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Assessing the influence of farm fertility amendments, field management, and sorghum genotypes on soil microbial communities and grain quality $^{\bigstar, \bigstar \bigstar}$

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ABSTRACT

It is imperative for sorghum breeders to focus on enhancing plant-microbial partnerships to help deliver efficient genotypes for sustainable agricultural systems. Alternative farm fertility amendments and field management practices may also improve sorghum yields and grain quality, especially in combination with appropriately bred genotypes. Therefore, we assessed four genotypes of grain sorghum (two commercial hybrids and two landrace open-pollinated cultivars) for their influence on soil microbial communities, particularly arbuscular mycorrhizal fungi, at two field sites. All genotypes were grown as sole crops, while one commercial hybrid and one openpollinated cultivar were also intercropped with cowpea to compare the two farm management practices. We established three treatments: non-amended control, commercial fertilizers (N and P), and worm compost (alternative fertility amendment). Grain production and nutritional quality were assessed to link belowground influences from genotypes and management on aboveground outcomes. Soil microbial communities were influenced by genotype, and higher fungal to bacterial biomass ratio was observed following worm compost amendment. Grain production was not significantly different between genotypes or treatments; however, percentage mycorrhizal root colonization was significantly greater for plants grown in soil amended with worm compost, as compared to commercial fertilizers. Genotype also had a significant effect on grain nutritional quality, while 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. Selective breeding of sorghum for agricultural systems designed around these alternative practices may further benefit grain nutrition and soil microbial communities.

1. Introduction

Grain sorghum (*Sorghum bicolor*) 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 over 15 million Mt in 2015 (USDA, 2016). Sorghum is also an attractive model crop for the study of functional crop genetics and insights gained from studying sorghum are readily transferable to several other crops (Paterson et al., 2008; Kumar et al., 2011), and its drought tolerance will be important for the future of food security (Hadebe et al., 2016).

The major goals of sorghum breeding and genetics programs are

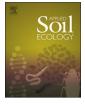
often to develop superior genotypes with improved grain production and disease resistance. While it is a food staple in many countries, it does suffer from nutritional deficiencies such as reduced protein digestibility (Duodu et al., 2003) and low lysine levels (Belton et al., 2006). Enhancing grain sorghum nutritional quality is an important breeding consideration (Taylor et al., 2014), as this benefits global farming communities. Additionally, understanding the variation in starch characteristics between different genotypes is key to improving sorghum use in various food products (Kaufman et al., 2013).

Sorghum cultivation provides opportunities to address global nutrition security through breeding and biofortification strategies to

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produce the most beneficial genotypes for sustainable food production. In particular, genotypes developed in the context of high-input farming may not be appropriate in low-income countries where farmers lack access to commercial fertilizers (Njeru, 2013). Our previous research indicates that modern sorghum genotypes are dependent on fertilizers rather than microbial partners (Cobb et al., 2016). Conversely, selective breeding can be utilized to develop sorghum genotypes that are more responsive to microbial partners, potentially improving soil stability and nutrient use efficiency in agroecosystems (Wissuwa et al., 2009; Bender et al., 2016).

Dwindling phosphorus reserves are a looming crisis for agriculture (Herrera-Estrella and López-Arredondo, 2016) because supplies may be within decades of exhaustion (Chen and Graedel, 2016). Additionally, in many countries, soil erosion is far outpacing natural replenishment (Pimentel, 2006; Oliver and Gregory, 2015; Tully et al., 2015). Fortunately, improved partnerships between agronomic crops and soil fungi could help solve these issues (Andrews et al., 2012; Denison, 2012).

Arbuscular mycorrhizal (AM) fungi form beneficial associations with up to 80% of land plants (Smith and Read, 2008) including most agricultural crops. In natural ecosystems, AM fungi can improve plant nutrition, disease resistance, and water use efficiency (see Gosling et al., 2006), and they present a great opportunity to enhance phosphorus use efficiency in agricultural systems (Ellouze et al., 2014; Rodriguez and Sanders, 2015; Köhl and van der Heijden, 2016). Additionally, AM fungi can improve soil aggregation, stability, and/or soil water-holding capacity (Zhu and Miller, 2003; Wilson et al., 2009; Willis et al., 2013; Mardhiah et al., 2016).

It is critical to assess not only the grain yield and quality of sorghum genotypes, but also their influence on soil microbial communities (particularly AM fungi), as microbial communities may be key to agricultural sustainability. Previous assessment of sorghum genotypes indicated a significant difference in their reliance on AM symbiosis for acquisition of soil phosphorus, with landrace open-pollinated (OP) sorghum cultivars nearly three times as responsive to AM root colonization for grain production and nutrition compared to fertilizer responsive, sorghum hybrids (Cobb et al., 2016). 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; Gutjahr, 2014; Bonfante and Genre, 2015; 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 explain the difference in mycorrhizal responsiveness between modern hybrids and OP cultivars.

There are also several farm methods that may expand the abundance and benefits of AM fungi in agroecosystems. A review of previous mycorrhizal research on agricultural practices concluded that additions of compost typically maintained or increased the AM fungal root colonization of many agricultural crops (Zhang et al., 2012; Cavagnaro et al., 2015). In many low income countries, farmers are utilizing worm compost to reduce local reliance on fertility amendments from external sources (Misra et al., 2003). Chaoui et al. (2003) reported that high rates of earthworm-based composts (vermicomposts) improved cropnutrition on par with commercial fertilizers, with the additional benefits of slow nutrient release and reduced leaching. Additionally, 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). Improved plant nutrition (Zuo and Zhang, 2009, 2011) and reduced soil erosion (Zougmoré et al., 2000) have also been associated with intercropping. A meta-analysis suggested intercropping could increase incomes and yields in Africa; however, more research involving leguminous intercrops is needed (Himmelstein et al., 2017).

Our research aims to determine if sorghum OP and hybird genotypes respond similarly to farm management practices, and how they influence microbial communities under different field conditions. We compared sorghum genotypes, cultivated under conventional (sole crop) or alternative planting systems (intercropped with cowpea, *Vigna unguiculata*), while treated with commercial (N and P fertilizers) or alternative fertility amendments (worm compost). Farm management is a key consideration, as many agricultural practices reduce soil microbial community diversity and total microbial biomass as compared to native systems (Richardson et al., 2011; Montecchia et al., 2011). Our results may help breeders and farmers utilize sorghum genetic traits and agricultural practices to increase soil microbial biomass and enhance the benefits of AM fungi in agroecosystems, potentially improving grain production and nutrition, decreasing the need for phosphorus amendments, and stabilizing farm soils.

2. Materials and methods

2.1. Experimental setup

A split-plot within complete block design was established with each whole plot consisting of four sole crop sorghum genotypes (commercial hybrids were Dekalb [DK 54-00] and Pioneer [P84G62]; landrace openpollinated [OP] cultivars were Ajabsido and Macia), and two genotypes (DK 54-00 and Macia) that were also intercropped with the cowpea cultivar Risina del Trasiorfino. The seeds of sorghum OP cultivars were acquired from the Kansas State University sorghum-breeding program. All sorghum genotypes were planted in April 2014 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). Three treatments: a non-amended control, commercial fertilizers (N and P), and worm compost were randomized within each of the six whole plots (blocks) at each field site. Collection of sorghum grain, root, and soil samples occurred in August 2014; however, cowpea production was not assessed as part of this experiment.

Sorghum genotypes were planted in 16 m rows (10 cm seed-spacing, 76 cm row-spacing) with an east/west orientation. Additional rows of DK 54-00 and Macia were planted as buffers to separate open-pollinated and hybrid genotypes as well as whole plot borders. Cowpea was planted in two 16 m rows (20 cm seed-spacing, 76 cm row-spacing) between additional rows of Macia and DK 54-00 for the intercrop portions. Therefore, 12 total rows (hybrid buffer, DK 54-00, P84G62, hybrid buffer, OP buffer, Ajabsido, Macia, Macia, Cowpea, Cowpea, DK 54-00, hybrid buffer) were planted within each whole plot using an Earthway 1001-B Precision Seeder^{*}. Treatment applications were completely randomized as 4.0 m x 9.12 m split-plots across each plot, with 2.0 m buffers separating treatment blocks. Sampling was confined to the middle two meters of each row within each split-plot (18 total factorial combinations).

2.2. Field conditions and treatments

Eight soil samples (each the composite of three randomly distributed subsamples) were collected (from 0 to 10 cm depth) by spade from both field locations before experimental set up to determine baseline relative abundance of microbial functional groups and total microbial biomass at both sites. 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 (SWAFL) at Oklahoma State University. Soil NO₃–N and NH₄ were extracted by 1 M 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

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