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Impact of Fertilizer, Corn Residue, and Cover Crops on Mycorrhizal Inoculum Potential and Arbuscular Mycorrhizal Fungi Associations

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Influence of forage radish or annual ryegrass cover crops, corn residue removal, and fertilizer type on mycorrhizal inoculum potential

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Introduction

Rationale:

- Productivity, soil structure, nitrogen and phosphorus uptake, and pathogen resistance improve when agricultural crops associate with **Arbuscular Mycorrhizal Fungi (AMF)** (4). The presence of AMF can be assessed through bioassays or *in situ* measurements.
- The majority of crops and cover crops (e.g., corn, soybean, annual ryegrass) form associations with AMF, while a few species (i.e., forage radish) do not (2,9). Little research has been done to assess if cover crops like forage radish impact AMF.
- AMF die or become dormant without a host (5). Harvesting corn residue may impact overall soil biology, but specific impacts on AMF have not been studied.
- Big bluestem, a native prairie species, is an obligatorily mycorrhizal plant, which will not survive to reproductive maturity without being associated with mycorrhizal fungi in soil (10,11). Manure and commercial fertilizer application may impact these associations (12), but little research has established the impact of fertilizer form or rate on AMF associations with big bluestem.
- Therefore, three studies were designed to assess impacts on AMF: 1) cover crop assessment, 2) residue removal assessment, and 3) fertilizer assessment.

Hypotheses:

1) Annual ryegrass cover crops will have a higher soil **Mycorrhizal Inoculum Potential (MIP)** compared to forage radish or no cover.
2) Leaving all corn residue (No removal) in place will have a higher MIP compared to aggressively harvesting corn (Full removal).
3) Full or half recommended rates of fertilizer application will decrease AMF associations with big bluestem roots.

Methods

For each study, bulk soil was collected from the surface 0-6" (0-15 cm).

1) April 2016 from a wheat field with forage radish, annual ryegrass, and no cover crop treatments
2) October 2015 from plots with or without history of corn residue removal, both in the soybean phase of the rotation
3) May 2016 from a perennial system with big bluestem

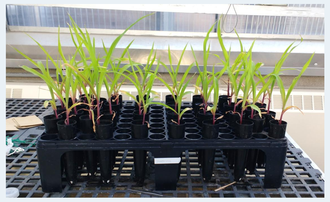

Figure 1. Corn growing in field collected soil for MIP bioassay

Figure 2. Collecting field grown corn to look for presence of AMF

Soil Mycorrhizal Inoculum Potential (MIP):

Bioassay (1,3) with bulk soil:

- In greenhouse, grow corn (studies 1 & 2) or big bluestem (study 3) ~30 days (Fig. 1)
- Remove roots from soil
- Clear with 5% KOH and stain with Trypan blue (6) to preferentially stain fungal arbuscules, hyphae and vesicles within root tissue (Fig. 3)
- Count AMF using grid-line intersect method

In situ MIP assay of field grown plants

- Collect corn at 3-4 leaf stage (June 2016) (Fig. 2) and big bluestem at maturity (September 2016)
- Separate and wash soil from roots
- Clear, stain and count AMF as described for bioassay

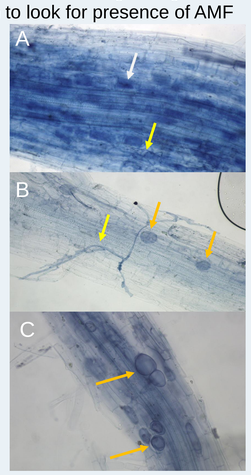

Figure 3. Corn roots infected with mycorrhizae, a. arbuscules (white arrows) and hyphae (yellow arrows), b. vesicles (orange arrows) and hyphae, and c. vesicles.

Statistical analysis were run in software program R (8):

- Equality of variances and normality were tested with Bartlett and Shapiro-Wilk
- Hypotheses were tested with two-way ANOVA with repeated measures using treatment and time as fixed effects

Results

Cover crop assessment

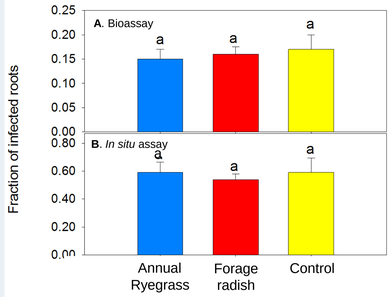

Figure 4. Fraction of infected roots across treatments from the a) bioassay and b) *in situ* assay.

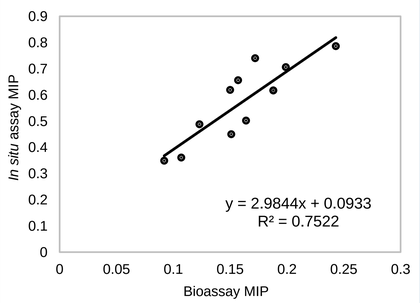

Figure 5. Regression of fraction of infected roots from bioassay versus *in situ* assay.

- No differences occurred among treatments within either assay (bioassay (p = 0.90), *in situ* assay (p = 0.88)), but fraction of infected roots were significantly different between assays (p < 0.001) (Fig 4). Regression indicated a positive correlation between the two assay methods (Fig 5).

Residue removal assessment

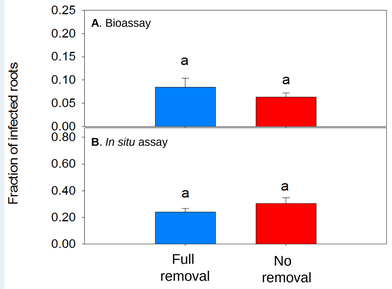

Figure 6. Fraction of infected roots across treatments from the a) bioassay and b) *in situ* assay.

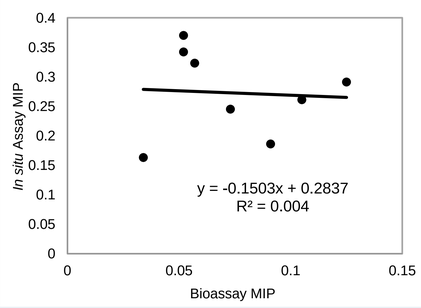

Figure 7. Regression of fraction of infected roots from bioassay versus *in situ* assay.

- No difference in fraction of infected roots between treatments in either assay (bioassay (p = 0.25), *in situ* assay (p = 0.46)), but a significant difference between assays was found (p < 0.001) (Fig. 6). Regression indicated fraction of infected roots from both assay methods were uncorrelated (Fig. 7)

Fertilizer assessment

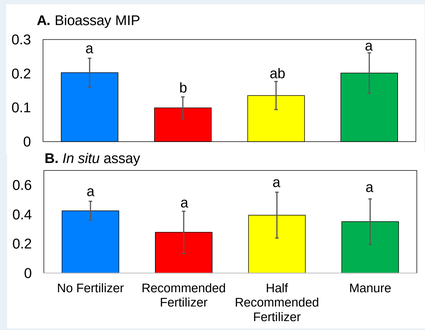

Figure 8. Fraction of infected roots across treatments from the a) bioassay and b) *in situ* assay.

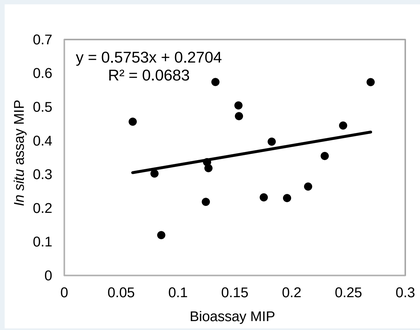

Figure 9. Regression of fraction of infected roots from bioassay versus *in situ* assay.

- A significant difference in fraction of infected roots occurred among treatments in the bioassay (p = 0.015), but not among treatments using an *in situ* assay (p = 0.47). Also, fraction of infected roots were significantly different between the assays (p < 0.001) (Fig. 8). A weak, positive correlation between the assay methods was indicated by regression analysis (Fig. 9).

Discussion

Cover crop assessment:

- Forage radish and no cover crop did not result in a lower soil MIP than annual ryegrass, suggesting that these treatments did not influence the mycorrhizal community. This is surprising given the amount of evidence supporting ryegrass associations with AMF (2,7,9). These results may reflect the short term nature of the study.
- MIP values from the spring *in situ* assay were significantly greater than the bioassay from soil samples taken earlier in the spring. There are two possible explanations:
  1. MIP is influenced by different assessment methods (bioassay vs. *in situ*).
  2. Possible timing/seasonal effects.
- A five-yr cover crop study in Japan showed that AMF associated with soybean regardless of cover crop treatment, suggesting that temperature or other environmental factors played a bigger role (4). Since corn was planted in the warmer spring months, it may have become a host for dormant AMF, thus positively influencing soil MIP (5).

Residue removal assessment:

- This study provided no evidence to support the hypothesis that residue removal influences MIP.
- MIP values from the spring *in situ* assay were significantly greater than the bioassay from soil sampled in the fall after soybean harvest. These results suggest that seasonal variables altered soil MIP.
- Fall soil conditions, such as death and dormancy of AMF after harvest, inhibit inoculum potential of the soil, and these conditions likely changed after corn is planted and AMF break dormancy (5).

Fertilizer assessment:

- This study provided evidence that different fertilizer treatments influenced AMF associations with big bluestem.
- MIP values from the fall *in situ* assay were significantly greater than the bioassay soil sampled in the spring. This may be explained by a greater time period allowed for colonization. Compared to corn *in situ* methods, which were sampled at juvenile or after 30 days of growth, big bluestem *in situ* samples were sampled in the fall at full maturity.

Conclusion

The assay method and sampling time had a greater impact on MIP compared to either cover crop or residue management. Sampling both soil and *in situ* corn roots at the same time needs to be done to determine if the observation is due to methodology or to conditions when soil was collected. Fertilizer rate and type influence big bluestem associations with AMF. Method or timing also had a significant impact on MIP.

References and Acknowledgements

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