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Research Paper

Long-term nitrogen fertilization reduces extraradical biomass of arbuscular mycorrhizae in a maize (*Zea mays* L.) cropping system

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with most plant roots in natural and agroecosystems. The benefit of the relationship to AMF, host plant and soil is realized through the production of extraradical hyphae important to nutrient acquisition and soil carbon (C) storage. Maize grown in rotation with soybeans is a major agroecosystem in the Midwest and northern Great Plains of the United States and its potential to sequester C is the subject of much research. The goal of this study was to determine the impact of crop rotation and N rate on the production of extraradical AMF biomass in a long-term, minimal till field in eastern Nebraska (USA) where maize had been grown continuously (**M**-M; current crop in bold) or in rotation with soybeans (**M**-S; **S**-M) and treated with five nitrogen (N) rates (0, 50, 100, 150 and 300 kg N ha^{-1}) for 12 years. The amount of extraradical AMF biomass was measured in the top 20 cm of soil using fatty acid methyl ester (FAME) analysis of the AMF biomarker C16:1*cis*11. AMF biomass was highest under **M**-M with significant rotation by date by N rate interactions. At peak crop biomass in August the AMF biomarker under 0 N addition declined from 37.5 nmol g^{-1} in **M**-M to 16.7 nmol g^{-1} in **M**-S to 8.0 nmol g^{-1} in soybean following maize (**S**-M). In **M**-M extraradical AMF biomass declined sharply as N rate increased from 0 to 100 kg ha^{-1}. A similar trend was found for **M**-S, but significant only at $p < 0.1$. Declining soil pH with increasing N rate could not account for this difference indicating a more direct effect of N on soil AMF biomass. This is in contrast to maize roots sampled the same year from the same field site where intraradical AMF structures were non-responsive to N rate (Tian et al., 2013). During soybean growth (**S**-M), there was no relationship between soil AMF biomass and the previous year's maize N rate. The effect of crop rotation and long-term N application on the extraradical AMF biomass in soil has implications for nutrient cycling, crop growth promotion, soil tilth, and C storage.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) in soil are obligate biotrophs that form associations with most plant roots in natural and agroecosystems. The fungus obtains all of its carbon (C) from the host plant and produces extensive extraradical mycelia that promotes nutrient uptake from the soil and improves the host's resistance to stress (Smith and Read, 2008). Nutrients known to have a mycorrhizal pathway into the plant are phosphorous (P), nitrogen (N), copper and zinc (Fellbaum et al., 2012; Smith and Smith, 2011). On a larger scale, AMF play an important role in ecosystem processes through the direct effects of their mycelia (Rillig, 2004). Extraradical AMF hyphae contribute to the maintenance of soil structure through the formation of water-stable macroaggregates (Bethlenfalvay et al., 1999; Miller and Jastrow, 2000; Rillig et al., 2010) and there is a strong positive correlation between the amount of AMF extraradical biomass in soil and soil organic C and N content (Wilson et al., 2009). Fellbaum et al. (2012) state that AMF are responsible for "massive nutrient transfer and global carbon sequestration", the latter in part through deposition of the AMF glycoprotein, glomalin (Rillig and Steinberg, 2002). Thus, the benefit of the AMF symbiosis to both plant and soil comes largely from the production of extraradical mycelia (Kabir et al., 1998).

Soil fertility exerts a profound effect on the production of extraradical AMF biomass. The production of AMF hyphae and storage lipids is dependent on C availability from the plant (Gavito and Olsson, 2003). Marshcner et al. (1996) found that nutrient enriched plants allocated less C to roots and AMF, and C allocation to spore and hyphae production is more sensitive to fertilization than C allocation to

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intraradical structures such as arbuscules and coils (Johnson et al., 2003; Tian et al., 2013). The individual and combined effects of soil N and P availability have been shown to influence the production of extraradical AMF hyphae (Edgerton-Warburton et al., 2007; Johnson et al., 2003; Treseder and Allen, 2002). Where P is not limiting, N enrichment generally results in a decline in AMF extraradical biomass in soil (Johnson et al., 2003). This has been demonstrated in grassland (Bradley et al., 2006; Johnson et al., 2003) and in forest (Waldrop et al., 2004; van Diepen et al., 2010) ecosystems. In P deficient soils, N enrichment was shown to increase C allocation to AMF (Johnson et al., 2003) and increase AMF biomass in the soil (Treseder and Allen, 2002).

Where soil fertility is intensively managed as a tool for crop production, the addition of N will impact the extraradical biomass of AMF in soil on a large scale. Maize planted in rotation with soybeans is the largest cropped ecosystem in the mid-western US (Russell et al., 2009) with an average of 135 kg ha^{-1} N applied. Both maize and soybeans have important associations with AMF. Grigera et al. (2007b) demonstrate that AMF are abundant during the reproductive stages of maize and may facilitate P uptake when maize is grown at high yield. Detection of AMF-derived alkaline- and acid-phosphatase and polyphosphate in maize roots provided indirect evidence for P uptake via AMF in highly productive maize agroecosystems (Tian et al., 2013). Frey and Ellis (1997) found that soybean shoot dry weight, root length, shoot P and Zn were increased by AMF colonization. Thus, N rate and crop rotation have the potential to impact the production of AMF extraradical biomass in the soil with implications for soil C storage, tilth and plant nutrient uptake.

In a long-term field trial of continuous maize and maize in rotation with soybean under minimum-till management we found no impact of N fertilization rate or rotation with soybean on AMF colonization of maize roots at any growth stage (Tian et al., 2013). Herein, we use this same field study to determine whether these findings extended to the production of extraradical AMF biomass in soil. The signature fatty acid C16:1*cis*11 (Olsson, 1999) was used to estimate AMF biomass in the soil as it has been shown to correlate well with extraradical AMF hyphae in field soil (Gryndler et al., 2006) and in laboratory studies (Olsson and Johansen, 2000). This biomarker is extracted from neutral, glyco- and phospho-lipids of AMF mycelia and other structures using the fatty acid methyl ester (FAME) method (Drijber et al., 2000; Grigera et al., 2007a). We hypothesized that the amount of AMF biomass in soil would decline with increasing N rate and that the decline would be greater in continuous maize compared to maize in rotation with soybean due to greater overall N fertilizer inputs.

2. Materials and methods

2.1. Study area

This study was conducted from spring 2009 to spring 2010 at the University of Nebraska Agricultural Research and Development Center near Mead Nebraska (N41.2°, W96.5°). Plots were established in 1988 on this field site containing Fillmore (fine, smectitic, mesic, vertic Argialboll) and Sharpsburg (fine, smectitic, mesic, typic, Argiudoll) silty clay loam soils. Current rotations, N rate and minimum till management (plots were disked twice with a tandem disk each spring) had been in place since 1997. Crop rotations sampled for this study were continuous (monoculture) maize (*Zea mays* L.) (**M**-M), maize following soybeans (*Glycine max* (L.) Merr.) (**M**-**S**) and soybeans following maize (**S**-M). The first letter (in bold) represents the crop sampled during the 2009 growing season. In 2010, the soil was sampled prior to planting of the current year's crop, so it is the same crop as what was harvested in fall 2009. Five rates of N (0, 50, 100, 150 and 300 kg N ha^{-1}) were applied to the maize crop in the form of urea on April 22, 2009 and incorporated with a disk. These nitrogen rates have been in place since 1997. Although Bray P is low at this site, P is sufficient to meet crop demand so no additional P was added in 2009. Maize (Pioneer 31N30) was planted on April 23 at a density of 86,487 plants ha^{-1}. Soybeans (Asgrow 3005) were planted on May 7 with a population of 449,732 plants ha^{-1}. Herbicide (Harness Extra, 46.3% Acetochlor, 2-chloro-*N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenly) acetamine, 18.3% Atrazine, 2-chloro-4(ethylamino)-6-(isopropylamino) s-triazine and related triazines) was applied to maize on May 1, 2009 at a rate of 5.39 L ha^{-1}, and post-emergence (Roundup Power Max, Potassium salt of *N*-(phosphonomethyl) glycine 48.7%) was applied to soybean at a rate of 1.61 L ha^{-1} on June 3, 2009. Plots were irrigated on July 8, 9, 27, 28 with an application of 3.2 cm of water each time. As the irrigation water contained nitrates, the contribution was estimated to be 56 kg N ha^{-1} over the growing season. Soybeans were mechanically harvested on 13 October, 2009. Maize was hand harvested for grain yield from two rows in each plot on 16 October, 2009. The remaining maize was harvested by combine after 6 November, 2009. The growing season in 2009 was unusually cool and moist with 54.13 cm of rainfall occurring between April and November. Maize stalks remained standing well into November. Weather information for this site was obtained from wunderground.com for the site KNEITHAC2, UNL Beef Research Feedlot, Ithaca NE. In 2010, maize was planted on May 4 prior to fertilizer application.

This study was set up in a randomized complete block design with three replications per N rate/crop rotation combination. Plots measure 6.1 by 12.2 m with a 6.1 m alley in between each block of treatments and a 9.1 m alley between each replicate.

2.2. Soil sampling and chemical analysis

For measurement of baseline soil properties, soils samples were collected on May 20, 2009 (post N fertilizer application) and May 5, 2010 (pre-N fertilizer application; to assess background pH and EC prior to fertilizer addition). For seasonal measurement of AMF biomass and soil pH, soil samples were collected in 2009 on May 20 (post N fertilizer application, maize at four-leaf vegetative growth stage ~V4), August 24 (maize at reproductive growth stage R2 to R3), and November 6 (post soybean harvest, but pre-maize harvest by combine), and on May 5, 2010 (pre-N fertilizer application) to assess the legacy effect of the previous year and overwintering on AMF biomass and pH at the beginning of the new growing season. Soil samples were taken to a depth of 20 cm using a 2 cm diameter soil probe. Ten soil cores were taken from the planting row in each plot. The samples were composited and stored in a cooler for transport to the lab. The samples were then stored at 3 °C for no more than one week until they could be sieved to 4 mm and frozen at −20 °C for FAME analysis or air-dried for soil chemical analyses. Visible roots were removed at the time of sieving.

Air-dried soil samples were analyzed for pH and electrical conductivity (EC) (Smith and Doran, 1996), total C (Nelson and Sommers, 1996), total N (Bremner, 1996), and Bray P (Bray and Kurtz, 1945; Murphy and Riley, 1962). Baseline soil properties are presented in Table 1.

2.3. Extraction and quantification of AMF fatty acid biomarker C16:1cis11

Microbial fatty acids were extracted from 10 g moist soil using 0.2 M KOH in methanol (Grigera et al., 2007b). This method does not extract the biomarker from AMF spores unless the soil is ground or milled (Grigera et al., 2007a; Olsson and Johansen, 2000). After 1 h at 37 °C, the extracts were neutralized using 1 N acetic acid. The FAMEs were partitioned into hexane, filtered through an Arcodisc® CR 13 mm syringe filter with 0.2 µm PTFE membrane and evaporated to dryness. Dried extracts were resuspended in hexane containing 0.05 mg ml^{-1} methyl-nonadecanoate as an internal standard. All solvents were HPLC grade.

FAMEs were separated using a Hewlett Packard 5890 gas chromatograph fitted with an HP-Ultra 2 (Agilent) capillary column (50 m, 0.2 mm I.D., 0.33 µm film thickness). Helium was used as the carrier