Arbuscular Mycorrhizal Fungi and Glomalin Presence in Fumigated and Natural Soils

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Summary

Arbuscular mycorrhzal fungi (AMF) have strong associations with some crops such as corn (*Zea mays* L.) while other crops have little or no association. This study was conducted to determine whether plant nutrients and yield reduction in corn grown on soil fumigated 19 m prior to seeding was caused by a reduction in AMF. The effects on AMF colonization, glomalin concentration, and grain yield were investigated near New Hradec, ND on a Moreau silty clay loam soil. Percentage of corn roots colonized by AMF were higher in natural soils but no significant difference in glomalin was detected. This study did show fumigation impacted AMF and grain yield. However a larger study including other locations and more replications with the same treatments and tissue testing for nutrient content is needed to make conclusions applicable to a larger area.

Introduction

AMF are ubiquitous in soils and are affected by crop rotations and tillage but their importance in western North Dakota is unknown. Extensive tillage, low cropping diversity and intensity in spring wheat (*Triticum aestivum* L. emend. Thell) – fallow systems has reduce soil organic carbon and soil test nitrogen levels(Peterson, et al., 1998). Wheat following wheat is a common practice on approximately 65% of the area seeded to wheat in southwest North Dakota (McMullen, 2004). Soil-borne root disease is a concern in intensive wheat rotations.

Soil fumigation has been used to reduce the incidence and severity of soil-borne disease in wheat, barley (*Hordeum vulgare* L.), and sugarbeet (*Beta vulgaris* L.) rotations (Cook et al., 1987; Jawson et al., 1993; Eckoff et al., 2003; Ashley et al. 2004). In addition to reducing soil-borne root pathogens, fumigation is also known to reduce beneficial fungi and bacteria (Rovira and Ridge, 1979). Fumigation with methyl bromide (CH_3Br) will temporarily reduce bacteria populations involved which convert ammonium to nitrate and is especially toxic to AMF (Menge, 1982). This fumigant is used to eradicate mycorrhizal fungi from experimental soils and will still severely reduce AMF even after 13 m. Fumigation with CH_3Br has been shown to stunt growth and cause nutrient deficiency symptoms in corn (Jawson, et al., 1993), pepper (*Capsicum annuum* L.) (Haas et al., 1987), and soybean (*Glycine max* (L.) Merr.) (Ellis, et al., 1995).

The objectives of this study was to determine in corn tissue nutrient levels and yield was the result of the elimination or severe reduction in AMF.

Materials and Methods

A field that had been in continuous no-till since 1995 located SW ¼, SE ¼, Sec 26, T141N, R96W near New Hradec, ND was the location for a winter wheat seed treatment trial which included a fumigated soil check. The cropping history of the field prior to fumigation was 1995-2000 spring wheat, 2001 sunflower and 2002 durum. The soil is a Moreau silty clay loam with 42 kg ha^{-1} NO_3-N, 27 mg kg^{-1} P, 545 mg kg^{-1} K, 113 kg ha^{-1} SO_4-S, and 32.5 kg ha^{-1} Cl according to analysis performed by NDSU Soil Testing Laboratory. Organic matter content at the site was 3.6% and pH was 6.9.

A randomized complete block design with six replications of winter wheat seed treatment was established in 2002-2003. Plots were 3 m by 13.7 m. Fumigated plots were covered with six-mil clear plastic sheet with edges buried in trenches 10 to 15 cm deep and CH_3Br in cans were placed in trays and released under the plastic sheet at the rate of 50g m^{-2} of surface area on 16 Sep 2002. The fumigated plots remained covered for 48 hours. After fumigation, winter wheat seed treatment plots were sown on 16 Sep 2002 using no-till methods and harvested 6 Aug 2003. After harvest, the plots and border areas were disked to destroy any unharvested areas of the plots to comply with the protocol established for managing the seed treatment trial.

Urea was broadcast applied 6 May 2004. Corn was planted in 2004 by the producer using a JD MaxEmerge planter equipped with row cleaners on 15 May 2004. The corn variety seeded was Pioneer 3980 and 7.4 Kg ha^{-1} N, 35Kg ha^{-1} P_2O_5, 1.9 Kg ha^{-1} Zn, and 1.9 Kg ha^{-1}

S was applied at planting in a separate band about 1 cm to the side and 1 cm below the seed. The producer contacted the area extension cropping systems specialist and reported corn plants grown in the fumigated plot areas as being "stunted and purpled" on 21 Jul 2004 (Fig. 1).

Corn plants and soil samples were taken on 5 Aug 2004, refrigerated and submitted to the Northern Great Plains Research Laboratory, Mandan, North Dakota for AMF analysis.

Ears from a single 3 m long row was harvested on 5 Oct from each rep of the fumigated plot and a single row of the natural soil plot. Samples were dried and then shelled by hand prior to weighing.

Soil processing

Roots were manually separated from rhizosphere soil using a series of stacked sieves. Soil collected on the bottom two sieves (0.250 and 0.053 mm) was placed on trays at room temperature. Dried soil was subsampled for glomalin extraction. Roots were collected on the top sieve (1 mm) washed with tap water to remove rhizosphere soil, cut into ~2 cm lengths, and mixed to created a homogeneous sampling pool. Half of the sample was placed in a container for storage in water:lactic acid:glycerol (1:1:1) and the other half was processed for mycorrhizal root colonization.

Percentage root colonization

Roots were submerged in 10% potassium hydroxide (KOH) and incubated at room temperature for four days. After removing the KOH, roots were rinsed with reverse osmosis (RO) water until neutralized. A 1% hydrochloric acid (HCl) solution was poured over the roots to acidify them prior to adding the 0.05% trypan blue stain in water:lactic acid:glycerol (1:1:1). After staining, roots were transferred to RO water for storage until colonization was measured by the gridline intersect method (Giovannetti and Mosse, 1980).

Glomalin extraction

Three 2-g subsamples were removed from each air-dried sample and placed into 50 ml tubes for glomalin extraction. Soil was extracted with 8 ml 50 mM sodium citrate, pH 8.0, for eleven 1-hr cycles at 121^{o}C or until the solution was straw-colored (Wright et al., 1996). Following extraction with sodium citrate, soil was extracted with 8 ml 100 mM sodium pyrophosphate, pH 9.0, for six 1-hr cycles at 121^{o}C to remove a recalcitrant fraction of glomalin (Nichols and Wright, 2004).

After each 1-hr cycle, samples were centrifuged at 2500 rpm for 20 min or 3400 rpm for 15 min to pellet the soil and remove extract. All eleven citrate extractions or six pyrophosphate extractions were combined, and the volumes of the citrate or pyrophosphate extractions were measured.

Glomalin concentration was measured by a colorimetric Bradford protein assay. The Bradford assay consisted of adding a Coomassie® Brilliant Blue G-250 dye reagent (Bio-Rad Protein Dye Reagent, Bio-Rad, Hercules, CA) to a measured aliquot of sample diluted to a standard volume (200 ul) in phosphate buffered saline (PBS). The optical density is measured and compared to a standard curve created by known concentrations (1.25 to 5.0 ug) of bovine serum albumin (BSA) diluted in BPS. For samples extracted with pyrophosphate, an aliquot of 100 mM pyrophosphate was added to the diluented BSA standard at a volume equivalent to the volume of the sample diluted in PBS to correct for interference by the pyrophosphate extractant. (Sodium citrate has been checked and does not interfere with the protein dye reagent, but sodium pyrophosphate does.)

Results and discussion

The roots from the natural soil plots were more heavily colonized with almost the whole root filled with mycorrhizal material (hyphae, arbuscules, vesicles, or spores) whereas in the fumigated plots, the level of colonization appeared to be more immature with fewer arbuscules and almost no spores (Fig. 2). This would indicate that these roots were colonized recently, and that fumigation either severely reduced or eliminated the number of mycorrhizal propagules in the soil and/or hindered the germination of these propagules and the subsequent colonization of the corn seedlings. This would explain the stunted growth pattern and the phosphorus deficiency (i.e. purple) of these plants.

There was no difference in either the citrate (Fig. 3) or pyrophosphate (Fig. 4) extracted glomalin between the fumigated and natural soil treatments. This is not surprising because the concentration of glomalin in the soil is something that builds up over time and is not always measurable the first year after treatment application. Converting from conventional till to no-till or changing in cropping history, such as a

not very complex rotation, the use of a non-mycorrhizal crop or reducing cropping intensity by including a fallow may result in a significant change in glomalin concentration. In addition, mycorrhizal colonization levels are typically higher later in the season and plants sampled at this time may show differences in glomalin levels.

Corn grain yield (Table 1) was significantly less for corn grown on fumigated soils compared to corn grown on natural soil. Jawson et al. (1993) found a reduction in corn grain yield and a visual and tissue analysis P deficiency was associated with low mycorrhizal root infection even with adequate soil P. In our plots we noted a distinct purpling pattern characteristic of phosphorous deficiency (Fig. 1B) but this was not verified with tissue analysis.

Wheat yield the previous year in fumigated plots yielded significantly more grain than the natural soil check plots (Ashley et al., 2004). The reason for this may be attributed to two factors: 1. when wheat is grown without rotation, disease levels will be relatively high compared to those found after fumigation or diverse rotations (Cook, 1990; Cook and Veseth, 1991) and 2. wheat has a low dependency upon AMF compared to corn and therefore yield loss as a result of eliminating or severely reducing AMF does not occur. Mozafar et al. (2000) suggested root pathogens need to be taken into consideration in mycorrhizal studies and the lack of yield differences in wheat indicate that eliminating disease propagules in soil using CH_3Br may have little risk.

This study did show that fumigation may impact mycorrhizal colonization and has the potential to impact long-term accumulation of glomalin. A larger study including fields at other locations with the same treatments as well as a larger sampling pool and sampling throughout the season including plant tissue analysis for P may give a better picture of the impact of fumigation on crop yield, AMF, glomalin, P absorption, and the structure and stability of these soils.

Implications of Demonstration

This demonstration illustrates the important relationship of AMF, a fungus, has with corn. When AMF is eliminated or occurs at low levels in soils in southwest North Dakota, corn develops phosphorous deficiencies which limit growth and yield. Practices such as rotations with AMF host crops and no-till are expected to improve productivity of fields compared to the mono-culture wheat and wheat-fallow systems.

Acknowledgments

We would like to thank Ryan Kadrmas, Dickinson, ND for observing and reporting his observations on corn growth in his field and then allowing us to collect samples for this study.

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Table 1. Grain yield of corn grown on fumigated and natural soil plots, New Hradec, ND, 2004.

Treatment	Grain yield
	kg ha-1
Natural	4019
Fumigated	956
Mean	2487
CV%	7.5
LSD 0.05	277

Figure 1. Corn plants in a field where the soil was fumigated (three rows in the center) or not fumigated (outer rows) (A) showed signs of phosphorus deficiency (B) which may be linked to the level of mycorrhizal colonization.

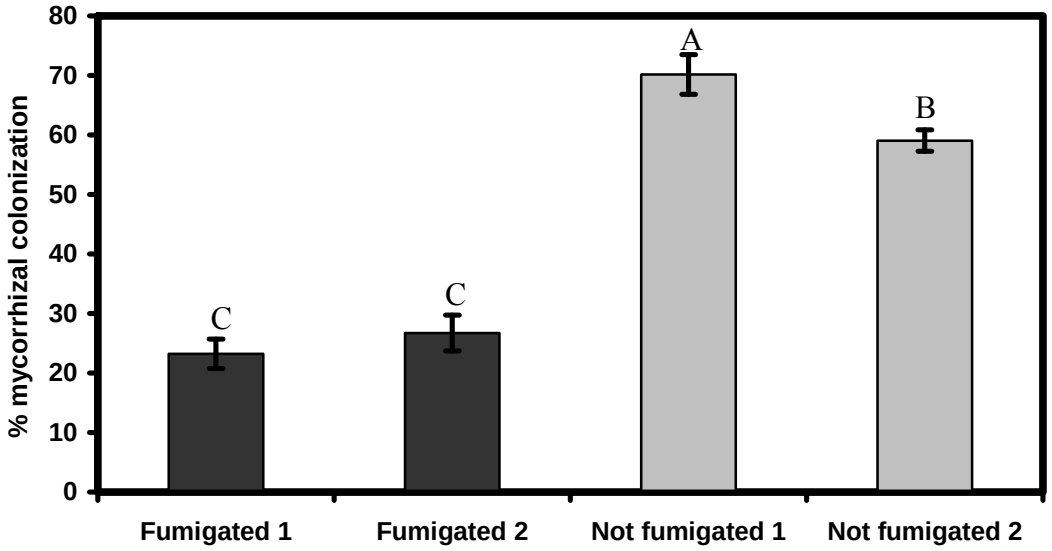

Figure 2. Percentage root colonization by arbuscular mycorrhizal fungi measured in corn grown in soil that was fumigated or not fumigated. Bars (means ±SE) with different letters are significantly different ($P \leq 0.01$).

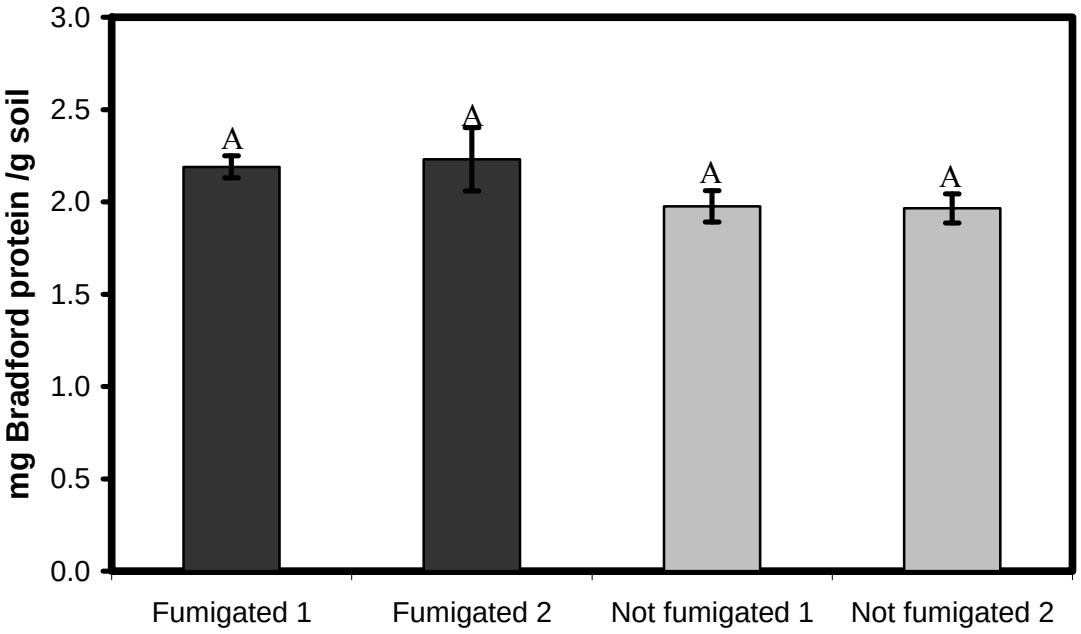

Figure 3. Concentration (mg g^{-1}) of Bradford measured glomalin extracted with 50 mM sodium citrate, pH 8.0, at 121°C from soil that was fumigated or natural soil plots prior to planting corn. Bars (means ±SE) with different letters are significantly different ($P < 0.05$).

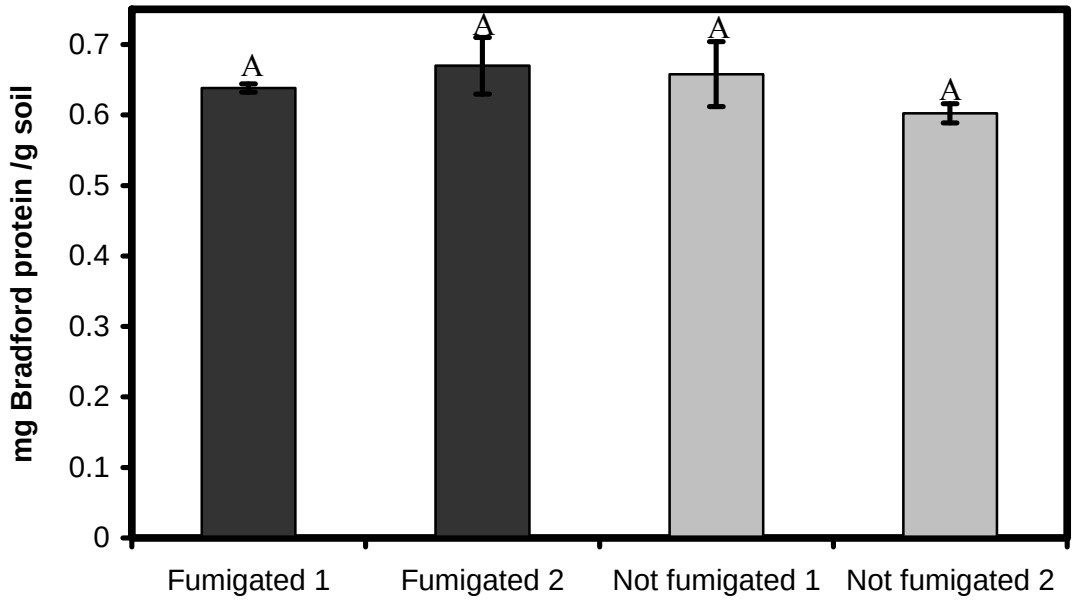

Figure 4. Concentration (mg g^{-1}) of Bradford measured recalcitrant glomalin extracted with 100 mM sodium pyrophosphate, pH 9.0, at 121°C from soil that was fumigated or natural soil prior to planting corn. Bars (means ±SE) with different letters are significantly different ($P < 0.05$).