ARBUSCULAR MYCORRHIZAL FUNGI: CROP MANAGEMENT SYSTEMS ALTER COMMUNITY STRUCTURE AND AFFECT SOYBEAN GROWTH AND TOLERANCE TO WATER STRESS

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Dedico esta tesis a mi Padre, por que su amor eterno todo lo cree y todo lo espera de mi. Su vision es mas amplia de lo que yo puedo imaginar.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

ABSTRACT

Zubieta, Lisseth M.S., Purdue University, December 2018. Arbuscular mycorrhizal fungi: crop management systems alter community structure and affect soybean growth and tolerance to water stress . Major Professor: Lori Hoagland.

Arbuscular mycorrhizal fungi (AMF) are best known for their potential to help plants acquire nutrients, especially phosphorous. These microbes improve soil health by promoting soil aggregation and carbon sequestration, and further benefit plants by helping them withstand biotic and abiotic stress. Currently, there are 200 recognized species of AMF within the phylum Glomeromycota. Recent studies indicate that individual AMF species differ in the benefits they provide, with some even acting as parasites. Moreover, AMF community composition can be altered by soil and crop management practices, but the effect of these changes on the benefits conferred by AMF are still not well understood. Consequently, the goal of this study was to determine how two widely used crop management systems can alter the composition of AMF species, and affect the potential for these communities to promote the productivity and drought tolerance. To accomplish this goal, we collected AMF inoculum from a long-term crop systems trial comparing organic and conventional management for use in greenhouse trials where we subjected plants to drought. We collected AMF inoculum during mid-summer when differences between the two management systems were likely cause larger effects on AMF communities, and again in autumn after harvest to see if differences in AMF communities would persist. We determined AMF species composition using next generation sequencing. Results of this study confirm that soil-building practices commonly used in organic farming systems can improve soil health and increase the productivity of food-grade soybeans. They also demonstrate that AMF communities in Indiana croplands are highly diverse, and

some of these taxa can improve soybean growth and help plants tolerate water stress. Although the overall diversity of AMF communities did not differ between the organic and conventional management systems in mid-summer, individual AMF taxa did differ between the systems, which were likely responsible for the greater tolerance to water stress observed when plants were amended with inoculum from the organic system. AMF communities present during autumn were significantly different between the two crop management systems, but did not result in differences in drought tolerance of soybeans, indicating that the loss of key AMF taxa in the organic system from the first relative to the second experiment was likely responsible. Finally, plants grown using inoculum from both crop management systems in autumn had greater tolerance to water stress than plants that received a AMF commercial inoculum. This provides further evidence that individual AMF species vary in the benefits they provide, and that the presence of a diverse consortium of AMF species is needed to optimize plant health and productivity in agricultural systems. Agricultural producers should consider incorporating soil-building practices that are commonly used in organic farming systems such as planting winter cover crops, to improve the health of their soil and enhance the productivity of their crops.

1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are one of the most well studied symbioses between plants and soil microorganisms. In this obligate relationship, the fungal partner receives carbon (C) synthesized from its host plant in exchange for nutrients. AMF are considered one of the most important microorganisms in agriculture [1], because in addition to aiding in nutrient adsorption they can improve plant growth by facilitating water uptake, while making soil less prone to degradation by promoting stabilization of soil aggregates [2, 3] and increasing soil carbon content [4]. Moreover, AMF could further benefit agroecosystems by promoting crop resilience under stress [2, 3, 5] [1] There are currently approximately 200 recognized species of AMF within the phylum Glomeromycota [6], though this AMF diversity might be greater, since there is bias due to the amount of AMF species that are culturable [7]. Greater diversity of AMF species is expected to benefit agricultural systems by providing functional complementarity in utilization of different niches [7]. Biotic and abiotic factors such as plant species, soil pH, moisture and fertility are well known for their potential to affect the functional benefits provided by AMF [8], though differences among individual AMF species could also play a role. For example, variations among AMF species has been noted in the distance forged by fungal hyphae to obtain phosphorous [9,10], ability to exploit phosphorus [11], uptake inorganic vs. organic nitrogen (N) compounds [12], improve soil structure via stabilization of soil aggregates [13], and promote water uptake [7,14]. Moreover, differences in C allocation strategies among AMF species have also been noted including total amount extracted from the host [15], investment in sporulation [16,17], and investment in storage vs. nutrient uptake [18]. Consequently, while some AMF species enhance soil and plant health, others could act as parasites, negatively affecting plant productivity [7, 19]. Development of agricultural systems based on the intensive use of inputs such as inorganic fertilizers has substantially

increased the productivity of crops [20]. These practices have also caused soil organic matter to decline however, resulting in soil erosion and compaction, as well as nutrient imbalances that can lead to nutrient loss [21]. Moreover, when nutrient availability is high, plants are expected to reduce resource allocation to roots, thereby increasing competition for limited resources among microbial symbionts, which could potentially favor species that have changed the functions that provide services to the plants, for those that improve their capacity for reproduction and survival [7,22–24]. This could also favor AMF species and genotypes that are highly competitive though less beneficial to the host, by increasing C allocation to reproduction and storage structures rather than hyphal networks [7]. It could also result in competitive dominance by individual AMF taxa such as *Glomus intraradices* (now classified as *Rhizophagus irregulares*), which has been noted to be nitrophilic [25]. Long fallow periods, which are common during winter in temperate cropping systems, are also expected to favor species with greater investment in storage and reproductive structures [26]. In contrast, fertility practices that provide nutrients as well as organic matter, such as animal and green manures, have potential to improve the diversity and functional benefits of AMF in agricultural systems. These practices are commonly employed in organic farming systems, which have been shown to have higher organic matter [27], lower nutrient availability [28], and greater AMF diversity [16, 29, 30] in comparison to their conventional counterparts. In particular, winter cover crops reduce fallow periods, allowing plant hosts to sustain AMF communities and potentially limit the need for AMF species or genotypes to invest more heavily in storage and reproductive structures. However, whether such practices alter the functional potential of AMF communities is less clear. Several studies have reported AMF ability to confer plants tolerance to water stress. In this respect, the increases of ROS (reactive oxygen species) during water stress overcome plants antioxidants capacity [31], causing lipid peroxidation, degradation of proteins, cell membranes and death of cells [32]. Nevertheless, AMF can significantly decrease accumulation of ROS such as H_2O_2, decreased electrolyte leakage, improve wholeness of cell membranes, antioxidants (such as SOD,

CAT or GR) and decrease lipid damage compared with plants non-inoculated with AMF [31, 33]. Soybeans is an important source of vegetal oil and protein, is grown in several regions worldwide. However, this crop is very susceptible to drought since it reduces plant growth and yields. In particular drought during flowering can decrease soybean yields in 46% as a result of reduction in photosynthesis rate, stomatal conductance [34, 35]. In addition, other studies indicate that this crop is highly dependent on AMF in limited nutrient conditions [36, 37]. In this context, the goal of this study was to determine how these types of agricultural production practices alter the composition of AMF communities and their potential to help soybean plants withstand water stress. To accomplish this goal, AMF inoculum was collected from a long-term crop systems trial comparing organic and conventional management during summer, when soybean plants were expected to be influenced by previous management practices and in autumn following harvest to see if potential differences in AMF communities caused by the alternative management practices would persist. The inoculum were added to a sterile sand:soil mix, planted with soybeans and subjected to water stress in a greenhouse experiment, and composition of AMF in roots determined using high-throughput sequencing of the small subunit (SSR) rRNA gene region.

2. MATERIALS AND METHODS

2.1 Soil and crop management history

The greenhouse experiments were conducted using soil collected from a long-term crop systems trial carried out at Purdues Meigs Horticulture Research Farm (lat. 401721 N. long. 865302) located in Tippecanoe County, IN, U. S. A. Soil at this site is classified in the Drummer soil series (fine-silty, mixed, mesic Typic Endoquall), which typically contain approximately 3. 2% organic matter and a neutral pH. The crop systems trial was established in 2011 on adjacent tracts of land with uniform topography that had been managed using either organic or conventional farming practices since 2001. The systems trial was arranged as a split-block design with three replicates for each crop system given constraints at this site. Within each crop system, four cash crops (tomato (*Solanum lycopersicum*), carrot (*Daucus carota*), popcorn (*Zea mays everta*) and soybean (*Glycine max*) were grown annually and managed using standard practices for each system. This included application of inorganic fertilizers and synthetic pesticides in the traditional conventional system, and inclusion of cover crops and organic fertilizers derived from animal manure in the organic systems. Cash crops were rotated in all systems annually in the following order: tomato -¿ carrot -¿ popcorn -¿ soybean, with the exception of the soil-building organic system, which included a summer cover crop in odd years and the cash crop in even years. Soils from two of these crop systems were used in the greenhouse trials described in this manuscript. Crop systems representing traditional conventional (CNV) and soil-building organic (ORG) farming practices were selected for use in the greenhouse trials. In the CVF treatment, popcorn cv. Red Beauty (Johnnys Seeds, Winslow, ME) was cultivated during the previous growing season and a pre-emergent herbicide (Select Max, Valent BioSciences, Libertyville, IL) was applied at

a rate of 2. 98 l ha-1 prior to soybean planting. In the ORG treatment, *Sorghum sudangrass* (Sorghum X drummondi) cv. BRM (High Mowing Seeds, Wolcott, VT) was cultivated during the previous growing season as a summer cover crop, followed by a custom fall green manure mix (winter rye (*Secale cereale* L.), hairy vetch (*Vicia villosa*), winter pea (*Pisum sativum*), annual rye (*Lolium perenne*), and timothy grass (*Phleum pratense*) (Cloverland Seed, Millersburg, OH). The winter cover crop, with aboveground biomass estimated at $5,380 kgha^{-}1$, was mowed and disked into the soil approximately 30 days prior to soybean planting. No fertilizer was applied in either crop system since the cash crop was a legume and would have the potential to fix nitrogen, and preliminary soil tests indicated that other macro- and micro-nutrients (ie. P, K, Ca and Mg) were sufficient (data not shown). Untreated soybean cv. 21F3 (Blue River Hybrids, Ames, IA) was inoculated with *Bradyrhizobium japonicum* (N-Dure, Verdesian Life Sciences, Cary, NC) at the recommended rate and planted in May 2014 at a rate of $370 Kseedsha^{-}1$ in all plots. Soybean plots were harvested with a plot combine in October and weighed to quantify grain yield.

2.2 Soil collection and analyses

Before planting in the field trial, 15 soil samples were randomly collected from each replicate plot using a 2. 0-cm diameter soil probe to a depth of 30 cm, pooled and homogenized to determine baseline fertility and soil quality parameters. Subsamples were submitted to Midwest Laboratories (Omaha, NE) for standard nutrient analyses using common methods for this region [38]. Briefly, soil organic matter was determined using loss of weight on ignition; available P was extracted as Weak Bray (readily available P) and Strong Bray (potentially available P) and analyzed colorimetrically; exchangeable K, calcium (Ca), and magnesium (Mg) were extracted with neutral ammonium acetate (1 N) and quantified by inductively coupled argon plasmamass spectrometry detection; and base saturation and cation exchange capacity $[mmol(+)\ kg1]$ were estimated from the results of exchangeable minerals [38]. Ac-

tive carbon was quantified using the permanganate oxidizable carbon (POXC) technique [39]. Reacted samples and standards were measured on a plate reader (BioTek Epoch; BioTek, Winooski, VT) at 550 nm. Soil inoculum was collected from the field plots for use in the greenhouse trials when soybean plants were at 50% flowering in mid-June, and following harvest in October. At each sampling time, soil was collected from 15 randomly selected locations within each plot and pooled. In each location, soil was collected immediately adjacent to the root system of soybeans plants to a depth of 20 cm. Soil samples were stored at 4° C until inoculum preparation described below, which occurred within one week following each collection time. To estimate AMF diversity in the soil inoculum collected during mid-summer for use in the greenhouse trials, subsamples of soil from each plot were subject to the sucrose density gradient centrifugation extraction method [40]. Briefly, 20 grams of soil was combined with tap water and passed through a series of sieves (500, 125 and 45 m). The material retained on the125 and 45 m sieves was collected and placed in a 50 ml sterile tube using a fine tipped spatula and wash bottle containing sterile water [41]. 30 ml of the mixture was combined with 20 ml of sucrose (20% and 70%) [42], and centrifuged at 3350 rpm for 4 m. The resulting supernatant was poured over a 45m sieve and washed with tap water to remove sucrose. AMF spores collected from soil inoculum collected in mid-summer were transferred to a petri dish and visualized under a microscope at 40X magnification [43], and AMF spores were classified based on morphological characteristics. AMF spores collected from each crop system during both mid-summer and autumn were subject to DNA extraction, PCR amplification and Illumina sequencing using techniques described below.

2.3 Soil inoculum preparation

Soil from each soil system and sampling time was sieved (2 mm) to separate roots from the soil, and roots were cut into 1 cm pieces before being mixed back into the sieved soil. The resulting soil and root mixtures were air dried at room temperature

(21° C) for two weeks to stabilize AMF fungal spores. To obtain microbial inoculum without AMF spores, 150 ml each of the CNV and ORG soil mixtures were pooled, combined with 500ml of ultrapure water and filtered with Whatman No. 4 (20-25 um) filter paper following methods described in **Verbruggen et al. (2012**).

2.4 Greenhouse experiment and plant analysis

Greenhouse experiments were carried out in the Purdue University Department of Horticulture and Landscape Architecture Greenhouse Complex (lat. 86° 54' 53, long. 40 ° 25' 15"), located in West Lafayette, IN, U. S .A. The experiments were set up in a split-plot design with moisture regime as the main plot and soil treatment as the split plot. Each moisture regime and soil treatment was replicated six times when using soil collected at soybean flowering during the first experiment, and eight times using soil collected following soybean harvest during the second experiment. In each experiment, soil treatments included: 1) a negative control (NCT) that received the AMF spore-free suspension of microbes from pooled CNV and ORG treatments, 2) a positive control (PCT) that in addition to receiving the AMF spore-free suspension of microbes from the pooled CNV and ORG treatments was amended with a commercial AMF inoculum containing *R. irregulares* (Myke, Premier Biotechnologies, Quebec, Canada), 3) soil inoculum from CNV, and 4) soil inoculum from ORG. Inoculum from each of the four treatments was added to 7. 6 L pots containing 4 L of a 1:1 sand: soil mixture that had previously been autoclaved at 120° C for 4 hours two times within 24 hours. The AMF spore-free suspension was added at a rate of 75 ml per pot, and the soil inoculum was added to CNV and ORG treatments at a rate of 200 ml per pot. The inoculum in each pot was covered with 200 ml of the sterile sand: soil mixture. Soybean cv. 21F3 (Blue River Hybrids, Ames, IA) seeds were surface sterilized in a 30% bleach solution and a drop of polysorbate for 20 m, washed three times with sterile water and left to air-dry for 30 m on sterile paper towels. Once dry, seeds were inoculated with *Bradyrhizobium japonicum* (N-Dure, Verdesian Life Sciences,

Cary, NC) at the recommended rate. Three seeds were planted in each pot and pots were thinned to one plant per pot following emergence. Each pot was watered daily to saturation and fertilized weekly using a modified Hoagland nutrient solution that was low in P. The solution included P (20% of recommended P) (0. 14gKH_2PO_4), 70g KNO_3, 150$gCa(NO_3)$, 2, 11$gMgSO_4$, 1. 6g K_2SO_4, 15g EDTA, 3. 1g H_3BO_3, 0. 16g $ZnSO_4$, 0. 17g $CuSO_4$, 1. 18g $MnSO_4$, 0. 08g MoO_3 and 60g $(NH_4)_2SO_4$) per liter following recommendations described in **Taffuou et al. (2014)** for soybean. The soil moisture regimes were initiated once soybean seedpods began to fill, with half of the plants being kept at field capacity and the other half at 70% field capacity until harvest. Soil water holding capacity was determined prior to the start of the experiment. Pots were saturated and then left to dry for 9 days. After this time the volumetric water content (VWC) was calculated (water divided by volume of 6 pot 1400ml). Soil moisture was monitored in the pots using Decagon EC-5 sensors (Decagon Devices, Pullman, WA, USA) and Priva Office Direct Version 8. 2. 3, and water was applied using drip irrigation when needed. Once pots were subject to the two water regimes, stomatal conductance was measured weekly in the third fully expanded leaf of three plants per treatment using a leaf porometer (Decagon Devices, Pullman, WA, USA), and leaf water potential was measured twice using a pressure chamber (PMS instruments, OR, USA). After senescence, plant biomass was collected from each pot, separated into roots, shoots and pods, and dried at 70° C for two days prior to weighing.

2.5 Root AMF characterization

Once soybean plants reached 50% flowering, which was expected to represent the greatest percentage of AMF colonization in soybean roots [44], secondary roots from three plants in each treatment were collected to quantify root AMF infection and diversity. Soybean root colonization rates were determined by thinning roots to $\leq$ 1mm diameter pieces using methods described in **Charoenpakdee et al. (2010**).

Briefly, roots were placed in tissue cassettes (and bleached by immersing roots in a 10% KOH solution and autoclaving twice at 230° C per 20 min. After bleaching, roots were washed with tap water and immersed in a solution of 1% HCl for 5 m. Tissue cassettes were then placed in a 0. 05% trypan blue solution and autoclaved for 1 h at 230° C per 20 min, and stored in lactoglycerol 1:1:1 (water: lactic acid: glycerol) [45]. The AMF colonization rate was observed using the gridline intersect method described in **McGonigle et al. (1990)**. Dyed roots were cut into 1 to 1. 5 cm pieces and placed in parallel on microscope slides for observation under the microscope at 40X magnification (Olympus CX31, Center Valley, PA, USA). These observations were recorded from 10 roots in triplicate. Soybean roots or AMF spores isolated from soil as described above, were ground in liquid nitrogen (N) and genomic DNA was extracted using PowerLyzer Ultraclean Microbial DNA Isolation kits (MoBio Laboratories, CA, USA), with a modified beat-beating protocol, in duplicate. The two replicates were pooled and analyzed for purity and concentration of total extracted ds-DNA determined using a NanoDrop 2000c Spectrometer (Thermo Scientific, USA). Primers pairs AMV4. 5NF (AAG CTC GTA GTT GAA TTT CG) and AMDGR (CCC AAC TAT CCC TAT TAA TCA T) [46], modified to contain an adapter region for sequencing on the Illumina MiSeq platform, were used to amplify the small subunit (SSR) rRNA gene region to characterize AMF community diversity. PCR reactions were performed in triplicate (including positive and negative controls) with 12. 5 ul of GoTaq colorless master mix (Promega), 0.5 ul of each primer (each at 10 um), 1 ul of DNA template, and 10. 5 ul filter-sterilized ultra-pure H_2O, for a total volume of 25 ul. All amplifications were conducted on a BioRad T100 Thermocycler thermocycler (BioRad Inc. , Hercules, CA) with the following program: an initial denaturation step at 94C for 2 m, followed by 39 cycles of denaturation at 94C for 60 s, annealing at 50C for 45 s, and a final elongation at 72 C for 7 m. PCR products from each triplicate reaction were checked for amplification specificity on a 1% agarose gel, pooled, and purified using UltraClean PCR Clean-Up Kits (MoBio Laboratories, CA, USA). Cleaned PCR products were subjected to a second PCR reaction, with specific

tag encoded primers for each sample. The same thermocycling conditions described above were used, with the exception of 5 amplification cycles instead of 30. Resulting PCR products were checked for amplification specificity on a 1% agarose gel, purified using UltraClean PCR Clean-Up Kits, and quantified using a Ultra-violet NanoDrop 2000c Spectrometer (Thermo, USA). Exactly 20 ng of each sample were pooled and submitted to the Purdue Core Genomics facility for dilution and sequencing on an Illumina MiSeq (Illumina Inc. , San Diego, CA). Quality trimming, (Phred ¿= Q20), filtering and adapter trimming of raw sequence reads generated using Illumina MiSeq was performed using cutadapt (version 1. 9. 1) [47]. Read-pairs were merged using FLASH (version 1. 2. 11) [48], and merged reads were converted to fasta format using the FASTX-toolkit (version 0. 0. 14) [49]. Sequence reads were processed using the QIIME open-source bioinformatics pipeline [50]. Operational taxonomic unit (OTU) picking and taxonomic assignments were carried out using the open-reference OTU picking module UCLUST [51]. Sequence reads were clustered against the MaarjAM database (version 0. 8. 1) of published Glomeromycota SSU rRNA gene sequences [52], and reads that failed to hit the reference were subsequently clustered de novo. High quality sequences were taxonomically classified using the Ribosomal Database Project (RDP) classifier with a cutoff of 97% [53]. The OTUs were screened to remove any chimeric OTUs using ChimeraSlayer algorithm [54]. After removing chimeric OTUs, the output OTU table, corresponding phylogenetic tree file generated using FastTree2 [55], and a self-created metadata file were used for downstream statistical analyses.

2.6 Statistical analyses

Statistical analyses of soil chemical properties prior to planting in the field trial, AMF spores isolated from field plots, soybean yield in the field trial, and soybean biomass and physiological characteristics in the greenhouse trials were carried out using tests for normality, homogeneity of variance and analysis of variance (ANOVA) in

SAS 9. 2 (SAS Institute, Cary, NC, USA). All data were initially checked for normality according to the Shapiro-Wilk test using PROC UNIVARIATE, and non-normal data were Box-Cox transformed using PROC TRANSREG. Levenes test was used in order to test for homogeneity of variance when a two-way ANOVA model was used. One-way ANOVA was used to test impacts of treatments within each greenhouse trial, and a two-way ANOVA was used to compare experiment-wide differences between treatments and the two greenhouse trials, and their interaction, using PROC MIXED. Data are reported separately by greenhouse trial because of significant treatment by greenhouse trial interactions. Relationships between leaf water measurements, soybean performance and soil chemical properties were determined using Pearson correlation coefficients using PROC CORR. Differences were determined as significant at the $p \leq 0.05$ probability level, unless otherwise stated. All multiple comparisons were made using Tukey-adjusted least squared means when the ANOVA F-test was statistically significant. For statistical analysis of AMF sequence data in soybean roots grown in the greenhouse experiment, QIIMEs filter scripts were used to generate OTU tables and retain OTUs where 25% of the samples in groups being compared have OTUs. The alpha diversity script was used to estimate within-sample species richness and evenness based on Faiths phylogenetic diversity index [56]. The alpha rarefaction script was used to estimate species richness for a given number of sequences by the number of observed phylotypes and the Chao1 richness estimate [57], and to generate alpha rarefaction plots for each sample. The beta diversity script was used to generate the Bray-Curtis distance matrix, and results were used to generate 3D principle coordinate analysis (PCoA) plots [58]. The make distance boxplot script was used to further assess the community differences, including two-sample t-tests for all pairs of boxplots. Using the Bray-Curtis distance matrix as input, the compare categories script was used to evaluate differences between treatments by performing statistical tests using the ADONIS [59] and ANOSIM [60] methods. In order to evaluate differential abundance for specific OTUs between groups among the different comparisons, the phyloseq (phyloseq_1. 19. 1) software package [61] implemented in

Bioconductor [62,63] was used to provide a platform for statistical analysis and figure generation in R (R Core Team, 2013; RStudio, 2013). Using the DESeq2 package (DESeq2_1. 14. 1) [64], phyloseq determined differentially abundant OTUs and assigned adjusted p-values for each OTU, MA plots were generated us to visualize the OTU abundance data model fit (p ¡ 0. 05), and 2D PCoA plots (non-phylogeny based) were generated to illustrate community differences based solely on OTU abundances. Finally, for each comparison, phyloseq was used to generate ggplot2 summary plots of the significantly differentially abundant OTUs (p ¡ 0. 01 and 0. 05).

3. RESULTS

3.1 Soybean yield, soil chemical properties and AMF communities in the crop systems trial

Soybean seed yield was significantly greater in the ORG relative to the CNV system during summer 2014 (Figure 1). Soil chemical properties collected prior to seeding were also significantly different between the two crop management systems. In particular, total soil organic matter (Table 1) and active soil carbon (Figure 2) were greater in the ORG relative to the CNV system. In contrast, soil in the ORG system had less Bray-1P (readily available P) than soil in the CNV system, while Bray-2P (potentially available P), K, Mg, Ca, CEC, and percentage base saturation of K, Mg, and Ca were not significantly different (Table 1). The number of AMF spores, Shannon diversity, richness and evenness were greater in the ORG relative to the CNV system, but none of these factors were significantly different (Table 2). Rarefaction curves using the Faiths Phylogenetic Diversity Index to estimate whether sequencing depth was sufficient enough to cover all of the AMF diversity among spores in field soil inoculum failed to plateau, indicating that we did not fully capture all of the diversity within these samples (Figure 3). Estimates of alpha diversity using observed species, Chao1 and ACE (abundance-based coverage estimator) indicated that AMF diversity in ORG soils appeared to be greater than the CNV soils collected during mid-summer when soybean plants in the field were at 50% flowering, but did not appear to be different between the two management system when soils were collected in autumn after soybean plants were harvested (Figure 4). Estimates of alfa diversity using Shannon, Simpson and Fisher indexes indicated that AMF diversity in the ORG soil collected in mid-summer and autumn was greater than the CNV system (Figure 4). On the other hand, to analyze beta diversity we used Bray-Curtis

distance matrix to generate 3D principle coordinate analysis (PCoA) plots [58]. This allowed us to identify differences among AMF between treatments using statistical tests the ADONIS [59] and ANOSIM [60] methods.

3.2 Soybean physiological characteristics and yield in greenhouse trials

During the first experiment conducted using AMF inoculum collected during mid-summer, stomatal conductance in plants receiving the adequate water treatment were significantly affected by the soil inoculum treatments during weeks 1, 2, and 3, and when averaged across the 4 weeks of the trial (Table 3). Specifically, during the first three weeks, the PCT, CNV and ORG treatments all had greater stomatal conductance than the NCT treatment, and in week 3, the PCT treatment was greater than the CNV and ORG. However, when averaged across the 4 weeks of the study, only the CNV and ORG treatments were greater than the NCT. When plants were under water stress during the first experiment, results of stomatal conductance were more variable (Table 3). During week 1, plants in the PCT treatment were lower than all the other treatments, while plants in the ORG treatment were greater than the CNV. During week 3, stomatal conductance in the PCT, CNV and ORG treatments were greater than the NCT treatment, and the PCT treatment was greater than the CNV and ORG treatments. Finally, when averaged across the 4 weeks of the trial, the PCT, CNV and ORG treatments were greater than the NCT. During the second experiment conducted using AMF inoculum collected during autumn, there were no significant differences in stomatal conductance among the soil inoculum treatments in the absence or presence of water stress (Table 3). When comparing leaf water potential, there were no significant differences among the soil inoculum treatments during the first or second experiment, though there was an interaction between the first and second experiment indicating that all plants were under greater stress during the first experiment than in the second (Figure 5). Total soybean biomass was significantly different among the soil inoculum treatments during the first experiment in both the

absence and presences of water stress (Table 4). In the absence of water stress, the PCT, CNV and ORG treatments were all greater than the NCT treatment. When plants were subject to water stress, plants in the ORG treatment were significantly greater than the NCT and CNV treatments, and the PCT treatment was greater than the NCT. There were no significant differences in shoot and root weight alone, or in the root to shoot ratio in the first experiment (Table 4). During the second experiment, the only difference in plant biomass occurred in root weight when plants were under stress (Table 4). In particular, root biomass in the ORG and CNV were greater than the PCT and NCT. The total number of pods and pod weight were significantly affected by the soil inoculum treatments during the first experiment in both the absence and presence of water stress (Figure 6). Specifically, in the absence of water stress, the PCT, CNV and ORG all had greater number and weight of pods than the NCT treatment. In contrast, when plants were subject to water stress, pod number and weight were greater in the ORG than the PCT, CNV and NCT treatments, and the PCT and CNV treatments were greater than the NCT treatment. During the second experiment, pod number and yield were only affected by the soil inoculum treatments when the plants were subject to water stress (Figure 6). Specifically, pod number and weight in the ORG and CNV treatments were greater than the PCT and NCT treatments.

3.3 Diversity of AMF communities in the roots of soybean plants grown in greenhouse trials

No AMF structures were observed in the roots of soybean plants in the NCT treatment during the first or second experiment, confirming the absence of AMF in these treatments (Figure 7). Roots from the PCT treatment during the first experiment were lost during processing and therefore could not be visualized. AMF structures in soybean roots grown in the CNV appeared be greater (56%) than those in the ORG treatment (16%) during the first experiment. During the second experiment,

there did not appear to be any difference in AMF colonization between the PCT, CNV (65,3%) or ORG (58,6%) treatments (Figure 7). Rarefaction curves using the Faiths Phylogenetic Diversity Index to estimate whether sequencing depth was sufficient enough to cover all of the AMF diversity in soybean roots grown during the first and second greenhouse experiments failed to plateau, indicating that we did not fully capture all of the diversity within these samples (Figures 8 and 9). Estimates of alpha diversity using observed species, Chao1 and ACE in the roots of soybean plants grown in the first experiment indicated that AMF diversity was not significantly different in the CNV and ORG compared PCT treatment (Figure 10). Alpha diversity using these metrics also indicated that AMF alpha diversity was not different between CNV and ORG treatments. In the second experiment, estimates of alpha diversity using observed species, Shannon and Simpson in the roots of soybean plants indicated that CVN had greater diversity compared ORG treatment. In this parameter, we observed that ORG had greater diversity compared PCT (Figure 11). Estimates of beta diversity of AMF communities between the roots of soybean plants grown during the first experiment indicated that diversity was significantly greater in the ORG relative to the PCT treatment (p¡0.001), and diversity in the CNV treatment was marginally greater than the PCT treatment (p¡0.10), but there was no difference between the CNV and ORG treatments (Table 5; Figures 10, 12, 13 and 14). During the second experiment, estimates of beta diversity of AMF communities between the roots of soybean plants indicated that diversity was significantly greater in the CNV relative to the ORG treatment (p¡0.05), and while both the CNV and ORG appeared to have greater diversity than the PCT treatment, they were not significantly different (Table 5; Figures 11, 15, 16, 17).

3.4 Differences in AMF families, genera and individual OTUs in soil inoculum and roots of soybean plants grown in two greenhouse trials

The majority of AMF observed in the soil and roots of soybean plants grown in inoculum from the CNV and ORG systems collected during summer and autumn belonged to the families *Glomeraceae* and *Claroideoglomeraceae*, demonstrating the dominance of these family in agricultural soil in this region (Figures 18 and 19). A small amount of AMF from the *Paraglomeraceae* family was present in the CNV and ORG soil and roots of soybean plants grown in inoculum collected during mid-summer, but AMF from this family were negligible in soils collected during autumn indicating that they are not well adapted to reproduce in soybean plants. AMF representing the families *Diversisporaceae, Archaeosporaceae* and *Gigasporaceae* were present in the ORG soil inoculum and roots grown in this inoculum during the first but not the second experiment. AMF that were unable to be unassigned were present in both experiments, but to a lesser extent in the second experiment. In both experiments and treatments using field soil inoculum, *Glomeraceae* were lower in roots relative to soil while *Claroideoglomeraceae* were greater in roots relative to soil, indicating that while AMF in the *Claroideoglomeraceae* family are adapted to living in soybean roots, AMF in the *Claroideoglomeraceae* family are uniquely adapted to thrive in the roots of this crop species (Figures 18 and 19). Differences in the relative abundance of AMF families were observed in the CNV and ORG soil inoculum collected during summer and autumn, and in the soybean roots grown in this inoculum. For example, during the first experiment, within the CNV treatment *Claroideoglomeraceae* represented 64.7% of AMF roots and 21.8% in soil, *Glomeraceae* represented 10.5% of roots and 61.9% in soil, and *Paraglomeraceae* represented 0.3% of roots and 3.4% in soil. In contrast, within the ORG treatment during the first experiment, *Claroideoglomeraceae* represented 53.9% of AMF in roots and 7.13% in soil, *Glomeraceae* represented 31.5% of roots and 48.6% in soil, *Paraglomeraceae* represented 0.3% of roots and 0.03% in soil, *Diversisporaceae* represented 0.2% of roots

and 0.01% in soil, *Archaeosporaceae* represented 0.2% of roots and 0% in soil, and finally *Gigasporaceae* represented 0% in roots and 0.15% in soil. During the second experiment, within the CNV treatment *Claroideoglomeraceae* represented 54.3% of roots and 12.2% in soil, and *Glomeraceae* represented 4.5% of roots and 81.2% in soil. Within the ORG treatment during the second experiment, *Claroideoglomeraceae* represented 92.4% of roots and 11.7% in soil, and *Glomeraceae* represented 4.5% of roots and 81.2% in soil. In both experiments, soybean roots grown in the PCT treatment were both dominated by *Glomeraceae* with 98.5 and 98% in the first and second experiments respectively. At the genera level, a wide diversity of AMF taxa were present in the soil and soybean roots grown in the soil inoculum collected from the CNV and ORG systems collected during mid-summer and autumn (Figures 20 and 21). During the first experiment, individual AMF taxa that were significantly different in the roots of soybean plants between the CNV and PCT treatments, the ORG and PCT treatments, and the CNV and ORG treatments are presented in Figures 22, 23 and 24 respectively. During the second experiment, individual AMF taxa that were significantly different in the roots of soybean plants between the CNV and PCT treatments, the ORG and PCT treatments, and the CNV and ORG treatments are presented in Figures 25, 26 and 27 respectively.

3.5 Differences in the relative abundance of AMF families and individual taxa between the CNV and ORG crop systems in the first and second experiment

The relative abundance of some AMF families changed from summer to autumn (Figures 18 and 19). For instance, in the CVN treatment, the relative abundance of AMF in the family *Glomeraceae* increased from 61. 9% in summer to 87. 8% in autumn, while the relative abundance of AMF in the families *Claroideoglomeraceae* and *Paraglomeraceae* decreased from 21. 7% in summer to 12. 2% in autumn, and from 3. 4% in summer to 0% in autumn, respectively. In contrast, in the ORG treat-

ment, the relative abundance of AMF in the family *Glomeraceae* also increased from 48. 6% in summer to 81. 2% in autumn, and AMF in the family *Paraglomeraceae* also decreased from 0. 03% in summer to 0. 00001% in autumn, the relative abundance of AMF in the family *Claroideoglomeraceae* increased from 7. 13 % in summer to 11. 7% in autumn. When comparing individual taxa that were significantly different in the soil treatments between the first and second experiment, we observed that the number of AMF species that were more abundant in the ORG compared to PCT treatment decreased from 21 in summer (21) to 12 in autumn (Figure 23 and Figure 26). However, (Glomus_sp_vtx00248,Glomus_sp_VTX00365,Glomus_Liu2012b phylo12_VTX00143,Archaeospora_trappei_VTX00245,Glomus_sp_VTX00143,-Glomus_Algualcil11dGb-G10_VTX00222,Glomus_sp_VTX00067,-Glomus_sp_VTX00063,Glomus_sp_VTX00280,Glomus_Yamato09-A2_VTX00248,-Paraglomus_Para2_VTX00308,Glomus_sp_VTX00092, Glomus_Yamato08-B_VTX00113) were significantly different between these soil treatments in both experiments, indicating that these taxa could have played a role in soybean tolerance to water stress observed in the greenhouse trials. In contrast, when comparing the CNV and PCT soil treatments in the two greenhouse experiments, we observed the number of individual AMF taxa that differed between the systems dropped substantially between the first to second experiment (Figures 22 and 25). This would indicate that the presence of some of the individual AMF taxa that were significantly greater in the CNV treatment relative to the PCT treatment during the first experiment, this could indicate that AMF taxa in PCT have acted more as parasites, thereby preventing greater soybean growth when plants were under water stress compared with CNV and ORG. Alternatively, AMF taxa that could not be assigned at the species level that were significantly greater in the CNV treatment relative to the PCT treatment in the second experiment could have been responsible for the tolerance to water stress observed. Finally, when comparing the CNV and ORG soil treatments between the two experiments, we observed that 10 AMF taxa (Glomus_sp_vtx00248, Glomus_sp_VTX00222, Glomus_sp_VTX00365, Glomus_Liu2012b

phylo_12_VTX00143, Archaeospora_trappei_VTX00245, Glomus_sp_VTX00409, Glomus_sp_VTX143 and Glomus_Algualcil11dGb G10_VTX00222) that were significantly different between these soil treatments in the first experiment, were not were not present during the second experiment (Figures 24 and 27). Consequently, these taxa could have been responsible for the greater tolerance to water stress observed in the ORG relative to the CNV treatment observed during the first experiment.

4. DISCUSSION

In recent years, the importance of maintaining soil health has received increasing attention, as healthy soils are known to provide many beneficial agroecosystem services. Soil health has been defined as the capacity of soil to function as a vital living system, within ecosystems and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water quality, and promote plant and animal health [65]. Soil microbial communities present in healthy soils in particular, can play a key role in maintaining the health and productivity of plants [66]. For example, arbuscular mycorrhizal fungi (AMF) are well known for their potential to help plants acquire nutrients [7, 67], and more recently they have been demonstrated to help plants tolerate pathogens [68] as well as water stress [69]. Consequently, the broad goal of this experiment was to determine whether differences in AMF communities present in the long-term crop systems trial comparing organic and conventional management could have contributed to the dramatic increase in soybean yield observed in the organic system during summer 2014 (Fig . 1). Because prolonged periods of drought have become more frequent in this region, we also sought to determine whether differences in AMF communities in these systems could help soybean plants withstand water stress and thereby contribute to enhanced crop performance.

4.1 Effects of crop management practices on soil health and AMF communities

Results of this study provide further evidence that management practices commonly employed in organic farming systems can improve soil health [70]. In particular, soil in the organically managed system evaluated in this study had greater total as well as active soil organic matter pools as indicated by the permanganate

oxidizable carbon (POXC) test (Table 1; Figure 2). Greater levels of active organic matter could have contributed to the significantly greater soybean yield (1. 4X) (Fig. 1), as soil with greater levels of POXC and total organic matter have previously been correlated with increased crop productivity and biomass. For instance, earlier studies found that use of conservation tillage practices increased soil organic matter by 1 Mg ha-1, and corresponded with an increase in soybean yield of 2. 5 times [71] Soil organic matter is a critical component of many soil processes including nutrient cycling and availability of nutrients for plant uptake, as well as soil aggregation and carbon (C) storage [65]. Soil organic matter is composed of several fractions that include a labile or active fraction that can be decomposed by soil organisms in a matter of days, an intermediate fraction that can persist in soil for years to decades, and a stable fraction that can persist in soil for decades to centuries. Previous studies have demonstrated that measurements of the labile or active soil organic matter pool, such as POXC, reflect rapid changes in the accumulation of soil organic matter in agroecosystems, and can therefore serve as a sensitive indicator of how management practices are influencing soil health [72]. For example, several studies have reported that management practices such as amending soil with compost and biochar, no-till and conservation tillage, and planting cover crops resulted in greater POXC levels in soils, indicating that these practices were improving soil health [71] [72]. Moreover, these studies demonstrated that there is often a correlation between POXC with common indicators used to measure soil microbial activity, such as microbial biomass and substrate-induced respiration, among others [71] [72]. Since labile or active soil organic matter includes plant residues, manures and dead microorganisms, other microorganisms such as AMF could easily decompose these substances to derive nutrients and thereby improve the health of their symbiotic plant partners [8, 73]. Because the conventional and organic crop management systems evaluated in this study had similar soil tillage regimes, we expect that greater total and active soil organic matter in the organic system was likely a result of the inclusion of the winter (*Secale cereale* L, *Vicia villosa* R, *Pisum sativum*, *Lolium multiflorum* and *Phleum*

pratense L.) cover crops in this system. Previous studies have demonstrated that cover crops can increase soil fertility, reduce the presence of weeds, pests and diseases, improve soil porosity, prevent loss of topsoil, improve water infiltration, and provide a source of plant biomass to build soil organic matter [74]. For example, earlier studies have reported that cover crops can reduce soil erosion by 62% in comparison with soils that were not planted with cover crops [74]. *Vicia villosa* R. cover crops in particular have been demonstrated to prevent soil erosion during autumn, while *Secale cereale* L, *Pisum sativum* and *Lolium multiflorum* reduced soil erosion during winter [74]. In addition, previous studies have demonstrated that *Secale cereale* L. , can produce large amounts of plant biomass with high a C:N ratio that decomposes slowly and thereby contributes to organic matter accumulation, while legumes such as *Vicia villosa* decompose more quickly releasing nitrogen (N) for the subsequent cash crop [74]. Inclusion of fall cover crops in intensive agricultural systems has previously been demonstrated to increase the abundance of AMF [75], which as stated above, are well known for their potential to contribute multiple benefits in agricultural systems. AMF are obligate symbionts that require an appropriate host to survive. Consequently, the absence of a host crop during winter fallow periods, as well as inclusion of a non-mycorrhizal host such as canola, can decrease AMF colonization, richness and diversity [32,76]. In contrast, including cover crops in crop rotations can increase the presence of AMF spores [75, 76]. For instance, previous studies have found that winter cover crops can increase the presence of *Gigasporaceae* and *Paraglomeraceae* families in soybean [37]. Others have demonstrated that crop systems with greater diversity of plant species (such as cover crops), can modify AMF diversity [77]. However, as discussed in the introduction, while some AMF species can enhance soil and plant health, others could act as parasites, negatively affecting plant productivity [7,19]. Consequently, learning more about factors that affect AMF diversity, and the benefits they provide, is essential to realizing the benefits of these symbionts in agricultural systems. Though not statistically significant, assessments of AMF abundance and diversity based on evaluation of AMF spores in soil samples

collected during mid-summer when soybeans were at 50% flowering, indicated that AMF communities in the organic systems were likely more abundant and diverse than the conventional system (Table 2). Subsequent evaluation of these soils using NGS sequencing provided further evidence that the AMF communities in these two crop systems appeared to be different. As discussed above, these differences could have been due to the inclusion of cover crops in the organic system, as well as the greater levels of total and labile soil carbon (Table 1; Figure 2). In addition, differences in AMF communities between these two crop systems could have been related to differences in nutrient availability. For example, Bray-1P indicates the amount of phosphorus that is available for plant uptake (Midwest Labs). According to soybeans requirements, the organic system had adequate levels of Bray-1P at 27. 7 ppm for soybeans (15–30 ppm), while the conventional system had excessive levels of Bray-1P ($\geq$ 40 ppm) at 52.3 ppm. Previous studies have demonstrated that some AMF species/families are only present in soils with low fertility, whereas others are adapted to soils with high levels of P or N [78, 79]. In particular, previous studies have reported that high levels of nutrients such as nitrogen (N) can increase the abundance of AMF within the *Glomeraceae* family such as *G. intraradices* [80]. This AMF species is also frequently found to colonize the nodules of leguminous plants [80]. In contrast, high levels of N can reduce the frequency of other AMF species [80]. For example, in alpine meadow ecosystems, high doses of N and P fertilizers (Diammonium phosphate ($(NH_4)_2HPO_4$)) decreased spore density, total AMF colonization, % of arbuscules, and AMF richness in E. nutans roots [81]. This fertilizer treatment also decreased overall plant diversity. The authors in this study suggested that under high doses of fertilizers, AMF species compete with plants for allocation of photo-assimilates. This is because under sufficient nutrient conditions, plants allocate more carbohydrates towards increasing shoot height and shoot biomass and reduce allocation of carbohydrates to AMF. Furthermore, they reported that competition for plant carbohydrates under high fertilizer doses could also result in the reduction of AMF species richness by half. This is because

AMF species vary in their ability to compete for plant carbohydrates. As a result, species like *G. intraradices/fasciculatum* or *A. trappei* were present in plants under high fertilizer doses, while other AMF species were only present in plants that received low fertilizer doses [81]. Similarly, another study reported that high doses of fertilizers can decrease AMF activity, measured as the length of AMF extra-mycelium, which allow AMF to uptake water and nutrients [81]. In contrast, low input or sustainable farming practices increase AMF extra-mycelium (AMF activity) [82–85]. However, the increase in AMF activity does not guarantee that in low fertilizer conditions plants will improve their performance. This is because AMF species differ in their cost-benefit ratio. Nevertheless, it is possible that in soils with more diverse AMF species, that there is more likely to be species that can contribute beneficial AMF functions, such as stimulation of plant growth, resulting in greater plant performance [83]. The presence of many of the same AMF species in both the CVN and ORG treatments in this study, indicate that these species could be generalists. For example, AMF belonging to the family *Glomeraceae* are often described as generalists, since they can colonize many different host plant species and survive in disturbed environments [8, 26, 32]. Likewise, other studies have found that *Glomus spp.* can colonize new hosts quickly, since they can produce abundant extra-radial mycelium, and can colonize plants via mycelium fragments as well as spores and infected plant roots [8, 86–89]. AMF belonging to the *Glomeraceae* family can produce anywhere from one to multiple spores in soils and roots depending on various environmental factors [8, 86, 88]. In particular, *G. mosseae* (also known as *Funneliformis mosseae*) has been shown to produce anywhere from a single spore, to up to 20 in soils and within roots throughout the year [90]. This variability allows *Glomeraceae* to survive in conditions were other AMF families cannot [8, 86, 87]. For instance, species such as *G. intraradices* and *G. aureum* (also known as *Dominikia aurea*) have been found to survive in several environments from natural forests to soils managed with intensive practices, regardless of their degree of disturbance or host plants present [8, 32, 84]. Species such as *G. mossaea* and *G. intraradices* can

also perform anastomosis, which is the fusion of hyphae from different spores [90, 91]. Furthermore, several studies indicate that AMF in this family, such as *G. mosseae*, can colonize several plant hosts, and are therefore widespread worldwide [90, 92]. AMF belonging to the family *Claroideoglomeraceae* also tend to be considered generalists. AMF in this family were previously classified as Glomus Group B, since their morphology is similar to AMF in the *Glomeraceae* family. However, recent studies using molecular methods have now classified these AMF into their own family [88]. AMF species in this family, such as *C. etunicatum*, have been observed throughout the year in a wide range of habitats including prairies as well as agricultural soils in Illinois, Missouri and Florida [93]. AMF in this family can produce a large number of spores in the dying roots of plants, which allows them to proliferate rapidly in the autumn [88]. Like AMF in the *Glomeracea*, AMF belonging to the *Claroideoglomeraceae* have been observed to colonize several plant hosts including *Andropogon scoparius, Zea mays, Allium cepa, A. sativum, Capsicum frutescens* var. longum*, Geditsia triacanthos* var. inermis Willd and *Trifolium repens* [93]. The AMF species, *C. lamellosum* has been found in association with grasses near the Great Lakes (Nottawasaga bay in Georgian bay, and Baileys Harbor in Wisconsin) [94]. This AMF species was demonstrated to produce abundant extra-radial mycelium, arbuscules, vesicles and spores in soils and roots, indicating that it can proliferate rapidly. Interestingly, its spores can differentiate from *C. etunicatum* to produce three persistent layers [94]. Like AMF species belonging to the *Claroideoglomeraceae, Paraglomeraceae* spores are also similar to *Glomeraceae*, and for that reason, they were also previously classified within the *Glomeraceae* family. However, recent studies using molecular tools has revealed substantial differences between the *Paraglomeraceae* and *Glomeraceae* families, and for that reason, these AMF are now classified as their own family (Schler and Walker 2010). Unlike, other AMF species *Paraglomeraceae* after long periods of time does not form vesicles and its intraradical colonization is very scarce in spite of producing abundant spores 40–320 spores/cm^3 [95]. In addition, *Paraglomeraceae* family have been

reported in many natural ecosystems such as native grasslands, forests and semiarid climates [8, 95, 96]. Although the presence of AMF in this family are uncommon in agricultural soils, they have been observed in soils with low organic matter contents [96]. AMF in this family have also been found in association with a wide variety of plant species including *T. pratense, Z. mays, S. sudanense, Avena sativa, Oryza sativa, Theobroma cacao, Quercus spp. , Ischaemum L. , Triticum L.* and *Malus pumila*, indicating that they are also generalists, though they are generally low in abundance [95]. The fact that AMF species belonging to the *Claroideoglomeraceae* and *Glomeraceae* families including *C. etunicatum, C. lamellosum, G. intraradices, G. mosseae*, and one species in the *Paraglomeraceae* family were observed in both the CVN and ORG treatments in this study (Claroideoglomus_etunicatum_VTX00193, Claroideoglomus_Glo7_VTX00193, Claroideoglomus_lamellosum_VTX00193, - Claroideoglomus_sp_VTX00193, Glomus_Glo1c_VTX00067, - Glomus_mosseae_VTX00067, Glomus_sp_VTX00063, Glomus_sp_VTX00067, Glomus_sp_VTX00248, Glomus_sp_VTX00280, Glomus_sp_VTX00409, - Glomus_yamato08-B_VTX00113, Paraglomus_Para2_VTX00308), provides further evidence that these are generalist species that are widely adapted to multiple habitats. This provides further support that these species likely have an r type life history strategy, which allows them to abundantly sporulate and propagate by mycelial fragments, allowing them to be successful under a wide range of conditions [8, 32, 84]. Similar results have been reported in other studies that compared local AMF species within the Maarja global database, where *G. mosseae*and *C. Claroideoglomus* were present across broad ecosystems [97]. In contrast, other AMF families and species are considered specialists, because they only are present in particular environments or are associated with a particular host species [32, 84]. Consequently, specialist AMF species are more vulnerable to extinction after disturbance and loss of their specific host species [32] and could therefore be considered as indicator species. For example, AMF species such as *G. fasciculatum/G. intraradices* (Glomus_Yamato08 B_VTX00113) and *Claroideoglomus lamellosum*, were considered indicator species in

forested environments [97]. Other studies comparing the effects of crop management on AMF diversity have varied in their conclusions. For example, some have found that conventional management does not affect AMF diversity, while others reported that constant soil disturbance and fertilization commonly observed in these systems can modify the abundance of *Glomus spp* and other AMF species, either increasing or decreasing them [98]. Other studies have provided evidence that some species can persist under conventional management practices. For example, in a long-term study evaluating the effects of 50 years of monoculture, the authors reported that taxa similar to *G. intraradices* were abundant in soils managed using standard conventional practices and in soils not cultivated [98]. Likewise, these authors found that use of fertilizers increased the abundance of *G. irregulare, G. Claroideum (Claroideoglomus claroideum), G. Claroideum (Claroideoglomus claroideum), G. etunicatum (Claroideoglomus etunicatum), Paraglomeraceae* and *Archaesporaceae.* However, these families were less abundant than other groups [98] The incorporation of corn stalks by plowing and avoiding fertilizer applications increased the abundance of AMF species such as *Glomus mossae, G. viscosum,* and *Glomus constrictum* among others species. In contrast, this treatment decreased the abundance of *G. intrarradices, G. etunicatum (Claroideoglomus etunicatum)* and *G. claroideum (Claroideoglomus claroideum)* [98]. Consequently, these studies conclude that conventional management practices tend to increase the abundance of AMF species that can tolerate soil disturbance, application of fertilizers, low plant diversity, and can also tolerate many plant hosts [83]. For this reason, greater relative abundance of Glomus_irregulare_VTX00114 and Claroideoglomus_etunicatum_VTX00193 in the CVN treatment in this trial, indicate that these taxa are more tolerant to conventional management practices than those used in the ORG system [84].

4.2 Effects of AMF inoculum on soybean growth and tolerance to water stress

There were two objectives for our greenhouse trials. One was to determine how differences in AMF communities induced by the two crop management systems would affect soybean growth and tolerance to water stress, and thereby could have contributed to the greater soybean yield observed in the field trial. This objective was determined by collecting soil inoculum during mid-summer when soybean plants were at 50% flowering and likely to be heavily influenced by the previous crop management conditions for use in a greenhouse trial. The second objective was to determine whether differences in AMF communities between the two systems would be sustained over time, or if the presence of a single crop species, in this case soybean, would override differences caused by the management systems. This second objective was determined by collecting soil inoculum in autumn after the soybean plants were harvested for use in a second greenhouse experiment. Both greenhouse trials included a negative control that contained no AMF, and a positive control containing only *R. irregulares.* This allowed us to document the extent that AMF can aid in plant growth and help soybean tolerate water stress and determine how much the presence of diverse AMF community assemblages present in field soils could affect these results. During the first experiment, the presence of AMF in all soil treatments increased stomatal conductance (Table 3), as well as total plant biomass and pod yield (Table 4; Figure 6), in both the presence and absence of water stress confirming the beneficial effects of AMF on plants. Not surprisingly, we did not observe differences in plant growth allocation, as previous studies in soybean have found that drought conditions did not cause significant changes in the root/shoot ratio [34]. Interestingly, when plants were subject to water stress, those grown in pots that received the organic inoculum had greater pod number and weight than the other treatments, indicating that differences in the abundance or composition of AMF in this treatment likely helped the soybean plants tolerate stress. During the second experiment, pod

number and yield were greater in both the CNV and ORG treatments relative to the positive and negative control treatments (Figure 6), providing further evidence that the abundance of diverse AMF assemblages including unique taxa present in field soils can help soybean plants tolerate water stress. Because the CNV and ORG treatments no longer differed from each other as they did in the first experiment, we can conclude that the presence of a single plant species, in this case soybeans, might override the effects of previous crop management practices. Consequently, to maintain AMF diversity as well as individual taxa with the potential to promote plant growth under stress, growers should diversify their production systems by including other plant species such as winter cover crops. Maintaining plant biomass in the presence of drought conditions is relevant, since drought is one of the main reasons for yield losses. This reduction of crop yield occurs when plants cannot compensate for water losses that occur as a result of transpiration [99–102]. Other studies have provided evidence that AMF can compensate for water losses causes by transpiration [100, 103, 104] , while non-AMF plants have lower transpiration rates indicating that they are more susceptible to drought [105]. AMF can help plants tolerate water stress via several mechanisms. For example, under drought conditions, AMF can keep plant tissues hydrated and increase plant biomass by increasing root surface area, which allows plants to take up more water and nutrients [2,100,101,103]. Other studies have found that AMF can increase plant biomass under drought stress by osmotic adjustment. This allows plants to reduce their water potential and maintain a positive flow of water from soil to roots [100]. In order to do this, plants accumulate organic solutes and inorganic ions such as soluble sugars, non-structural carbohydrates (NSC), proline, $K^+, Ca^{2+}, and Mg^{2+}$ [69, 106]. AMF can also improve accumulation of proline and carbohydrates to reduce migration of carbohydrates from shoots to roots, resulting in greater plant biomass than plants that are not inoculated with AMF [69, 100, 103, 107] The increase of proline reduces the osmotic potential by creating a gradient that allows plants to uptake water under drought. In addition, proline regulates pH in the cytosol and the ratio of NDA/NDAH, which reduces

the accumulation of ROS (hydroxyl radicals) and stabilizes proteins [108]. One of the most common AMF species, *Glomus intraradices* (now classified as *Rhizophagus irrelgulares*), which is widely available as a commercial inoculant, has been demonstrated to improve drought tolerance in plants using many of these mechanisms. For example, inoculating plants with *G. intraradices* has been demonstrated to improve the absorption of nutrients (P, K, Ca and Mg), as well as increase the accumulation of antioxidants such as APX (ascorbate peroxidase) and GR (glutathione reductase), which protect plants from oxidative damage caused by ROS induced under water stress [108]. In addition, the increase in K protects plant chloroplasts from oxidative damages, directly increases water uptake and maintains CO2 fixation, resulting in greater accumulation of biomass in roots and shoots under drought conditions [108]. In another study, AMF plants decreased their osmotic potential by accumulating soluble sugars, soluble starch and NSC, resulting in an increase of plant biomass and enzymes, and protecting membranes and cells from dehydration [106]. In contrast, plants without AMF altered their carbohydrate distribution between shoots and roots, by reducing leaf expansion and shoot biomass to transfer carbohydrates to roots and support key functions, such as osmotic adjustment, to improve water uptake and maintain biomass [69, 100, 103, 107, 109–113]. Finally, reduction in soil moisture can decrease the availability of phosphorus for plant uptake. Consequently, increases in AMF colonization and hyphal length observed during dry seasons compared with rainy seasons, could also help plants tolerate water stress by maintaining phosphorous uptake [114].

4.3 Differences in AMF community assemblages that could have been responsible for improved soybean growth and tolerance to water stress

While we did not consistently observe differences in AMF diversity between the treatments, we did observe differences in individual AMF taxa among the treatments, which were likely related to the differences in plant growth observed.

For example, during the first experiment AMF belonging to *Archaeosporaceae, Diversisporaceae, Glomeraceae*, and *Paraglomeraceae* families were more abundant in the ORG compared to the CVN treatment (Figure 18). In particular, the CVN treatment had a lower relative abundance of the following taxa than the ORG treatment: Glomus_sp_VTX00248,Glomus_sp_VTX00222,Glomus_sp_VTX00365,-Glomus_Yamato09-A2_VTX00248,Glomus_sp_VTX00063,Glomus_Liu2012b-Phylo-12_VTX00143,Archaeospora_trappei_VTX00245,Glomus_sp_00409,-Glomus_sp_VTX00143, andGlomus_Algualcil11dGb-G10_VTX00222, (Figure 24). This is interesting, given that many of these taxa have been reported under a wide range of conditions, and therefore tend to be characterized as generalist species. Nevertheless, differences within the crop systems described above, such as the presence of cover crops, greater labile organic matter pools and lower P in the organic system could have altered AMF communities in the organic system. Individual AMF taxa that differed between the ORG and CNV treatments, as well as the ORG and CNV treatments in comparison with the PCT, could have been responsible for the differences in plant growth and tolerance to water stress observed in this study. For example, Glomus_sp_vtx00248 has been associated with drought tolerance in species of Moringa [115], and other plants from *Fabaceae* family (Sophora fraseri) (as Sf8B. 1 and Sf9A) [116]. This AMF taxa has also been observed in association with the roots of *O. europea* and *Prunus Africana* (referred as NF02) in a dry forest in Ethiopia [117], providing further evidence that it could play a role in helping plants tolerate water stress. Glomus_sp_vtx00248 is thought to be closely related to *G. fasciculatum/G. intraradices* [115]. Similarly, Glomus_sp_VTX00063 was found in symbiosis with drought resistant *Moringa spp.* [115], as well as within the following grass species: *Stipa krylovii, Leymus chinensis, Allium bidentatum*, and *Astragalus brevifolius* (referred as Glo6) [118,119]. The authors in this study indicated that Glomus_sp_VTX00063 is closely related to*Glomus constrictum/Glomus viscosum* [115]. Other specific AMF taxa that differed among treatments in this study have not specifically been evaluated for

their potential to help plants withstand water stress, but they have been found in systems receiving fertilizer applications indicating that they are widely adapted. For example, Glomus_sp_VTX00409 (referred as Glo3), has been observed colonizing a natural grass (*Stipa krylovii*) [118], and in soils fertilized for 6 years with N ($10gNm^{-2}y^{-1}$), $P(5gP_2O_5m^{-2}y^{-1})$, and NP ($10gNm^{-2}y^{-1}y5gP_2O_5m^{-2}y^{-1}$) (referred as GLO13, Glo18 and Glo22) [120]. These studies concluded that this taxa is also closely related to *G. intraradices/G. irregulare* [118]. Glomus_Liu2012b Phylo-12_VTX00143 was detected in the roots of an alpine meadow ecosystem (grasslands) fertilized with low ($30gm^{-2}yr^{-1}$) and high ($90gm^{-2}yr^{-1}$) doses of ($(NH_4)_2HPO_4$), though this taxa was more abundant in *Elymus nutans* roots at high doses of fertilizer ($90gm^{-2}yr^{-1}(NH_4)_2HPO_4$) [81] Glomus_sp_VTX00365 was detected in fertilized and tilled soils as well non-fertilized and tilled soils, though it only colonized maize roots in the non-fertilized and tilled soils [83]. Glomus_sp_VTX00222 only colonized the roots of grassland plants at low doses of fertilizer ($30gm^{-2}yr^{-1}of(NH_4)_2HPO_4$), while Glomus_Algualcil11dGb-G10_VTX00222 was present in a wide range of environments including agricultural soils, forest, grasslands and shrublands. Colonizing several plant host such as *Prunus persica, Oxalis acetosella* and *E. nutans*. Both Glomus_sp_VTX00222 and Glomus_Algualcil11dGb-G10_VTX00222 are closely related to *Glomus indicum* [81]. The uncultured Glomus_sp_VTX00143 has been reported in several environments such as agronomic soils, forests, grasslands and shrublands, including soils from organically managed farms in Canada [121]. Archaeospora_trappei_VTX00245 was observed colonizing *Elymus nutans* roots only under high doses of fertilizer ($120gm^{-2}yr^{-1}of(NH_4)_2HPO_4$) (referred as Phylo-32) [81]. Archaeospora_trappei_VTX00245 (referred as VTX00245) has also been found in grasses, intensive and sustainable managed soils, indicating that it can be broadly adapted to many environments [97]. Spores and infected roots are thought to be the main propagules of Archaeospora_trappei_VTX00245, which have allowed it to colonize diverse species including*G. cinerea, R. officinalis, T. mastichina* and *T. zygis* [122]. However, unlike other AMF families,

this species require around four months to produce single spores in soils and occasionally within roots, indicating that it is slower growing [123]. In order to identify AMF taxa that might have been responsible for improved tolerance to drought in the CVN and ORG treatment, we compared the relative abundance of AMF families in these treatments with the PCT across the two experiments. For example, the ORG treatment had a greater relative abundance of the following species in comparison with the PCT treatment: Archaeospora_trappei_VTX00245,-Glomus_Algualcil11dGb-G10_VTX00222,Glomus_Liu2012b-Phylo-12_VTX00143,-Glomus_sp_VTX00092,Glomus_sp_VTX00143,Glomus_sp_VTX00222,-Glomus_sp_VTX00365,Glomus_Yamato09-A2_VTX00248) (Figure 23 and 26s). Even though we found that both CVN and ORG treatments had a greater abundance of Glomus_sp_vtx00248,Glomus_sp_VTX00409 andGlomus_sp_VTX00063 compared the PCT (Figures 22, 23, 25 and 26), the relative abundance of these taxa was far lower in the CNV than the ORG treatment (Figures 24 and 27), indicating that they could have played a role in helping the soybean plants tolerate water stress. We also detected that the relative abundance of the following taxa were greater in the ORG compared with the PCT: Glomus_Liu2012b-Phylo-12_VTX00143,-Glomus_sp_VTX00143,Glomus_sp_VTX00365,Glomus_Algualcil11dGb-G10_VTX00222,Glomus_sp_VTX00222, Archaeospora_trappei_VTX00245) (Figures 23 and 26), indicating that they also could have been related to improved soybean performance. In contrast, they were not significantly abundant in the CVN treatment compared to the PCT. Although we do not know with certainty, which practices increased or decreased the abundance of specific AMF taxa within the CVN and ORG treatments, it is important to consider how differences in AMF composition between these systems might have affected the potential for these AMF to help plants tolerate water stress. For example, while earlier studies have indicated that AMF promote many plant functions including growth, nutrient uptake, carbohydrate cost for plants and drought tolerance among others, these functions can vary among species and even within strains of the same AMF species [33]. Previous studies have sought to

determine how differences in AMF taxa could affect plant tolerance to water stress. For example, in a recent study using a metadata analysis, the authors reported that AMF from the families *Claroideoglomareaceae* and *Glomeracerae* increased stomatal conductance in similar proportions [124]. In particular, it was found that *Claroideoglomus etunicatum, F. mosseae, R. intraradices* and *G. deserticola* could all significantly increase stomatal conductance under drought conditions [124]. Other studies have observed similar results. For example, *G. deserticola, G. etunicatum, G. intraradices* and *G. fasciculatum* were found to help *Lactuca sativa* plants tolerate water stress [125]. In this study, all of these AMF species significantly increased stomatal conductance under moderate drought, though in addition to improving stomatal conductance *G. etunicatum* and *G. intraradices*, also increased the photosynthetic activity of plants in drought conditions [125]. Other studies have also observed differences among individual AMF taxa in their potential to help plants tolerate water stress along with other beneficial effects. For example, different strains of *G. microcarpum* isolated from a desert ecosystem, affected the potential for plants to prevent water vapor loss [115]. For example, one of the strains kept plant stomas open, allowing plants to assimilate more CO2 under drought conditions [115]. This effect is important under drought conditions, because drought can significantly reduce CO2 assimilation. In another study, single inoculations with native AMF species such as *A. trappei* and*P. ocultum* increased biomass of *Lavandula dentata* by (336%) and (264%) respectively, under drought stress [33]. In addition, these native AMF species differed in their potential to help plants uptake Fe, Zn, Mn and Cu. For instance,*S. constrictum* increased the uptake of Fe, *P. ocultum* improved Zn uptake, and *D. aunantia* improved Mn and Cu uptake, while *A. trappei* increased Mn, Cu, Fe, and Zn uptake [33]. While there may be differences in the potential for individual AMF taxa to help plants tolerate water stress, this beneficial effect could also result from the presence of a combination of AMF species. For example, the combined presence of *Septoglomus constrictum, Diversispora aunantia, Archaeospora trappei, Glomus versiforme,* and *Paraglomus ocultum* were most

effective in promoting drought tolerance in *Lavandula dentata* [33]. Similarly, in another study, inoculation with a native consortium of AMF species along with *Bacillus thuringiensis*, improved drought tolerance in *Trifolium repens* [108]. The authors in this study concluded that the presence of the natural consortium of AMF species and their potential mycorrhizal helper bacteria, improved plant biomass, P, K, Ca, Mg and B uptake, relative water content, and accumulation of glutathione reductase (GR) compared with a negative control [108]. Finally, a natural consortium of *Glomus aggregatum, Glomus deserticola, G. geosporum, Glomus microaggregatum* and *Sclerocystis coremioides*, improved fruit dry weight of C. annuum L. cv San Luis by198% compared to a control treatment under well watered conditions [126]

4.4 Differences in plant responses between the two greenhouse trials

It is possible that the absence of Glomus_sp_VTX143,Glomus_sp_VTX00365,-Glomus_sp_vtx00248,Glomus_sp_VTX00222,Glomus_sp_VTX00063,-Glomus_sp_00409-Glo3,Glomus_Liu2012b-phylo-12_VTX00143,-Glomus_Algualcil11dGb-G10_VTX00222 andArchaeospora_trappei_VTX00245 in the ORG treatment during the second experiment relative to the first (Figures 24 and 27), might be associated with the absence of significant differences in soybean tolerance to water stress between the CNV and ORG during the second experiment (Table 4 and Figure 6). However, it is unclear whether differences in plant growth responses between the first and second experiments were due solely to the presence of different AMF communities when the soil inoculum was collected, or if they were due to the season in which the greenhouse experiment was conducted. For example, despite the fact that both experiments were conducted in the greenhouse where air temperature and lighting could be controlled and kept constant, the first experiment was conducted in autumn when external day length was getting shorter, while the second experiment was conducted in the spring when day length was getting longer. These difference in photo-period could have been responsible

for the lower above-ground biomass observed across all treatments (Table 4), and the greater overall plant stress in the first experiment as indicated by the leaf water potential measurements (Figure 5). This seasonal difference in when the experiments were conducted may also have been responsible for the lack of clear response across soil treatments to the water stress during the second experiment, as all plants in this experiment were stressed due to shorter day-length. Previous studies have provided evidence that seasons can affect plant-AMF symbiosis [127]. For example, while this can be species-specific, overall, AMF species are affected by soil moisture content, soil nutrient availability, and a decrease in the photosynthetic light affect [128]. This likely occurs because AMF symbioses involve the exchange of plant carbohydrates, nutrients and water, and therefore, the benefits of these symbioses vary if production of plant carbohydrates change [128]. These factors are likely to vary given photoperiod length, and in fact previous experiments have demonstrated that photoperiod-length can affect plant responsiveness to AMF. For instance, researchers in a study investigating the effects of three different photoperiods on the growth, photosynthesis and stomatal conductance of citrus plants inoculated with *G. fasciculatus*, found that that long-day periods increased AMF colonization, plant height, plant biomass, leaf area, and P uptake [129]. At the same time, long-day length conditions reduced stomatal conductance compared with short days, possibly in response to water stress, though plants inoculated with AMF produced more photoassimilates under long-day than under short-day conditions [129]. Thus, it is possible that differences in photoperiod lengths between our first and second experiment altered plant responsiveness to AMF and affected our results. At the same time, the phenology of AMF species can vary during the year [130]. For instance, in a study that extracted AMF spores from grasses on an abandoned farm during different seasons, researchers found that spores of *Glomus spp.* were most abundant from January to May, and decreased from July to September [130]. In contrast *Acaulosporas spp.* spores were constant from January to November [130]. Other studies have also observed seasonal variations

in AMF communities. For example, under semiarid conditions, where certain AMF produced spores when the host plants produced fruit, the spores remained present in autumn, decreased in winter, and recovered in spring [131]. In another study, AMF spores decreased in autumn, and the authors theorized that the presence of other fungal species such as those from the Ascomycota division displaced the AMF [80]. However, they also suggested that other environmental and physiological factors could have reduced AMF diversity in autumn, such as reductions in day-light period, plant growth, plants nutrient requirements, photosynthesis and production of plant carbohydrates [80]. In a study conducted in Algeria, spores in the rhizosphere of *Tamarix articulate* and *T. gallica* and spores from the AMF speces *Septoglomus constrictum, F. mosseae, F. geosporum, R. fasciculatus* and an uncultured*Glomus spp.* , were more abundant in autumn than in summer [127]. However, AMF root colonization was higher in spring for both plant species [127]. Finally, difference in freezing tolerance between AMF species have also been observed. For example, freezing (-5° C) did not affect the colonization rate of *G. intraradices,* but it did affect the colonization rate of *G. etunicatum* (21%), *G. mosseae* (19%), *Scutellospora calospora* (82%) and *Acalospora denticulata* (43%) [130]. The authors of this study concluded that the presence of vesicles of *G. intraradices* present in roots might help might this species resist freezing, whereas AMF species in *Scutellospora* and *Acaulospora* families do not form vesicles and therefore could not survive.

4.5 A potential confounding effect in this study

While new molecular techniques such Illumina sequencing have dramatically improved the potential to detect the presence of AMF species in comparison with observations of morphological characteristics of spores, the AMF are still a difficult group of microbes to study. For example, many species that inhabit plants roots do not produce spores, or their sporulation depends on seasons and their physiological stage, which can make studies of their abundance in soil misleading [80, 84]. Other

studies have corroborated these findings. For example, a previous study conducted in a vineyard indicated that AMF species in soils did not correlate with AMF species observed within roots [76, 78]. In particular, the intra-radical mycelium of *G. intraradices* in roots was often far more abundant than the extra-radical mycelium in soil. In contrast, other species such as *Acaulospora* have scarce extra-radical or intra-radical mycelium [80]. In addition, not all AMF species in soils colonize plant roots [84], since plants might only allow colonization by particular AMF species. Likewise it was suggested that other AMF species, despite being present in the soil, do not colonize plant roots [132]. These results were observed in vineyards, where several AMF species found in soils did not colonize vine roots. The authors suggested that those species that did not colonize vine roots, maintain AMF propagules and survived colonizing cover crops and weeds as alternative hosts [76, 78].

4.6 Conclusions

Results of these studies support earlier studies demonstrating that AMF can improve the health and productivity of soybean plants, particularly when plants are subject to water stress. Based on the results of our studies, it seems plausible that AMF taxa differ in their potential to help plants withstand stress, and this should be explored further in future studies. In addition, it is also plausible that the presence of a consortium of AMF taxa better protects plants from water stress than individual AMF species. Because individual AMF taxa can competitively exclude others, future research should explore the extent to which commercial AMF inoculants that contain rapidly proliferating species could disrupt the presence and activity of native AMF consortiums that are more sensitive. Results of our field study also clearly indicate that including soil-building practices that are commonly used in organic farming systems, such as integrating winter cover crops in a crop rotation, can increase soil health. Such improvements in soil health are likely to directly and/or indirectly affect the health and productivity of plants through various mechanisms. One of these

enrichment in the abundance and diversity of AMF species. While we did not detect significant differences in AMF diversity between the organic and conventional management system evaluated in this trial, we did observe distinct differences in the composition of individual AMF taxa between these two farming systems, which likely played a role in improving soybean growth and tolerance to water stress in our greenhouse trials. Although our experimental design does not allow us to identify which specific management practices improved the presence of these AMF taxa in the organic managed system, we suspect that the presence of winter cover crops likely played a role, and this should be explored further in future research trials.

5. FUTURE DIRECTIONS

During this research we determined that organic management practices could promote the presence of a diverse set of AMF taxa that can improve drought tolerance, and increase soybean biomass, pod number and pod dry weight. This has important implications for maintaining the health and productivity of agricultural systems, particularly as weather patterns become increasingly unstable. However, most of the AMF taxa identified in this study have not yet been cultured, and thus the specific mechanisms that these taxa use to enhance crop performance cannot be determined. Moreover, many of the AMF taxa identified in this study belong to the same virtual taxa, such as Glomus_sp_VTX00248 and Glomus_Yamato09 A2_VTX00248, (Glomus_Liu2012b Phylo-12_VTX00143 and Glomus_sp_VTX00143, and Glomus_sp_VTX00222 and Glomus_Algualcil11dGb G10_VTX00222. This indicates that these taxa are likely related and could even be the same species, though they are genetically distinct and therefore may behave differently. Others have confirmed that there is large genetic heterogeneity among single AMF isolates and even within individual spores. This lack of homogeneity among AMF isolates and single spores cultures indicates that AMF diversity and their functions in natural conditions are even more complex than previously thought [133, 134]. For instance, some of these studies indicate that genetic difference between the ribosome gene (rRNA) of the same AMF isolate and single spore cultures within a given location are so large, that there could be more similarity in the AMF genome of isolates from different continents. The dramatic genetic variation among individual AMF isolates identified in recent studies could be caused by different distribution of alleles, since alleles are physically separated among nuclei [133]. Differential distribution of alleles may occur during sporulation, when the multinucleate hyphae transfer nuclei to spores, which is known as heterokaryosis. While heterokaryosis is common in several fungi, AMF can transfer mutations that occur during the lifetime

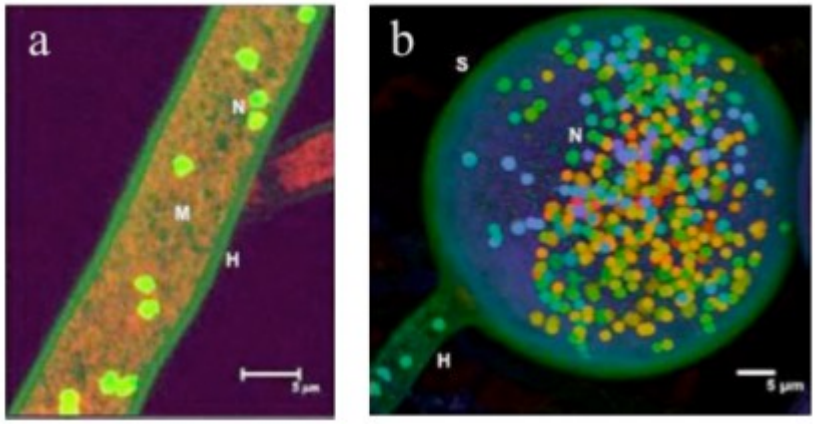


Fig. 5.1. Figure 5.1 Multinucleate hyphae (lack of septa that separate nuclei (green dots)) and a AMF multinucleate spore. Source: (Boon and Hijri 2012)

of nuclei to the developing spore [133]. Other studies indicate that genetic variation develops when closely related AMF species exchange nuclei in a process known as anastomosis. During anastomosis hyphae fuse, transferring nuclei from one hyphae to another [133]. Consequently, both anastomosis and heterokaryosis might cause different distribution of alleles within an individual AMF isolate.

Genetic differences within AMF isolates and individual spores have been noted in many studies. For example, genetic differences within the ITS region of single spore cultures of *G. mosseae* and *Acaulospora colossica* have been observed [134, 135]. In another study that compared the LSU region of 7 isolates of *G. coronatum* with other related AMF species (*G. constrictum, G. mosseae* and *G. geosporum*), the authors found that within the same AMF isolate there were 12 different patterns of PCR-single stranded conformational polymorphisms (PCR-SSCP) [134]. Moreover, they found that most of the isolate sequences were unique, though different AMF species shared a small proportion of sequence, indicating that this could be caused by the exchange of nuclei within closely related species (anastomosis) Given these differences, it will be important to identify intraspecific genetic variation among AMF species that can induce drought tolerance in future studies, to understand how soil management

practices affect the presence, dynamics and functionality of these species [136]. Many of the tools that are currently being used to quantify AMF diversity, such as high throughput sequencing using 454 pyrosequencing or Illumina Miseq, might overlook intraspecific variation since AMF spores or hyphae may contain nuclei from other taxa/species resulting in misidentification of species [134]. In addition, most of the AMF sequencing studies to date have used ribosomal markers in the SSU, ITS and LSU regions. In particular, the SSU is one of the most commonly used markers in AMF studies, even though it does not allow researchers to distinguish between closely related species [137]. The ITS marker does differentiate more species than the SSU, though the information available for Glomeromycota in databases with this marker is very limited [137]. There are primers available that can cover the entire SSU-ITS-LSU region, which can increase distinction between closely related AMF species [137] SSU-ITS-LSU not only facilitates distinction between closely related species, but it n also make it easier to assemble sequencing reads. This is particularly relevant when there is no assembled genome [133]. However, these primers have a long sequence length (1500 bp), so they cannot be used with 454 pyrosequencing (allow sequence length of only 800 bp) or Illumina MiSeq (allow sequence length of only 500 bp).New third-generation sequencing technologies such as SMRT (single molecule real-time) machines, can sequence long DNA fragments up to 40000 bp, allowing detection of variations in the sequences of closely related species at a cost that is similar to 454 pyrosequencing or Illumina Miseq [137], and therefore could be used to provide greater resolution in AMF studies. For example, the SSU-ITS-LSU primer and SMRT technology was recently used to trace the presence of *F. mosseae* and *R. irregulare* in field soils and wheat roots after inoculation [137]. This allowed the researchers to identify the effects of AMF inoculation on native AMF communities and detect genetic differences within the same AMF species. For example, they were able to detect the presence of two closely related species *Rhizoglomus irregulare* and *Rhizoglomus intraradices* within the roots and soils evaluated in this study. The subtle distinction between R. species is important, since differences among *Glomeraceae* and *Diver-*

sisporaceae families have little genetic variation within the SSU region [137]. Even within a single spore culture of *R. intraradices,* there can be 16 different sequences (intra-spore genetic variation) [137], further demonstrating the need for greater resolution in studies of AMF. The studies described above provide evidence that SSU-ITS-LSU and SMRT technology has the potential to differentiate between closely related taxa/species, whereas other technologies such as 454 pyrosequencing and Illumina MiSeq are less valuable because while they produce high quality read, they can only provide data on short sequences [137]. However, at this time, SMRT technology is not widely used because it has low throughput and quality of reads in comparison with 454 pyrosequencing or Illumina MiSeq. For example, with three passes SMRT technology produces low quality reads with an error rate of 68%, while 454 pyrosequencing and Illumina Miseq have an error rate of less than 2%. To overcome this challenge, researchers could consider using five passes instead of three, which can stabilize and reduce the error rate similar to 454 pyrosequencing or Illumina MiSeq [137]. In addition, the high error rate that has been observed when using SMRT technology to sequence bacterial communities would not be as high when sequencing AMF communities, because they are not as diverse as bacteria [137]. Moving forward, researchers should consider applying SMRT sequencing technology using the adjustments described above to identify intraspecific variation within AMF taxa present in organically managed soils that can induce drought tolerance in plants. In addition, they could use these tools to identify specific farming practices such as inclusion of individual cover species or mixtures of these species, application of organic fertilizer amendments, or the lack of pesticide inputs, that increase the abundance of these species. This technology could also be used to determine how seasonal changes affect the abundance of AMF communities in these soils, and determine if individual AMF species/taxa induce drought tolerance or if they act together in a consortium to help plants tolerate water stress. Finally, they could use this technology to identify the mechanisms individual species use to help plants tolerate water stress, as well as the exact isoforms in which these species contribute these benefits. Results of these stud-

ies would help all agricultural producers adopt management practices that preserve and protect the diversity of this important microorganism.

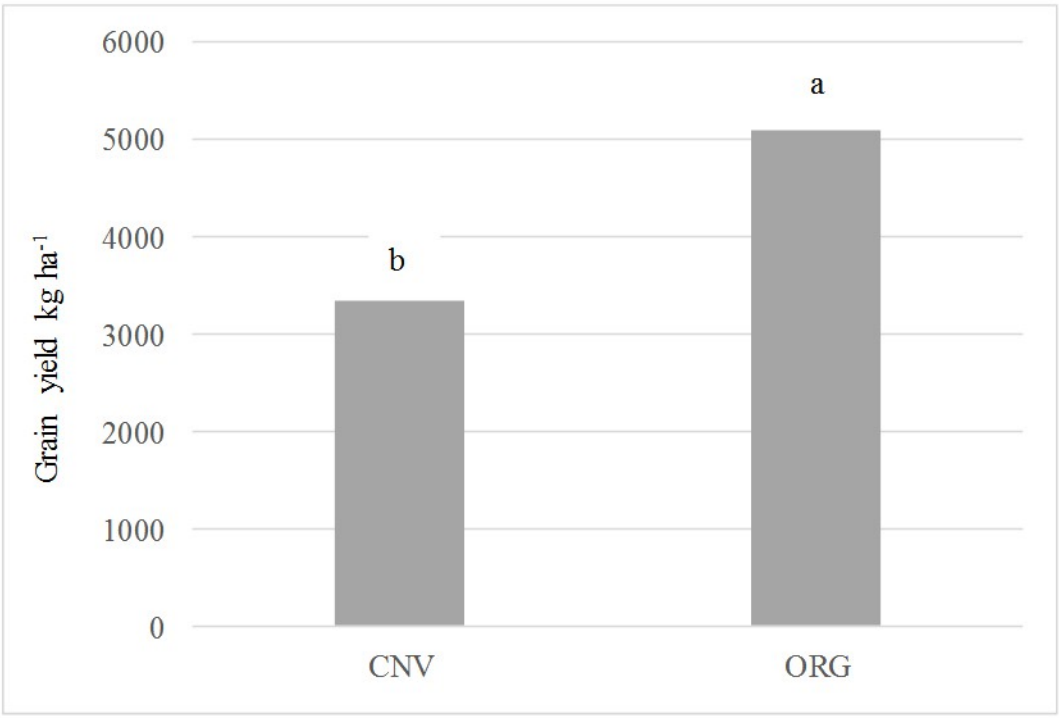


Fig. 5.2. Soybean yield during summer 2014

* Different letters within column indicates a significant difference (P¡0.05) We observed that use of organic farming practices such as cover crops and crop rotation (ORG) significantly increased soybean yield compared conventional farming practices (CVN) during summer 2014.

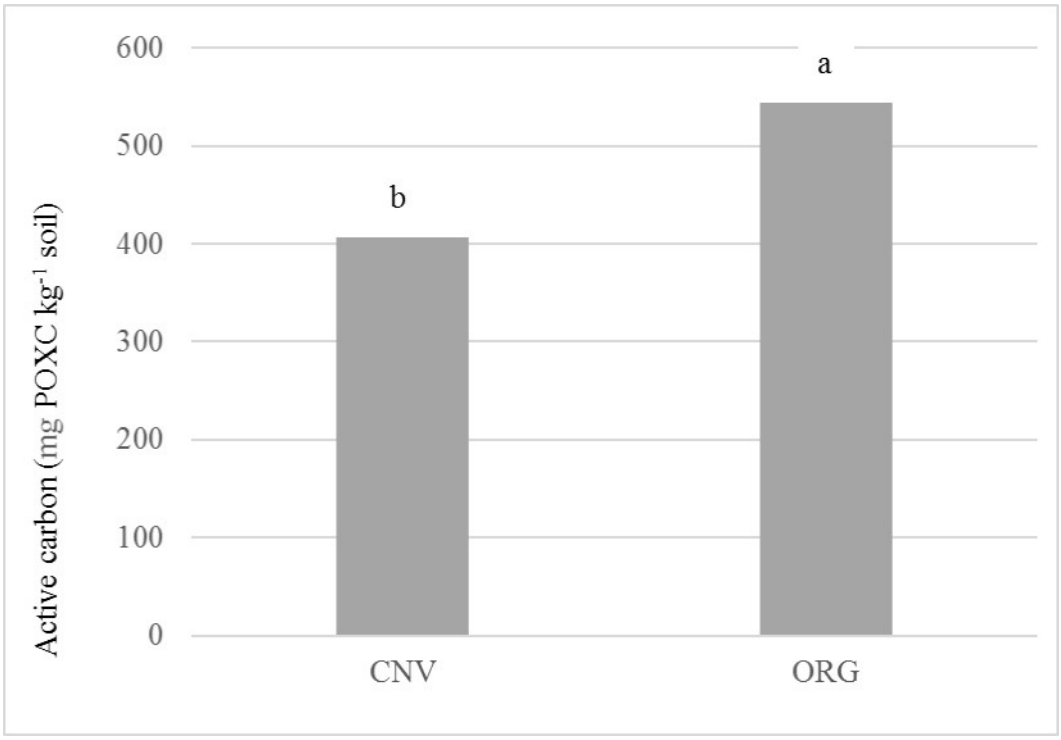


Fig. 5.3. Active soil carbon just prior to soybean planting during summer 2014.

* Different letters within column indicates a significant difference (P¡0.05) Active carbon measured by POXC is a measurement of the labile fraction of organic matter in soils. In this regard, we observed that the use of organic farming practices (ORG) significantly increased the labile fraction of organic matter compared with conventional farming practices (CVN).

Table 5.1.

1. Soil chemical properties in two crop systems just prior to planting soybean during summer 2014

					ppm				% base saturation		
Crop System	pH	% total OM	Bray-1 P	Bray-2 P	K	Mg	Ca	CEC (meq/100g)	%K	%Mg	%Ca
CNV	6. 5	2. 3 b*	52. 3a	63. 3	204. 7	307. 0	1853. 3	13. 4	3. 9	19. 1	69. 2
ORG	6. 7	3. 4 a	27. 7 b	49. 0	188. 3	368. 7	2410. 7	16. 9	2. 9	18. 1	71. 2

* Different letters within column indicates a significant difference (P¡0.05) We observed that organic farming practices (ORG) significantly increased the percentage of total organic matter compared conventional farming practices (CVN). This total organic matter is composed by the labile (also known as active) fraction, that can be discomposed by soil microorganism in days. The intermediate fraction that can persist for years-decades. The stable fraction that can remain unaltered for decades to centuries.

We observed that the Bray-1 P or P that is available for plants was significantly higher in conventional managed soils (CVN) compared soils managed with organic farming practices.

We did not observe significant differences in pH, Bray-2 P, K, Ca, Mg, CEC, percentage base saturation of K, Mg and Ca between conventional (CVN) and organic (ORG) managed soils.

Table 5.2.

Table 2. Total abundance, diversity, richness and evenness of AMF spores in soil collected just prior to planting in 2014

System	Total amount of AMF spores (50g of soil)	Shannon diversity (H)	Richness	Evenness(E)
CNV	29	1.16	15	0.54
ORG	61	1.87	21	0.8

* Different letters within column indicates a significant difference (P¡0.05) The use of organic farming practices (ORG) increased total number of AMF spores, the diversity index Shannon, richness (Total number of AMF species identified per treatment) and evenness compared conventional farming practices (CVN). However, we did not find significant differences between treatments.

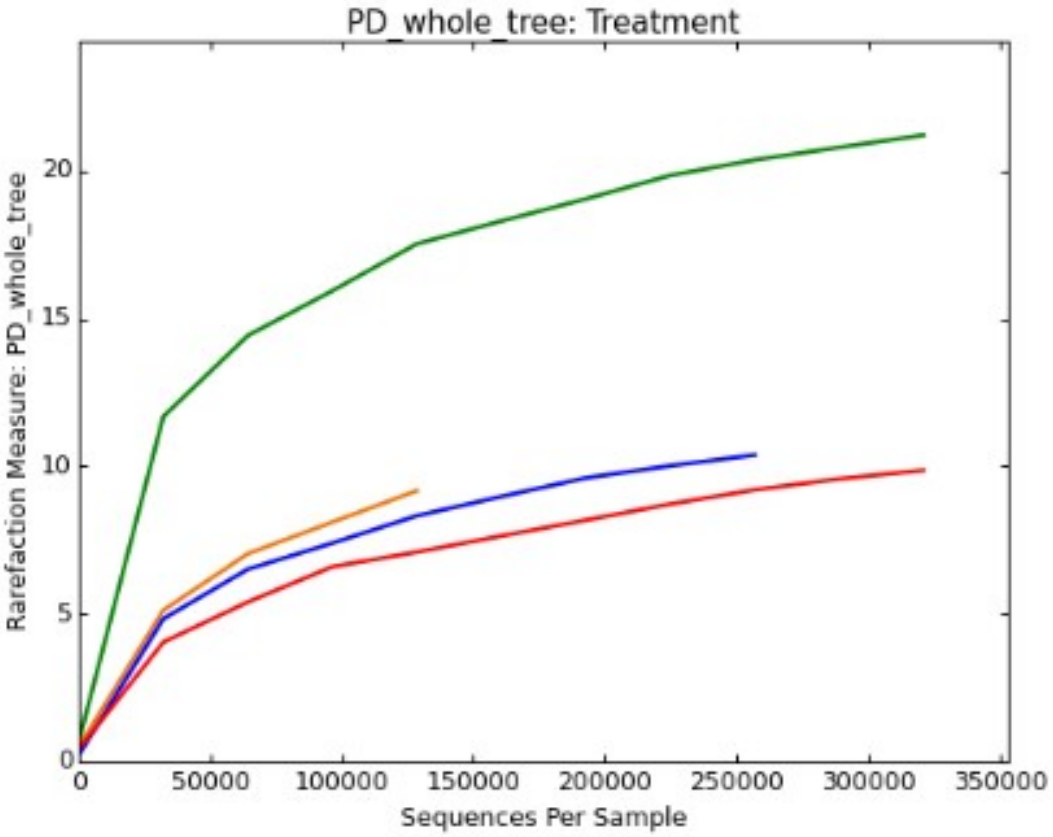


Fig. 5.4. Rarefaction curve generated using the Faiths Phylogenetic Diversity Index of AMF communities in soil collected from long-term research plots managed using conventional and organic practices in summer and autumn in 2014. (orange line CNV (summer), green line ORG (summer), red line CNV (autumn), and blue line ORG (autumn)

We analyzed the rarefaction curved of AMF sequences in soils collected from long-term trials to elucidate if sequencing depth covered the total diversity in these samples. In this respect curves that reach plateau cover all AMF diversity. In this respect, our results indicate that we could not cover all the diversity in these samples.

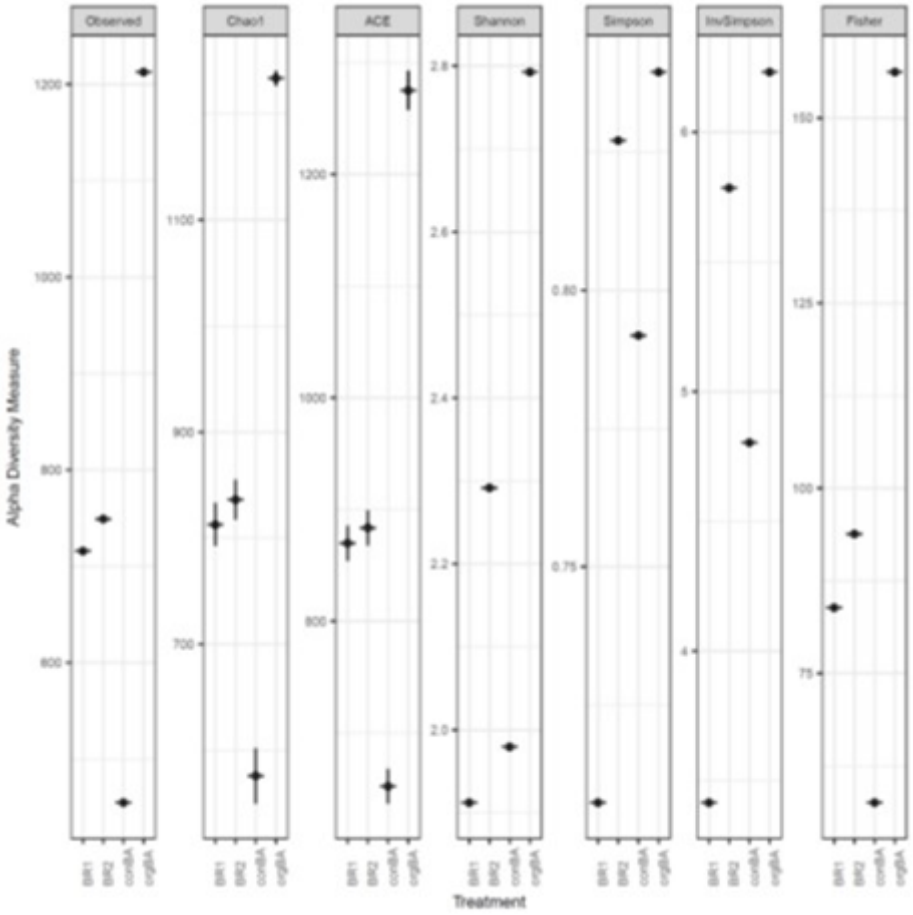


Fig. 5.5. Alpha and beta diversity estimates generated using several indexes for AMF communities in long-term research plots managed using conventional and organic practices in summer and autumn in 2014. (BR1 CNV autumn; BR2 ORG autumn; conBA CNV summer; or orgBA ORG summer).

We compared α diversity of AMF present in soils, this allow us to compare the diversity of AMF communities present in conventional and organic managed soils. This diversity can be estimated with qualitatively measurements (that consider the presence or absence of taxa Chao 1 or ACE (abundance-based coverage estimator)), or with quantitatively measurements that consider the abundance of each taxon (Shannon or Simpson).

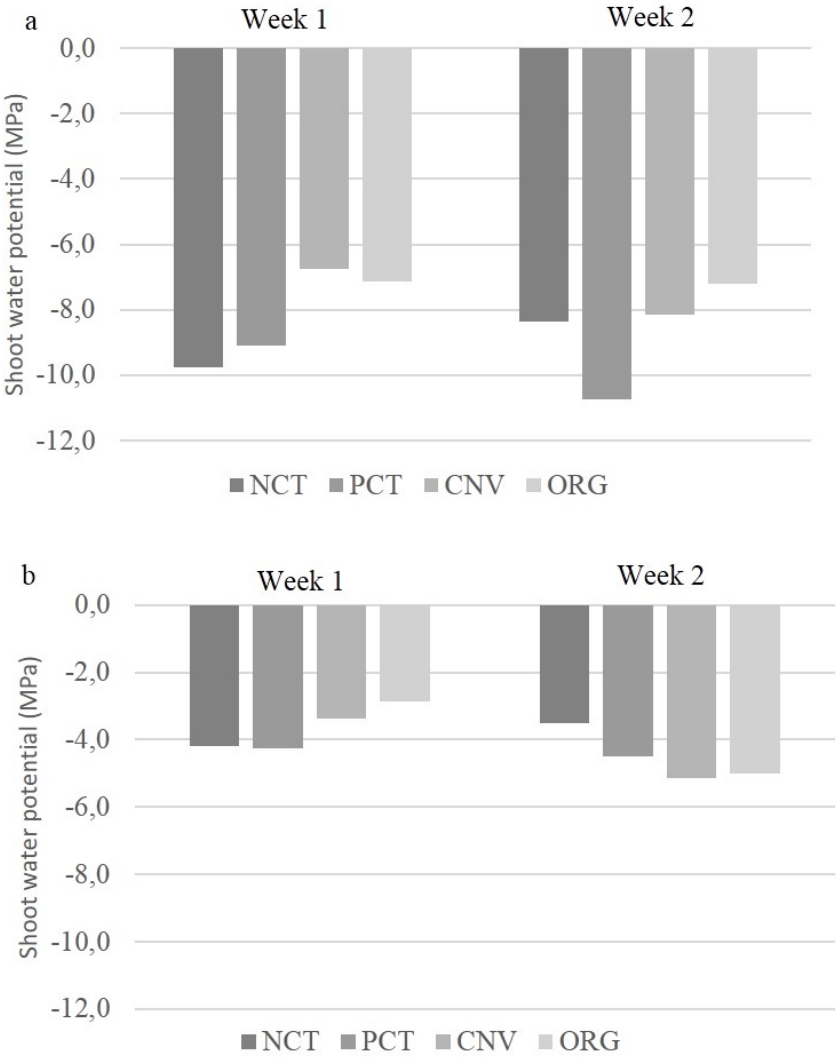


Fig. 5.6. Leaf water potential in soybean leaves subject to four soil treatments and water stress, one and two weeks after initiating water stress in experiment #1 (a) and experiment #2 (b).

Despite that, we performed the experiments under the same controlled conditions of light and air temperature. We observed that during summer the shoot water potentials were more negative (lower) than those obtained in autumn. These results indicate that during the summer the plants suffered greater stress compared to the plants that were grown in autumn.

Table 5.3.
Stomatal conductance of soybean leaves subject to four soil treatments using field soil AMF inoculum collected at two time points for use in separate greenhouse experiments

Experiment #1 (AMF soil inoculum collected in summer)																				
Soil treat.	Week 1				Week 2				Week 3				Week 4				Average			
	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D
	gs ($mol^{-2}s^{-1}$)																			
NCT	290.9b	29	473.9ab	44	351.4b	97	503.2	127	383.2c	60	379.8c	83	462.1	243	639.5	57	454.7b	82	416.3b	43
PCT	550.1a	109	321.6c	79	574.7a	88	533.8	87	682.4a	60	708.3a	53	595.9	18	516.6	126	539.9ab	40	580.9a	36
CNV	506.9a	50	454.3b	79	552.4a	85	726.4	114	536.9b	62	539.3b	64	670.7	27	619.7	55	597.7a	52	553.9a	58
ORG	507.1a	56	547.2a	84	565.1a	38	636.0	82	502.32b	66	571.0b	55	762.5	96	660.0	28	629.2a	49	558.6a	28
Experiment #2 (AMF soil inoculum collected in autumn)																				
Soil treat.	Week 1				Week 2				Week 3				Week 4				Average			
	Adeq. w	St. dv	W. stress	St. dv	Adeq. w	St. dv	W. stress	St. dv	Adeq. w	St. dv	W. stress	St. dv	Adeq. w	St. dv	W. stress	St. dv	Adeq. w	St. dv	W. stress	St. dv
	gs ($mol^{-2}s^{-1}$)																			
NCT	399.8	108	389.7	71	526.0	72	471.0	115	491.8	38	400.0	122	440.0	97	511.3	33	425.2	67	482.2	25
PCT	427.6	160	413.2	108	505.8	98	475.5	101	481.5	84	386.6	104	427.7	89	509.2	92	425.7	68	481.0	78
CNV	404.4	165	339.9	114	485.1	152	466.3	170	387.6	104	308.2	266	472.7	146	520.3	108	396.8	163	449.3	84
ORG	397.2	166	452.4	144	576.1	145	508.6	129	416.5	166	357.8	221	481.5	133	604.5	101	450.0	136	498.6	7

* Different letters within column indicates a significant difference (P¡0.05) AMF affect increase opening of stomas and with this AMF increase transpiration and photosynthesis (reduce stomatal resistance of water). Earlier studies indicate that AMF increase the uptake of K^+ allowing stomas to maintain open [69]. In this regard, during the first experiment we observed that AMF increased stomatal conductance in well and water stress conditions

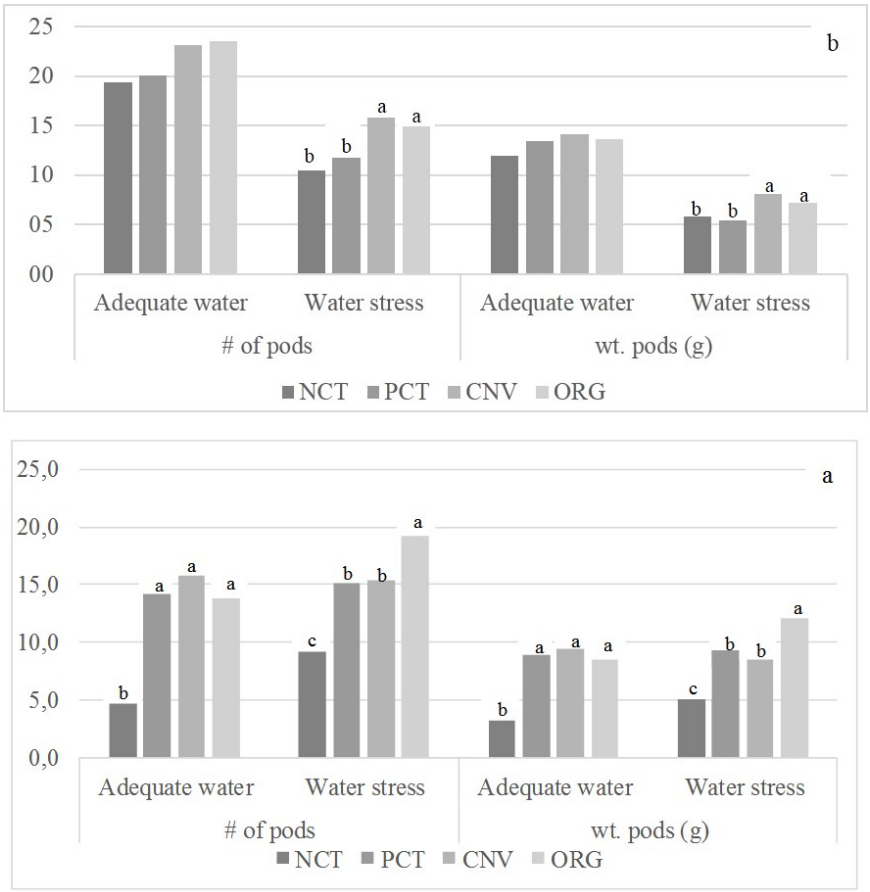


Fig. 5.7. Soybean yield in in plants subject to four soil treatments using field soil AMF inoculum collected at two time points for use in greenhouse experiment #1 (a) and experiment #2 (b).

* Different letters within column indicates a significant difference (P¡0.05) We observed that during the first experiment AMF increased the number of pods and pod dry weight in well water and water stress conditions. However, the use of inoculum from organic managed soils (ORG) significantly increased the number and pods dry weight in drought conditions compared the positive control (PCT) and AMF inoculum from conventional farming practices (CNV). On the other hand, we did not observe differences between ORG and CNV during the second experiment. Since both treatments increased the number of pods and pods dry weight.

Table 5.4.
Soybean above and belowground biomass and root to shoot ratio in plants subject to four soil treatments using field soil AMF inoculum collected at two time points for use in separate greenhouse experiments

Experiment #1 (AMF soil inoculum collected in summer)																
Soil treat.	Total biomass (g)				Shoot weight (g)				Root weight (g)				Root/Shoot ratio			
	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D
NCT	8.0b	2.8	9.8c	1.6	2.4	1.0	2.1	0.2	1.7	1.0	1.8	0.6	0.68	0.1	0.83	0.2
PCT	14.2a	1.4	14.9ab	3.3	3.3	0.6	3.1	1.0	1.9	0.4	2.1	0.9	0.57	0.0	0.68	0.2
CNV	14.9a	2.9	12.4bc	3.4	3.1	1.1	2.3	0.5	2.0	1.3	1.1	0.3	0.58	0.2	0.47	0.1
ORG	13.2a	2.5	17.4a	2.7	2.5	0.9	2.7	0.3	1.9	1.2	2.2	1.4	0.72	0.4	0.78	0.4
Experiment #2 (AMF soil inoculum collected in autumn)																
Soil treat.	Total biomass (g)				Shoot weight (g)				Root weight (g)				Root/Shoot ratio			
	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W. stress	S.D	Adeq. w	S.D	W stress	S.D	Adeq w	S.D	W. stress	S.D
NCT	40.8	27.1	16.3	18.5	29.8	20.6	8.8	14.9	2.8	0.6	1.7 b	0.7	1.69	1.2	0.83	0.9
PCT	44.6	31.1	30.6	33.5	33.0	26.4	21.4	29.4	3.9	1.9	1.9 b	0.7	1.87	1.5	1.71	1.8
CNV	30.2	17.3	27.5	17.4	13.1	12.2	14.9	15.3	3.0	1.5	3.0 a	0.9	0.68	0.5	1.13	0.9
ORG	30.8	14.5	23.0	11.5	14.0	10.7	11.4	10.1	3.3	0.9	2.7 a	0.7	0.76	0.4	0.90	0.7

*SD: Standart deviation

Table 5.5.
AMF colonization rate in soybean roots grown in greenhouse trials using soil inoculum collected from field trials managed using conventional and organic practices during in summer and autumn 2014 (roots from PCT in summer are missing)

Total AMF Colonization rate (%)			
Season	NCT	CVN	ORG
Summer	0%	56%	16%
Autumn	0%	65%	58%

We confirmed that negative control (NCT) was not colonized with AMF. We lost roots samples of positive control (PCT) treatment. During the first experiment we observed that AMF colonization rate in plants inoculated with conventional managed soils (CVN) was higher than plants inoculated with organic managed soils (ORG). However, we did not detect large differences in AMF colonization rate between plants inoculated with conventional managed soils (CVN) and organic managed soils (ORG) during the second experiment.

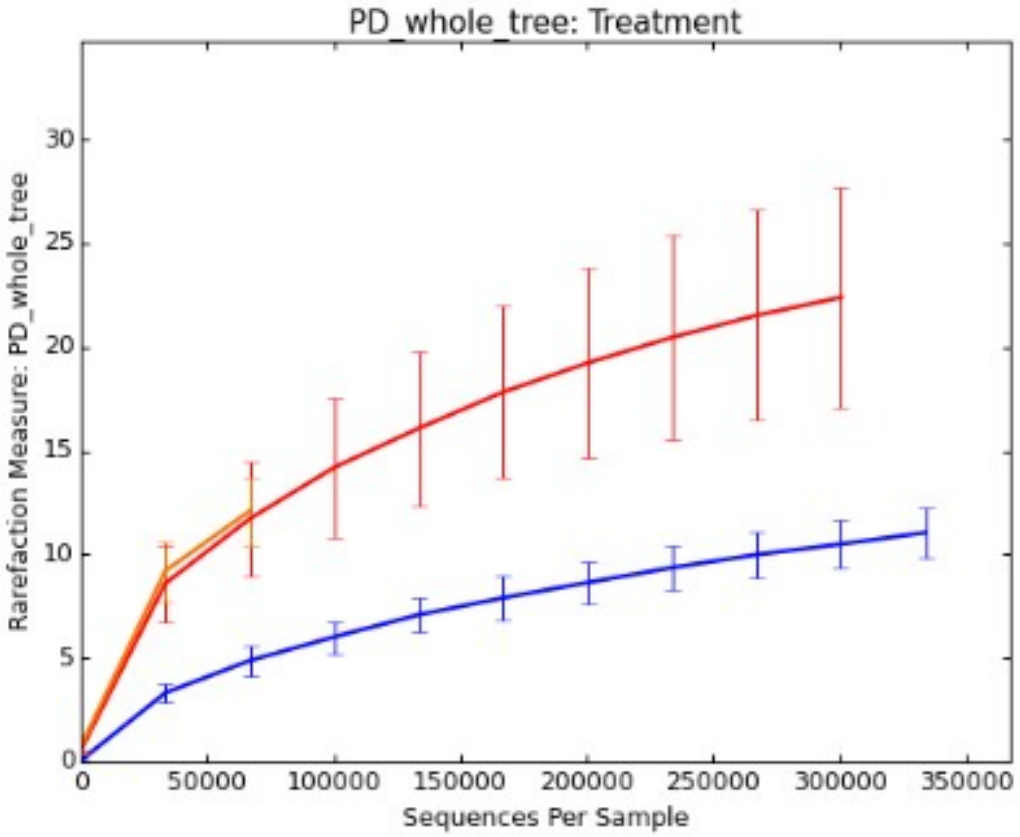


Fig. 5.8. Rarefaction curve generated using Faiths Phylogenetic Diversity Index of AMF communities in soybean roots of plants grown using soil inoculum collected in summer 2014. (Red line CNV; Orange line ORG; Blue line - PCT)

During the first experiment we analyzed rarefaction curves of plants inoculated with conventional managed soils (CNV), organic managed soils (ORG) and positive control (PCT), to determine if sequencing depth covered the entire AMF diversity. In this regard, we observed that the samples did not cover all AMF diversity since rarefaction curves did not reach the plateau.

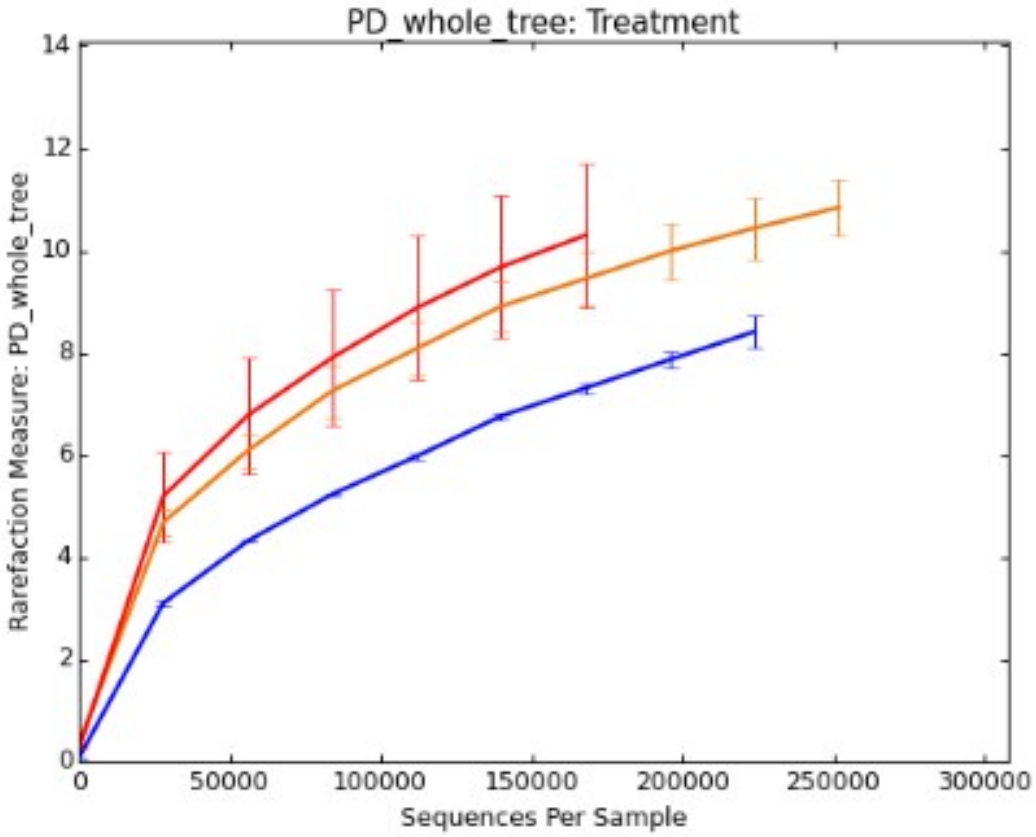


Fig. 5.9. Rarefaction curve generated using the Faiths Phylogenetic Diversity Index of AMF communities in soybean roots of plants grown using soil inoculum collected in autumn 2014. (Red line CNV; Orange line ORG; Blue line - PCT)

During the second experiment we analyzed rarefaction curves of plants inoculated with conventional managed soils (CNV), organic managed soils (ORG) and positive control (PCT), to determine if sequencing depth covered the entire AMF diversity. In this regard, we observed that the samples did not cover all AMF diversity since rarefaction curves did not reach the plateau.

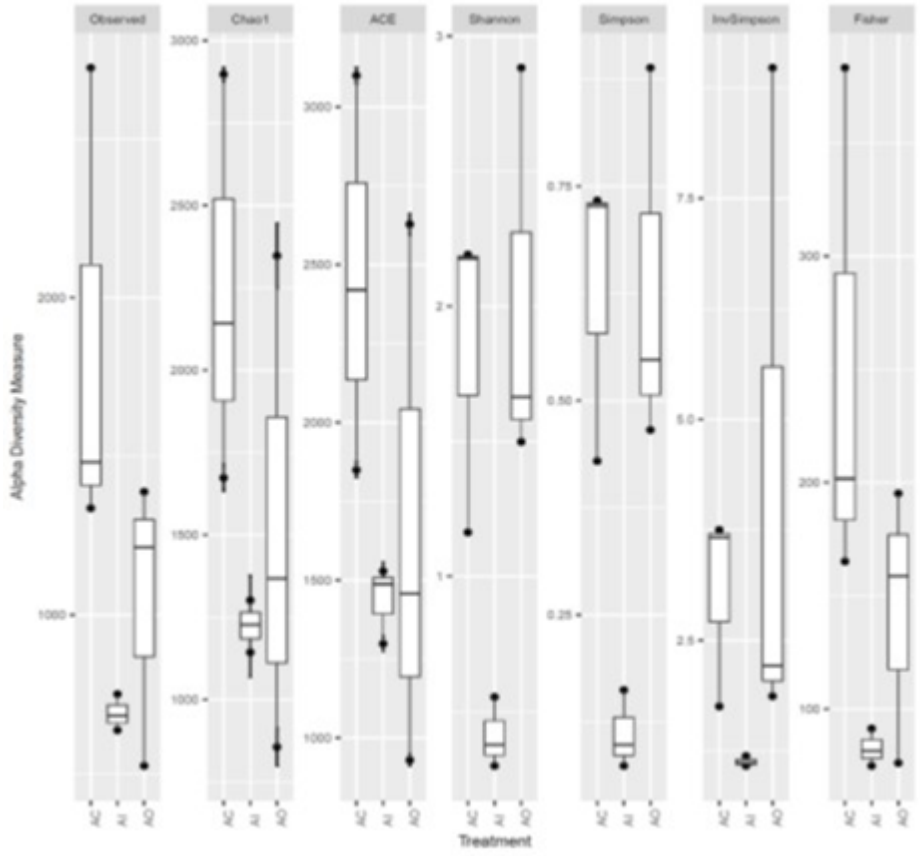


Fig. 5.10. Alpha and beta diversity of AMF communities in soybean roots of plants grown in soil with inoculum collected from field plots in summer 2014 using several indexes. (AC CNV; AI PCT; AO ORG).

During the first experiment we compared α diversity in plants inoculated with organic managed soils (ORG), conventional managed soils (CVN) and positive control (PTC), in order to analyzed AMF diversity of each treatment. We analyzed α with qualitatively measurements such as Chao 1 or ACE (abundance-based coverage estimator), that consider the presence or absence of taxa. We also analyzed diversity with quantitatively measurements such as Shannon or Simpson indices that consider the abundance of each taxon. We found that AMF diversity was higher in CVN and ORG than in PCT. However, AMF diversity was not different between CVN and ORG treatments.

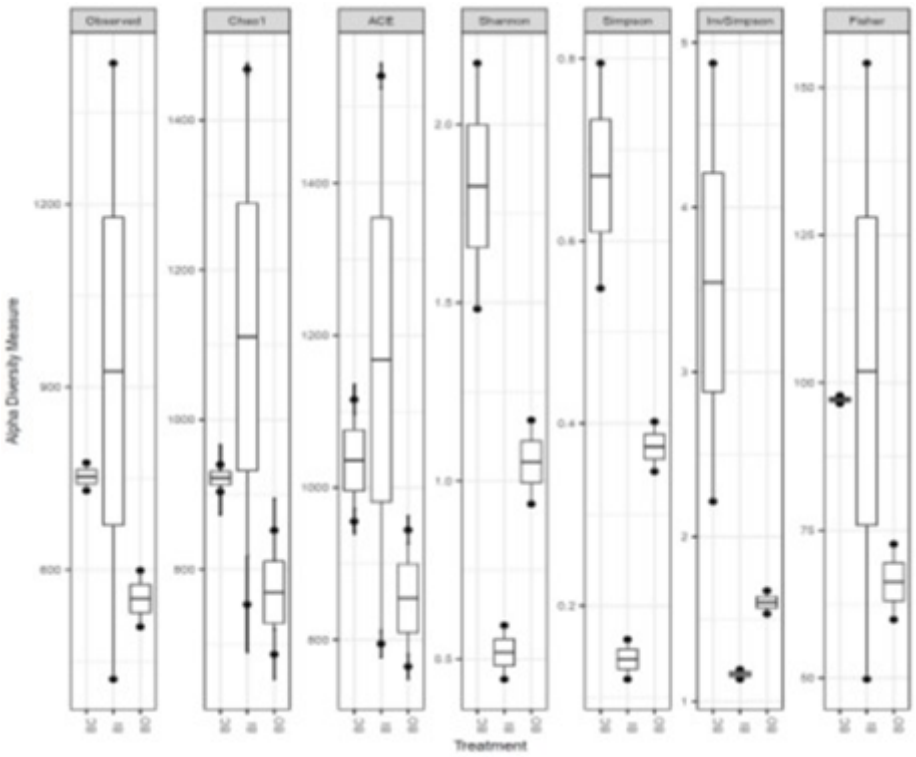


Fig. 5.11. Alpha and beta diversity of AMF communities in soybean roots of plants grown in soil with inoculum collected from field plots in autumn 2014 generated using several indexes. (BC CNV; BI PCT; BO ORG).

During the second experiment we compared α diversity in plants inoculated with organic managed soils (ORG), conventional managed soils (CVN) and positive control (PTC), in order to analyzed AMF diversity of each treatment. We analyzed α with qualitatively measurements such as Chao 1 or ACE (abundance-based coverage estimator), that consider the presence or absence of taxa. We also analyzed α diversity with quantitatively measurements such as Shannon or Simpson indices that consider the abundance of each taxon. In this respect during the second experiment, AMF diversity was higher in CVN compared with ORG. Likewise, AMF diversity in ORG treatment was higher than in PCT.

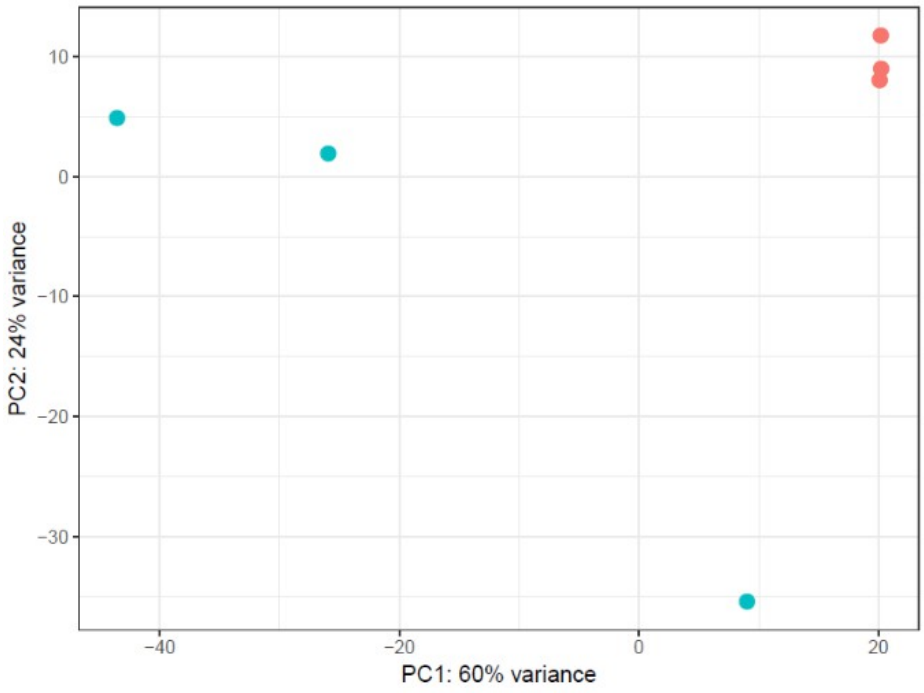


Fig. 5.12. Principal component plot representing differences based on Bray-Curtis Dissimilarity between AMF communities in soybean roots grown in field soil collected from CNV (blue dots) during summer 2014 and PCT (red dots).

We evaluated measurements of β diversity such as principal component plots based on Bray-Curtis Dissimilarity, to determine differences in AMF diversity between plants inoculated with conventional managed soils (CNV) and positive control (PCT) [138]. We found that AMF communities in CNV were different to AMF communities in PCT (PC1:60% of variance).

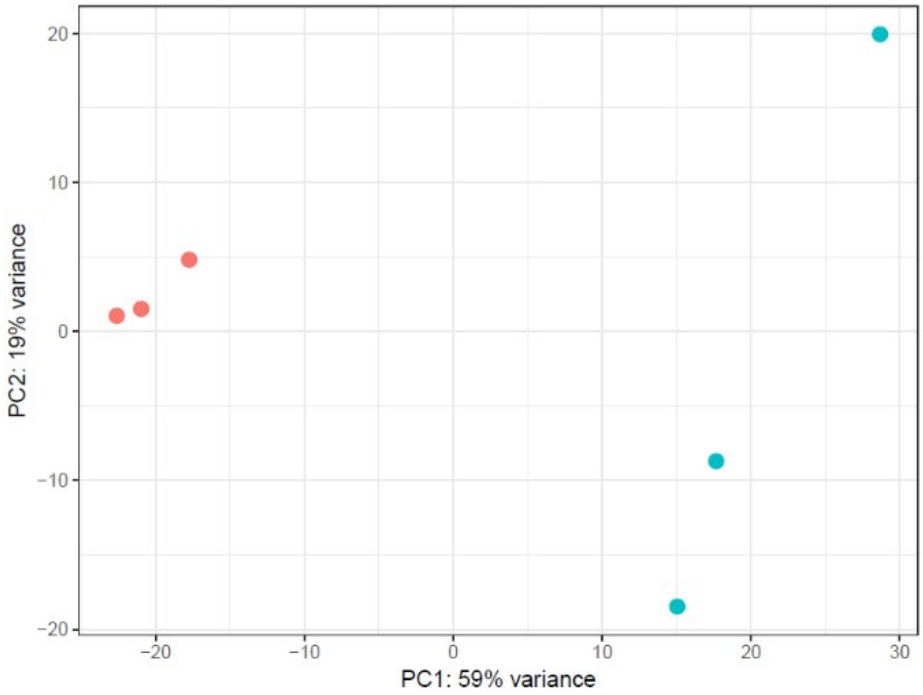


Fig. 5.13. Principal component plot representing differences based on Bray-Curtis Dissimilarity between AMF communities in soybean roots grown in field soil collected from ORG (blue dots) during summer 2014 and PCT (red dots).

We evaluated measurements of β diversity such as principal component plots based on Bray-Curtis Dissimilarity, to determine differences in AMF diversity between plants inoculated with organic managed soils (ORG) and positive control (PCT) [138]. We found that AMF communities in ORG were different to AMF communities in PCT (PC1: 59% of variance).

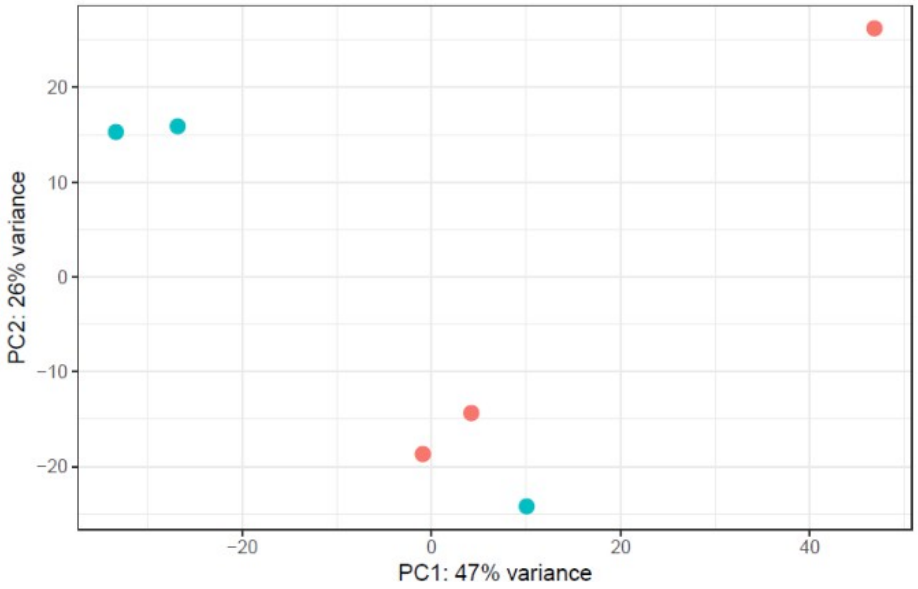


Fig. 5.14. Principal component plot representing differences based on Bray-Curtis Dissimilarity between AMF communities in soybean roots grown in field soil collected from CNV (blue dots) and ORG (red dots) during summer 2014.

We evaluated measurements of β diversity such as principal component plots based on Bray-Curtis Dissimilarity, to determine differences in AMF diversity between plants inoculated with conventional managed soils (CNV) and organic managed soils (ORG) [138]. We found that AMF communities in CVN were different to AMF communities in ORG (PC1: 47% of variance).

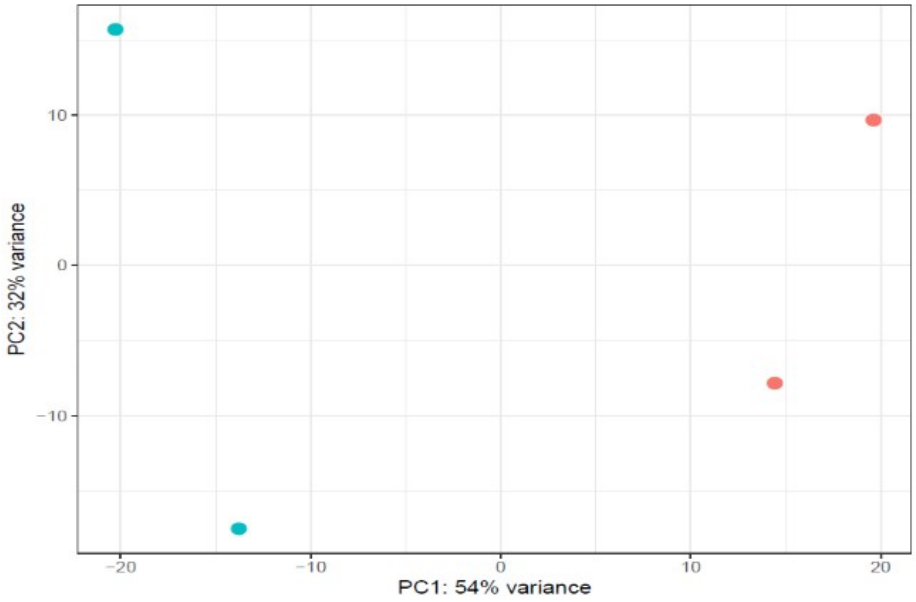


Fig. 5.15. Principal component plot representing differences based on Bray-Curtis Dissimilarity between AMF communities in soybean roots grown in field soil collected from CNV (blue dots) and PCT (red dots) during autumn 2014.

We evaluated measurements of β diversity such as principal component plots based on Bray-Curtis Dissimilarity, to determine differences in AMF diversity between plants inoculated with conventional managed soils (CNV) and positive control (PCT) [138]. We found that AMF communities in CNV were different to AMF communities in PCT (PC1: 54% of variance).

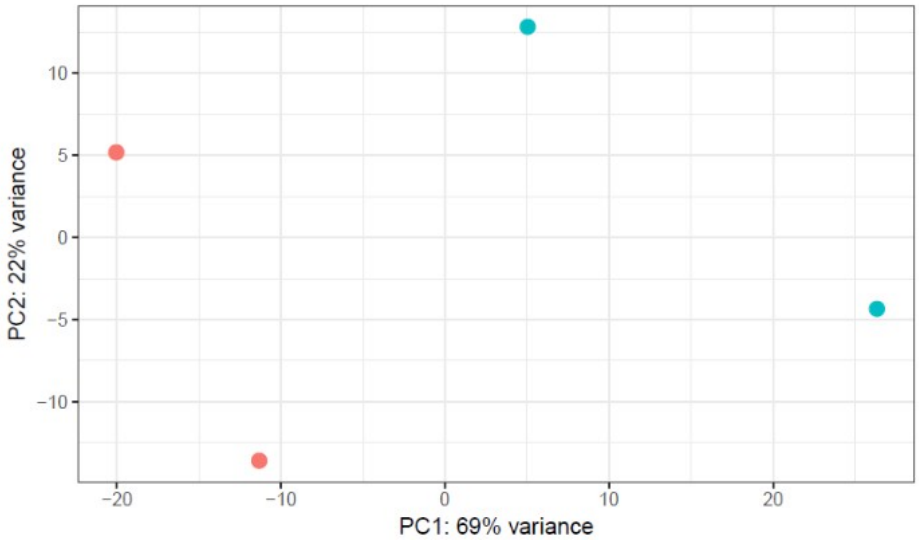


Fig. 5.16. Principal component plot representing differences based on Bray-Curtis Dissimilarity between AMF communities in soybean roots grown in field soil collected from ORG (blue dots) and PCT (red dots) during autumn 2014.

We evaluated measurements of β diversity such as principal component plots based on Bray-Curtis Dissimilarity, to determine differences in AMF diversity between plants inoculated with organic managed soils (ORG) and positive control (PCT) [138]. We found that AMF communities in ORG were different to AMF communities in PCT (PC1: 69% of variance).

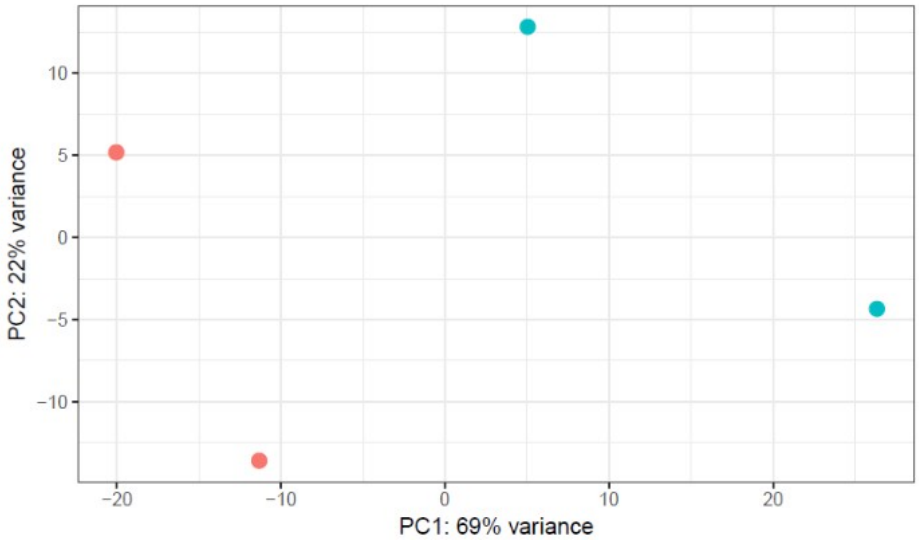


Fig. 5.17. Principal component plot representing differences based on Bray-Curtis Dissimilarity between AMF communities in soybean roots grown in field soil collected from CNV (blue dots) and ORG (red dots) during autumn 2014.

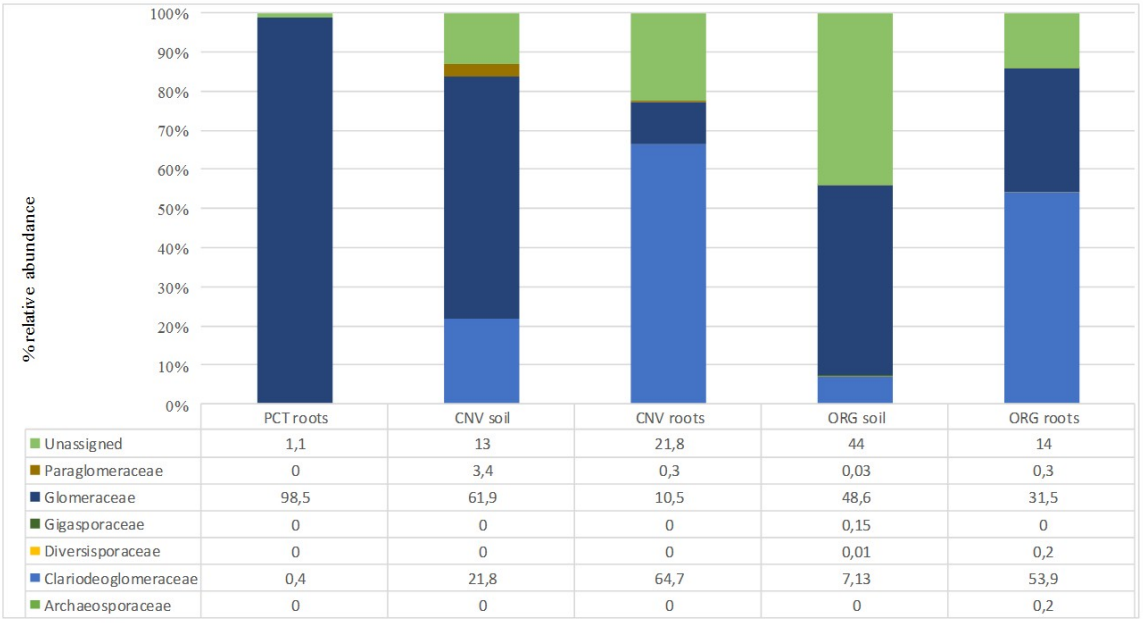
We evaluated measurements of β diversity such as principal component plots based on Bray-Curtis Dissimilarity, to determine differences in AMF diversity between plants inoculated with conventional managed soils (CNV) and organic managed soils (ORG) [138]. We found that AMF communities in CVN were different to AMF communities in ORG (PC1: 71% of variance).

Table 5.6.
Statistical comparison between AMF communities in soybean plants subject to four soil treatments using field soil AMF inoculum collected at two time points for use in separate greenhouse experiments

Comparison	ADONIS		ANOSIM	
	Stat	p-value	Stat	p-value
Experiment #1 (AMF soil inoculum collected in summer)				
CNV vs. PCT - roots	0.8218	0.1	1	0.094*
ORG vs. PCT - roots	0.7651	0.001*	1	0.093*
CNV vs. ORG - roots	0.3181	0.101	0.33	0.201

Comparison	ADONIS		ANOSIM	
	Stat	p-value	Stat	p-value
Experiment #2 (AMF soil inoculum collected in autumn)				
CNV vs. PCT - roots	0.507	0.333	0.75	0.344
ORG vs. PCT - roots	0.7359	0.333	1	0.298
CNV vs. ORG - roots	0.6102	0.042*	0.5	0.305

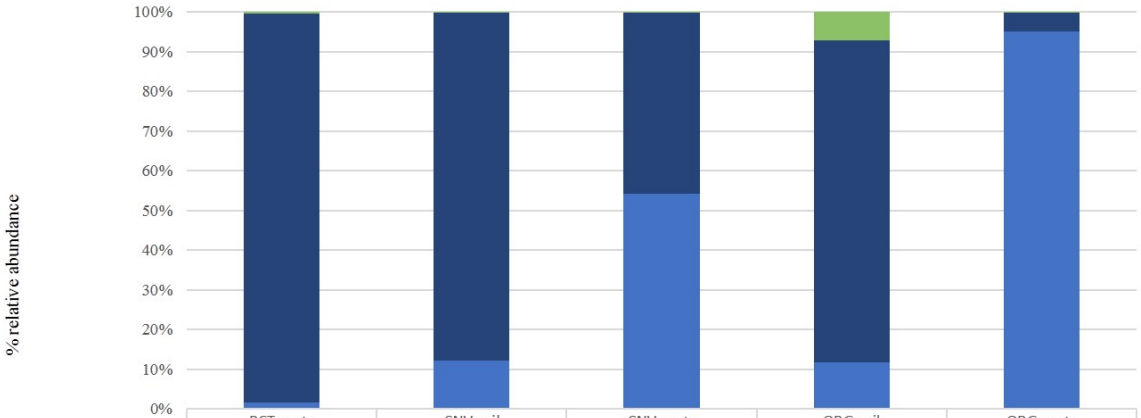
Although during the first experiment we found differences between AMF diversity between CVN - PCT (PC1:60%), ORG - PCT (PC1:59%) and CVN - ORG (PC1:47%). Analysis with Adonis indicated that only ORG PCT were significantly different (p¡0.001). On the other hand, analysis with ANOSIM indicated that CNV PCT and ORG-PCT were significantly different, during the first experiment. We found differences between AMF communities in CVN - PCT (PC1:54%), ORG PCT (PC1:69%) and CVN ORG (PC1:71%). However, we found that only CNV ORG were significantly different (p¡0.042).



	PCT roots	CNV soil	CNV roots	ORG soil	ORG roots
Unassigned	1,1	13	21,8	44	14
Paraglomeraceae	0	3,4	0,3	0,03	0,3
Glomeraceae	98,5	61,9	10,5	48,6	31,5
Gigasporaceae	0	0	0	0,15	0
Diversisporaceae	0	0	0	0,01	0,2
Clariodeoglomeraceae	0,4	21,8	64,7	7,13	53,9
Archaeosporaceae	0	0	0	0	0,2

Fig. 5.18. Relative abundance of AMF families in field soil and roots subjects to soil treatments in greenhouse experiment #1.

We observed that AMF families *Glomeraceae* and *Claroideoglomeraceae* were the more abundant families. In addition, we observed the presence of *Paraglomeraceae* family in smaller proportion in plants inoculated with conventional (CNV) and organic (ORG) managed soils. In particular, we observed that plants inoculated with ORG contained *Diversisporaceae*, *Archaeosporaceae* and *Gigasporaceae*. On the other hand, we found that *Glomeraceae* family was more abundant in soils compare with soybean roots inoculated with CNV. While *Claroideoglomeraceae* family was more abundant in soybean inoculated with CNV and ORG compared with soils. This indicate that *Claroideoglomeraceae* is more adapted to soybean roots than *Glomeraceae* family.



	PCT roots	CNV soil	CNV roots	ORG soil	ORG roots
Unassigned	0,4	0,06	0,2	7,1	0,2
Paraglomeraceae	0	0	0	0,00001	0
Glomeraceae	98	87,8	45,5	81,2	4,5
Gigasporaceae	0	0	0	0	0
Diversisporaceae	0	0	0	0	0
Clariodeoglomeraceae	1,6	12,2	54,3	11,7	92,4
Archaeosporaceae	0	0	0	0	0

Fig. 5.19. Relative abundance of AMF families in field soil and roots subjects to soil treatments in greenhouse experiment #2.

We observed that AMF families *Glomeraceae* and *Claroideoglomeraceae* were the more abundant families. In particular, we observed that only soils managed with organic managed practices (ORG) contain the *Paraglomeraceae* family. On the other hand, we found that *Glomeraceae* family was more abundant in conventional (CNV) and organic managed soils (ORG) compare with soybean roots of these treatments. On the other hand, *Claroideoglomeraceae* family was more abundant in soybean roots inoculated with CNV and ORG compared with soils. This might indicate that *Claroideoglomeraceae* is more adapted to soybean roots than *Glomeraceae* family.

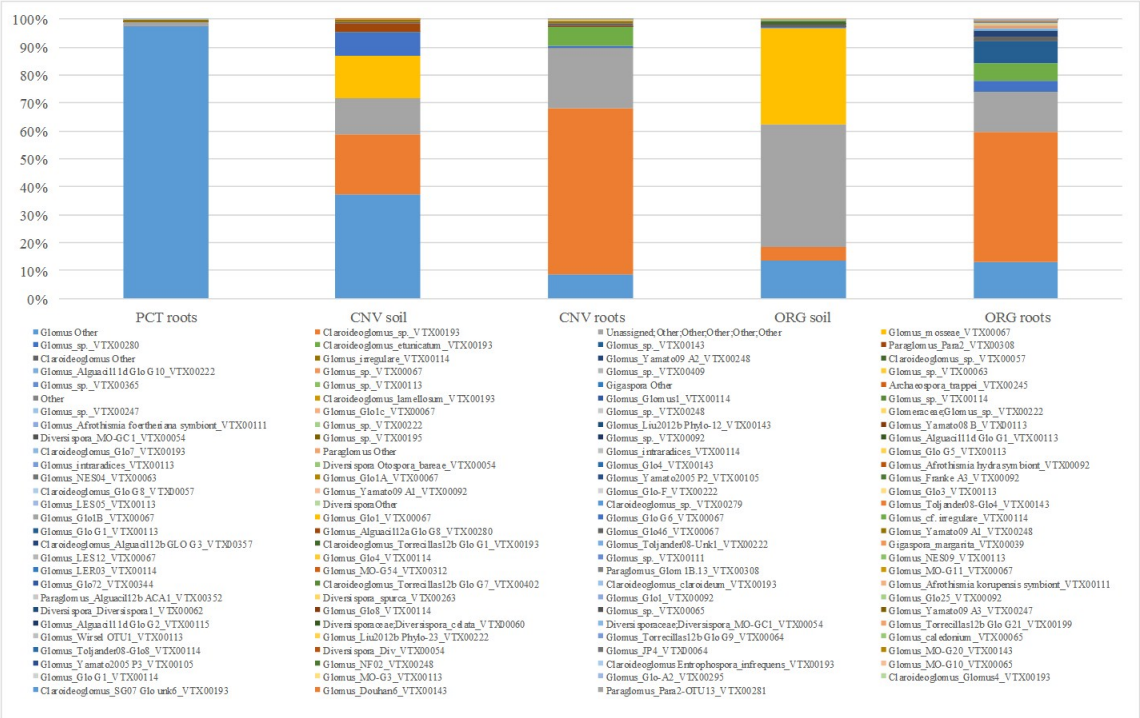


Fig. 5.20. Relative abundance of AMF genera in field soil and roots subjects to soil treatments in greenhouse experiment #1.

We detected that in summer the use of conventional managed practices (CNV) significantly increased the abundance of seventeen (17) AMF different virtual taxa. Whereas virtual taxa such as Glomus_Glomus1_VTX00114, Glomus_irregulare_VTX00114 and Glomus_sp_VTX00114 were more abundant in positive control (PCT).

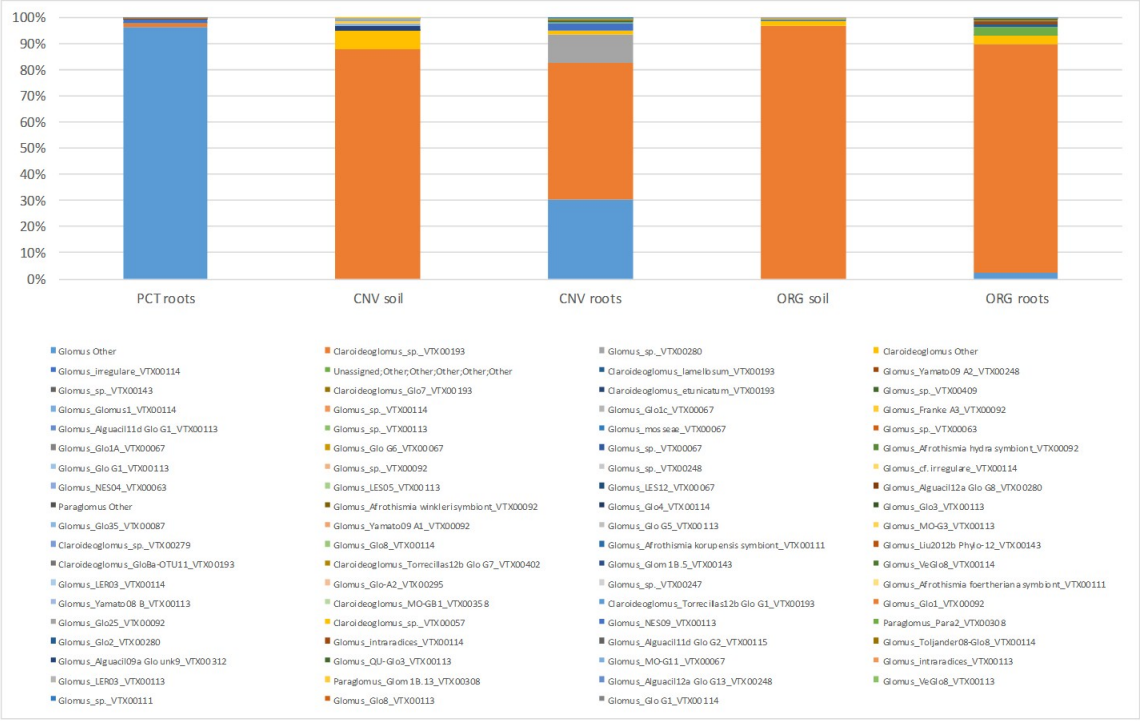


Fig. 5.21. Relative abundance of AMF genera in field soil and roots subjects to soil treatments in greenhouse experiment #2.

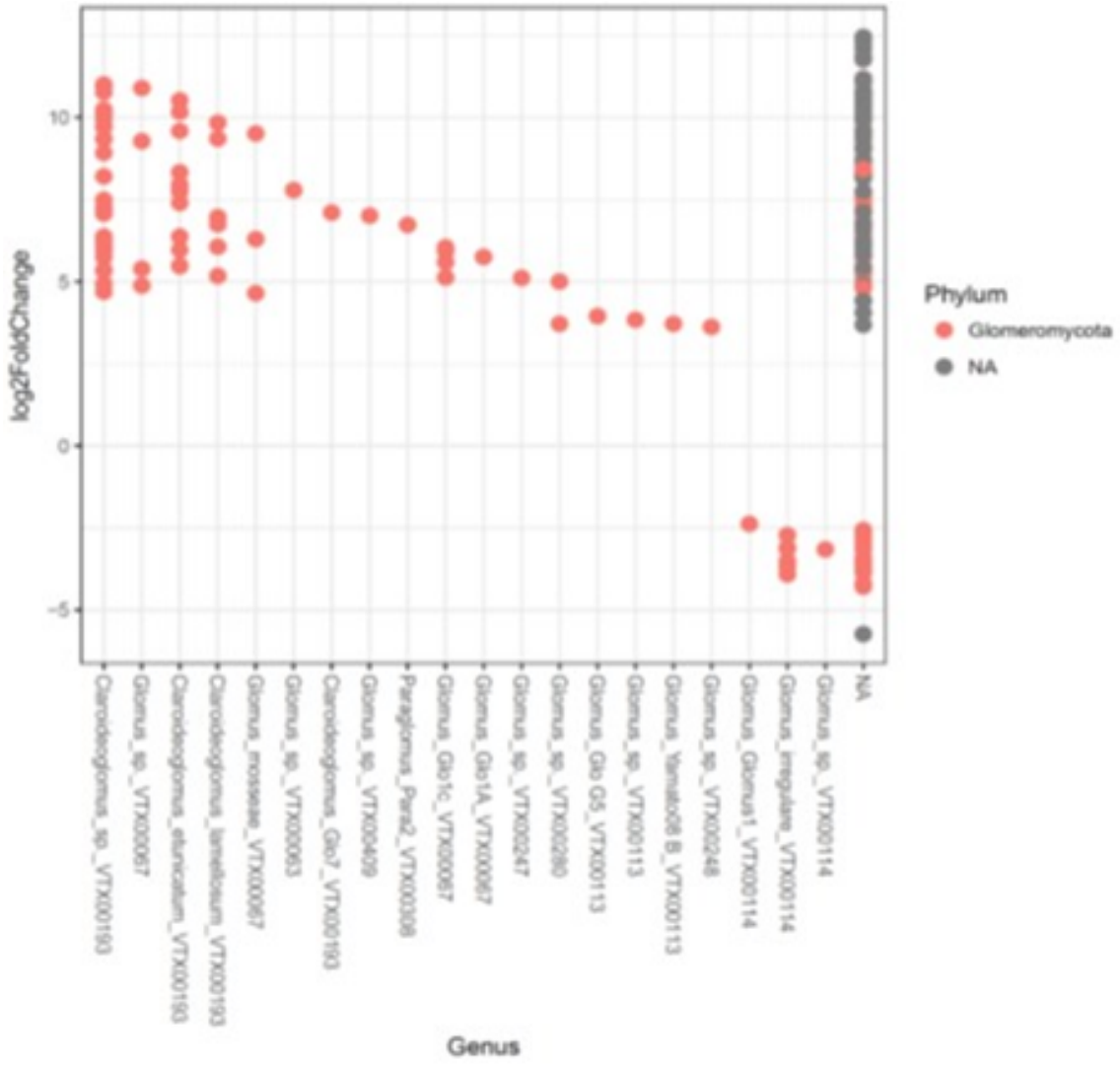


Fig. 5.22. Individual OTUs that were significantly different between roots subject to AMF inoculum from CNV and PCT treatments in experiment #1.

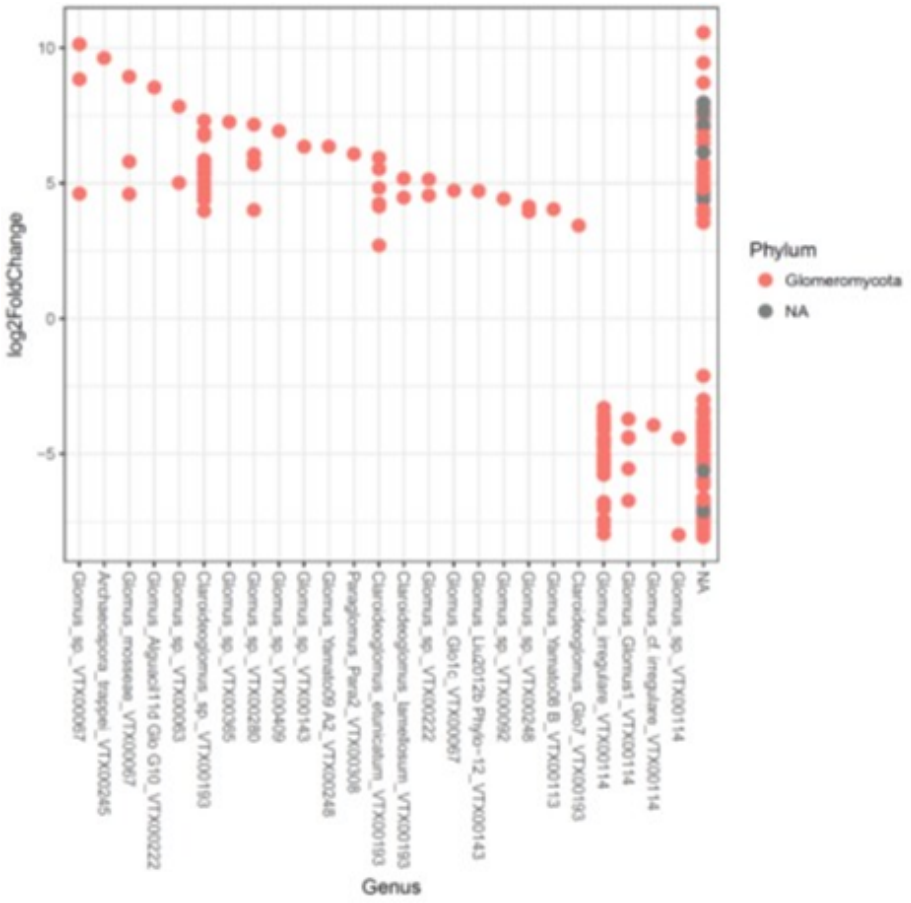


Fig. 5.23. Individual OTUs that were significantly different between roots subject to AMF inoculum from ORG and PCT treatments in experiment #1.

We detected during summer that the use of organic managed soils (ORG) significantly increased the relative abundance of twenty-one (21) different virtual taxa. On the other hand, we observed that positive control (PCT) increased the abundance of Glomus_irregulare_VTX00114, Glomus_Glomus1_VTX00114, Glomus_cf. irregulare_VTX00114 and Glomus_sp_VTX00114.

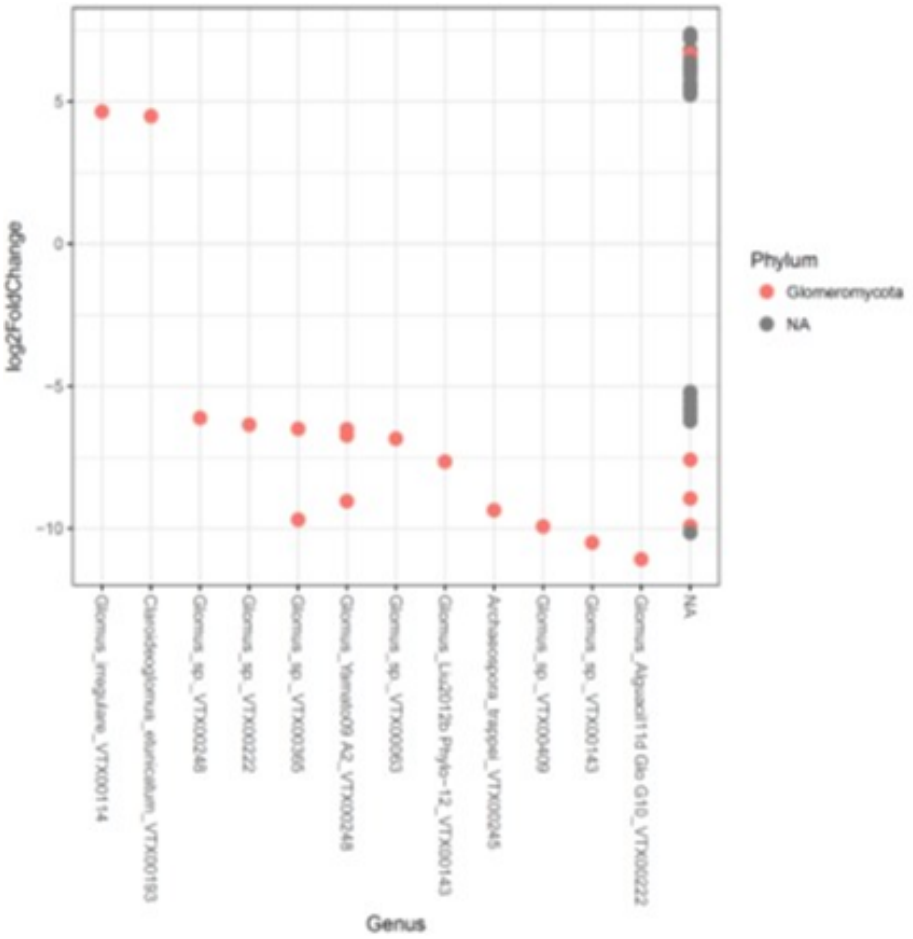


Fig. 5.24. Individual OTUs that were significantly different between roots subject to AMF inoculum from CNV and ORG treatments in experiment #1.

During summer we observed that the use of conventional farming practices (CNV) significantly increased the abundance of Glomus_irregulare_VTX00114 and Claroideoglomus_etunicatum_VTX00193. On the other hand, this treatment significantly reduced the abundance of ten (10) AMF virtual taxa, that were more abundant in soils managed with organic farming practices (ORG).

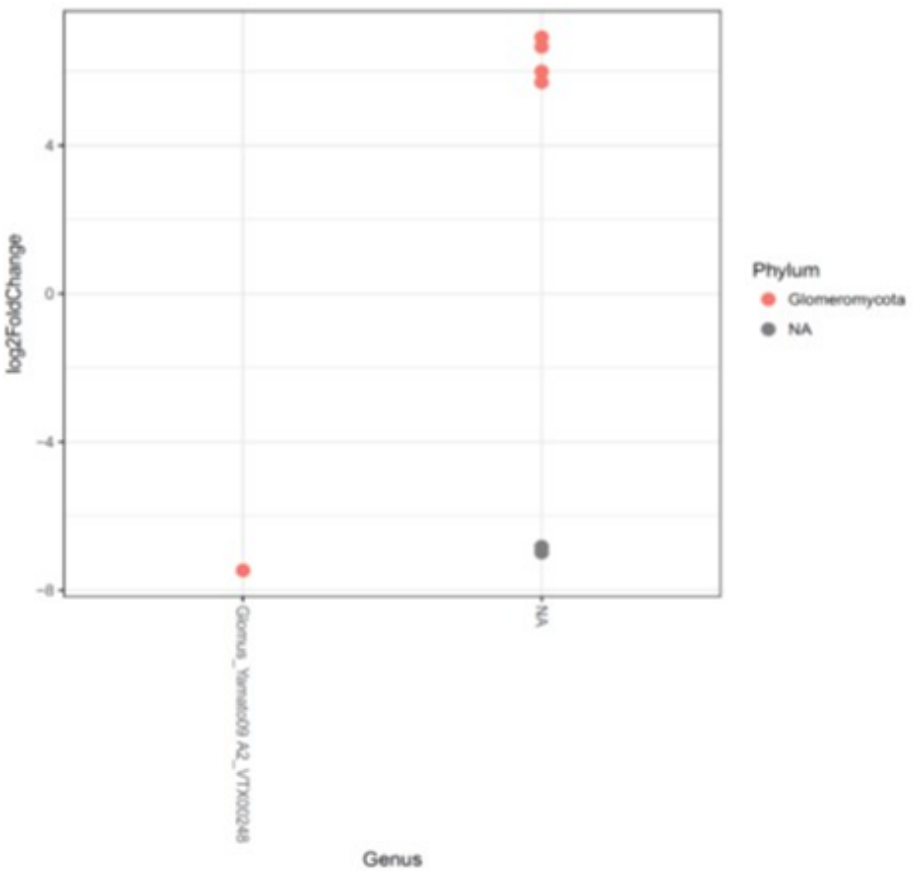


Fig. 5.25. Individual OTUs that were significantly different between roots subject to AMF inoculum from CNV and PCT treatments in experiment #2.

We noticed that in autumn, after soybean harvest soils managed with conventional managed practices (CNV) significantly decreased the abundance of Glomus_Yamato09 A2_VTX00248 compare the positive control (PCT)

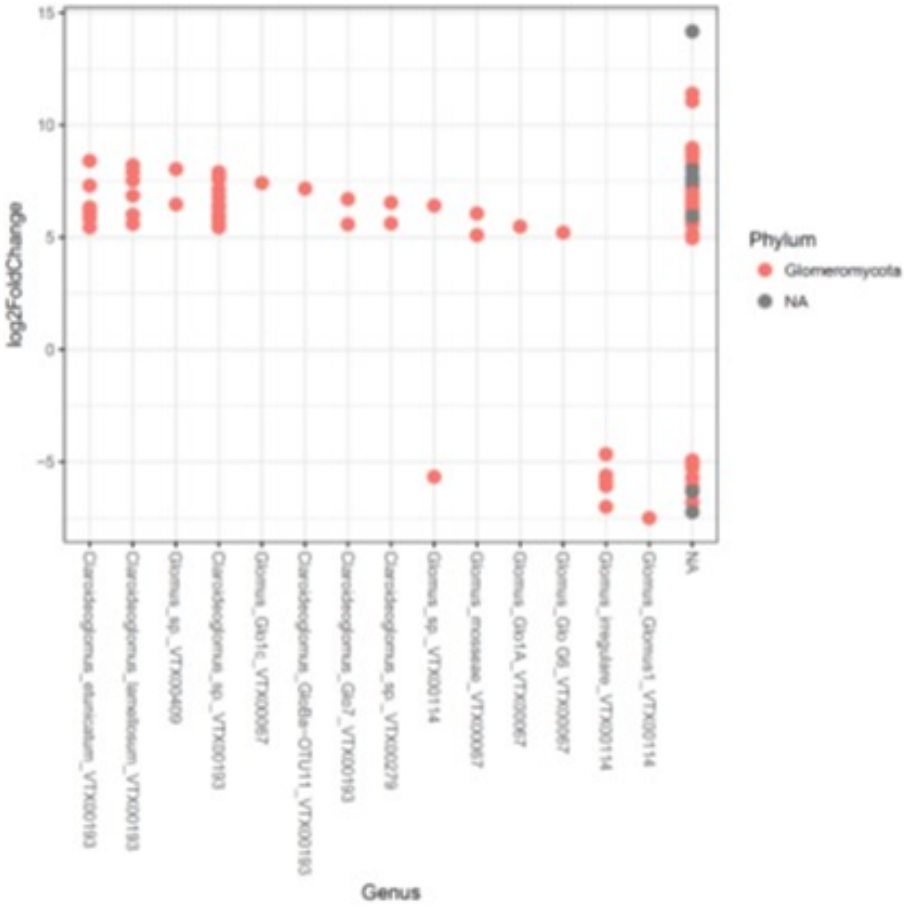


Fig. 5.26. Individual OTUs that were significantly different between roots subject to AMF inoculum from ORG and PCT treatments in experiment #2.

In autumn, we observed that organic managed practices (ORG) significantly increased the relative abundance of twelve AMF virtual taxa compared to the positive control (PCT). On the other hand, the use of ORG significantly decreased the abundance of Glomus_irregulare_VTX00114 and Glomus_Glomus1_VTX00114.

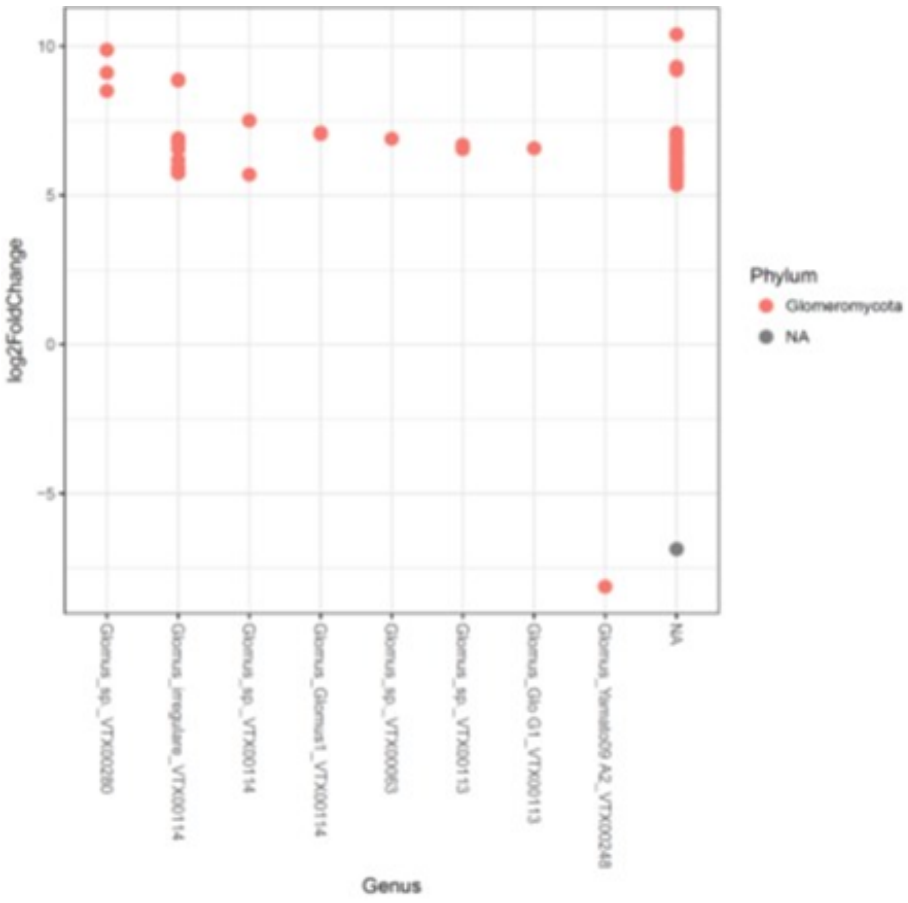


Fig. 5.27. Individual OTUs that were significantly different between roots subject to AMF inoculum from CNV and ORG treatments in experiment #2.

In autumn, we observed that the use of conventional farming practices significantly increased the relative abundance of seven (7) different AMF virtual taxa. However, CNV significantly decreased the abundance of Glomus_Yamato A2_VTX00248 compared with soils managed with organic manage practices (ORG).

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