminating dietary gluten and an increase of celiac disease is also driving current sorghum research. Sorghum cultivation in many

developing countries provides opportunities to address global nutrition security by improving breeding and biofortification strategies to produce the most beneficial and locally appropriate genotypes and farm systems for sustainable sorghum production.

Fertilizers are critical to improving yields in sub-Saharan Africa (SSA) but represent a substantial cost to farmers (Thurow 2013), so alternative inputs like worm compost may provide a way to improve yield and soil health while reducing costs. Chaoui et al. (2003) reported that earthworm-based composts (vermicomposts) reduced nutrient leaching, as compared to commercial fertilizers, while maintaining yield.

Arbuscular mycorrhizal (AM) fungi form beneficial associations with as many as 80% of land plants. A review by Gosling et al. (2006) reported AM fungi improve plant nutrition, disease resistance, and water use efficiency in natural ecosystems, with the potential to improve agricultural production while reducing the rate of fertility inputs. Arbuscular mycorrhizal fungi present a great opportunity to make global agriculture more efficient, more sustainable, and more productive (Ellouze et al. 2014; Rodriguez and Sanders 2015).

The major goals of sorghum breeding and genetics programs are often to develop superior seed-lines with improved grain production and disease resistance. However, breeding efforts to improve sorghum genotypes for grain production may have inadvertently reduced positive plant-fungal relationships. It is critical to assess not only the productivity and grain characteristics of sorghum genotypes, but also their influence on soil microbial communities, including interactions with AM fungi, as microbial communities serve as a link between soil health and agricultural sustainability.

An analysis assessing soil erosion suggests that losses are occurring between 30 and 40 times faster than natural replenishment in many countries (Pimentel 2006). The contribution of AM fungi to soil aggregate formation, soil stability, and soil carbon sequestration could be as important as its contribution to crop nutrition, because AM hyphal structures can physically entangle soil particles and improve structure and carbon sequestration (Willis et al. 2013). The fragile state of many tropical and subtropical soils infer an increase in AM fungal abundance through selection of responsive crop genotypes and best management practices, may globally stabilize food production systems by enhancing soil organic matter and plant nutrient use efficiency (Andrews et al. 2012; Ellouze et al. 2014).

Reactive nitrogen pollution is projected to more than double current levels by 2050, and strategies to improve the efficient use of nitrogen in agriculture are critical to environmental conservation efforts (Bodirsky et al. 2014; Tilman et al. 2002). There is evidence that mycorrhizal association with legumes improves the fixation of atmospheric nitrogen and reduces leaching of mineralized nitrogen (Veresoglou et al. 2012). A review by Cavagnaro et al. (2015) reported AM fungi can promote wide-scale sustainable nutrient cycling and use-efficiency by facilitating plant uptake and by intercepting and incorporating excess nutrients into soil microbial networks.

Many experts calculate world phosphorus reserves to be within 5-10 decades of exhaustion (Chen and Graedel 2016). The agricultural practice of intercropping may increase root and soil microbial interactions and the exploration of diverse soil horizons; thereby efficiently mobilizing more soil phosphorus (Hinsinger et al. 2011). Phosphorus is a key soil nutrient provided to plants through the mycorrhizal pathway (Smith and

Read 2010), and soil phosphorus limitation has been reported to increase sorghum AM fungal root colonization (Yoneyama et al. 2007) while plant benefits diminish with high phosphorous inputs (Richardson et al. 2011). Our research seeks to harness the benefits of AM fungi to improve crop nutrient-use efficiency. Previous assessment of these grain sorghum cultivars (Chapter 1) indicated a significant difference in their reliance on the AM symbiosis for acquisition of soil phosphorus and other limiting resources, with open-pollinated African sorghums nearly 3 times as responsive to AM root colonization for grain production and nutritional content. The current study will extend our understanding of the farm-scale effects of sorghum genotypes (two hybrid and two open-pollinated) on the abundance of inter- and extra-radical AM fungi.

Additionally, we will assess whether alternative planting systems (intercropping) and fertility amendments (worm compost) maintain or enhance plant-microbial partnerships, crop yields, and grain quality. We established three treatments: a non-amended control, commercial fertilizers (N&P), and worm compost. All genotypes were grown as sole crops, while one hybrid and one open-pollinated genotype were also intercropped with cowpea (*Vigna unguiculata*) to assess these two farm management systems. Intercropping legume and grain crops has been reported to increase iron and zinc concentrations in seeds (Zuo and Zhang 2009). Globally, iron and zinc deficient diets affect as many as 35% of all children aged 0 to 5 and have severe negative health consequences (Yang et al. 2007).

The primary objectives of this study were to (1) assess the productivity and grain quality of hybrid and open-pollinated sorghum genotypes grown in low nutrient soil with commercial fertilizers and worm compost, (2) to assess yield and grain quality

differences between sole crop sorghum and sorghum intercropped with cowpea (3) to assess the relative abundance of microbial community functional groups, including AM fungi, associated with each genotype, treatment, and cropping system combination using phospholipid/neutral lipid fatty acid (PLFA/NLFA) analysis, and (3) to assess the potential role of AM fungal colonization on sorghum grain production and nutritional quality.

Because previous research suggests worm compost improves AM fungal diversity, abundance, and plant-benefits (Cayuela et al. 2013; del Mar Alguacil et al. 2009; Zhang et al. 2012), we hypothesized the worm compost amendment would increase the abundance of intra- and inter-radical AM fungi associated with each sorghum genotype as compared to commercial fertilizers and non-amended control. We also hypothesized an increase in intra- and inter-radical AM fungi associated with the genotypes intercropped with cowpea compared to corresponding sole crops of those genotypes. Additionally, we hypothesized increased intra- and inter-radical abundance of AM fungi associated with open-pollinated genotypes, as compared to hybrid genotypes; we further hypothesized any significant increase in AM fungal abundance would result in significantly increased yield and grain nutritional quality, but not in changes to grain physical and starch characteristics.

MATERIALS AND METHODS

Experimental Setup. A modified completely randomized split-plot design, with four genotypes and three treatment combinations, was established for four sole crop sorghum

genotypes (hybrid genotypes were Dekalb [54-00] and Pioneer [84G62]; open-pollinated genotypes were Ajabsido [MNO9-7018] and Macia [PRO9110-4319]), while two genotypes (Dekalb and Macia) were also intercropped with the cowpea genotype Risina del Trasiorfino. The sorghum breeding and genetics program at Kansas State University provided all sorghum seed. All genotypes were grown in low-fertility soils at Oklahoma State University Wes Watkins Research and Extension Center (Lane, Oklahoma, USA) and the Samuel R. Noble Foundation (Ardmore, Oklahoma) were planted in April 2014. Three treatments: a non-amended control, commercial fertilizers (N&P), and worm compost, each with six replications were established at both field sites. Collection of grain, root, and soil samples occurred in August 2014.

Sorghum genotypes were planted in 16m rows (10 cm seed-spacing, 76 cm row-spacing) with an east/west orientation. Additional rows of Dekalb and Macia were planted as buffers to separate open-pollinated and hybrid genotypes as well as treatment borders. Cowpea was planted in two 16 m rows (20 cm seed-spacing, 76 cm row-spacing) between Macia and Dekalb for the intercrop portions. Six replicated plots of 12 seeded rows (Buffer, Dekalb, Pioneer, Buffer, Buffer, Ajabsido, Macia, Macia, Cowpea, Cowpea, Dekalb, Buffer) were planted at each field site using an Earthway 1001-B Precision Seeder®. Treatment applications were completely randomized as 4.0 m x 9.12 m blocks across the twelve rows in each plot, and 2.0 m buffers separated treatment blocks. Sampling was confined to the middle two meters of each row.

Soil and treatments. Eight soil samples containing 5 g were taken from both fields before experimental set up to determine baseline relative abundance of microbial functional groups and total microbial biomass at both sites (Figure 1 & Figure 2). Field

soils were analyzed for plant available nutrients, pH, and organic matter (Lane site: pH = 6.3, N = 14 ppm, P = 17 ppm, K = 80 ppm, OM = 1.4%; Ardmore site: pH = 5.9, N = 10 ppm, P = 5.1 ppm, K = 128 ppm, OM = 1.8%) by the Soil, Water, and Forage Analytical Laboratory (SWOFL) at Oklahoma State University. Soil NO_3-N and NH_4 were extracted by 1M KCl solution and analyzed using the Lachat Quickchem 8000 Flow Injection Autoanalyzer (Kachurina et al. 2000). Two grams of soil were extracted with 20 ml Mehlich 3 solution for plant available P and K (Mehlich 1984), and the concentrations of P and K in the extract were measured by an inductively coupled plasma emission spectroscopy (ICP)(Pittman et al. 2005). Soil pH was measured using a pH electrode in a 1:1 soil to water suspension. Soil organic matter (SOM) was determined by dry combustion using the LECO Truspec CN analyzer (Nelson et al. 1996).

The Ardmore site was converted from perennial rangeland two years prior to our study (Wilson silt loam, Alfisol); the Lane was in agricultural production for > 30 years prior to establishing our study (Bosville fine sandy loam, Alfisol). Commercial fertilizer treatments consisted of diammonium phosphate (DAP; 18-46-0) applied with a total target rate of 60 kg per ha phosphate combined with ammonium nitrate (34-0-0) applied with a total target rate of 100 kg per ha nitrogen. Potassium was applied to all plots as potash (0-0-60) with a total target rate of 132 kg per ha, to ensure uniform potassium for all plants. Worm compost was applied at a rate of 2100 kg per ha (pH = 7.2, total N = 0.77%, P = 0.3%, K = 0.3%, total C = 9.96%, moisture = 27.9%), adding an equivalent of 16.17 kg per ha total N, 6.3 kg per ha total P, and 6.3 kg per ha total K. The Oklahoma State SWAFL analyzed compost samples to determine composition using the methods of Peters et al. (2003).

All seeds were treated with Poncho™ (clothianidin) systemic agent for pest protection and Concep® (fluxofenim) herbicide antidote to allow the use of DUAL® (S-metolachor) as a pre-emergent weed control applied at rate of 1.52L per ha. The Oklahoma Mesonet (Brock et al. 1995; McPherson et al. 2007) stations at Ardmore and Lane were used to track minimum levels of plant available water at soil depths from 10-80 cm throughout the season (Table 1). Grain was harvested after maturation, and average production of each sample row was determined by collecting multiple grain heads and dividing grain dry weight by total number of plants harvested.

Quantification of grain and starch characteristics. The USDA-ARS Center for Grain and Animal Health Research in Manhattan, Kansas, USA determined, grain hardness, moisture, diameter, weight, protein, protein digestibility, starch granule size distribution, and amylose/amylopectin ratios. Hardness was determined (Single Kernel Characterization System) by crushing the grain, which then was then recovered and utilized in the other quality evaluations, see Kaufman et al. (2013b). Subsamples of the crushed material were used to determine total protein using N combustion (Leco N combustion analyzer) and total starch (Megazyme total starch analysis). Sorghum protein digestibility was assessed using methods of Wong et al. (2009) and completely hydrolyzed starch percentage was evaluated using modified methods from Zhao et al. (2009). Starch was isolated from the crushed grain using the method of Park et al. (2006). Starch granule size distribution (laser diffraction analysis) (Wilson et al. 2006) and amylose/amylopectin ratios (dual wavelength iodine binding) (Kaufman et al. 2015) were assessed. Correlations of all protein and starch analyses were evaluated against variations in total digestibility.

Quantification of mineral concentrations. The USDA-ARS Children's Nutrition Research Center in Houston, Texas assessed grain mineral concentrations for some of the minerals that are critical for human and animal health (Ca, Cu, Fe, K, Mg, P, and Zn). Using methods of Farnham et al. (2011), two subsamples (~0.25 g dry weight) of each ground sample were digested and processed for elemental analysis. Elemental analysis was performed using inductively coupled plasma–optical emission spectroscopy (CIROS ICP Model FCE12). Tissue mineral concentrations were determined on a dry mass basis (μg g–1 or mg g–1), and an average value was derived from the two sub-samples of each replicate.

Quantification of Soil Microbial Communities. Relative abundances of soil microbial functional groups (gram- positive and negative bacteria, AM and saprophytic fungi), and total microbial biomass were assessed using phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA). At harvest, soil was collected from the rhizosphere of each plant sampled for grain measurements. Phospholipid fatty acid and neutral lipid fatty acid (NLFA) analyses were extracted from the soil using a modification of the Bligh and Dyer (Bligh and Dyer 1959) extraction (White and Ringelberg 1998). Total lipid extracts were separated into PLFA's and NLFA's using silicic acid chromatography; the fatty acids cleaved from the glycerol backbone using KOH saponification; and the harvested fatty acids methylated to form fatty acid methyl esters (FAME) (Allison and Miller 2005; White and Ringelberg 1998). The FAME's were then analyzed by gas chromatography and mass selection detection using a GCMS unit Agilent MS 5975C/GC 7890A. Biomarkers used to select for the functional group of gram-positive bacteria consisted of i-15:0, a-15:0, i-17:0, and i-16:0. For gram-negative bacteria, selected biomarkers were

16:1ω7, cy19:0, cy17:0ω9, 2-OH 14:0, 2-OH 16:0, 3-OH 14:0, and 18:1ω9 trans. For inter-radical AM fungal biomass, biomarkers consisted of 16:1ω5c, 20:1ω9, and 22:1ω13. Biomarkers selected for the functional group of saprophytic fungi were 18:2ω9,12 and 18:1ω9c. The abundances associated with these biomarkers were used to calculate a total nmol per gram of soil for each functional group and for total microbial biomass when all functional groups were added with non-specific markers (14:0, 15:0, 16:0, 17:0, 18:0, and 20:0).

Quantification of intra-radical AM abundance. Live roots were subsampled, washed, stained with trypan blue, and scored for AM colonization using the magnified gridline intersect method (Mcgonigle et al. 1990). This method uses a digital microscope (Hirox KH 7700) to measure the percentage root length colonized by hyphae, vesicles, coils, and arbuscules, which were combined to determine total percent colonization.

Statistical Analysis. Soil PLFA/NFLA profiles, AM root colonization, productivity, grain protein concentration, protein digestibility, and mineral concentration response variables were analyzed using mixed models methods. Grain characterization and starch characteristics were analyzed using generalized linear mixed models methods. The Tukey multiple comparison method was utilized for significant effects, and results are reported as least square means. All tests of significance were performed at the nominal 0.05 level. The data analysis for this paper was generated using SAS® version 9.4. Copyright © 2013 SAS Institute Inc. SAS and all other SAS Inc. product of service names are registered trademarks of trademarks of SAS Institute Inc., Cary, NC, USA.

RESULTS

Baseline Soil Microbial Communities. Pre-planting PLFA data showed similar live microbial abundances for each field with a difference between means of 0.02 nmol per gram of soil for AM fungi (Figure 1). Baseline NLFA data were more variable for spore biomass of saprophytic fungi than those of AM fungi with a difference between means of 0.38 nmol per gram of soil for AM fungi (Figure 2). Therefore, the sites were considered to have similar AM fungal abundances.

Soil Microbial Communities at Grain Harvest. Soil in proximity to roots of the open-pollinated (OP) genotype Ajabsido were significantly lower in relative abundance of AM fungi, saprophytic fungi, and gram-negative bacteria, as compared to soil in proximity to roots of the intercropped hybrid Dekalb (Figure 3). Dekalb soil, in the sole crop, was also significantly lower in abundance of gram-positive bacteria, as compared to intercropped Dekalb. Non-amended soil (control) and soil amended with worm compost had significantly more extra-radical AM fungi (nmol per gram of soil) than soil amended with commercial fertilizers (control = 1.9221a, compost = 1.9137a, commercial fertilizers = 1.5076b) (data not shown). Overall, there was also significantly more extra-radical AM fungi, gram-negative bacteria (GNB), and saprophytic fungi associated with hybrid genotypes as compared to OP genotypes when grown as a sole crop, regardless of treatment.

There were no significant differences between sorghum genotypes, cropping systems, or soil treatments for total microbial biomass (range: 31.726 to 40.527 nmol per gram of soil) or for neutral lipid biomarkers (range: 2.104 to 2.300 nmol per gram of soil

for AM fungi) (data not shown). There were significant differences in fungal:bacterial biomass (F:B) ratios by sorghum genotypes (Figure 4). Treatment also had a significant effect, with higher F:B in soils amended with worm compost as compared to soils amended with commercial fertilizers or non-amended soils. There was also significantly greater F:B associated with hybrid as compared to OP genotypes regardless of treatment and with sole cropped genotypes as compared to corresponding intercropped plants.

Root Colonization. There was not a significant interaction between treatment and genotype for AM fungal root colonization (Figure 5). However, there was a significant treatment effect regardless of genotype, with the highest percentage of root colonization by AM fungi in soil amended by worm compost (control = 32.5ab, compost = 35.2a, fertilizer = 29.1b) (data not shown).

Grain Production. There were not significant differences in sorghum grain productivity by treatment, genotype, or cropping system (Figure 6).

Grain Characterization Data. There were no significant differences in grain physical characteristics between genotypes, except for grain moisture (Table 3). Grain moisture was significantly greater for Macia grown as a sole crop and amended with commercial fertilizers (10.56% ± 0.22) as compared to Macia grown as an intercrop in non-amended soil (9.20% ± 0.27) (data not shown).

Grain Starch Analysis. There were significant differences in amylose percentage and completely hydrolysable starch percentage for the interaction of genotypes with treatments (Figures 7 and 8). Overall, there was significantly greater grain amylose in OP

genotypes, but significantly greater completely hydrolysable starch in hybrid genotypes, regardless of treatment or cropping system.

Grain Protein Concentration. There were significant differences in grain protein concentration between genotypes (Figure 9), and OP genotypes had significantly greater grain protein compared to hybrid genotypes. Additionally, amendment with commercial fertilizers resulted in significantly greater grain protein concentrations compared to compost amendment, but neither of these treatments were significantly different from non-amended control.

Grain Protein Digestibility. There were significant differences in grain protein digestibility for the interaction of genotypes with treatments (Figure 10). While there were not significant differences between OP and hybrid genotypes grown as sole crops, intercropped Macia had significantly more digestible protein as compared to intercropped Dekalb, regardless of treatment.

Grain Mineral Concentrations. There was not a significant interaction between treatment and genotype for grain mineral concentrations of copper, iron, potassium, and zinc, but there were significant interactions for calcium, magnesium, phosphorus, and sulfur (Table 2). Grain iron concentration was significantly higher for genotype Ajabsido than for genotype Macia, regardless of treatment. There was also significantly higher copper concentration in sole cropped sorghum as compared to plants intercropped with cowpea. Grain calcium potassium, magnesium, phosphorus, and sulfur concentrations were significantly higher for OP genotypes compared to hybrid genotypes.

DISCUSSION

Many of the observed soil microbial differences between genotypes were specifically associated with Ajabsido (OP genotype) and Dekalb (hybrid). Ajabsido and Dekalb may have specific root exudates that alter rhizosphere microbial communities. Additionally, there may be an exudate interaction between Dekalb and cowpea (when intercropped) that increased gram-positive bacteria, compared to Dekalb grown as sole crop. Root exudates influence multiple soil microbial community responses (Bertin et al. 2003), and sorghum genotypes have been shown to each produce a different array of rhizosphere exudates (Tesfamariam et al. 2014). The diversity of plant/soil/microbial interactions between the different genotypes in our study indicate an opportunity to breed sorghum genotypes for specific rhizosphere microbes.

A long-term experiment by Cong et al. (2015) found greater total root biomass and subsequent increases in soil C and N in intercrop systems, as compared to sole crops. Our results indicated similar or slightly improved microbial abundances for each microbial functional group associated with Macia and Dekalb genotypes when intercropped with cowpea. This increase in microbial biomass may contribute to greater soil C storage, as reported in Cong et al. (2015). Similarly, increases in fungal biomass has been shown to be tightly correlated with soil aggregation and C storage in tallgrass prairie (Wilson et al. 2009).

Phosphorus fertilizers have been shown to decrease plant production of signaling hormones that are critical for AM root colonization (Yoneyama et al. 2013). In our study, amendments of commercial fertilizers (N&P) significantly reduced intra- and inter-

radical AM fungal abundance, compared to the worm compost amendment. Lagerlöf et al. (2014) reported a range of microbial community metrics that did not significantly change in farm systems in Kenya, even following 20 years of improved practices. This suggests that soil microbial communities can be extremely slow to recover from farm management effects such as tillage and over-fertilization. In our study, worm compost generally resulted in similar or improved productivity and grain quality as compared to commercial fertilizers, but improved intra- and inter-radical AM abundance in one season. Use of compost as an alternative fertility amendment may result in even more sustainability benefits over time, through improved soil organic matter and soil structure.

A review by Willers et al. (2015) reported soil phospholipids are a dependable method to determine shifts in microbial communities. Fungal:Bacterial (F:B) ratios are used to describe effects of various experimental variables (Frostegård et al. 2011). For example, organic amendments (e.g. manure) have been reported to increase F:B; tillage has been reported to decrease F:B (Frostegård and Bååth 1996). Differences in worm compost and commercial fertilizer amendments assessed in our study support that of Romaniuk et al. (2011) and Bragazza et al. (2015), as their studies found F:B ratios increased with increases in soil organic carbon. The high F:B ratio associated with sole cropped Dekalb in our study is due to decreased gram-positive bacterial abundance associated with sole cropped Dekalb compared to all other genotypes.

Generally, sorghum genotypes explained differences in grain nutrition, starch, and physical characteristics, rather than soil amendments or AM fungi. Intercropping typically resulted in similar or improved grain qualities as compared to sole croping. Food nutritional (Zuo and Zhang 2009; Zuo and Zhang 2011) and soil conservation

(Zougmoré et al. 2000) benefits have been associated with intercropping, and our study also indicates intercropping is a promising sustainable farm method. While hybird genotypes had greater completely hydrolyzable starch, OP genotypes had greater grain amylose and higher concentrations of several minerals and protein. However, neither genotype origins had significantly more digestible protein. This suggests that breeding programs can utilize genetic material from OP genotypes without sacrificing grain nutritional quality, and further selective breeding may improve the hydrolysable starch in these African OP genotypes.

Yield and root colonization did not significantly differ between genotypes, regardless of treatment or cropping system. In a previous study (Chapter 1), greater mycorrhizal responsiveness in OP genotypes significantly improved grain yield and nutrition, as compared to less responsive hybrid genotypes, when grown in low-fertility soil. Differences in phosporhus-availability and water-limitation between our previous greenhouse study and and our current field study presumably led to these differences. Plant-avaliabe soil phosphorus was lower in the greenhouse (4 ppm vs 17 ppm at Lane & 5.1 ppm at Ardmore), and greenhouse plants were watered every 2-3 days, ensuring no water deficit.

While average plant-available soil moisure was adequate throughout the growing season, as recorded by Mesonet stations at Lane and Ardmore, (Table 1), between emergence and harvest, there were eight distinct periods of 6 - 12 days with less than 0.5 mm of precipitation, and average temperatures > 25 °C at each site. Periods of moisture limitations, in combination with the sandy soil profile of the field sites, suggest periodic dry soil regularly occurred in the top 10-20cm. Roots were subsampled from the top 10-

20 cm of soil and assessed for inter-radical AM fungal colonization. The sampled roots may have been relatively more adapted to dry soil conditions, compared to the deeper root system. Spatical differnces in AM colonization of root systems have been well documented for nutrient-providing mycorrhizal partnerships (Bever 2015; Bever et al. 2009), but less is know about the spatical variablitity of AM fungi for plants under water-limited conditions. Upregulation of AM colonization (inter-radical abundance) for water acquisition may not require that plants have an overall water deficit, but rather that localized roots respond to dry soil conditions.

Propster and Johnson (2015) studied the interacting effects of water and phosphorus limitation on AM fungi associated with maize plants grown in soil collected across the Serengeti. Mycorrhizas provided the most benfit to host plants when the experimental limitation (water or phosphorus) matched the conditions common to the region of the Serengeti where that soil was collected, such that arid soil mycorrhizas assisted more with water uptake, and P-limited soil mycorrhizas assisted more with phosphorus uptake. Southern Oklahoma was selected for this trial because of charactristic low-P soils, however, frequent drought and sandy soil may have played a large role in shaping the AM communities. Likewise, AM communities of high clay, more mesic soils collected in Stillwater, OK, used for the greenhouse study, could differ substantially from those of the field soils in southern OK and partially explain observed differences in AM benefit between our greenhouse and field studies.

It was beyond the scope of our current study to assess AM fungal genetic diversity. Mycorrhizal taxa have been shown to vary in value as plant mutualists and in physical forms within plant roots (Treseder 2013). Sangabriel-Conde et al. (2015)

assessed AM richness associated with both hybrid and landrace maize genotypes in Mexico, and found that native maize landraces associated with a more diverse AM community, as compared to hybrid maize. Furthermore, a local landrace genotype was more successful at capturing phosporous through the pathway, as compared to the hybrid genotype (Sangabriel-Conde et al. 2013). Though there is some ambiguity in genomic and phenotypic assessment of AM fungi (Angelard et al. 2014; Sanders and Croll 2010), including soil community genomic analyses in future studies will improve our understanding of the productivity and efficiency tradeoffs between different sorghum genotypes.

CONCLUSION

Due to finite reserves, the availibilty of phosphorus fertilizers will decrease while costs increase. It is critical to understand the impact of selective breeding on belowground microbial partnerships and crop nutrient use efficiency to produce genotypes most appropriate for sustainable agriculture (Wissuwa et al. 2009). It is also critical to assess strategies for improving microbial abundance in agricultural soils, as many agricultural practices reduce soil microbial community diversity and total microbial biomass as compared to native systems (Montecchia et al. 2011). The restoration of these microbial communities on farms is an important strategy to enhance soil sustainability.

Our results indicate worm compost amendments and the intercropping of sorghum with cowpea maintained or enhanced AM fungal abundance both in sorghum roots and rhizosphere soil. Arbuscular mycorrhizal symbiosis with agricultural crops has global

implications as mycorrhizas are an important contributor of soil carbon storage (Soudzilovskaia et al. 2015). Selection of sorghum genotypes for agricultural systems designed around these alternative practices may also improve microbial benefits to crop nutrition, soil health, and farmer livelihoods.

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TABLES AND FIGURES

Table 1. Minimum plant available water (mm)* in soils at both field sites throughout the growing season (4/20/14 to 8/10/14).

Soil Depth (cm)	Low (mm)	High (mm)	Average (mm)
Lane (Top 10cm)	7	28	22
Ardmore (Top 10cm	2	20	11
Lane (Top 40cm)	33	96	78
Ardmore (Top 40cm	17	83	53
Lane (Top 80cm)	81	188	158
Ardmore (Top 80cm	40	161	97

*Data acquired from Oklahoma Mesonet.

Table 2. Grain physical characteristics* by genotype.

Genotypes: African open-pollinated genotypes (Ajabsido, Macia), hybrid genotypes (Dekalb, Pioneer), intercropped (IC) genotypes

Genotype	Hardness	Moisture (%)	Diameter (mm)	Weight (mg)
Ajabsido	51.21d	10.06a	3.01a	33.79a
Macia	81.04a	10.10a	2.45c	25.21c
Dekalb	70.30b	10.13a	2.54c	28.25bc
Pioneer	62.20c	10.18a	2.70b	29.08b
Macia (IC)	81.90a	9.77a	2.46c	24.81c
Dekalb (IC)	69.26b	10.06a	2.49c	27.95bc

Within a column, means that do not share a letter are significantly different ($P < 0.05$).

*Single-kernel characterization system

Table 3. Grain mineral concentrations (ppm) for all genotypes by treatment, including sorghum intercropped (IC) with cowpea.

Treatments: CON = control, NP = fertilizer, WC = worm compost

Treatment	Genotype	Ca	Cu	Fe	K	Mg	P	S	Zn
CON	Ajabsido	212a	8.1a	39.6a	3296a	1408abcd	2596abc	1247ab	26.7a
	Macia	172abc	11.1a	35.2a	3084a	1206cd	2441bc	1214abc	28.3a
	Pioneer	192ab	9.9a	42.4a	2963a	1292bcd	2300bc	1159bcd	31.8a
	Dekalb	164abc	10.6a	38.1a	2908a	1200cd	2197c	1105bcd	25.7a
	Macia (IC)	202ab	12.0a	38.6a	3189a	1428abc	2932ab	1242ab	31.5a
	Dekalb (IC)	154bc	6.4a	35.0a	3206a	1199cd	2249bc	1069bcd	24.3a
NP	Ajabsido	211a	9.0a	45.9a	3424a	1727a	3217a	1406a	31.6a
	Macia	162abc	11.1a	38.6a	3161a	1359bcd	2812ab	1186abc	29.0a
	Pioneer	132c	11.7a	36.3a	2804a	1126cd	2023c	961cd	26.2a
	Dekalb	148bc	8.2a	40.9a	2971a	1378bcd	2532bc	1186bc	29.8a
	Macia (IC)	151bc	8.2a	30.6a	2867a	1110d	2218bc	1054bcd	24.1a
	Dekalb (IC)	166abc	9.7a	41.1a	3058a	1443abc	2625abc	1241ab	28.2a
WC	Ajabsido	188ab	6.8a	39.4a	3248a	1535ab	2793abc	1186bc	26.5a
	Macia	171abc	11.2a	34.8a	3141a	1245cd	2492bc	1165bc	27.8a
	Pioneer	150bc	9.9a	36.2a	2911a	1121d	2042c	940d	26.2a
	Dekalb	165abc	10.2a	36.9a	3074a	1188cd	2146c	1099bcd	23.8a
	Macia (IC)	160bc	9.5a	32.6a	2994a	1228cd	2539abc	1096bcd	25.4a
	Dekalb (IC)	141c	6.9a	33.6a	2948a	1169cd	2082c	1101bcd	23.3a

Within a column, means that do not share a letter are significantly different ($P < 0.05$).

Figure 1

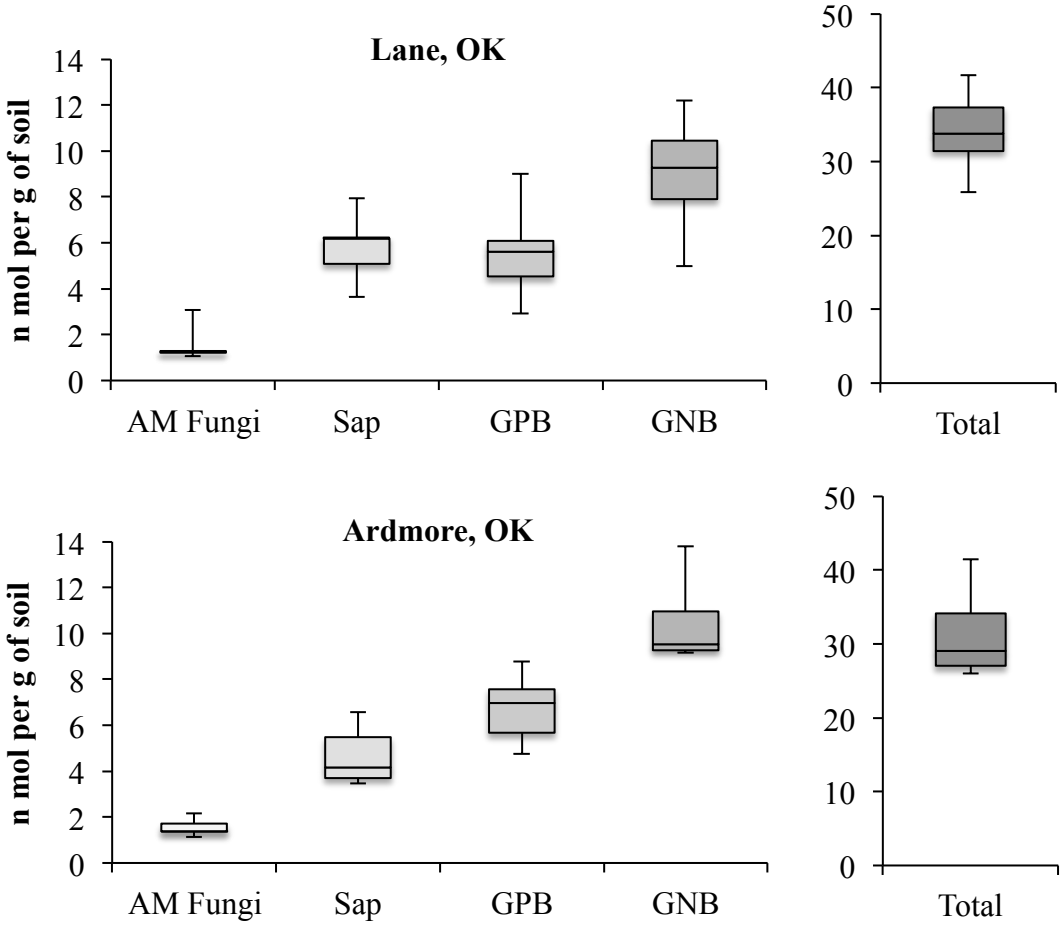

Figure 1. Lane and Ardmore baseline total microbial biomass and microbial functional groups: AM fungi, saprophytic fungi (Sap), gram-positive bacteria (GPB), and gram-negative bacteria (GNB), as determined by phospholipid fatty acid analysis (PLFA). Minimum, 1st Quartile, Median, 3rd Quartile, and Maximum values are represented by box & whisker (n = 8).

Figure 2

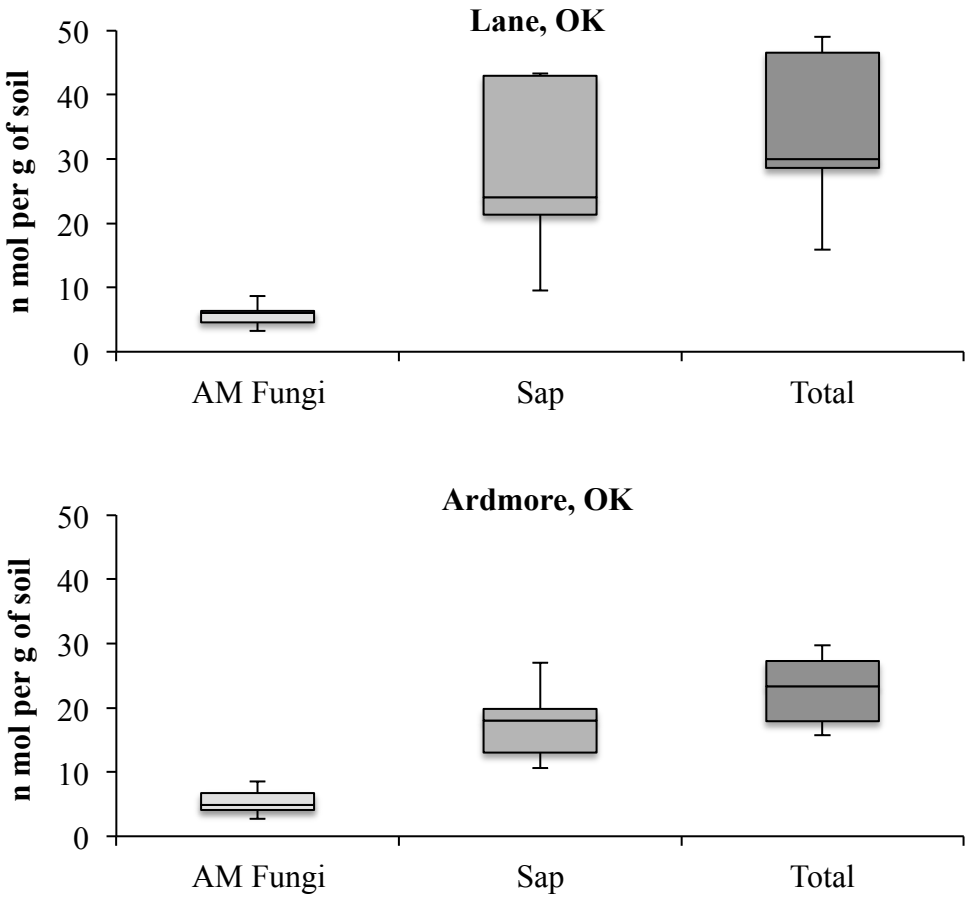

Figure 2. Lane & Ardmore field soil baseline total fungal biomass and microbial functional groups: AM fungi, and saprophytic fungi (Sap), as determined by neutral lipid fatty acid analysis (NLFA). Minimum, 1st Quartile, Median, 3rd Quartile, and Maximum values are represented by box & whisker (n = 8).

Figure 3

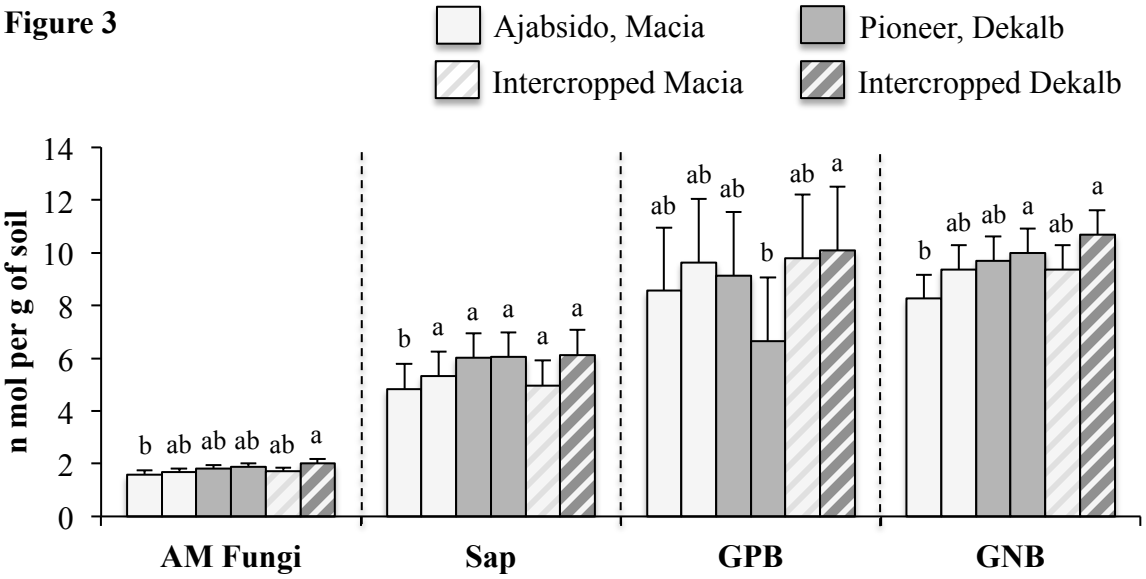

Figure 3. Soil microbial functional groups: AM fungi, saprophytic fungi (Sap), gram-positive bacteria (GBP), and gram-negative bacteria (GNB) associated with rhizosphere soil of sorghum genotypes, as determined by phospholipid fatty acid analysis. From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 36). Bars that do not share a letter are significantly different ($P < 0.05$).

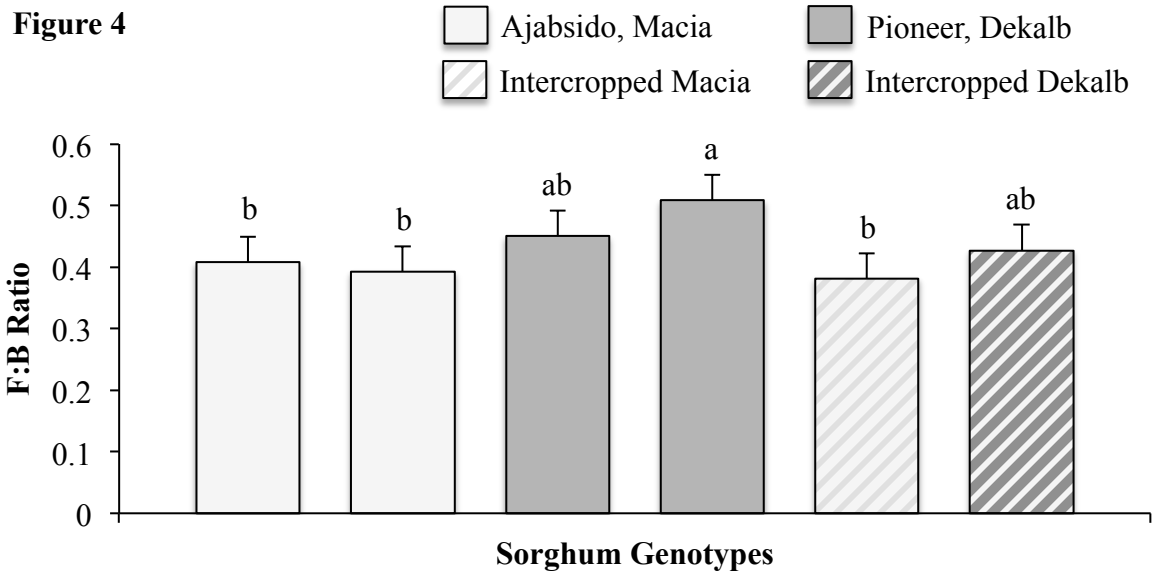

Figure 4. Fungal to bacterial ratio associated with rhizosphere soil of sorghum genotypes, as determined by phospholipid fatty acid analysis. From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 36). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 5

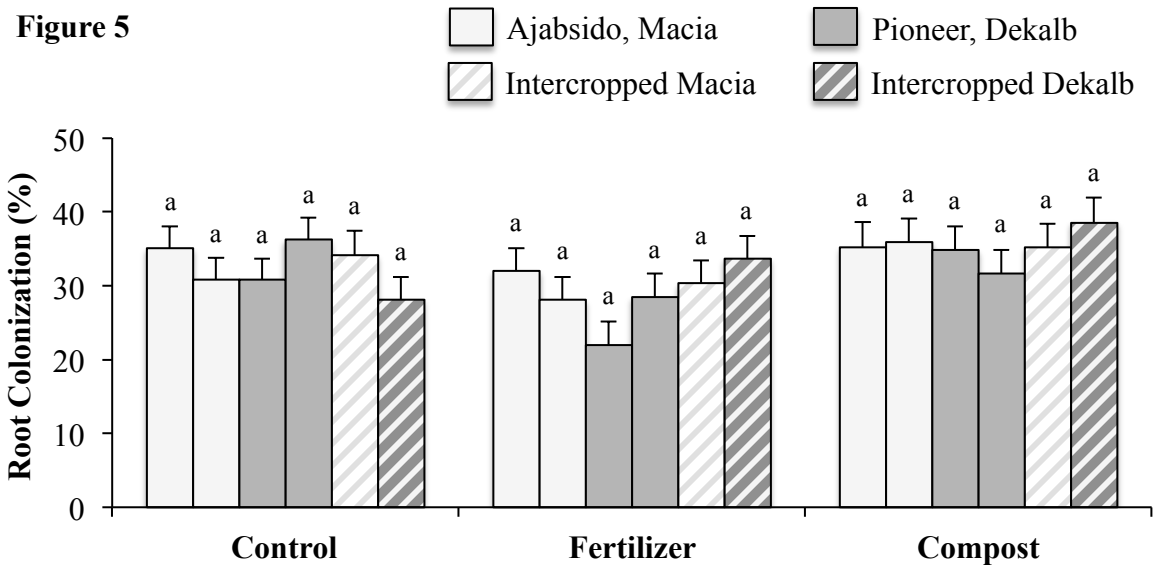

Figure 5. Arbuscular mycorrhizal fungal root colonization (%) for sorghum genotypes x treatment (non-amended control, commercial fertilizers, or worm compost). From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 12). Bars that do not share a letter are significantly different ($P < 0.05$).

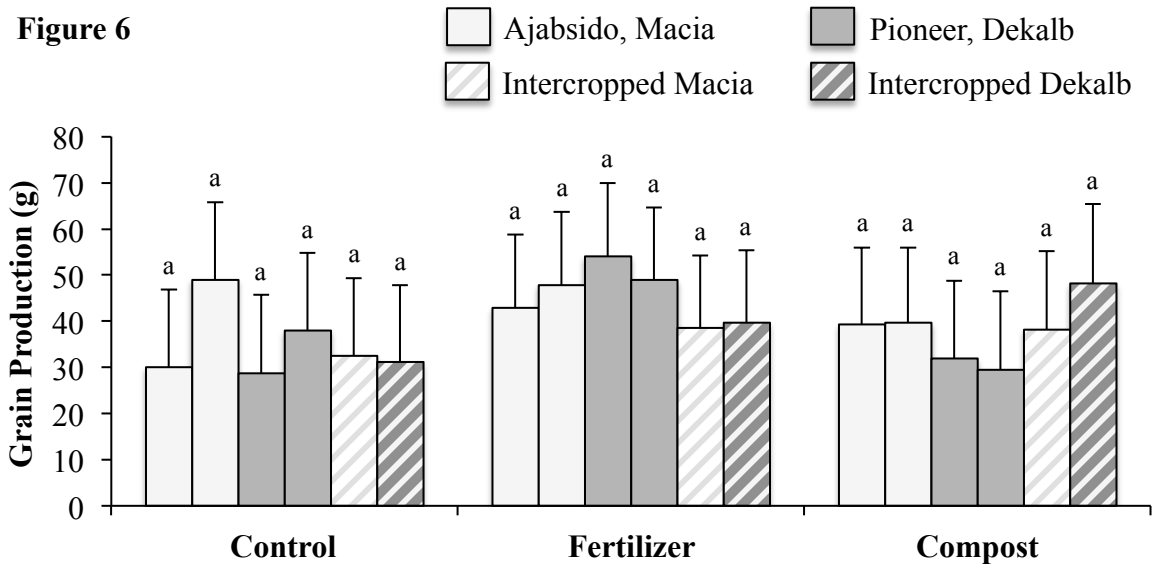

Figure 6. Total grain yield for all sorghum genotypes x treatment (non-amended control, commercial fertilizers, or worm compost). From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 12). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 7

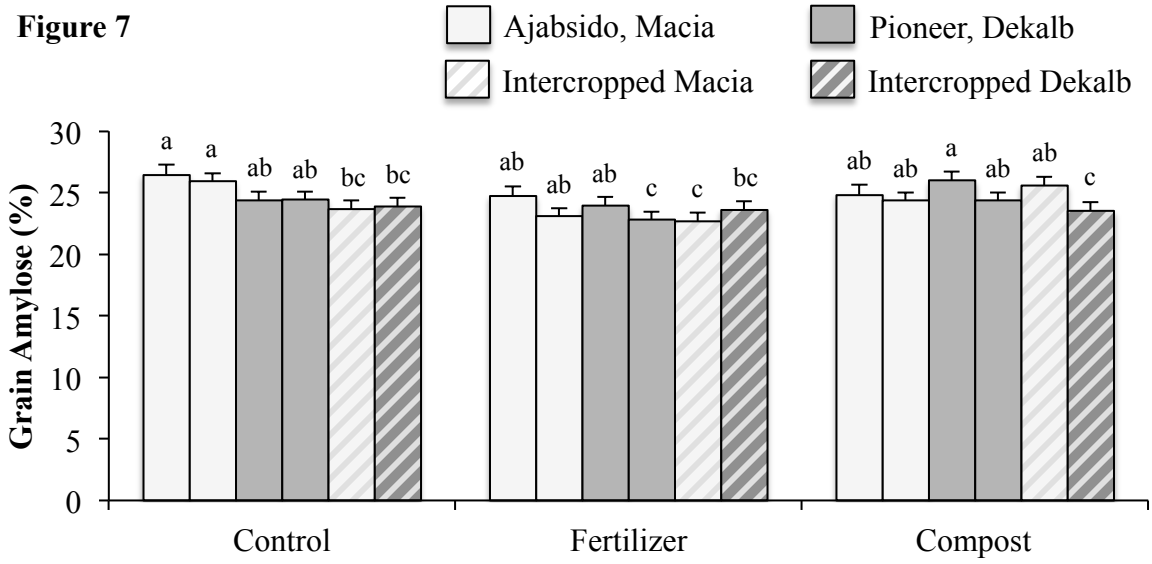

Figure 7. Grain amylose (%) for sorghum genotypes. From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 12). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 8

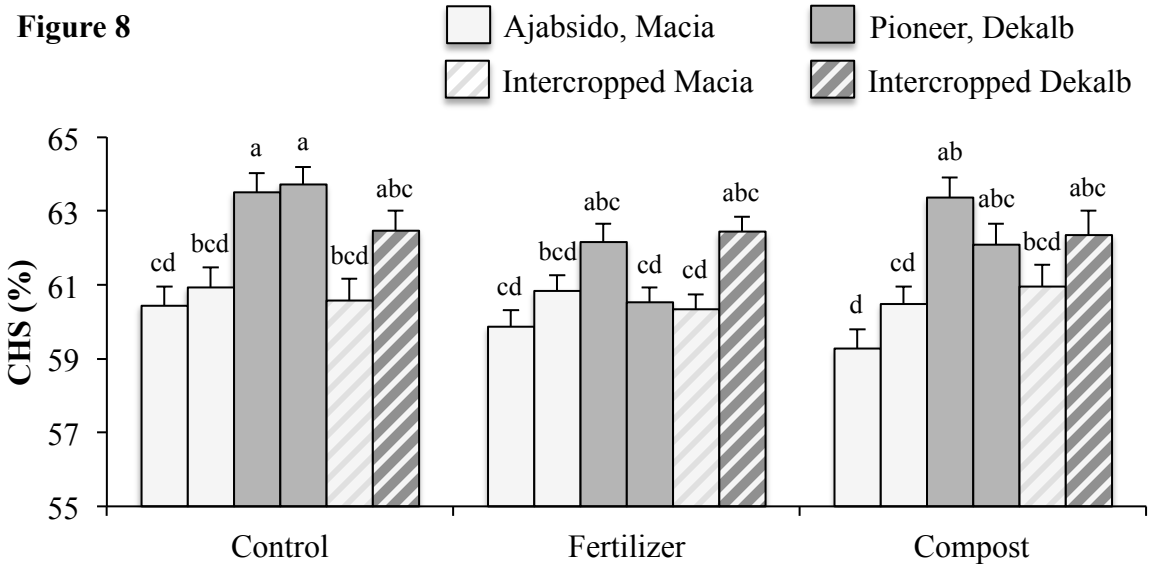

Figure 8. Grain completely hydrolysable starch (%) for sorghum genotypes. From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 12). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 9

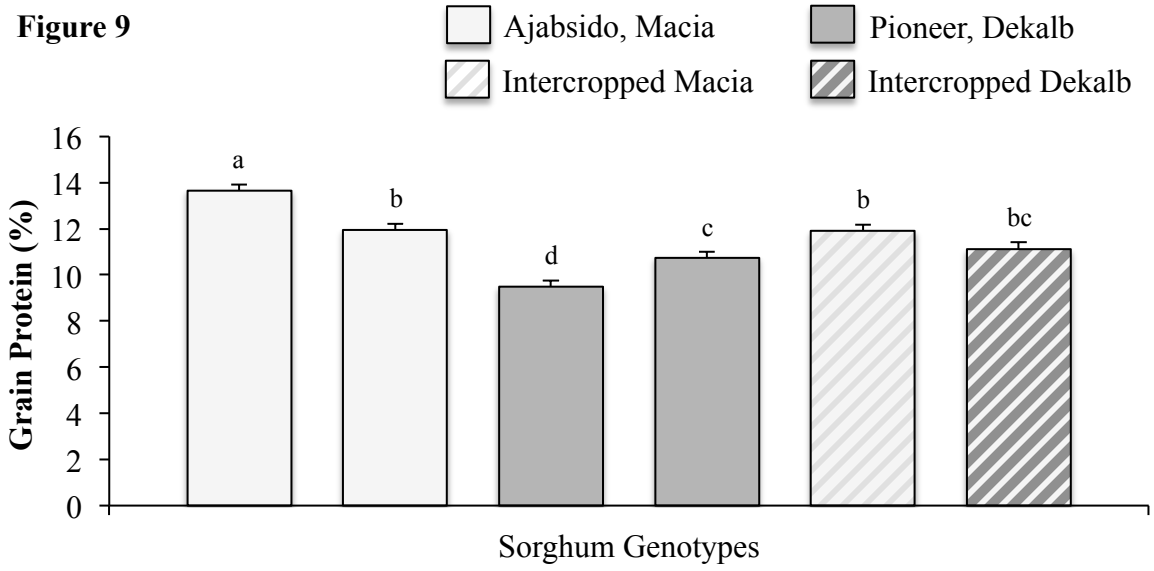

Figure 9. Grain protein concentration (%) for sorghum genotypes. From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 36). Bars that do not share a letter are significantly different ($P < 0.05$).

Figure 10

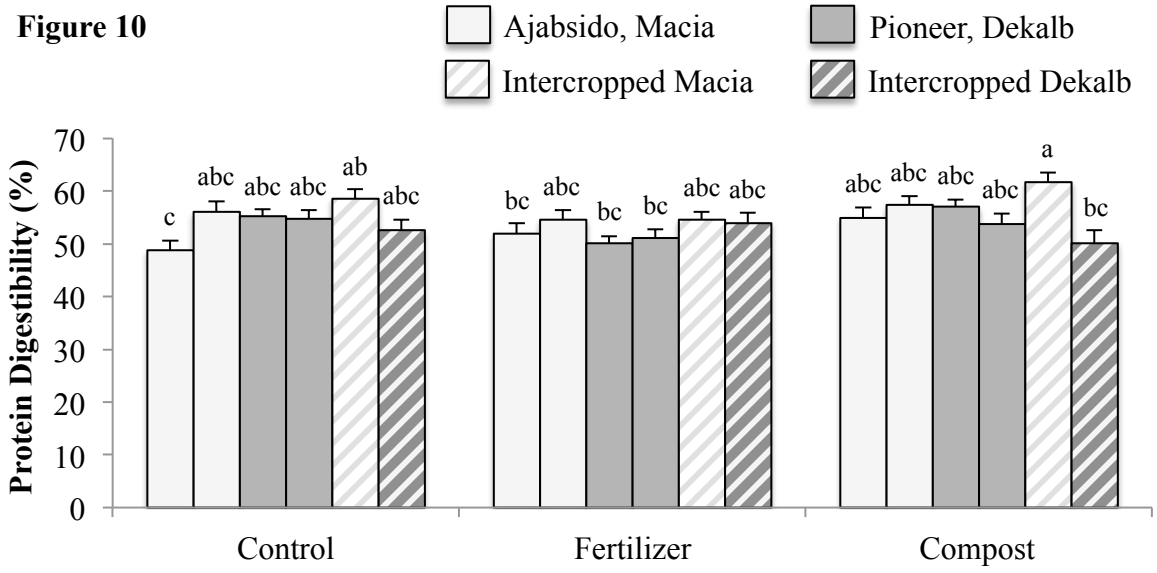

Figure 10. Grain protein digestibility for sorghum genotypes (% of total grain protein). From left to right genotypes are: Monocrop (open bars) Ajabsido, Macia, Pioneer, Dekalb & intercrop (hatched bars) Macia and Dekalb. African open-pollinated genotypes are the light-grey bars, and US hybrid genotypes are the dark-grey bars. Bars represent means, + SE (n = 12). Bars that do not share a letter are significantly different ($P < 0.05$).

CHAPTER IV

Dissertation Synthesis, Implications, and Conclusions

INTRODUCTION

It is imperative to bridge research disciplines if the goal is developing sustainable, community-based food systems. Our research was inspired initially by discussions with scientists from sub-Saharan Africa (SSA) and their concern that US crop genotypes were ineffective for local farmers, often because they lacked sufficient access to commercial fertilizers. We explored belowground mechanisms that may alleviate the issues they observed. One method of increasing farm sustainability while maintaining productivity is utilizing an ecological driver that reduces the need for commercial fertilizers.

Arbuscular mycorrhizal (AM) fungi are beneficial soil microbes that partner with the majority of agricultural crops. This association allows an exchange of resources between the plants and AM fungi – primarily phosphorous and carbon (Smith and Read 2010). In natural ecosystems, these mutualistic fungi help ensure ecosystem health by stabilizing soil structure (Wilson et al. 2009), limiting nutrient runoff (Cavagnaro et al. 2015), and reducing soil greenhouse gas emissions (Bender et al. 2014). Enhancing the

effectiveness of AM fungi in farm systems presents an opportunity to utilize them as "natural biofertilizers" (Berruti et al. 2015)(p. 1) that can reduce costs and improve health in farming communities (Oruru and Njeru 2016). This idea is also gaining popularity as a way to mitigate some of the human impacts on natural ecosystems and the planet as a whole (Bender et al. 2015; Gosling et al. 2006).

The overall purpose of my dissertation research is to harness the benefits of AM fungi for sustainable food production and nutrition – reducing farm dependence on ecologically harmful chemical inputs. Agricultural fertilizers are recognized widely as off-farm pollutants, with water quality and natural ecosystem function negatively impacted by nutrient runoff (Daigle 2003). Sustainable agriculture is essential for environmental preservation, and the multiple benefits of the AM symbiosis are likely to play a pivotal role – maintaining soil fertility and enhancing plant nutrient uptake, food nutritional value, and soil structure.

As crop genetics are developed in breeding programs, selective pressures may create inadvertent trade-offs that reduce plant/microbial partnerships (Denison 2012). This would be an unintended consequence that reduces farm system efficiency. It is critical for agronomic researchers to consider the vast implications of Everett Rogers' (2003) theory to avoid a pro-innovation bias that can lead to unintended consequences and innovations that are not appropriate for developing countries. Rogers' innovation adoption framework provides guidance for scientific inquiry to improve local food systems. Additionally, it is vital to utilize his theory to investigate effective ways of diffusing sustainable agriculture by facilitating farmer adoption through understanding their personal and cultural characteristics.

The answer includes transdisciplinary collaboration and research projects that assess both soil ecology and human social dynamics in the context of local food production. Navarro (2008) suggested that effective solutions to the issues and causes of poverty should link local people, institutions, and their pooled indigenous knowledge with outside facilitators as "an interactive and integrative model of shared knowledge and joint discovery" (p. 75). This suggests that any innovation a change agency considers introducing to a community must be reimagined through the local prism and implemented in ways that are appropriate for that population.

SOIL AND SECURITY

Many developing countries are struggling to improve public health, education, and reduce poverty while needing to protect their natural resources – such as productive soils – that are major drivers for social and agricultural progress. A review of soil decline in SSA has indicated food production demands are leading to loss of soil fertility, loss of ecosystem services, and the inability of small-scale farmers to produce enough food for their families (Tully et al. 2015). Throughout history, loss of soil fertility has been linked with social instability and even the collapse of vast empires (Rimas and Fraser 2010).

Worldwide, soil resources are being degraded and lost at an alarming rate (Pimentel 2006), and this loss is linked to reduced food security as well as human health issues (Oliver and Gregory 2015). Mismanaged soils create an environmental condition where important micronutrients like iron and zinc are scarcely available to growing plants and therefore are not present at sufficient levels in human food (Yang et al. 2007).

Chronic malnutrition of protein, iron, and zinc impact about one-third of the world's population, and these concerns have inspired a biofortification campaign through crop genetics and improved farm soil management (Carvalho and Vasconcelos 2013).

Lal (2009) suggested that, "ecologically restored and judiciously managed soil resources are adequate to meet the essential needs of the present and future populations" (p. 54). Because AM fungi can stabilize and enhance soil while benefiting most of our crops with increased water and nutrients, they are crucial to unlocking the power of living soil. Our research provides unique insights into the link between plant partnerships with AM fungi, alternative soil fertility amendments, and the resulting yield and food nutritional quality derived from these belowground interactions.

AGROECOLOGY AND NUTRITION

Numerous agencies and organizations work to reduce the rates and effects of poverty in underdeveloped countries throughout the world. Varied approaches include health and nutrition programs (Colecraft et al. 2012), the expansion of local agricultural and economic capacity (Mmari and Kileo 2015), ecological sustainability projects (Lewis et al. 2011), and social equity interventions through the education and empowerment of women and other marginalized populations (Gates 2014).

An emerging paradigm of interconnected problems related to community malnutrition, poor agricultural management, and environmental degradation is being called econutrition (Blasbalg et al. 2011; Deckelbaum et al. 2006). This approach suggests that groups interested in finding solutions for any of these problems within a

community must integrate interventions to improve all three, rather than addressing a single issue (see Figure 1). Healthy soil is a foundation for multisectoral community programming, and links innovations in agriculture, environment, and nutrition.

SUMMARY OF RESEARCH FINDINGS

Our research indicates sorghum genotypes that are more responsive to AM fungi produce more grain (with equivalent or improved nutrition) when grown in marginal soil, as compared to less symbiotic genotypes (Chapter 1). If we seek to protect our global ecosystems and meet the needs of a growing population, we need to breed crops specifically for more efficient and sustainable agriculture. Our results also indicate that soil carbon sequestration and crop production/nutrition can be enhanced through the utilization of alternative farming inputs such as biochar and worm compost (Chapter 2). At the same time, farmer fertility input costs (Hoornweg et al. 1999) and environmental fallout from greenhouse gases can be reduced (Thomazini et al. 2015). We also found agricultural methods such as intercropping grains with legumes can result in similar or improved grain quality and productivity as compared to a sole grain crop while potentially benefitting soil health (Chapter 3). However, each soil type, crop type, and climate can alter outcomes in ways that are difficult to forecast. It is important to understand these elusive dynamics and operationalize systems that add to soil health and food quality. We can reduce poverty, hunger, and malnutrition while developing systems that are built on ecological foundations – regenerating soils while meeting human needs.

DIFFUSION OF INNOVATIONS THEORY

Distilling the theoretical framework of Rogers (2003) starts with the understanding that the diffusion of a new idea occurs as a process over time as individuals form attitudes about the innovation by communicating with people in their social system or with change agents about the advantages or disadvantages of adopting the innovation (see Figure 2). This means that the way a change agent (such as an NGO or extension professional) communicates and manages the perceptions of potential adopters can speed up or slow down the process of diffusion, and that the social system's characteristics can influence the receptivity of community members to new ideas in general and to the specific innovations being suggested by the development group (Rogers, 2003).

The scale and design of technologies used in development may have a large impact on their rate of adoption and sustained use over time (Rogers, 2003). This can be overlooked by agricultural researchers, resulting in innovations that are not appropriate for communities in developing countries. It is imperative to connect agricultural scientists with local change agents so these groups can collaborate on appropriate innovations.

IMPLICATIONS AND RECOMMENDATIONS

Beneficial soil fungi present an opportunity to make global agriculture more efficient, more sustainable, and more productive (Ellouze et al. 2014). Crop symbiosis with AM fungi provides a potential path to maintain or improve food production and nutrition with fewer economically and environmentally costly fertilizer inputs. Some crop genotypes

rely strongly on AM fungal partnerships in low-nutrient soils, while other genotypes do not. Therefore, seed selection is critical to farming success in local communities.

When encouraging the use of new seed varieties or cropping techniques, Rogers (2003) would suggest an important role for the characteristics of *observability* and *trialability* in the innovation decision process for potential adopters. If the change agent wants to speed up the rate of adoption of these new crop varieties and methods, planting fields as demonstration plots – displaying the new seeds and cropping techniques – will allow potential adopters to observe the outcome of the innovations. Another strategy could be providing support and incentive for local farmers to plant a small portion of their fields with the new seeds and using the new methods. If the farmers can observe better production because of the innovations, it may help them form a positive attitude about the relative advantages and improve their odds of implementing the change agent's system. Examples like the One Acre Fund (Thurow 2013) demonstrate that improved soil management and enhanced crop genotypes can successfully diffuse through communities via Farmer Interest Groups (FIGs) that facilitate sharing the resource burden and the innovation's benefits (La Rovere et al. 2009).

Even though mycorrhizal association strategies and ecologically beneficial cropping systems have the potential to embody the econutrition paradigm by improving agricultural production, enhancing soil conditions, and impacting the nutritional quality of food without high input costs, it still represents a potentially large innovative shift from the current systems employed around the world. To effectively transfer these ideas, change agents need to employ Rogers' (2003) theory base in communicating the importance of health soils to farmers.

Agroecosystems are inherently complex. Richards (1989) criticized the fallacy of envisioning discreet farm system components that can be researched separately and then recombined to form an optimized mechanism. In reality, research involving AM fungi in agroecosystems needs to address the multiple aspects of plant genetics, farm environment, and soil management, simultaneously (see Figure 3).

The scientific literature contains numerous contradictory results for the study of AM fungi in agroecosystems. In some of these cases, the inconsistency may have arisen from researchers ignoring key aspects of AM research. For example, genetic differences in crops (leading to differences in AM benefit) may appear less pronounced if the field site is relatively depleted of fungal biomass because of previous farm management. Additionally, some research into management effects has ignored crop genetics. Other studies also have utilized complex technology or costly inputs that would not be applicable on small-scale farms in developing countries. This highlights the need for transdisciplinary teams that plan integrated sustainable farm system research.

CONCLUSIONS

Crops selected for microbial partnership and bred in low-nutrient soils may increase local production and food quality in developing countries. The resulting seed would provide relative advantage (Rogers 2003) compared to fertilizer-dependent genotypes which should increase the rate of adoption. These strategies will be conceived and implemented best if agricultural researchers consult with international extension professionals, and

develop together a mechanism to receive feedback from local communities (Navarro 2008).

If agronomic research ignores the lessons of Rogers (2003) and other change theorists, it may produce methods, technologies, and other innovations that are inappropriate and/or detrimental for farmers in developing countries. Unique issues related to fertilizer inputs, infrastructure, and the knowledge base of local farmers should be considered in research design (Navarro 2008). Our results demonstrate that carefully assessed microbial partnerships present an opportunity to enhance the sustainability of local food systems through suitable crop genetics, improved farm soil management, and other appropriate innovations.

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FIGURES

Figure 1. Adapted from Deckelbaum et al. (2006) and Blasbalg et al. (2011).

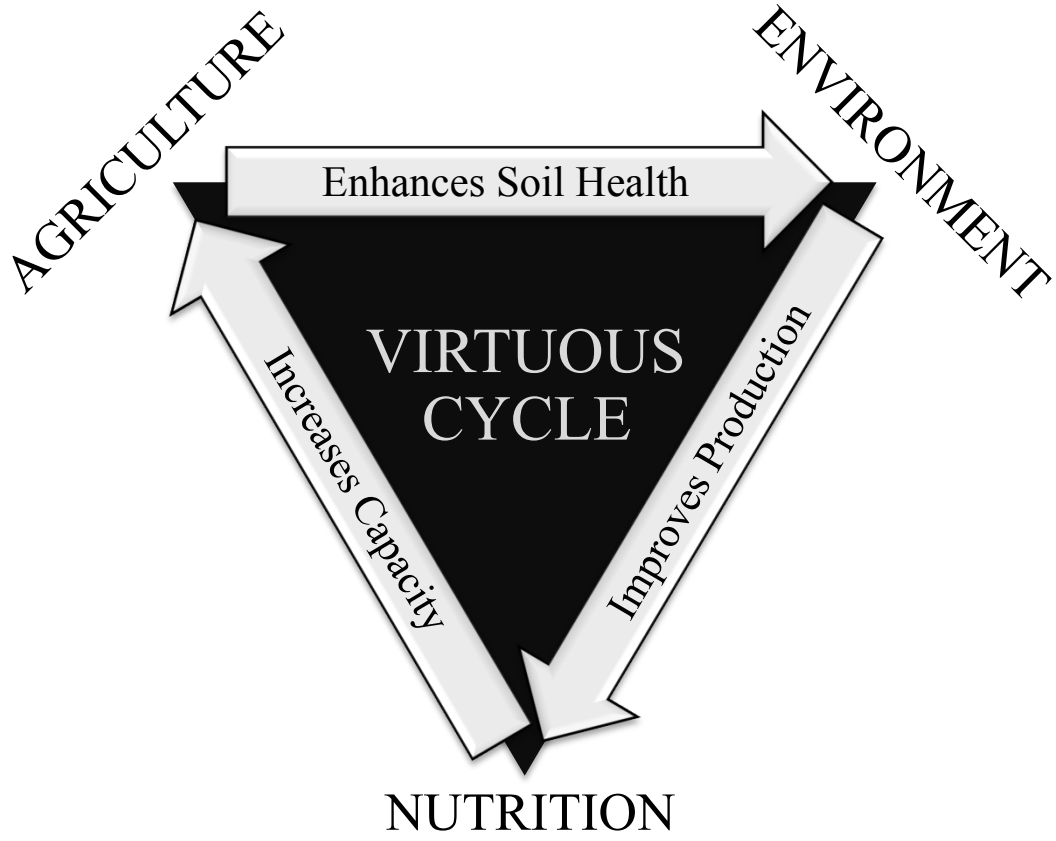

Figure 1. According to the econutrition framework, the interconnectedness of agriculture, environment, and nutrition explains the vicious cycle of community poverty. Poor agricultural management results in soil erosion and degrades the environment, leading to declines in food productivity and worsening local malnutrition, resulting in decreased human capital and feeding back on another cycle of debilitated farming. Improving agricultural practices and outcomes can result in a virtuous cycle of community development, especially if the farm innovations are compatible with environmental and nutritional objectives.

Figure 2. Adapted from Rogers (2003).

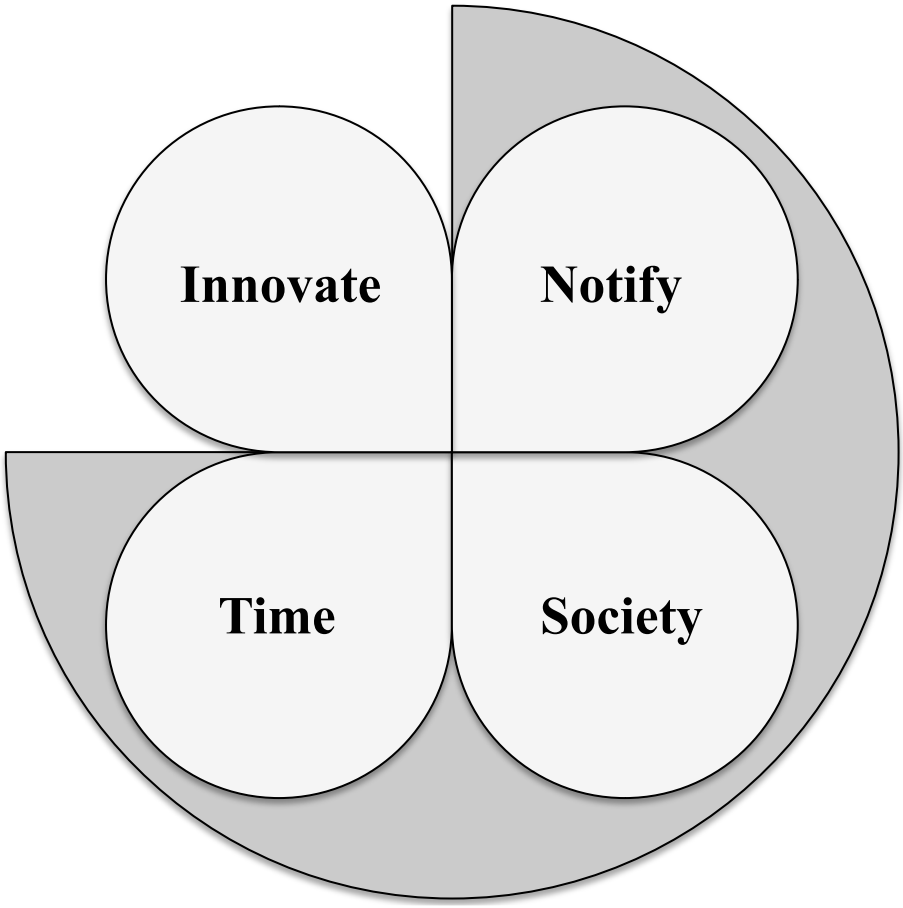

Figure 2. When a researcher is able to innovate a new technology, method, or scientific idea, they must then notify potential adopters, who exist within a particular society, and who over time may accept the innovation. In the agricultural sciences the development of the innovation is the portion of the diffusion process that is typically conducted by an academic researcher, while understanding the local social context and communicating effectively to potential adopters is often the occupation of extension agents. However, linking these activities though collaborative networks can improve the process of appropriate innovation for the researcher, and the ability of change agents to understand and communicate the relative advantage of research findings.

Figure 3

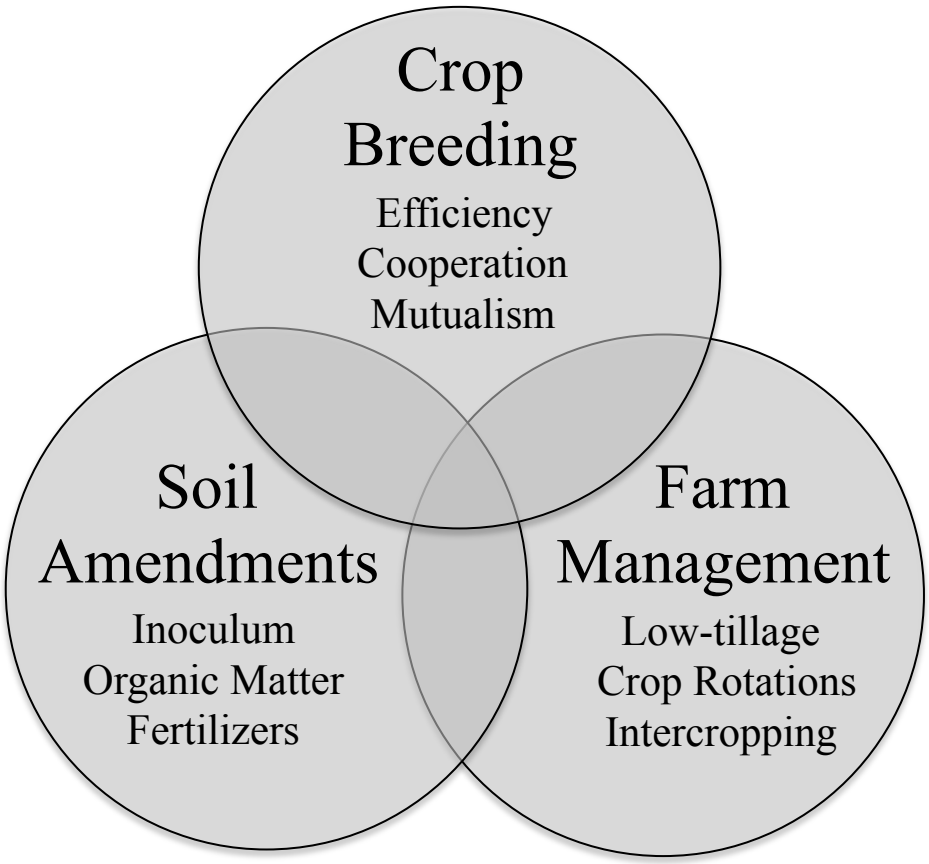

Figure 3. Crop breeding research includes identifying and selecting for genetics that facilitate the efficient use of system resources, cooperation between plants and neighboring crops, and mutualism between plants and beneficial soil microbes. Farm management research includes methods that reduce tillage, the incorporation of diverse crop rotations, and planting different crop types in polyculture. Soil amendment research includes culturing and utilizing beneficial soil microbe inoculum, the integration of materials to enhance soil organic matter, and optimization of chemical fertilizer farm inputs. It is critical to unify these often-disparate lines of inquiry and design agricultural systems that are informed by all three areas.

VITA

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