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A study of *Glycine* max (soybean) fungal communities under different agricultural practices

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ABSTRACT

Both *Glycine* max (soybean) and *Thlaspi arvense* (pennycress) are valuable resources for renewable biofuels and industrial products. Pennycress, a member of the Brassicaceae, can be grown over the winter months providing soil cover and thus reducing erosion. In addition, pennycress does not compete with food production making it an appealing option for biofuels. The objective of this study was to determine the effect of different agricultural practices on fungal communities associated with soybean roots and their interactions with an emerging crop/cover crop, pennycress. We examined roots collected in the summer of 2013 and 2014 from three treatment plots (organic, conventional soybeans, and conventional soybean with pennycress as a cover crop). Root DNA was amplified using fungal primers followed by 454 pyrosequencing. A total of 560,948 sequences were obtained with Ascomycota as the dominant fungal phylum in all plot treatments. Soybean roots in all treatments were dominated by the order Hypocreales with *Fusarium* as the most abundant genus. Fungal community structure was affected by host and farming practice. The magnitude of the effect was determined by sampling year. The cover crop, pennycress, had a distinct fungal assembly from soybean and did not affect the structure of fungal communities in soybean when used as a cover crop. The organic soybean fungal community was significantly different and contained lower diversity than the conventional soybean, with the greatest differences observed in 2014. This study shows that the structure of fungal communities associated with plants in agroecosystems are regulated by a complex number of abiotic and biotic factors including weather conditions, farming practice, and host.

1. Introduction

The United States produces approximately 84 million tons of soybeans annually (Grassini et al., 2014). The majority of the soybean crop (80%) is grown in the upper Midwestern states where corn-soybean crop rotations are the principal farming practice. Soybeans grown in the United States account for 35% of the total production worldwide and are one of the world's leading sources of animal feed protein and the second largest source of vegetable oil (Grassini et al., 2014). The USDA forecasts in 2016 a $700 million increase in soybean meal exports from the United States, increasing the economic benefit to $26.1 billion (http://www.ers.usda.gov/topics/crops/soybeans-oil-crops.aspx).

The human population is expected to grow to 9.7 billion by the year 2050 (Alexandratos and Bruinsma, 2012). Grain production must more than double to meet the food needs of this projected population (Oerke and Dehne, 2004). Production in modern agriculture is limited in part by the availability of suitable land, water shortages, conservation of fertile soils, an increasing number of pathogens, and little understanding of factors that regulate plant-microbial interactions. Microbial communities regulate plant diversity, composition, and productivity in terrestrial ecosystems (Van Der Heijden et al., 2008) but the biotic and abiotic factors regulating microbial community structure are poorly understood. The objective of this study was to evaluate the effect of crop management on the structure of fungal communities in a soybean agroecosystem. Three crop management systems (organic, conventional, and conventional with pennycress as a cover crop) were evaluated for two years.

Most cropping systems in the Midwestern United States are traditional corn-soybean rotations (Gesch et al., 2010). Conventional agroecosytems require intense application of fertilizers and pesticides to maintain high levels of productivity (Phippen and Phippen, 2012). The addition of cover crops to conventional rotation systems has been shown to provide both economic and ecological benefits (Gesch et al.,

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2010) since the added diversity can increase nutrient and water availability in the soil and help control pathogens (Kirkegaard et al., 2008; Krupinsky et al., 2006).

The use of cover crops and organic farming has the potential to increase microbial diversity, facilitate nutrient cycling, increase organic matter and nitrogen fixation, improve weed control, and ultimately increase crop yields (Altieri, 1999; Reddy et al., 2003; Sainju and Singh, 1997; Williams et al., 1998). Organic farming reduces the use of pesticides and fertilizers and can show positive effects on the diversity and abundance of fungi (Oehl et al., 2004). Cover crops can also play an important role in maintaining topsoil and enhancing the number of beneficial fungi in fallow fields by providing nourishment to obligate mutualists during winter months (Kabir and Koide, 2002). Boswell et al. (1998) reported an increase of extraradical hyphal density and increase of arbuscular mycorrhizal fungi (AMF) in maize when winter wheat was used as a cover crop. In soybean, cereal rye and Brassicaceae cover crops reduced soil compaction, facilitating the penetration of roots into the soil (Williams and Weil, 2004).

Thlaspi arvense L. (pennycress) is a member of the Brassicaceae family and can be used as a winter cover crop helping to prevent erosion (Phippen and Phippen, 2012). Plants within this family produce compounds left by the root residues that can reduce pests and disease in the soil (Sarwar et al., 1998). Pennycress is a short season winter annual that could potentially be used as a cover crop in the Midwest (Phippen and Phippen, 2012). Microbial communities associated with pennycress and their interactions with soybean are unknown, but AMF fungi have been reported as symbionts of the genus *Thlaspi* (Regvar et al., 2003; Vogel-Mikuš et al., 2005). No other studies on root-associated fungi have been reported for this plant and in general studies on root-associated fungal communities in agroecosystems are limited and mainly focus on AMF. Root-associated fungi (including endophytes or rhizosphere fungi) are complex communities that interact with the root system and are known to improve plant growth, facilitate resistance to drought and heat, and reduce the effects of pathogenic microorganisms (Porras-Alfaro and Bayman, 2011). These symbiotic interactions range from mutualistic to parasitic and are influenced by complex biotic and abiotic factors including available hosts, edaphic conditions, environmental change, among others.

The objectives of this study were to: 1, compare the root fungal communities associated with soybean grown under organic and conventional farming practices; 2, determine the effect of pennycress on soybean microbial communities when used as a winter cover crop; and 3, Observe how consistent the fungal-plant relationships remained across years. The fungal communities were assessed in pennycress and soybean roots (plants at stage *R*3, pod formation) for two consecutive years (2013 and 2014). Soybean plants were evaluated in three treatments: organic, conventional, and conventional with pennycress as a cover crop. Fungal communities were compared for soybean and pennycress.

2. Materials and methods

2.1. Site description — conventional soybeans and pennycress cover crop

Samples were collected in 2013 and 2014 at the Western Illinois University Agricultural Field Laboratory near Macomb, Illinois. Both soybean sites were characterized by Ipava silt loam soils (fine, smectitic, mesic Aquic Argiudolls) with 23 g kg^{-1} of organic matter with a pH range of 6.5 to 7.1. Average air temperatures at the field sites from July to October 2013 were 20 °C and 18.9 °C in 2014. The average volume of water content in soil was 20.2% and 45.7% in 2013 and 2014, respectively. The average soil temperatures from July to October within the plots for 2013 and 2014 were 20.6 °C and 18.6 °C, respectively.

Planting dates occurred from mid-May to mid-June in both years based on harvest times for pennycress. Corn was the preceding crop. Pennycress seed (Patton breeding line) was drilled in 19 cm rows in a cultivated field at a rate of 16 kg ha^{-1}. No herbicides were applied on pennycress and 50 lbs. N + 10 lbs. S were applied in granular form and incorporated prior to planting of pennycress. Traditional soybean production was followed with herbicide but not additional fertilizers.

Round-up ready (glyphospate-resistant) soybeans (Pioneer '93 M11') maturity group 3.1 were no-tilled drilled into the crop residue in the spring of 2013 and 2014. Before planting soybeans, pennycress was cut using a string trimmer with crop residue remaining in plots. Control soybean plots were planted on fallow ground. Soybeans were harvested in October for both years using hand clippers. Plants were processed with a mobile cylinder plant thresher and seed was screen-cleaned before yield determination. The total soybean yield was adjusted to a moisture content of 130 g kg^{-1}.

2.2. Site description — organic farm

Organic soybeans were collected from the Western Illinois University Allison Organic Farm located in southwest Warren County near Roseville, Illinois. This is a traditionally pesticide free farm with a long-term history of organic farming management practices. The Allison farm consists of Sable silty clay loam and Muscatune silt loam soils, and follows a corn-soybean-wheat/red clover rotation. Corn was the preceding crop in the experimental fields sampled.

Organic soybeans (Lakeview Farms LVF3507) were planted in tilled soil at a seeding rate of 170,000 seeds per acre. No pesticides or herbicides were used during the growing season. Rotary hoeing and row-crop cultivation were used for weed control management. Soybeans were harvested in October using a KEM plot combine. Following harvest, the grain was weighed and the length of the plot was measured to determine soybean yields.

2.3. Soybean yield

Soybeans grown in all treatments showed no indication of plant disease based on yield data and field observations. Organic soybeans produced 47.1 bushels per acre in 2013 and 48.6 in 2014. The conventional soybeans grown on fallow soil produced 51.3 bushels per acre in 2013 and 36.1 bushels per acre in 2014. Conventional soybeans with pennycress as cover crop produced 45.8 bushels per acre in 2013 and 34.2 in 2014.

2.4. Sample collection

Glycine max (L.) Merr. (soybean) and *Thlaspi arvense* roots were collected in 2013 and 2014 (08/29/13 and 09/09/14, respectively). Three 100 m transects were set and 10 plants were collected in a zig-zag pattern across the rows for each treatment plot: conventional soybeans (10 soybean plants), organic soybeans (10 soybean plants), soybean following pennycress (10 soybean and 10 pennycress plants). All soybean plants were collected when they reached the R3 stage (pod development). Roots were placed in a plastic bag and kept on ice until processed in the lab (for approx. 1 h).

Healthy roots were clipped and washed thoroughly with water to remove soil particles. The last rinse was done with distilled water. Ten one centimeter segments were randomly selected from each plant and placed into microcentrifuge tubes and stored at −80 °C until extraction.

2.5. DNA extraction, PCR, and sequencing

Plant root tissue was ground using liquid nitrogen prior to DNA extraction. DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. To confirm adequate DNA, product extract was amplified using internal transcribed spacer nuclear ribosomal RNA (ITS nrRNA) fungal specific primers, ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Gardes and Bruns, 1993; White et al.,

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