Response of Arbuscular Mycorrhizae to Soil Test Based Phosphorus Fertilization of Corn (*Zea mays* L.) in Eastern Nebraska

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Introduction

Abuscular mycorrhizal fungi (AMF) in soils and plants are dynamic, responding to environmental factors, crop growth stage, crop rotation and nutrient management. In corn grown at high yield, AMF are thought to facilitate P acquisition during reproductive growth (Grigera et al., 2007), a period of high P demand. In many parts of the Midwest corn belt, soils test low for Bray-1 P and additional P is added to boost P levels for maximum yield. Unfortunately, it is unknown whether maintaining a high soil test P is important to yield in enough years to justify the cost. High soil P can also reduce AMF colonization of corn roots and may lead to reduced P uptake via AMF. To investigate whether AMF are impacted by P fertilization of low P soils an experiment was initiated in 2011 on a silt-loam soil in eastern Nebraska with Bray-1 P levels averaging 5 ppm in the top foot of soil. Corn roots under no-till and reduced tillage were sampled in August 2011 and analyzed for percent colonization, spore density, phosphatase activity, AM biomass using fatty acid methyl esters (FAMEs) and community composition using PCR-DGGE.

Materials & Methods

1. Experimental design

1) Site management: Nitrogen and nutrients other than soil P were managed according to UNL recommendations. Hybrid choice, weed and pest management, and irrigation were in consultation with the respective site manager.

2) Main plot treatments: no-till compared with reduced tillage, e.g. disk tillage.

3) Sub-plot treatments:

(1) P0: no additional P applied;

(2) P15: UNL recommended annual application for continuous corn to maintain Bray-1 P > 15 ppm;

(3) P25: annual application to maintain Bray-1 P at 25 ppm. Each sub-plot replicated three times.

4) Soil and root sampling: composite of eight soil cores (1.5 x 8") plot at R3 stage in August 2011.

5) Measurements:

(1) AMF spore density in soil.

(2) The concentration of AMF biomarker, C16:1*cis11* in soil (soil FAME).

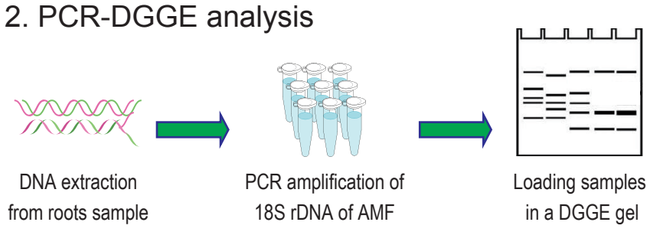
(3) AMF colonization of roots.

(4) ACP and ALP activity in roots (Data is not shown).

(5) The concentration of AMF biomarker, C16:1*cis11* in roots (root FAME).

(6) AMF communities in roots using PCR-DGGE.

2. PCR-DGGE analysis



DNA extraction from roots sample → PCR amplification of 18S rDNA of AMF → Loading samples in a DGGE gel

1) PCR : Nested PCR (Liang et al., 2008).

2) Primers:

(1) The AMF fungal specific primers NS31 and AM1 (Helgason et al., 1998) in first round PCR.

(2) NS31-GC and Glo1 (Cornejo et al., 2004) in second round PCR.

3) DGGE conditions:

(1) Performed with the DCode Universal Mutation Detection system (Bio-Rad, CA, USA).

(2) Denaturing gradients ranged from 35 % to 55 %.

(3) Separated by electrophoresis at 50 V for 16 h at a constant temperature of 55 °C.

(4) Gels were stained for 20 min in an 1: 10,000 SYBR Green solution.

3. AMF community analysis

(1) All statistical analysis were performed using the vegan package version 2.0-4 in R 2.15.1.

(2) Shannon–Wiener diversity index (*H'*).

(3) Redundancy analysis (RDA).

(4) A presence - absence matrix was built for statistical analyses.

Results

Table 1 AMF parameters in corn roots and soil at three soil P levels.

Tillage	Soil P levels	AMF colonization (%)	AMF spore density (No. g^{-1} dry soil)	Root FAME (mmol g^{-1} dry root)	Soil FAME (nmol g^{-1} dry soil)
No-till	P0[1)]	37.8	18.7	1.02	7.0
	P15[2)]	25.8	13.5	1.02	5.7
	P25[3)]	13.2	5.1	1.00	7.2
Tilled	P0	26.8	12.4	1.83	6.5
	P15	20.5	8.7	1.34	6.3
	P25	8.6	3.0	1.39	7.8
	Soil P levels (A)	***	***	ns	ns
	Tillage (B)	***	***	ns	ns
	A x B	*	*	ns	ns

ns, not significant.
*, *** significant at $P < 0.05$ and $P < 0.001$, respectively.

1) P0: no additional P applied
2) P15: UNL recommended annual application for continuous corn to maintain Bray-1 P > 15 ppm.
3) P25: annual application to maintain Bray-1 P at 25 ppm.

AMF colonization and spore density tended to decrease with increasing soil P level (Table 1) while the concentrations of root and soil FAMEs remained unchanged. The results of two-way ANOVA showed soil P levels, tillage and their interaction affected AMF colonization and spore density, but not root and soil FAMEs.

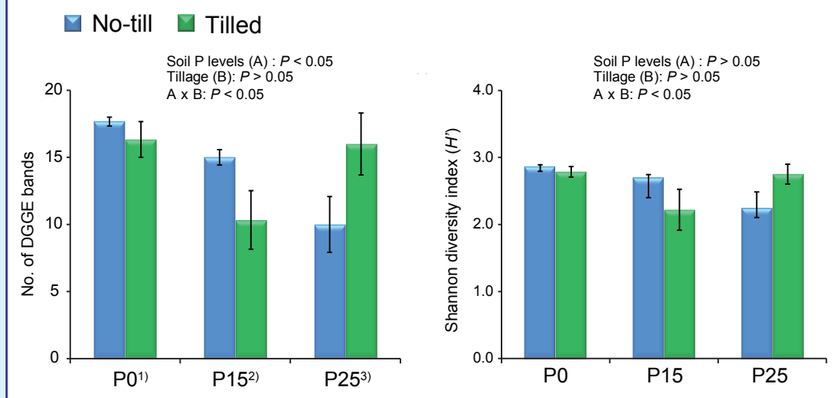


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Fig. 1. The number of DGGE bands and Shannon diversity index (*H'*) of AMF communities in the corn roots at three soil P levels. Vertical bars represent the mean (±S.E).

The number of DGGE bands decreased with increasing soil P level under no-till (Fig. 1), but not under reduced tillage. The Shannon index was similar among P levels and did not respond to tillage. The results of two-way ANOVA showed soil P levels, or their interaction affected the number of DGGE bands and Shannon index.

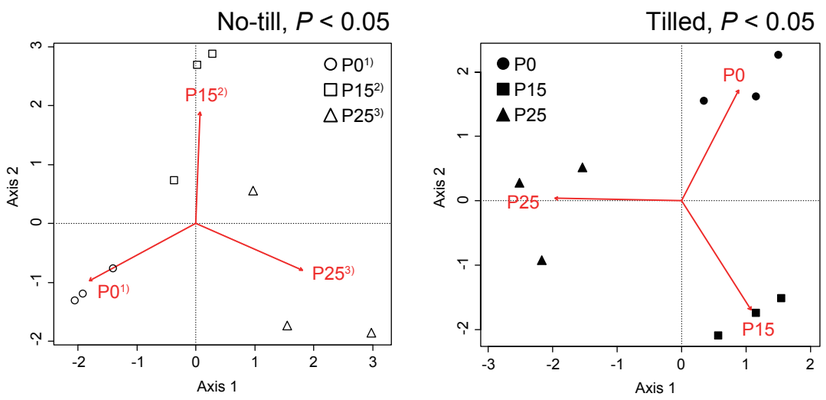

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Fig. 2. Redundancy analysis (RDA) biplot showing the relationship between AMF communities in corn roots and soil P levels under differing tillage.

RDA was used to identify relationships between AMF communities in the corn roots and soil P levels under no-till and reduced tillage (Fig. 2). A Monte-Carlo permutation test indicated that the relationship between the two datasets was significant under both no-till ($P < 0.05$) and reduced tillage ($P < 0.05$). Soil P level contributed significantly to the variation in AMF communities in corn roots under both tillage systems (Fig. 2).

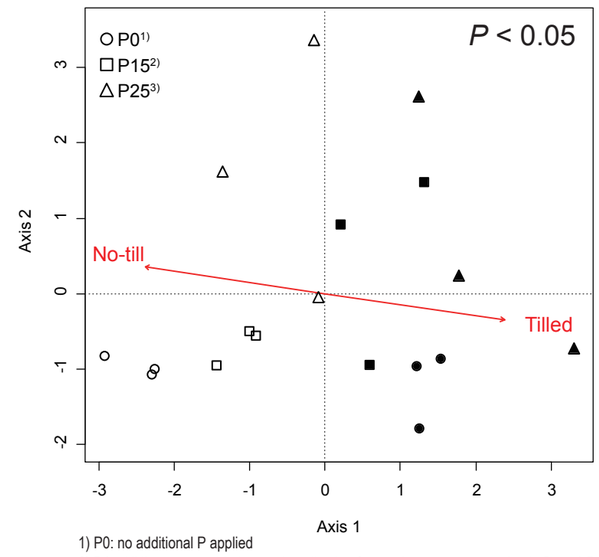

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Fig. 3. Redundancy analysis (RDA) biplot showing relationship between AMF communities in the corn roots and tillage management. Open symbols and black closed symbols represent no-till and reduced tillage, respectively.

RDA was used to identify relationships between AMF communities in corn roots and tillage management (Fig. 3). A Monte-Carlo permutation test indicated that the relationship between the two datasets was highly significant ($P < 0.05$). Tillage contributed significantly to the variation in root AMF communities (Fig. 3).

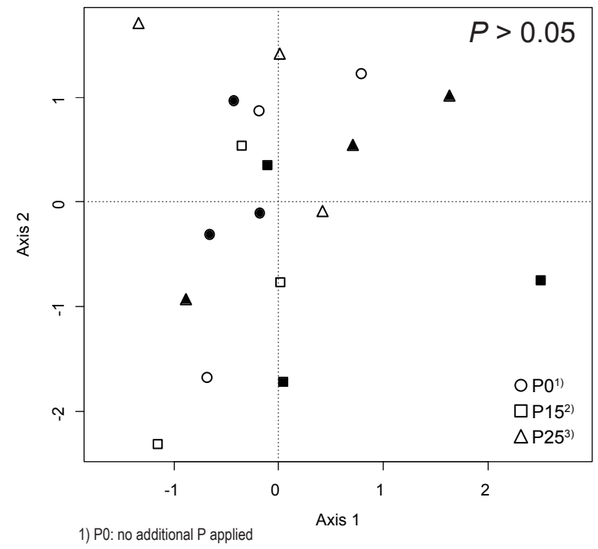

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Fig. 4.Redundancy analysis (RDA) biplot showing relationships among corn grain yield and yield components (dataset 1, biplot), and measured variables (dataset 2) and soil managements (dataset 2). Open symbols and black closed symbols represent no-till and tilled plots, respectively.

RDA was used to identify relationships between corn grain yield and yield components, and measured variables, soil managements (Fig. 4). A Monte-Carlo permutation test indicated that the relationship between the two datasets was not significant ($P > 0.05$). In addition, measured variables and soil managements did not contribute significantly to the variation in corn grain yield and yield components.

Conclusion

1. Root and soil FAMEs were unchanged under differing soil P levels and intensity of tillage.
2. Soil P level and tillage altered AMF communities in the corn roots.
3. Overall, no clear link could be established between corn grain yield, and measured variables and soil management despite alterations in AMF communities. Thus, further investigation into the functional aspects of AMF communities would be required to clarify relationships among soil management, corn growth, and P flow via AMF between soil and crop.

References
1) Grigera et al., 2007. Soil Biology & Biochemistry 39, 1401-1409.
2) Liang et al., 2008. Soil Biology & Biochemistry 40, 956-966.
3) Simon et al., 1992. Applied and Environmental Microbiology 58, 291-295.
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