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Influence of smallholder farm practices on the abundance of arbuscular mycorrhizal fungi in rural Zambia



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Keywords: Sub-Saharan Africa Agroecosystems Phospholipids Neutral lipids	While the effects of row crop agriculture on arbuscular mycorrhizal (AM) fungi have been studied in temperate climates, much less is known about the consequences of methods employed by smallholder farmers in Africa. We compared soils from 12 small farms in rural eastern Zambia with soil from nearby grasslands (control) to assess microbial abundances, including AM fungi, using phospholipid and neutral lipid fatty acid (PLFA/NLFA) analyses. We also measured soil pH, soil organic matter, and key nutrients, and categorized farms to link microbial abundances with agricultural practices including: low and high input cultivation, conservation farming, and agroforestry. Soil organic matter was significantly greater in grassland (control) soils, compared to any of the farms. Concentrations of N, P, and K were greater in farm soils compared to grassland soil. Extra-radical AM fungal biomass was significantly greater in grassland soils compared to farms, and grasslands contained more than double the total microbial biomass compared to any farm. Our results indicate agricultural practices in Zambia are negatively affecting AM fungi as well as other soil microbes. However, conservation farming shows promise in facilitating AM fungal recovery, with potential long-term benefits for soil stability via hyphal exudates and physical entanglement.

1. Introduction

Zambia and many other countries are struggling to improve public health, education, and reduce poverty while protecting natural resources, such as productive soils. Row crop agriculture in sub-Saharan Africa (SSA) is often linked with rapid soil erosion, losses of above- and belowground ecosystem services, and the inability of small-scale farmers to produce sufficient food for their livelihoods (Tully et al., 2015).

Improving soil stability and reducing erosion are critical pursuits for countries such as Zambia (Government of the Republic of Zambia, 2002). Fortunately, symbiotic partnerships between agronomic crops and soil microbes (particularly arbuscular mycorrhizal [AM] fungi) present opportunities to make global agricultural systems more sustainable and improve soil health (Andrews et al., 2012; Ellouze et al., 2014; Rodriguez and Sanders, 2015). Mycorrhizal fungi form beneficial associations with ~80% of land plants including most agricultural crops (Smith and Read, 2008). Increasing the abundance of AM fungi in agroecosystems may diminish the need for commercial fertilizers by improving plant nitrogen and phosphorus uptake and reducing leaching (Hodge and Fitter, 2010; Asghari and Cavagnaro, 2011). These symbiotic fungi also directly contribute to soil aggregate formation and

stability through physical entanglement of soil particles and glomalin production (Zhu and Miller, 2003; Wilson et al., 2009; Willis et al., 2013). However, mycorrhizal abundances and benefits can be depleted in agroecosystems due to farm management practices such as high phosphorus fertilizer application, fallow periods, and heavy tillage (Richardson et al., 2011; Bowles et al., 2016).

An increase in AM fungal abundance on farms may be of global importance; analyses of erosion suggests soil losses are occurring between 30 and 40 times faster than natural replenishment in many countries and are rapid across SSA (Pimentel, 2006; Masso et al., 2017), and this loss has been connected to reduced food security as well as human health issues (Oliver and Gregory, 2015). However, Mardhiah et al. (2016) found AM hyphal networks in combination with plant roots improve soil cohesion and helped prevent erosion by surface water flow. Enhancing the effectiveness of AM fungi may also allow farmers to utilize them as 'natural biofertilizers' (Berruti et al., 2015) that can reduce production costs and improve human health in rural communities (Oruru and Njeru, 2016). Therefore, understanding how farm management practices in SSA are impacting AM fungi and other soil microbial functional groups is an important step to unlocking potential benefits for local farmers.

Our field study was conducted in July 2014 near Navaruli village

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(Chadiza District) in the Eastern Province of Zambia. The Eastern Province is Zambia's most rural with the vast majority of farmers producing on less than one hectare of land; more than 60% of the population lives in poverty, and more than 60% of children exhibit signs of stunted growth due to malnutrition (Sitko et al., 2011). There is relatively little research comparing the impact of local agricultural practices on AM abundance in SSA smallholder farm soils (Lekberg et al., 2008).

We compared soils from small farms in rural eastern Zambia with soil from a nearby native grassland to assess relative abundance of soil microbial functional groups, particularly AM fungi using phospholipid (PLFA) and neutral lipid fatty acid (NLFA) analyses. We determined soil chemical properties and discussed field management methods with local farmers to categorize farms with an overall goal of assessing relative abundances of soil microbial communities and linking results with common SSA agricultural practices. We hypothesized that farms utilizing more conventional practices, such as high rates of P-fertilizer, dry season fallow, and heavy soil tillage, would have significantly reduced soil organic matter (Lal, 1999; Prasad et al., 2016) and AM abundance (Evans and Miller, 1990; Lekberg and Koide, 2005) as compared to local untilled grasslands. We further hypothesized improved sustainability practices (agroforestry and conservation agriculture) would support soil microbial biomass that is intermediate between conventional practices and native grassland soils. Under farm sustainability practices, greater AM abundance may follow trends of greater AM diversity observed in other studies of improved farming methods (Säle et al., 2015; Manoharan et al., 2017). Our research will help provide direction for international agricultural development and sustainable cropping systems research. By determining the impact of farming practices in eastern Zambia on soil microbial abundance, especially AM fungi, we can extend our understanding of the consequences of smallholder farm practices on the potential benefits of AM fungi in SSA, with regional and global implications for food security and sustainability.

2. Methods

2.1. Local conditions and experimental setup

This study occurred during the cold/dry season, when corn (*Zea mays*) crops had recently been harvested. According to soilgrids.org (Hengl et al., 2017), soils around Navaruli village are predicted to be Ustalfs/Haplic Luvisols, the top 15 cm are characterized by low plant-available N and CEC, with pH between 5.0–5.9, and are typically well-drained, composed of 70% sand, 11% silt, and 19% clay, with bulk density approximately 1.5 g cm⁻³. Local agriculture is primarily rainfed and planting is timed to the rainy season (November-April). Although farmers in the Eastern Province have reported the rainy season is starting later and ending earlier in the past decade, they typically benefit from around 1000 mm of precipitation each year (Mulenga and Wineman, 2014).

Field locations were selected through discussion with local farmers (Chewa language translation provided by Mr. Evan Brothers, Peace Corps Volunteer). We designated each selected farm site based on the description of agricultural methods employed by the owners. Typically, farmers in the region plant the majority of their land to maize (corn), tobacco (*Nicotiana tabacum*), or cotton (*Gossypium hirsutum*), with smaller areas planted to various pulse and local vegetable crops as well as grain sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum*). Farmers explained a preference for planting local maize cultivars for their personal household consumption, but using certified commercial hybrids for market sale. This inclination has also been noted by CIMMYT researchers, who explained it as a matter of the taste, storability, and the low risk farmers associate with landrace maize in eastern Zambia (Andersson and Setimela, 2014).

Fields selected for this research were used to produce hybrid maize

in the previous season, and no two farms were > 1.5 km apart. While exact fertilization applications (timing, rate, type) were not available, the government of Zambia subsidizes commercial N and P fertilizers, and all farmers indicated they applied commercial fertilizers. We selected 12 small farms (< 2 ha) utilizing the following agricultural practices: 1) relatively high rates (double annual cost compared with all other farms) of commercial fertilizer use (high input); 2) relatively low rates (half the annual cost of high input farms) of commercial fertilizer use (low input); 3) low input agroforestry (Howard, 2012) with approximately 10 trees ha⁻¹ local Miombo species (Brachystegia spp., Julbernardia spp., and Isoberlinia spp.) interspersed in the fields; 4) low input conservation agriculture techniques, referring to practices such as reduced tillage and mulching implemented for approximately 5 years (FAO, 2015); and we utilized 3 nearby native grassland areas as a control. Each selected farm had ~5 years of management history under its categorized agricultural practice. Three farms were selected to represent each of the agricultural practices along with three nearby grassland sites (15 total locations). Spatially randomized soil samples were collected (0-10 cm depth) from each site with one 100 g sample (combination of six subsamples) collected for soil nutrient analysis and two 10 g samples (each the combination of three subsamples) for soil microbial analysis.

2.2. Soil properties

The Soil, Water, and Forage Analytical Laboratory at Oklahoma State University analyzed all soil nutrient samples. Soil samples were dried at 65 °C overnight and passed through a 2 mm sieve. Soil pH was measured by glass electrode in a 1:1 soil:water suspension and Sikora buffer solution, respectively (Thomas, 1996; Sikora, 2006). Soil NO₃-N and NH₄-N were extracted with 1 M KCl solution and quantified by a Lachat Quickchem 8000 Flow Injection Autoanalyzer (LACHAT, 1994; Kachurina et al., 2000). Plant-available P, K, Ca and Mg were extracted using Mehlich 3 solution (Mehlich, 1984). P, K, Ca, and Mg in the extract were quantified by a Spectro Blue inductively coupled plasma (ICP) spectrometer (Soltanpour et al., 1996; Pittman et al., 2005). Soil organic matter was determined using a LECO Truspec dry combustion carbon analyzer (Nelson and Sommers, 1996). Plant available Zn and Fe were extracted by DTPA-Sorbitol and quantified by ICP (Gavlak et al., 2005).

2.3. Soil microbial communities

Relative abundances of soil microbial functional groups (gram-positive and gram-negative bacteria, AM and saprophytic fungi), and total microbial biomass were assessed using phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analyses (Olsson et al., 1995). Phospholipid fatty acids are constituents of biological membranes that can be used to estimate active biomass of bacteria and fungi, because biovolume and cell surface area are well correlated (Frostegård et al., 2011). Neutral lipid fatty acids are basic storage products of many fungi and serve as the primary energy reserve in AM fungi (Larsen and Bødker, 2001; Sharma and Buyer, 2015). Phospholipid fatty acids and neutral lipid fatty acids were extracted from the soil using a modification of the Bligh and Dyer (1959) extraction (White and Ringelberg, 1998). Total lipid extracts were separated into PLFAs and NLFAs 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 (FAMEs) (White and Ringelberg, 1998; Allison and Miller, 2005). The FAMEs were then analyzed by gas chromatography and mass selection detection using a GCMS unit Agilent MS 5975C/GC 7890 A. We utilized c:19 as an internal standard. Biomarkers detected for the functional group of grampositive bacteria consisted of i-15:0, a-15:0, i-16:0, and i-17:0. For gram-negative bacteria, detected biomarkers were 2-OH 14:0, 2-OH 16:0, $18:1\omega9$ trans, and cy19:0. For extraradical AM fungal biomass we

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