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Characterizing Arbuscular Mycorrhizal Associations in Corn Inoculated with Soil from Till or No-Till Farmlands

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Abstract

Many crops have mutualistic arbuscular mycorrhizal fungi(AMF) associations that positively affect plant and soil health. AMF likely mitigates agricultural problems like topsoil depletion and compaction from tilling. The literature review on AMF associations under different practices showed the need for more studies. This project studied an AMF community's impact on soil quality and plant health in conventional till(CT) and no-till(NT) managed corn and soybean plants. Data was gathered from these fields in West MI, using field soil to inoculate greenhouse plants in normal/drought conditions. Three types of data were collected for each case: 1)AMF community via spore type and presence, 2)Soil structure via aggregate size and nutrient quantity, and 3)Plant productivity via root-shoot biomass ratio and structure. This study will provide an update of our ongoing data collection and analysis. This study aims to describe the conditions where AMF benefit crop production and soil quality locally.

Background

One group of fungi known as arbuscular mycorrhizal fungal (AMF) symbionts have a large impact on both plant productivity and soil health in agriculture [Citation]. AMF have a direct connection to the plant via internal hyphal networks and have a direct impact on the soil environment via external hyphal networks. AMF have been shown to have a positive effect on drought tolerance, pathogen resistance, and nutrient uptake in the plants that they associate with, while also mitigating topsoil depletion and compaction of the soil. This extraordinary plant-fungal partnership seems to hold solutions for major agricultural problems including soil erosion.

One of the major agricultural practices that can adversely affect the benefits of AMF associations is tilling. Tilling is the mechanical disturbance of soil before planting, that has been shown to increase soil compaction, water runoff, and topsoil erosion [Citation]. Tilling breaks the hyphal network in the soil, severely stunting the growth of these fungi each planting season. These fungal networks naturally break through compacted soil, forming aggregates that stabilize the soil, reduce compaction, and increase organic matter and improve overall health of the soil. AMF formed aggregates are made through the glycoprotein, glomalin, a hyphal exudate that works like a glue to form small clumps in the soil. Aggregates are harder to wash away than loose soil, and naturally aerate the soil as the clumps leave pores that help allow better water infiltration and water holding capacity. AMF hyphal glomalin is an important part of the benefit of these fungi to agriculture and for soil health, but is still poorly understood.

Approach

In the Fall of 2019 we set out to better understand AMF, the effects of tilling, and the effect of AM associations on crops under normal and drought conditions in our local community of West Michigan. We spent Fall learning about AM fungi, discussing current literature, and developing our research project. We planned to monitor and sample two local fields with different tilling practices, one with a history of reduced tillage and the other with a history of intensive tillage. These samples would then be used to inoculate sterile soil and grow crops under controlled conditions in the greenhouse, allowing for testing of the effect that these inoculants would be able to have on the growth of plants under different watering conditions(see Appendix 1: Winter 2020 proposal).

While many frameworks and intentions were laid out, by the time the 2020 growing season had arrived, the pandemic of Covid-19 was upon us as well. Due to quarantines and

shutdowns of campus leading to limited access, remote work became necessary. This brought about a literature review, compiling existing information into a database that could then be analyzed. Additional pot experiments were also conducted at my personal residence to limit contact with individuals during this season.

Materials Methods

Field Work

Initial sample collection was done at my uncle's family farm mid-June when the field crops were [___] old. Under the supervision and aid of Graduate student Derek Bennett, composite random sampling was done at six locations in each field and each location was tested in triplicate for four different variables: water infiltration rates, soil compaction, soil moisture levels, and the collection of soil cores. Water infiltration rates were determined by inserting a 3 inch diameter PVC pipe 2 inches into the soil and measuring the time required for 1 inch of rainfall, simulated by 115mL of water, to filter through the soil. Soil compassion was determined using a compaction probe that recorded compaction levels every 3 inches as the probe was inserted into the ground. Soil moisture was determined by a moisture meter probe that went 3 feet into the soil. Soil cores were taken by a 1.5 inch diameter PVC pipe inserted 7.9 inches (20cm) into the ground. Triplicates of cores from each location were compiled and stored for later use.

Apartment Pots

After having exposure to a COVID-19 positive individual, it became necessary to have a remote plant study for me to participate with while quarantined. Accounting for space limitations at my apartment, 24 pots seeded with corn were grown on my porch. Potting soil and sand were autoclaved for sterility, before being mixed 1:1 by weight and put into each of 24 greenhouse pots. Pots were grouped and numbered by 3 variables, crop type, inoculant type, and watering conditions. Three seeds were planted in each pot at recommended planting depths. A volume of inoculum, or sterile soil for the controls, with approximately 2.5% weight of the total pot was added to each container. To account for microclimate variations, the potted seeds were arranged 4 X 6 in randomized order (via random number generator). All 24 pots were thoroughly soaked with water. As seedlings emerged, they were thinned to 1 plant per pot. After 1 month from planting, we began inducing drought conditions.

Figure 1 Grouping of Treatments

	Corn Plant, 1-24
CT, WW	1-4

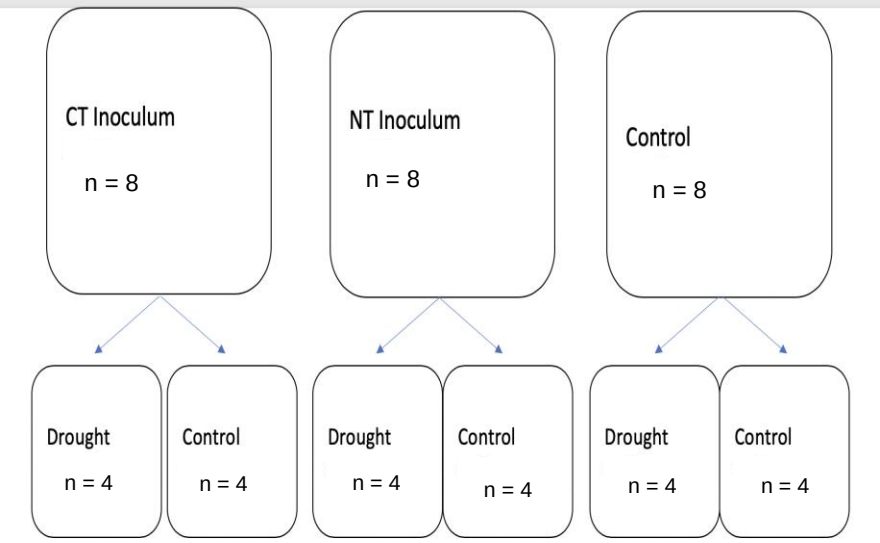


Table 1 Pot numbers and Condition Grouping

WW	9 -12
Control, D	13 -16
NT, D	17 - 20
CT, D	21 - 24

Harvesting of Plants

After two months of growth, pots were destructively harvested and analyzed. Shoot and root systems were separated, and soil was removed from the root systems and stored. We measured the length of the main stem, the diameter at the base of the stem, the above and below ground biomass at harvest, the above and below ground biomass when dry (1 week after harvesting), and if the plants had reached tassel stage or not.

Spore Counts

One way to characterize AMF in soil is by spore counts and morphology identification. Soil samples from the sampling locations for both the NT and CT field were prepared for spore extraction and counting. 200 grams for each CT and NT were shaken for 15 minutes through a series of stack of mesh sieves in the following top-down order: 4000 µm, 2000 µm, 500 µm, 250 µm, 125 µm, 63 µm, bottom (solid pan). The soils from the bottom and lowest 3 filters were pooled.

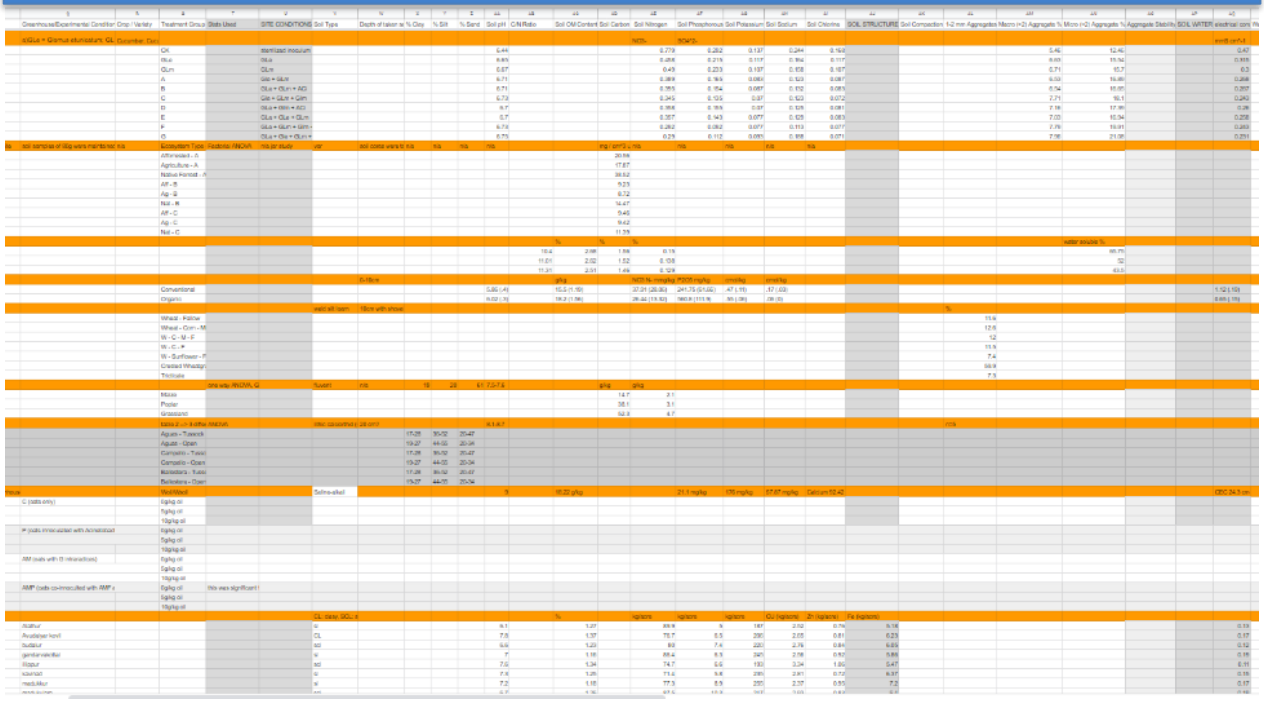
Two glucose solutions (60% and 20% table sugar) were made and carefully added to the same 50mL microfuge tube so that a gradient between the two solutions was still visible. 15mL of the filtered and pooled soil was added to each microfuge tube before being centrifuged. Sand, silt, and heavy organic matter was pushed into a pellet, while the supernatant contained the spores. To analyze large and small spores, the supernatant was again sieved through the 63 µm filter and a sterile coffee filter (approximately 20 µm) above the pan. The material on the filters was washed with DI water to remove the glucose solution and collected into separate petri dishes.

A total of 12 plates were made, three plates from NT 63 µm mesh, three plates from NT 20 µm mesh, three plates from CT 63 µm mesh, and three plates from CT 20 µm mesh. During microscopy each plate was placed on top a template with three, 1mm square areas. The area inside these boxes were meticulously analyzed for spores, which were counted and morphological characteristics were described.

Literature review

As the pandemic continued to complicate the plans our team had made for the summer, a transition to remote work was made. Our team determined that a comprehensive review of existing literature could be done, and we began to gather articles. To compile literature for this review we needed to find relevant journal articles that would provide data we could evaluate. Articles were determined viable by the presence of information around four general topics: Nutrients, Soil Structure, Glomalin, and AMF characteristics. Twenty three articles were found to have information on these topics to varying degrees. The data of these articles was sorted into 81 different defined factors in a large spreadsheet to give us the raw data from these relevant

Table 2, Excerpt from Compiled Database



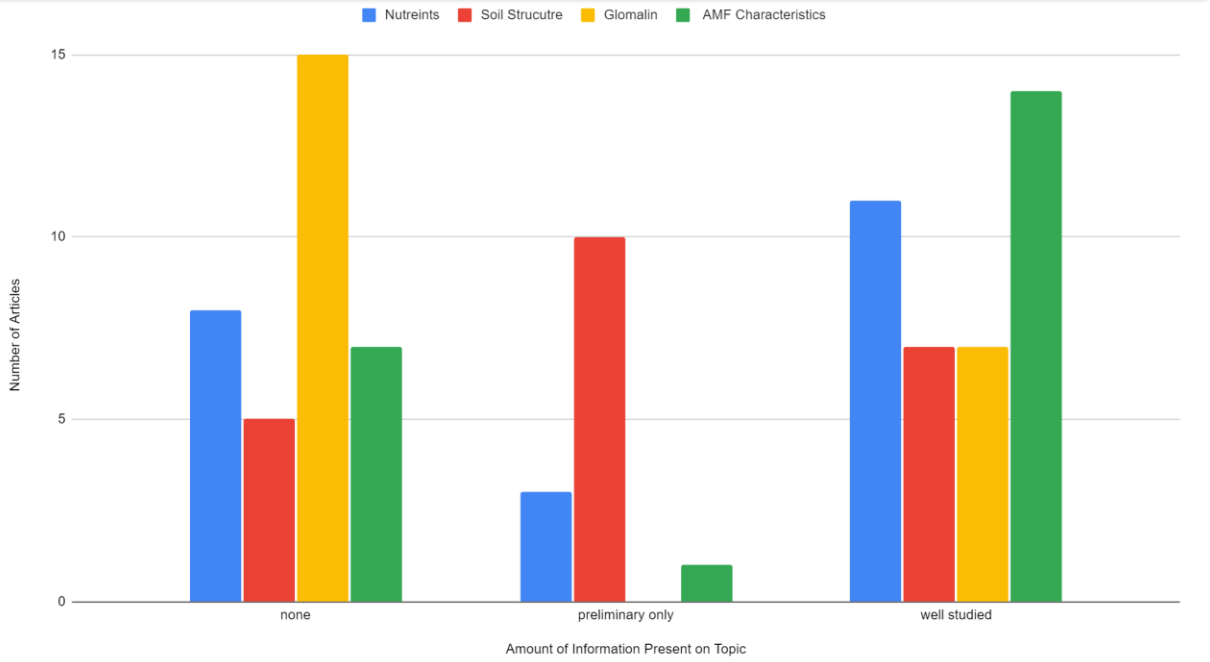
Results and Discussion

Literature review

While compiling articles for the literature review it became evident that many articles were very specific in the data they analyzed. It was common for an article to focus heavily on a few topics but have little to no data for many other aspects. Comparing data became increasingly complicated as some articles provided only preliminary data (before experimental conditions) while other articles monitored these factors as part of the study (Figure 2). As each study tested and analyzed different plants, fungal species, and used different experimental conditions, there was difficulty in organizing the raw data into something more concise. To further complicate the matter, the ways in which factors could be recorded also varied greatly. Many articles described how levels of specific compounds changed during the course of the experiment, with some studies analyzing compounds in the soil and others determining these values in plant tissues, while others yet determined these in the fungal tissues. Even when multiple studies had determined the content of a compound in the same locations, they would

report these numbers differently in regards to the form that the specific compound was in, as each study used a different extraction method that analyzed a specific form of the compound in question. This occurred often with many of the compounds we were studying; including glomalin, soil organic matter, and limiting nutrients like Nitrogen, Phosphorus, Potassium.

Figure 2: Article Information on Key Topics



While the data compilation was beneficial in my learning and expanded my understanding of the different ways that many of the factors we studied could be analyzed and affected, there was overall not enough similarity or consistency between studies to make accurate comparisons.

Field and Greenhouse Work

This study gave me the opportunity to engage with my community and work on communicating many scientific concepts in an accessible and meaningful way. Discussions on the topic of agriculture with people inside and outside of the scientific community broadened my understanding of how the many local traditions, scientific techniques, and the intermingling of the two form the basis for agriculture today. As the local community partner was also my Uncle, I was able to learn information about the business side of agriculture and how that impacted decisions everyday in his work, while also understanding the emotional aspect of his dedication to the farmland that our family has been working for multiple generations. This understanding will benefit my ability to further engage with farmers in communities around the world in my humanitarian efforts, being mindful and accepting of the beliefs and techniques that these people have been using, while also working with them to implement scientifically backed sustainable practices.

The time Derek and I spent working in the field and my time working with these research plants taught me about how to manage my time and duties in a way that was efficient but also

was mindful of the extremely hot and humid conditions we worked in, preventing literal burnout. Experiences in the greenhouse, while limited because of the pandemic, were beneficial for understanding the nature of greenhouse work. I gained valuable experience learning how to handle and care for plants with all the variables we worked to control, how to set up multivariate studies, and a better understanding of the growth of our two study crops. This work helped solidify my desire to pursue a career in agriculture and I am confident that these experiences have set me up with the skills I need to be successful in agricultural settings, both in field and in greenhouses.

Pandemic Planning and Future Work

Dealing with the pandemic caused us to manifest flexibility and graciousness as we dealt with constantly changing regulations and social distancing standards. It forced much of the laboratory aspects planned for this study to not be able to be performed in the time this study was supposed to occur. As a result of this, there is still further work to be done. The soil collected from the field and from all of our greenhouse plants is still stored and will later be analyzed for glomalin levels via the Bradford extraction method. Spore counts from the soils saved after harvest can also compared to the spore counts done earlier. The roots preserved can be rehydrated and evaluated for infection percentages. I plan to continue this work for my honor's thesis in CMB this winter.

Acknowledgements

This research was made possible with the help of my Uncle Ernest Sagman, who generously allowed his farmland to be studied and used for this research. Much of this work was done with the support of Derek Bennet, who was a graduate student. None of this would have been possible had it not been for my research mentor, Dr. Jennifer Winther. We received funding from GVSU's Office of Undergraduate Research Studies.

Appendix 1

S3 Winter 2020 Proposal for Project
Title: Characterizing the identity of fungal symbionts and their impact on soil glomalin levels in till and no-till corn fields.
Student: Catherine DeFouw
Faculty: Dr. Jennifer Winther
Project Goals - Most plants have a close association with fungi in their roots called an arbuscular mycorrhizal (AM) association. An AM association is a mutualistic symbiosis where the plant benefits by gaining access to limiting mineral nutrients from the fungus, and the fungus receives food in the form of sugars from the plant. In addition to providing required minerals to their plant host, AM associations have been shown to increase drought tolerance, provide pest resistance, increase plant growth, facilitate establishment of seedlings, and improve soil structure [1, 2, 3, 4]. Given these benefits (and many others), there has been growing interest in understanding AM associations in agricultural systems, in particular the impact of different agricultural management practices like till vs. no till on AM associations [5,6,7, 8]. The ultimate goal of these studies is to determine if, when, and how AM associations increase crop production and maintain (or even increase) soil quality over time [9].

Many current agricultural practices, including tilling, have resulted in major losses of topsoil (what plants grow in) and decreasing topsoil quality [10,11]. AM fungi play an important role in topsoil quality. AM fungi have a protein glomalin in their hyphae that is known to account for up to 60% of the organic component in a soil and AM hyphae (and the glomalin they contain) are critical in the structure and function of topsoil [12]. A quality agricultural topsoil that has a high organic component is fertile (can support plants without added inputs like fertilizer), has the proper amount of oxygen to facilitate root growth, and maintains proper water balance (absorbs and drains optimally). However, research into how AM associations impact soil structure and glomalin levels under different agricultural management practices is extremely limited. Even the most basic information, such the identity of the fungi that form AM associations with crop plants under different management practices, is poorly understood.

If funded by this grant, we will determine the identity of the AM symbionts in the corn fields using DNA sequences [13] and characterize the glomalin component via ELISA (enzyme linked immunosorbent assay[14]). We will be focusing on characterizing the AMF identity and glomalin in the soil in both field samples from till and no-till farms and in greenhouse experimental conditions (described in feasibility). Previous studies have demonstrated that AM fungi abundance varies between till and no till managed fields [15]. However, the identity of those AM fungi is poorly documented in agricultural studies. In addition, very little is known about glomalin levels under different management practices.

This study is enriching a study being conducted by Dr. Winther's graduate student Derek Bennet. Derek is conducting a much larger scale study on the impacts of till and

no-till farming practices in corn fields on macroscopic components of AMF communities (including abundance and percent root infection), soil structure (including aggregate size and nutrient quantity), and plant productivity (including root structure, and root-shoot biomass ratio). This complementary molecular-based study (making use of Derek's experimental design and samples) will provide a more complete picture of the role of AM fungi in corn till and no-till agricultural systems. To date, many AM studies in agricultural crops have only been conducted in greenhouses, making it difficult to apply the results to actual field conditions [16]. Our experimental design will allow us to contextualize both greenhouse and field data. Ideally, we will be able to determine the agricultural practice in corn with the most beneficial AM population in terms of soil structure and plant productivity. We predict no-till practices provide the most AM benefits and will further support education efforts to convince farmers to adopt no-till practices. Conducting this research will allow Catherine to apply the skills she has learned in her studies and help her develop a plan for the future the incorporates her love of science, agriculture, and environmental action.

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Appendix 2
List of articles used for literature review and the factors these articles were analyzed by

Article	Citation
1	Ji, L., Tan, W., & Chen, 0. (0019). Arbuscular mycorrhizal mycelial networks and glomalin-related soil protein increase soil aggregation in Calcaric Regosol under well-watered and drought stress conditions. Soil and Tillage Research, 185, 1-8.
2	Wright, S. F., & Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and soil, 198(1), 97-107.
3	Wang., Sun, Y., Cheng, Y., Liu, S., Chen, & Kuyper, T. W. (0018). Arbuscular mycorrhizal fungi negatively affect nitrogen acquisition and grain yield of maize in a N deficient soil. Frontiers in microbiology, 9, 418.
4	Zhang, L., 0u, M., Liu, Y., Zhang, F., Hodge, A., & Feng, G. (0016). Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytologist, 010(3), 1000-1030.
5	Marulanda, A., Azcon, R., & Ruiz-Lozano, J. M. (0003). Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by Lactuca sativa plants under drought stress. Physiologia Plantarum, 119(4), 506-533.
6	Verbruggen, E., Kiers, E. T., Bakelaar, P. N., Röling, W. F., & van der Heijden, M. G. (0010). Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant and Soil, 350(1-0), 43-55.
7	2ehl, F., Sieverding, E., 1neichen, K., Mäder, P., Boller, T., & Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Applied and environmental microbiology, 69(5), 0816-0804.
8	Verzeaux, J., Nivelle, E., Roger, D., Hirel, B., Dubois, F., & Tetu, T. (0017). Spore density of arbuscular mycorrhizal fungi is fostered by six years of a no-till system and is correlated with environmental parameters in a silty loam soil. Agronomy, 7(0), 38.
9	Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., ... & 2ehl, F. (0015). 1mpact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry, 84, 38-50.
10	Avio, L., Castaldini, M., Fabiani, A., Bedini, S., Sbrana, C., Turrini, A., & Giovannetti, M. (0013). 1mpact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. Soil Biology and Biochemistry, 67, 085-094.
11	Wang, P., Liu, J. H., 0ia, R. 0., Wu, Q. S., Wang, M. Y., & Dong, T. (0011). Arbuscular mycorrhizal development, glomalin-related soil protein (GRSP) content, and rhizospheric phosphatase activitiy in citrus orchards under different types of soil management. Journal of Plant Nutrition and Soil Science, 174(1), 65-70.
12	Sheng, M., Lalande, R., Hamel, C., & Ziadi, N. (0013). Effect of long-term tillage and mineral phosphorus fertilization on arbuscular mycorrhizal fungi in a humid continental zone of Eastern Canada. Plant and soil, 369(1-0), 599-613.
13	Rosner, K., Bodner, G., Hage-Ahmed, K., & Steinkellner, S. (0018). Long-term Soil Tillage and Cover Cropping Affected Arbuscular Mycorrhizal Fungi, Nutrient Concentrations, and Yield in Sunflower. Agronomy Journal, 110(6), 0664-0670.

14	Yan, L. 1., Ying-Long, C. H. E. N., Min, L. 1., 0ian-Gui, L. 1. N., & Run-Jin, L. 1. U. (0010). Effects of arbuscular mycorrhizal fungi communities on soil quality and the growth of cucumber seedlings in a greenhouse soil of continuously planting cucumber. Pedosphere, 00(1), 79-87.
15	Rillig, M. C., Ramsey, P. W., Morris, S., & Paul, E. A. (0003). Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. Plant and Soil, 053(0), 093-099.
16	Fokom, R., Adamou, S., Teugwa, M. C., Boyogueno, A. B., Nana, W. L., Ngonkeu, M. E. L., ... & Zollo, P. A. (0010). Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameroon. Soil and Tillage Research, 100, 69-75.
17	Lee, J. E., & Eom, A. H. (0009). Effect of organic farming on spore diversity of arbuscular mycorrhizal fungi and glomalin in soil. Mycobiology, 37(4), 070-076.
18	Wright, S. F., & Anderson, R. L. (0000). Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. Biology and Fertility of Soils, 31(3-4), 049-053.
19	Bedini, S., Avio, L., Argese, E., & Giovannetti, M. (0007). Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. Agriculture, ecosystems & environment, 100(0-4), 463-466.
20	Rillig, M. C., Maestre, F. T., & Lamit, L. J. (0003). Microsite differences in fungal hyphal length, glomalin, and soil aggregate stability in semiarid Mediterranean steppes. Soil Biology and Biochemistry, 35(9), 1057-1060.
21	0un, F., 0ie, B., Liu, S., & Guo, C. (0015). Effect of plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. Environmental Science and Pollution Research, 00(1), 598-608.
22	Sivakumar, N. (0013). Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. Annals of microbiology, 63(1), 151-160.

List of factors

Date	location	Environment type	Avg Rainfall	Avg Temp	Field conditions	Cropping history	Statistics used	Site conditions
Soil method	Soil type	Depth of sample taken	% clay	% silt	%sand	Soil pH	C/N ratio	SOM content
Soil Carbon	Soil Nitrogen	Soil Phosphorus	Soil potassium	Soil Sodium	Soil Chlorine	Electrical conducti-vity	Water infiltration rate	Soil watering
Soil Structure	Soil compac-tion	1-0mm Aggregates	Aggregate stability	Micro aggregate %	Macro aggregate %	Root length	Root volume	Cover crop used
Glomalin method	BSA amounts	ELISA amounts	Glomalin present	Bacteria present	Species richness	Herbivory	Experim-ental conditions	Treatment groups
Plant method	Crop/ variety	Harvest stage	Crop yield	biomass	Above ground biomass	Below ground biomass	Above: below ground biomass ratio	Plant nutrient density
Plant potassium	Plant Mn	Nutrients in plant	Plant fruit nutrient density	Herbicide/ fungicide use	Enzymes	Shoot height	Shoot dry weight	Soluble sugar content
AMF method	AMF taxa present	Spore abundance %	Spore diversity	% root infection	% colon-ization	Colonized diversity	AMF other	Spores present