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Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production

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ABSTRACT

Intensive agricultural practices, such as tillage, monocropping, seasonal fallow periods, and inorganic nutrient application have been shown to reduce arbuscular mycorrrhizal fungi (AMF) populations and thus may reduce benefits frequently provided to crops by AMF, such as nutrient acquisition, disease resistance and drought tolerance. We have evaluated the ability of different cover crops to elevate the native mycorrhizal inoculum potential of soils under soil–climatic conditions typical of the upper Midwest U.S. production agricultural region. We measured the number of soil AMF propagules at three sites in the late fall following cover crops that were seeded into summer-harvested small grains within a no-till rotation. At all three sites, soil AMF propagule numbers were generally low ($\leq$1 propagule g^{-1}). Fall cover crops significantly increased the mycorrhizal inoculum potential of the soils. Forage oats (*Avena sativa* (L.) Hausskn.), by itself or in mixtures, was most effective at both sites where it was planted. At the third site, a cover crop mixture doubled the inoculum potential of these soils. The effect of cover crop treatments on AMF propagules was corroborated at one site over two seasons by measuring AMF biomass with the neutral lipid fatty acid mycorrhizal biomarker, C16:1*cis*11. Identification of AMF-promoting cover crops for inclusion in diversified, no till cropping rotations in the upper Midwest U.S. will provide opportunity for reduced inorganic nutrient application with economic and environmental benefit.

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1. Introduction

The addition of cover crops into agricultural production systems reduces seasonal fallow and thus provides many benefits to the following cash crops and the health of the soil (Clark, 2007). The presence of a living plant provides a host for obligate mutualists like arbuscular mycorrhizal fungi (AMF) that can protect the host plant against pathogens, extend environmental tolerances, and provide substantial nutritional benefits, particularly improved phosphorus uptake (Rillig, 2004; Lekberg and Koide, 2005; Jansa et al., 2006). Studies evaluating the effect of cover crops on AMF in agricultural soils date back to at least 1995 (Galvez et al., 1995; Boswell et al., 1998; Kabir and Koide, 2000; Deguchi et al., 2007; White and Weil, 2010); however, much of this data is limited to soil-climatic regions or agricultural practices that are not representative of production agricultural systems for corn (*Zea mays* (L.)), soybean (*Glycine max* (L.) Merrill), and wheat (*Triticum aestivum*) that are dominant in the Midwest United States.

Because of dwindling phosphate reserves and soaring prices for phosphate fertilizer (Cordell et al., 2009; Van Vuuren et al., 2010), there is a renewed interest in the capability of AMF to efficiently supply plant-available phosphorous in production agricultural systems, especially for corn. AMF have been shown to support nutrient uptake in corn and may increase yield in some cases (Vivekanandan and Fixen, 1991; Murray, 2000; Kabir and Koide, 2002; Deguchi et al., 2007). Intensively farmed soils are often depleted of AMF (Douds et al., 1993; Mader et al., 2000; Oehl et al., 2004; Jansa et al., 2006) and a meta-analysis of published data has concluded that low AMF inoculum potential limits mycorrhizal colonization and plant performance (Lekberg and Koide, 2005). There are two possible strategies to increase AMF in agricultural soils: inoculation or selective management. Successful field-scale AMF inoculation for commodity crops is economically prohibitive using root cuttings, and only a single species, *Glomus intradices*, can be routinely produced in sufficient densities for spore applications (Atunes et al., 2009). Inoculation strategies are further constrained by the absence of information on which AMF strains are highly beneficial for a particular crop and which will be competitive in the ambient soil environment. With respect to management, many production agricultural systems rely on tillage, monocultures, seasonal or annual fallow, inorganic fertilizer and pesticide application, all of which

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can negatively impact AMF numbers and or diversity (Jansa et al., 2006).

We evaluated the ability of specific cover crops and cover crop mixtures to elevate the native AMF inoculum potential prior to corn planting within no-till, small grains–corn–soybean crop rotations under soil-climatic conditions representative of the upper Midwest U.S. The AMF inoculum potential of soils in agricultural fields with and without cover crops at three locations in South Dakota was determined by most-probable number enumeration of arbuscular mycorrhizal fungal propagules (spores, vegetative hyphae, infected root fragments) and by AMF biomass estimates using fatty acid analysis.

2. Materials and methods

2.1. Site 1: Brookings Research Farm

This 65 ha Eastern South Dakota Soil and Water Research Farm located in Brookings, South Dakota (44°19′N latitude; 96°46′W longitude) is operated by the North Central Agricultural Research Laboratory (USDA-ARS-Northern Plains Area) under a long-term lease agreement. The research farm is located at 500 m elevation with 58 cm mean annual precipitation (MAP) and a mean annual temperature (MAT) of 8 °C in the Big Sioux Basin of the northern glaciated plains (Bryce et al., 1998). The Mollisol soils are a Barnes sandy clay loam (fine-loamy, mixed, superactive, frigid Calcic Hapludoll) that are moderately drained, with a high total soil organic matter content, and a clay content of about 280 g kg^{-1} (Pikul et al., 2007). The experimental cover crop treatments were established on 20 August 2009 into spring wheat stubble following harvest; this area had been no-till for at least 3 years. The experimental area was divided into 3 m × 16 m plots arranged in a randomized complete block design with four replicates of eight cover crop treatments: no cover crop, forage oats (*Avena sativa* (L.) Hausskn.), hairy vetch (*Vicia Villosa* Roth), winter canola (*Brassica Napus* L.), and all possible combinations of the three cover crops. Treatments were planted at a depth of 1.9 cm with rows spaced 19 cm apart, using a JD1590NT Drill. Two days later the area was sprayed with Roundup Weathermax® (2.3 L ha^{-1}). Composite soil samples (six 30-mm diameter cores, 0–15 cm depth) were collected on 11 November 2009 from each plot to measure the number of arbuscular mycorrhizal propagules and for fatty acid analyses. In summer of 2010, a second experimental site was established on adjacent land that had been no-till for 10 years; on 12 August 2010 cover crop treatments were planted into oat (*Avena sativa* L.) stubble (not spring wheat) utilizing the same experimental design, treatment structure and agronomic practices. Fall soil sampling occurred 8 November 2010.

2.2. Site 2: White Lake Producer

A cooperating producer farm near White Lake, South Dakota (43°40′N latitude; 98°41′W longitude) was the second site. This farm is located at 502 m elevation with a 55 cm MAP and 9 °C MAT in the Southern Missouri Coteau of the Northwestern Glaciated Plains (Bryce et al., 1998). The Mollisol soils are a Houdek-Dubley complete (fine-loamy, mixed superactive, Mesic Typic Agriustolls) that are well drained, with a high total soil organic matter content, and a clay content of about 205 g kg^{-1}. On 18 August 2009, cover crop treatments (6.7 m × 40 m strips) were planted into spring wheat stubble in a rain-fed no-till field. The experimental design was a randomized complete block design with 3 replications. Experimental treatments consisted of no cover crop, winter canola, forage oats/winter pea (*Pisum sativum* (L.) subsp. *arvense*), crimson (*Trifolium incarnatum* (L.))/alsike clover (*Trifolium hybridum* (L.)), field pea (*Pisum sativum* L.)/timothy (*Phleum pretense* (L.)), forage radish (*Raphanus sativum* (L.) var. *niger* J. Kern)/field pea, and a slit-tillage treatment with no cover crop. Treatments were planted at a depth of 1.9 cm with rows spaced 19 cm apart, using a NT/JD Air Seeder. Four days prior to cover crop planting the experimental area was sprayed with Roundup Weathermax® (2.3 L ha^{-1}). Composite soil samples (six 30 mm-diameter cores, 0–15 cm depth) were collected on 12 December 2009 at two predetermined locations within each strip plot for AMF propagule enumeration.

2.3. Site 3: Ideal Producer

A cooperating producer farm near Ideal, South Dakota (43°33′N latitude; 99°54′W longitude) was the third site. This 4050 ha (1420 ha cropped; all no-till for over 20 years) farm is located at 575 m elevation with 60 cm MAP and 9 °C MAT in the subhumid Pierre Shale Plains of the Northwestern Great Plains (Bryce et al., 1998). The Vertisol soils are a Millboro silty clay (fine, smectitic, mesic Typic Haplusterts) that are well drained, with a high soil organic matter content, and a clay content of about 530 g kg^{-1}. On half of the total land (485 ha) where winter wheat had been harvested, this producer planted a cover crop mixture (cow pea (*Vigna sinensis*, *V. unguiculata*), winter pea, millet (*Pennisetum americanum* (L.) Leek), forage radish, turnip (*Brassica rapa* (L.)) into the wheat stubble at a depth of 2.5 cm with rows spaced 25 cm apart, using a NT/JD1895 Air Seeder on 27 July 2010. The other half of the harvested winter wheat was not planted with cover crops; all fields were sprayed with Roundup Ultramax (1.75 L ha^{-1}) mixed with 32% urea–ammonium–nitrate (94 L ha^{-1}) after wheat harvest, 2 days before cover crop seeding. Composite soil samples (six 30 mm-diameter cores, 0–15 cm depth) were collected on 11 November 2010 from six spatially paired sampling sites from adjacent fields with and without the cover crops.

2.4. Soils characterization

Basic soils characterization was performed on composite core samples collected from each of the sites prior to establishment of the cover crop treatments (Sites 1 and 2) or from the no-cover crop fields (Site 3) and analyzed by standard agronomic soil analytical practices at a contract laboratory. Soil pH was determined on a 1:1 soil:water mixture, total organic matter by loss of weight upon ignition, nitrate and ammonium were measured in 2 N KCl extracts by flow injection analysis, P was determined by the Mehlich-P3 method, major cations (K, Ca, Mg, Na) in ammonium acetate extracts, metals (Zn, Fe, Mn, Cu) in DTPA extracts, and boron in hot water extracts.

2.5. AMF inoculum potential

Arbuscular mycorrhizal propagules (spores, infected root pieces, vegetative hyphal fragments) were measured for each plot or sampling site using the most-probable-number (MPN) assay by serially diluting the soils in a sterile 1:1:1:1 (v/v) mix of quartz sand (4030 silica sand, 0.45–0.55 mm diameter, Unimin Minnesota Corp, Lesueur, MN), vermiculite (coarse, grade 2A, Therm-O-Rock, New Eagle PA), calcined clay (Turface All Sport Pro, Profile Products, Buffalo Grove, IL), and sterilized site soil (Douds et al., 2011). Dilution soils were sterilized by autoclaving twice for 60 min at 121 °C with a 24 h break in between. Five levels of serial dilution were performed in triplicate to create a five by three MPN matrix for each soil sample (Woomer, 1994). Bahia grass (*Paspalum notatum* Flugge) was the host species for the MPN assay and was planted into 65 cm^3 pots of the diluted test soils, grown in the greenhouse (day/night: 16/8 h, 25/18 °C) with weekly application of Hoagland's nutrient solution without P, and harvested after 4 weeks of growth. Pots with dilution soils alone were planted for negative controls. Washed roots