Title

Effects of Non-Native AMF Inoculum on Plant Quality and Soil Health Across Three Different Processing Tomato Agroecosystems

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Effects of Non-Native AMF Inoculum on Plant Quality and Soil Health Across Three Different Processing Tomato Agroecosystems

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Abstract

Climate-driven changes in temperature and precipitation patterns are translating to greater challenges for agriculture in the Western US, especially for summer-irrigated crops such as processing tomatoes. Farming practices that focus on building soil health can help growers to adapt to and to mitigate the effects of climate change. One practice under much scrutiny is the use of non-native microbial inoculums, such as arbuscular mycorrhizae fungi (AMF), an obligate biotroph that forms symbiotic associations with plant roots. It is well established that AMF can provide benefits to both plants, enhancing nutrient uptake and drought resistance, and soil, contributing to aggregate stability and to the formation and storage of soil organic matter (SOM). Yet, the value of AMF inoculants as effective inputs for sustainable agriculture is still inconclusive and much debated. Soil management practices such as fertilization, organic amendments and cover crops can alter both native microbial populations and the potential impacts of a non-native AMF inoculum on plants and soil. Here, we investigate the effects of a non-native AMF inoculum on the agronomic and nutritional traits of processing tomatoes and soil health indicators across a long-term soil management gradient. In a split plot factorial field study across three years, mycorrhizal root colonization increased in plots treated with a commercial non-native AMF inoculant in the first year but dropped in subsequent years in both control and inoculated plots. In the final year of the study, inoculation with non-native AMF led to a decrease in root colonization compared to control plots. Additionally, inoculation with non-native AMF did not improve plant nutrient uptake, or tomato yields, but did significantly increase both the SOM and the C:N of rhizosphere soils in conventional (synthetic N), organic (cover crop and compost) and mixed (synthetic N and cover crop) systems. Finally, inoculation had greater impacts on rhizosphere microbial communities during vegetative growth and fruit set than at harvest. Although more research is required to determine the mechanisms through which rhizosphere SOM increased and persisted, this study suggests that non-native microbial inoculants can increase SOM in the short term; potentially priming soil communities and soil health and C storage pathways for longer term benefits.

Table of Contents

Introduction 1
Materials and Methods 7
The Century Experiment and cropping systems 7
Experimental design and treatments 10
2019 Baseline Soil Ecosystem Parameters 10
Mycorrhizal Colonization 11
Biomass, Yield and Fruit Quality 12
Plant Nutrients 13
Soil Organic Matter 13
Soil Nutrients 14
Glomalin-Related Soil Protein 14
Microbial Communities 15
Statistical analysis 15
Results 16
Effects of Long-Term Management on Initial Soil Health 16
Effects of Non-Native Inoculation on Root Colonization 17
Root Colonization (2019-2021) 17
In-Season Root Colonization Dynamics (2021) 18
Plant Response to Inoculation Across Management Systems 19
Biomass, Yield, and Plant Nutrients (2019-2021) 19
Fruit Quality and Plant Nutrients (2021) 22
Rhizosphere Response to Inoculation Across Management Systems (2021) 25
Rhizosphere Nutrients 25
Soil Organic Matter 28
Bradford-Reactive Soil Protein 29
Rhizosphere Microbial Communities 30
Discussion 33
Conclusion 40
References 41

Appendix **51**
Supplemental Material **51**

Introduction

California leads the nation in the production and export of processing tomatoes (*Solanum lycopersicum*). In 2021, 92,268 ha were harvested, producing 9.8 million tonnes and approximately $905 million in total value (Ross, 2023). This crop is one of the leading commodities in Colusa, Fresno, Kings, Merced, Yolo, Sutter and Solano counties and California is the nation's sole exporter (supplying 99% or more) of processing tomatoes with $659 million generated in exports in 2021 (Ross, 2023) However, like much of the western United States, California is experiencing increasingly challenging production environments and dramatic effects of climate change with large implications for agriculture and processing tomato systems. Climate projections predict that both temperatures and drought frequency will increase while snowpack will decrease (California's Changing Climate 2018, n.d., www.climateassessment.ca.gov). In addition, winter rainfall variability is predicted to increase with heavy rain events and long periods of drought (Swain et al., 2018). Higher temperatures, reduced water availability for irrigation along with soil degradation, and shifts in herbivory and pathogens mean greater challenges for summer-irrigated crops such as processing tomatoes (Pimentel & Burgess, 2013). Growers must therefore quickly adapt and adopt sustainable practices to ensure a future for the California processing tomato industry in a changing climate.

A key area incentivized at the state level to both adapt to and to mitigate the effects of climate change is soil health and the adoption of soil health building farming practices. In the past few years, soil health has become an area of increasing research interest and debate (Janzen et al., 2021; Harris et al., 2022; Wood & Blankinship, 2022). The United States Department of Agriculture Natural Resources Conservation Service (NRCS) defines soil health as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans" ("Soil Health", n.d., nrcs.usda.gov/conservation-basics/natural-resource-concerns/soils/soil-health). Janzen et al. (2021) takes a more holistic approach to defining soil

health as “the vitality of a soil in sustaining the socio-ecological functions of its enfolding land.” At the basis of the multitude of definitions of soil health and the increasingly complex soil health frameworks are biogeochemical cycles, the pathways through which the elements necessary to life (i.e. carbon (C) and nitrogen (N)) are used, cycled, and replenished. These cycles are driven by soil microbiota. Therefore, it is critical to elucidate the structure and function of the soil microbial community to enhance the sustainability of cropping systems in a changing climate (Bender & van der Heijden, 2015; French et al., 2021).

Of particular interest are arbuscular mycorrhizal fungi (AMF), a well-known group of obligate biotrophic fungi from the *Glomeromycota* phylum that form symbiotic associations with nearly all plant phyla (Smith & Read, 2008). AMF scavenge the soil matrix for nutrients for their host plants and in return receive photosynthates and lipids from their hosts (S. E. Smith & Read, 2008; Keymer et al., 2017). AMF-associated plants can exploit a larger volume of soil than non-mycorrhizal plants as the mycelium of AMF extend beyond the rhizosphere (Smith & Read, 2008). The recent surge of interest in the use of AMF in sustainable agriculture revolves around AMF’s potential to increase the fertilizer use efficiency of crops, boost crop tolerance to pests and pathogens, or boost a crop’s ability to compete with weed species; however, these nutritional benefits may vary based on the resources available to the plant and fungi and on their identity (Walder et al., 2012; Thirkell et al., 2017).

AMF experiments in agricultural settings often involve manipulating the indigenous microbial community using both native and non-native inoculums. Non-native commercial inoculums allow researchers to introduce known species of AMF at quantifiable rates into systems. However, the degree to which mycorrhizae influences the crop depends on plant and fungal identity and compatibility and environmental conditions (Walder et al., 2012; Verbruggen et al., 2013). Inoculums developed from native, locally adapted mycorrhizae overcome these hurdles at the cost of possibly losing species-level identity of the AMF in the inoculant. Studies with both forms

of inoculum have shown that plants can benefit from root symbiosis with AMF through an increase in plant water (Auge, 2001) and nutrient uptake, especially phosphorus (P; Smith & Read, 2008) Both the extent of AMF colonization and the benefit of colonization to the plant have an inverse relationship with plant available P concentrations in soil (Treseder, 2004; Smith & Smith, 2013). Under low soil P concentrations, AMF root colonization increases, and plant benefit is observed more often; whereas under high soil P concentrations AMF may play a less important role in plant nutrition (Oliver et al., 1983; Lekberg & Koide, 2005). Though soil P is often the focus of mycorrhizal research, AMF can improve plant uptake of other nutrients such as N (Marschner & Dell, 1994; Smith & Read, 2008), copper (Cu; Smith & Read, 2008) and zinc (Zn; Cavagnaro et al., 2006; Cavagnaro, 2008) Smith and Read, 2008). Since soil nutrient contents vary widely between agroecosystems, the potential benefits of AMF on plant growth are uncertain and far from scientific consensus (Ryan & Graham, 2018; Rillig et al., 2019). Pellegrino and Bedini (2014) associated AMF inoculation of chickpea (*Cicer arietinum*) with increased root length colonization, greater yield and biomass, enrichment of protein within the seed, and greater N and P plant tissue concentrations. Positive impacts of mycorrhizal inoculation on agronomic traits have also been observed in meta-analyses of multiple studies across cereal, tubers and legume crops (Lekberg & Koide, 2005) such as wheat (*Triticum* spp.; Pellegrino et al., 2015), alfalfa (*Medicago sativa*; Gaur & Adholeya, 2002; Pellegrino et al., 2012), maize (*Zea mays;* Gaur & Adholeya, 2002), berseem clover (*Trifolium alexandrinum*; Gaur & Adholeya, 2002), oat (*Avena sativa*; Gaur & Adholeya, 2002), and cassava (*Manihot esculenta*; Fagbola et al., n.d.). Specifically, in processing tomatoes, AMF have been found to increase yield, water use efficiency, nutrient uptake (Bowles et al., 2016) and nutrient concentrations in tomato fruits (Pasković et al., 2021). In a controlled greenhouse study, Lazcano et al. (2014) found that processing tomatoes that formed symbiotic associations with AMF had greater adaptivity to changing soil moisture, increasing stomatal conductance sensitivity and photosynthetic rates in response to soil moisture levels. Despite these studies

demonstrating beneficial outcomes of AMF inoculation, a significant body of evidence suggests quite the opposite. Establishment of inoculated AMF isolates can be unreliable (Faye et al., 2013; Faye et al., 2020; Thomsen et al., 2021; Salomon et al., 2022). Variability of establishment success can be attributed to soil type, P source, and competition with native AMF(Bender et al., 2019; Faye et al., 2020). In fact, observations of non-native AMF providing no benefit to plants (Faye et al., 2020) or even negative impacts on plant growth under suboptimal soil conditions have been recorded (Dai et al., 2014; Püschel et al., 2016). Given the lack of consensus of AMF's potential benefits to agronomic crop traits, other avenues of AMF benefit or detriment in agroecosystems need to be explored.

Mycorrhizae also impact soil ecosystems and soil health. Mycorrhizal fungi play an important role in aggregate formation both directly through the hyphal enmeshment of soil particles (Cavagnaro et al., 2006; Rillig & Mummey, 2006; Lehmann et al., 2017) and indirectly through the effect of AMF on plant community composition, host root characteristics and soil community composition (Rillig & Mummey, 2006). Soil aggregation has been observed to increase in the presence of AMF-colonized tomato roots when compared to soils surrounding roots of a non-mycorrhizal tomato mutant in a field setting (Cavagnaro et al., 2006). In addition, researchers found that the percentage of small microaggregates was correlated with increased hyphal density (Cavagnaro et al., 2006) suggesting a direct impact of AMF on the formation of aggregates. Likewise, glomalin, a fungal protein of unknown function, has been hypothesized to 'glue' soil aggregates together, due to its hydrophobic nature and its strong correlation with soil aggregate water stability (Rillig, 2004; Wright & Upadhyaya, 1998). Lastly, mycorrhizal fungi can indirectly affect soil aggregation by influencing the composition of plant communities (van der Heijden et al., 1998) and by modifying water regimes, rhizodeposition patterns, and root decomposition (Rillig & Mummey, 2006). In turn, these mycorrhizae-driven changes to the soil system may also alter the soil organic C content.

Soil organic matter (SOM) may be impacted by the fungal-mediated shifts in rhizodeposition patterns, mycorrhizal biomass, and root decomposition mentioned above. Additionally, the priming of soil microbial communities via fungal exudates released by hyphae may also play a role in enhancing SOM formation and storage (Frey, 2019). SOM is the dynamic pool of organic material within soils under microbial control that is stabilized through interactions with soil minerals (Schmidt et al., 2011). The direct role of mycorrhizal fungi in the production, stabilization, and alteration of SOM is likely underappreciated. Mycorrhizae can funnel large amounts of C into the SOM pool either directly as exudates, necromass, or indirectly via nutrient foraging activities (Frey, 2019). Although plant C allocation is difficult to measure, estimates from culture and pot studies indicate up to 30% of net primary productivity (NPP) products are allocated by plants to AMF (Frey, 2019). In addition, fungal exudates are hypothesized to be a dominant route through which plant photosynthate C enters the soil (Kuzyakov, 2002; Sulman et al., 2017). Kaiser et al. (2015) were able to trace fungal exudation through AMF hyphae using ^{13}C labeling in wheat plants suggesting a proportion of plant photosynthate is deposited by fungal hyphae. AMF lack the ability to directly break down SOM but can indirectly stimulate mobilization of nutrients through priming saprotrophic microbial communities via fungal exudates (Tang et al., 2016; Tisserant et al., 2013). These saprotrophic microbes decompose SOM, transforming organic material into inorganic, plant-available forms that move more freely through soils. Though recent evidence suggests that labile C inputs and microbial by-products are important precursors to stable SOM (Schmidt et al., 2011; Whalen et al., 2022), this priming effect may stimulate soil C losses (Horsch et al., 2023). It has been hypothesized that these rapid cycling, inorganic nutrient economies encourage greater crop yields due to higher concentrations of available nutrients (Haynes, 2005). Due to the potential for soil C loss in these fast-cycling nutrient economies, appropriate management of AMF in agricultural systems will be necessary to achieve the multiple goals of increased crop yields while promoting soil health and SOM formation. This may be especially applicable to irrigated cropping systems located in semi-

arid ecoregion which may be undersaturated in soil organic matter, such as those in California (Romanyà & Rovira, 2011; Munoz-Rojas et al., 2012). Given the physical and ecological benefits AMF provide to soil ecosystems, the management of indigenous AMF or the application of non-native AMF may hold unforeseen benefits to agroecosystems.

Agricultural management deeply impacts fungal physical habitat, continuity, and the stoichiometry and abundance of resources available for their growth. This can influence both the abundance of native AMF communities and the establishment and persistence of AMF inoculum (Lekberg et al., 2008). Soil management practices such as the application of synthetic fertilizers, organic amendments, cover crops, and tillage alter AMF populations (Bowles et al., 2017) and can promote mycorrhizal colonization (Schonbeck et al., 2019). Tomato systems in California are managed with high fertilizer inputs and frequent tillage which could inhibit AMF colonization (Oehl et al., 2004; Cavagnaro & Martin, 2011; Bowles et al., 2017). Yet cover cropping and compost applications are becoming more common with recent growth in organic markets and new climate mitigation and conservation incentives (Mitchell et al., 2009; Bergtold et al., 2019). A meta-analysis (Bowles et al., 2017) found that reduced tillage and winter cover crops increased mycorrhizal colonization by approximately 30% in the subsequent cash crop. Importantly, cover crops resulted in a similar increase in AMF in both reduced and conventional tillage systems, suggesting the continuity of roots in the system was enough to overcome adverse effects of tillage. In a study on a long-term trial (Oehl et al., 2004) comparing farming systems with mineral inputs (conventional) to those with composted manure (organic), the number of AMF spores per gram of soil and the number of AMF species per plot were greater in organically managed systems as compared to conventional. This finding suggests that organic fertilizers such as composted manure can increase AMF diversity. However, compost applications often lead to high soil N and P which may inhibit AMF root colonization (Treseder, 2004; Bakhshandeh et al., 2017).

The influence of long-term agroecosystem management on the establishment and plant and soil benefits of non-native AMF has not been well documented. To address this knowledge gap, our study examined the impacts of a non-native AMF inoculum on the quality and soil health of processing tomatoes across a management gradient in the 25th, 26th, and 27th years of a long-term research experiment. We inoculated plants in organic (cover crop + poultry manure compost), mixed (cover crop + synthetic fertilizer), and conventional (synthetic fertilizer + winter fallow) management treatments. We collected plant roots, rhizosphere soil and foliar samples at three time points across a season as well as vine biomass, yield and fruit quality metrics at harvest. We hypothesized that 1) inoculation with non-native AMF would lead to an increase in root colonization and presence of mycorrhizae in the rhizosphere of processing tomatoes and 2) that this increase in root colonization would result in increased plant nutrient content, particularly plant P, increased yields, and improved fruit quality. We hypothesized that 3) the rhizosphere of inoculated processing tomato plants would have improved soil health indicators such as increased SOM, reactive C, soil nutrients, and glomalin content and a more diverse microbial community. Finally, we hypothesized that 4) organic management would enhance potential benefits to plant and soil health.

Materials and Methods

The Century Experiment and cropping systems:

This project was conducted over three years (2019, 2020, and 2021) at the Century Experiment at Russell Ranch Sustainable Agricultural Research Facility (RRSARF) in Winters, California (USA, 38.54˚N,121.87˚W). This experiment, started in 1993, was managed by the University of California, Davis until its termination after 30 years in 2022. The Century Experiment was a study aimed at understanding the long-term impacts of differing management systems on sustainability, including crop and soil health. The experiment was organized into a grid of 72 0.4

ha plots with three plots for each crop in each rotation (Wolf et al., 2018). Prior to 1993, this area was a native oak savanna and perennial grassland that had been replaced by annual row crops (Wolf et al., 2018). Farming operations used large-scale agricultural equipment typical of those used in commercial agriculture productions in California's Central Valley.

Each 0.4 ha plot contained a central 0.2 ha area where no manipulations to the long-term treatment were allowed. Short-term experimentations have been historically conducted in a 208.9 m^2 area to the west and east of the central area (10 beds on each side). Our study focused on three of the eleven management systems under investigation at the Century experiment: the organic maize-tomato system (OMT), the conventional maize-tomato system (CMT), and the legume-maize-tomato system (LMT; Fig. 1). All systems were transplanted with Heinz 1662 cultivar for all three years of the study. The OMT system received 10 tons ha^{-1} of chicken manure compost (approximately 20.0% C, 2.0% N and 1.4% P) from Foster Farms (Livingston, CA) applied annually after harvest (Wolf et al., 2018; Li et al., 2020). The LMT system received synthetic fertilizer at an annual input of approximately 200 kg N ha^{-1} (Li et al., 2020). The OMT and LMT systems were planted with winter cover crops (WCC) in the winter between cropping seasons. The cover crop was composed of 90 kg ha^{-1} *Vicia faba* (bell bean), 22.5 kg ha^{-1} *V. villosa* (hairy vetch), and 28 kg ha^{-1} *Avena sativa* (oats; Li et al., 2020). The CMT system received the same synthetic fertilizer input as the LMT system and lay fallow in winters, between cropping seasons. From 1993-2014, all systems received furrow-flood irrigation. After 2014, all systems had subsurface drip irrigation (Wolf et al., 2018). Cover crops were terminated in early spring via mower and beds were then disced and shaped in preparation for tomato transplant. Herbicides, insecticides, and fungicides were applied in conventionally managed plots. Roundup PowerMAX (Bayer Crop Sciences, Germany, https://bayer.com) and Shark EW (FMC Corporation, Philadelphia, PA, https://fmc.com) were applied in the winter at 110 oz ha^{-1} and 4 oz ha^{-1}, respectively, to manage weeds. In 2019, following transplant, two treatments of

Advise Four (Winfield Solutions LLC, Saint Paul, MN, https://winfieldunited.com) were applied at 10 oz ha^{-1} and 20 oz ha^{-1} for pest management. In 2020 and 2021, pesticide was switched to Macho 4.0 (Albaugh, LLC, Ankeny, IA, https://albaughllc.com) but application rates were kept the same. In 2019 and 2020, both Coragen (FMC Corporation) and Velum One (Bayer Crop Sciences) were applied in mid-summer at 18.75 oz ha^{-1} and 17.1 oz ha^{-1}, respectively. Organically managed plots were hand weeded.

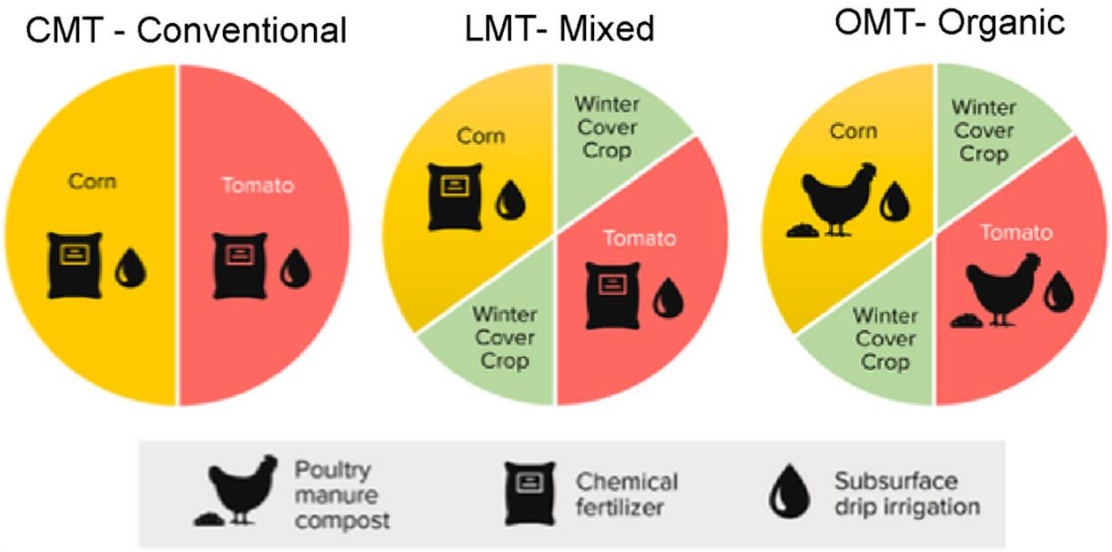

Figure 1. Three tomato systems at the Century experiment (1993-2022). Each pie chart represents the two-year rotation with alternating corn (yellow) and tomato (red) crops. All plots were irrigated with subsurface drip irrigation. Conventional systems (CMT and LMT) received chemical fertilizer. Organic systems (LMT) received composted poultry manure. OMT and LMT systems were planted with winter cover crops (green).

Tomato growing seasons lasted approximately 4 months with transplanting occurring in late May or early April and harvest occurring in late August or early September. The RRSARF is located in a Mediterranean climate with an average minimum temperature of 3.1˚C (37.6˚F) in December and an average maximum temperature of 34.2˚C (93.6˚F) in June (Climate At a Glance County Time Series, n.d., https://www.ncei.noaa.gov/access/monitoring/climate-at-a-

glance/county/time-series). The annual winter precipitation was 109.32 centimeters, 45.34 centimeters, and 60.96 centimeters for 2019, 2020, and 2021, respectively (Climate At a Glance County Time Series, n.d., https://www.ncei.noaa.gov/access/monitoring/climate-at-a-glance/county/time-series).

Experimental design and treatments:

Plots of the Century Experiment were arranged in a complete randomized block design and the experiment was conducted on a subset of nine 0.4 ha plots planted in tomatoes (n = 3 replicate plots of each system). Tomato transplants were inoculated by dipping transplant trays into an inoculant mixture of a concentration of 10 grams ha^{-1} prior to transplanting (Inoculation). Control sub-plots were treated in the same fashion with autoclaved mycorrhizal inoculant (Control). In 2019, the treatment and control were applied to randomized subplots of 13.7 meters on 3 beds per plot. In 2020 and 2021, the treatment and control were applied to the full length of 6 beds per plot and randomized (subplot = 3 beds per treatment). All measurements were conducted on the middle bed to control for edge effects.

2019 Baseline Soil Ecosystem Parameters:

In 2019, the Soil Health Institute (Morrisville, NC, https://soilhealthinstitute.org) conducted a survey of all plots of The Century Experiment. Total carbon (TC) and total nitrogen (TN) were measured using dry combustion (NC2100 Soil Analyzer CE Instruments). SOM was measured using loss on ignition after heating sample in a muffle furnace to 500°C (Heiri et al., 2001). Active C was measured following the Kellogg Biological Station (Michigan State University, MI, https:kbs.msu.edu) procedure for the determination of potassium permanganate oxidizable carbon (POxC) digestion and quantified colorimetrically (Weil et al., 2003). Respiration was measured after re-wetting soil samples to 50% water holding capacity and incubating for 4 days (Zibilski, 1994). Total microbial biomass was measured using phospholipid fatty acid (PLFA)

analysis (Quideau et al., 2016). Soil pH was measured using 1:2 mixture, by weight, of 1 part soil to 2 parts distilled H_2O (Eckert, 1988). P was measured using a Mehlich 3 extraction. Aggregate stability was measured using a vibratory sieve shaker with rainfall simulation (Moebius et al., 2007). Bulk density was calculated from the average dry weight of two 7.6cm soil surface cores divided by the volume of the hammer core used to collect the samples. Available water holding capacity was calculated from the difference of an intact sample under 33kPa and a repacked sample at 1500kPa. Soil hydraulic conductivity was measured using modified dual pressure head method (Saturo™ device).

Mycorrhizal Colonization:

In 2019 and 2020, all samples were taken at 6-weeks post-inoculation. In 2019, a total of 54 tomato plants were harvested for root sampling (n=9 samples/inoculation treatment). In 2020, 72 tomato plants were harvested for root sampling (n=12 samples/inoculation treatment). In 2021, samples were collected at three time points: 6-weeks post-inoculation (anthesis), 10-weeks post-inoculation (fruit set), and 17-weeks post-inoculation (harvest). At each time point, 108 plants were harvested (n=18 samples/inoculation treatment). Plants were uprooted by hand and severed at the base of the stem to separate belowground biomass from aboveground biomass.

One gram of fresh fine roots was subsampled randomly from each belowground biomass sample and stored in 50% ethanol at 4°C. To quantify root colonization, roots were rinsed, cut into approximately 2-centimeter segments and stained using a method modified from Vierheilig et al. (1998). In 2019 and 2020, root segments were cleared in a 10% KOH solution at 90°C for 40 minutes and stained in a 5% ink and vinegar solution for 5 minutes at 90°C. In 2021, roots were bleached in 10% bleach solution for 5 minutes as an additional step to increase root transparency following clearing with KOH prior to staining. After staining, roots were de-stained in acidified water and stored at 4°C until quantification. Quantification of AMF colonization was

performed using the gridline intersect method described in Giovannetti & Mosse (1980) on a dissecting scope at x 80 magnification (Fig. 2). A minimum of 100 root segments were counted for each plant with an average of 140 segments being counted in 323 samples.

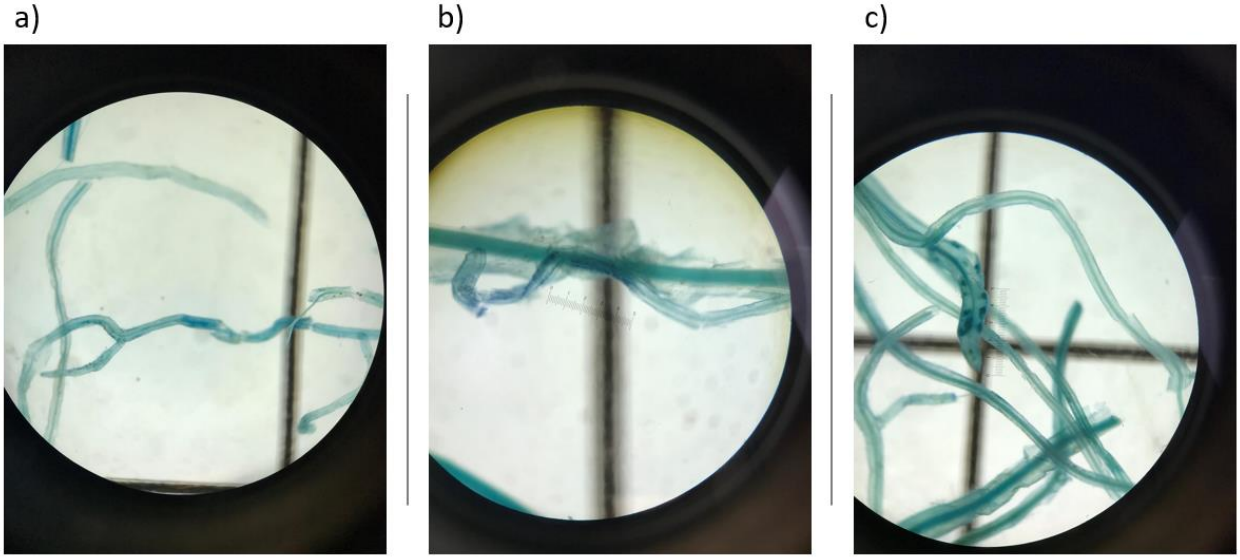

Figure 2. Photos of stained roots (blue) under dissecting scope at x80 magnification. Darker patches of blue staining indicate the presence of mycorrhizae within root. Black lines beneath roots are part of grid utilized in this method.

Biomass, Yield and Fruit Quality:

In 2019, 54 vines were collected (n=9/inoculation treatment/management). In 2020, 72 vines were collected (n=12/inoculation treatment/management). In 2021, 36 vines were collected (n=6/inoculation treatment/management). Vines were dried at 60℃ for approximately one month until weight was stable. Dried vines were then weighed for dry biomass.

In 2019 and 2021, hand harvest yield estimates for red and green fruit were collected while in 2020 machine harvest yield was collected. In 2021, after fruits were collected by hand, they were weighed by color and disposed of. A subsample of red fruits was collected from each subplot and taken to a Processing Tomato Advisory Board (http://www.ptab.org) grading station to measure total soluble solids, fruit color and fruit pH.

Plant Nutrients:

In 2019 and 2020, leaf samples were collected every two weeks until flowering and mixed by plot to form composite samples. More extensive measurements were taken over the length of the 2021 season to better understand the dynamic shifts in plant-AMF interactions and nutrient uptake with inoculation. In 2021, leaf samples were collected at three time points: 6-weeks post-inoculation (anthesis), 10-weeks post-inoculation (fruit set), and 17-weeks post-inoculation (harvest). Three leaflets were collected from each of 108 plants (n=6/treatment/plot) and mixed to form a composite sample representative of each plot. Samples were dried at 60℃, ground, and sent to the Agricultural Analytical Services Lab at Pennsylvania State University for nutrient analyses (State College, PA, https://agsci.psu.edu). Foliar tissues were analyzed for N using combustion (Elementar Vario Max N/C Analyzer) following (Horneck & Miller, 1998). P, potassium (K), calcium, magnesium, sulfur, manganese, iron, copper, boron, aluminum, zinc, and sodium were measured using acid digestion on a hot block following methods from (Huang & Schulte, 1985).

Soil Organic Matter:

Soil organic matter (SOM) and reactive C were measured in all rhizosphere samples collected throughout the 2021 season. SOM was sent to Ward Labs (Kearney, NE, https://www.wardlab.com) for measurement and was measured using the Loss-on-Ignition (LOI) method (Heiri et al., 2001). Active C was measured following the same method as in 2019 baseline sampling (Weil et al., 2002). C and N were measured in rhizosphere samples taken at harvest using elemental analysis combustion (Costech Analytical, Valencia, CA, https://costechanalytical.com).

Soil Nutrients:

In 2021, at 6, 10, and 17-weeks post-inoculation, rhizosphere soil was collected from the tomato plants harvested for leaf and root sampling. The rhizosphere soil collected from each subplot was mixed to form a representative composite sample. Bulk soil samples were collected for each subplot (n=6 samples/inoculation treatment). Both bulk and rhizosphere soil samples were stored at -20℃ until analyzed. Rhizosphere soil samples were sent to an external laboratory for soil pH and macro- and micronutrients (Ward Lab). Soil pH was measured using a 1:1 mixture, by weight, of 1 part soil to 1 part distilled H_2O (Eckert, 1988). Nitrate and ammonium were measured using by colorimetry on 2M KCl extracts (Keeney & Nelson, 2015). P was measured using an Olsen bi-carbonate solution extraction (Sims, 2000). K, calcium, magnesium, and sodium were measured using ammonium acetate extraction (Normandin et al., 1998). Boron was measured using hot water extraction (Jeffrey & McCallum, 1988). Zinc, iron, manganese and copper were measured with a DTPA extraction (Liang & Karamanos, 1993). Sulphite (sulfur trioxide) was measured using a Mehlich 3 extraction (Mehlich, 1984).

Glomalin-Related Soil Protein:

Glomalin-related soil protein (GRSP) was measured using a Bradford Quick-dye Reagent assay (Wright & Upadhyaya, 1998). Briefly, 1 gram of rhizosphere soil was aliquoted, mixed with sodium citrate, autoclaved, and centrifuged several times. Supernatant was removed after each round of autoclaving and centrifuging. Two technical replicates were performed for each sample. Following the nomenclature from Rillig (2004) the supernatant removed after the initial autoclaving will be referred to as EE-BRSP (Easily Extractable Bradford-Reactive Soil Protein). Any supernatant removed after subsequent autoclaves will be referred to as DE-BRSP (Difficulty Extracted Bradford-Reactive Soil Protein). The sum of the two will be referred to as BRSP (Bradford-Reactive Soil Protein).

Microbial Communities:

Microbial abundance and diversity and presence of mycorrhizae was measured in 2020 and 2021 seasons. Rhizosphere samples were sent to an external lab (Kearney, NE, https://www.wardlab.com) for phospholipid fatty acid (PLFA) analysis using a chloroform extraction and classification of microbial groups through gas chromatography (Buyer & Sasser, 2012).

Statistical analysis:

All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2022). Multi-seasonal data was modeled using a linear regression with inoculation, management, year and interaction terms as fixed factors and variance partitioned using a three-way analysis of variance (ANOVA). Statistical significance was determined using $\alpha = 0.05$. The Boxcox equation was applied to any non-parametric multi-seasonal linear regressions to determine the optimal transformation when required. Data from the 2021 season was modeled using linear mixed effects regression models with the lme4 R package (v1.1.31; (Bates et al., 2014). Differences in soil health indicators, fruit quality, agronomic traits, and plant nutrients within the 2021 season were assessed using a two-way ANOVA with fixed effects of inoculation, management and the interaction, and plot as a random effect. Residuals were checked for homogeneity and normal distribution using the package *redres* (v0.0.0.9; Goode et al., 2021). In the absence of significant effects of a treatment or an interaction of treatments, data were pooled across the non-significant treatment. Differences between means were measured using p-adjusted Tukey tests ($P \leq 0.05$). Correlations were determined using Pearson product-moment correlation coefficient. Principle Component Analyses (PCA) were built using the FactoMineR R package (v2.8; Lê et al., 2008). A distance matrix was built for each PCA using the Euclidean formula. This distance matrix was then tested for significance using a permutational multivariate ANOVA (PERMANOVA). Heatmaps were generated to visualize hierarchical clustering. Data was

normalized and heat maps were created using the heatmaply R package (v1.4.2; Galili et al., 2018).

Results

Effects of Long-Term Management on Initial Soil Health:

Twenty-five years of different management systems induced significant shifts in soil health metrics that might mediate the impacts and effectiveness of non-native mycorrhizal inoculation (Fig. 3).

Long-term management influenced the physical habitat. Organically managed systems (OMT) had 24.9% greater aggregate stability than under conventional practices (CMT: $P = 0.0433$). Though not statistically significant, aggregate stability was also greater (+23.2%) in LMT than in CMT plots ($P = 0.0565$) and bulk density dropped by 0.08 g cm^{-3} and 0.06 g cm^{-3} in OMT plots as compared to CMT and LMT plots, respectively ($P = 0.2173$, $P = 0.2839$; Fig. 3). No significant difference was identified in soil hydraulic conductivity amongst managements (Fig. 3).

Soil water and nutrient content were also impacted by long-term management practices. CMT and LMT plots had less available water holding capacity (-0.038 cm^3 H_2O $(cm^3)^{-1}$ and -0.018 cm^3 H_2O $(cm^3)^{-1}$) than OMT plots ($P = 0.0019$, $P = 0.0405$; Fig. 3). OMT and LMT plots had an average of 0.51% and 0.35% more total C ($P = 0.0011$, $P = 0.0161$) and 244.4 mg kg^{-1} and 145.6 mg kg^{-1} more active C ($P = 0.0030$, $P = 0.0331$) than CMT plots, respectively (Fig. 3). Long term use of cover crops and composts (OMT) lead to more SOM accumulation (+0.67%, $P = 0.0285$) and total N compared to conventional management (CMT; $P = 0.0039$; Fig. 3). LMT plots had a lower pH than both OMT and CMT plots, with an average pH of 6.6 in LMT plots as compared to 6.8 and 6.9 in OMT and CMT plots, respectively ($P = 0.0204$, $P = 0.0035$). Soil P

content was 105.2 mg kg^{-1} and 113.0 mg kg^{-1} higher in OMT compared to CMT and LMT plots (*P <0.0001, P <0.0001*, Fig. 3).

Conventional management (CMT) consistently lowered soil biological metrics compared to organic management (OMT). We measured greater biological activity under organic management (OMT, +0.225 mg g^{-1} 96 $hours^{-1}$ respiration, *P = 0.0125*) compared to conventional management (CMT). Although not significant, total microbial biomass trended higher in OMT and LMT compared to CMT (309.4 nmol g^{-1} and 307.2 nmol g^{-1}; *P = 0.0734, P = 0.0507*; Fig. 3).

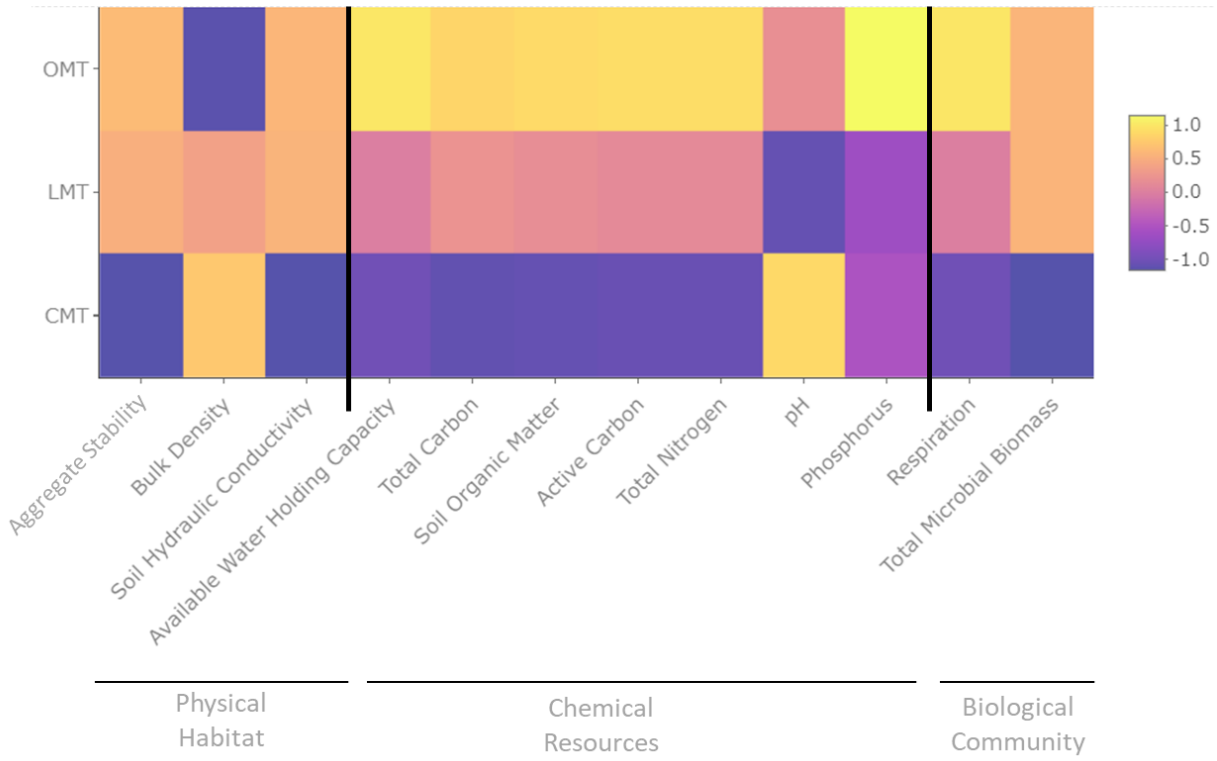

Figure 3. Z scores of normalized means of baseline soil health parameters (2019) across three management systems at the Century Experiment. Parameters are arranged into indicators of physical habitat, chemical resource availability, and biological community. CMT = Conventional Maize Tomato, LMT = Legume Maize Tomato, OMT = Organic Maize Tomato. Management details are given in Fig. 1.

Effects of Non-Native Inoculation on Root Colonization:

Root Colonization (2019-2021):

Root colonization in early development (6 weeks post-inoculation) was significantly higher in 2019 compared to 2020 or 2021 but remained low in all years (Fig. 4). Root colonization was similar across all managements in each season. In 2019, inoculated roots had an average of 9.5% more root length colonized than control plants (Fig. 4; *P = 0.0123*) but this effect was not consistently observed in 2020 or 2021 (*P = 0.1636*, *P = 0.0558*).

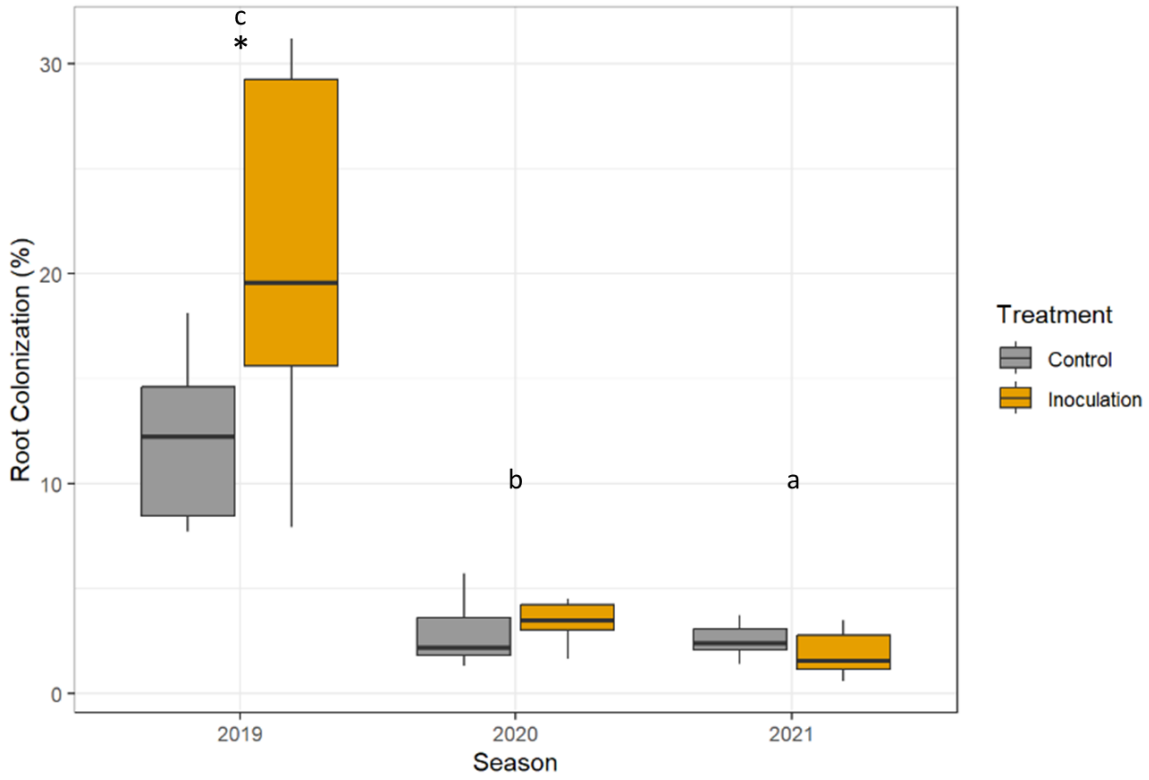

Figure 4. Root colonization across all seasons (n=9). (*) indicates significant inoculation effects within seasons *(P<0.05*), letters indicate seasonal effects.

In-Season Root Colonization Dynamics (2021):

In 2021, colonization increased over the 17-week season in all treatments, but the percentage of root length colonized remained low (Fig. 5). Root colonization at fruit set (10-weeks post-

inoculation) and at plant maturity (17-week post-inoculation) decreased with inoculation across all managements (*P = 0.0505, P = 0.0355,* Fig. 5). Earlier in development, root colonization showed the same trend, but these differences were not significant (*P = 0.2623*).

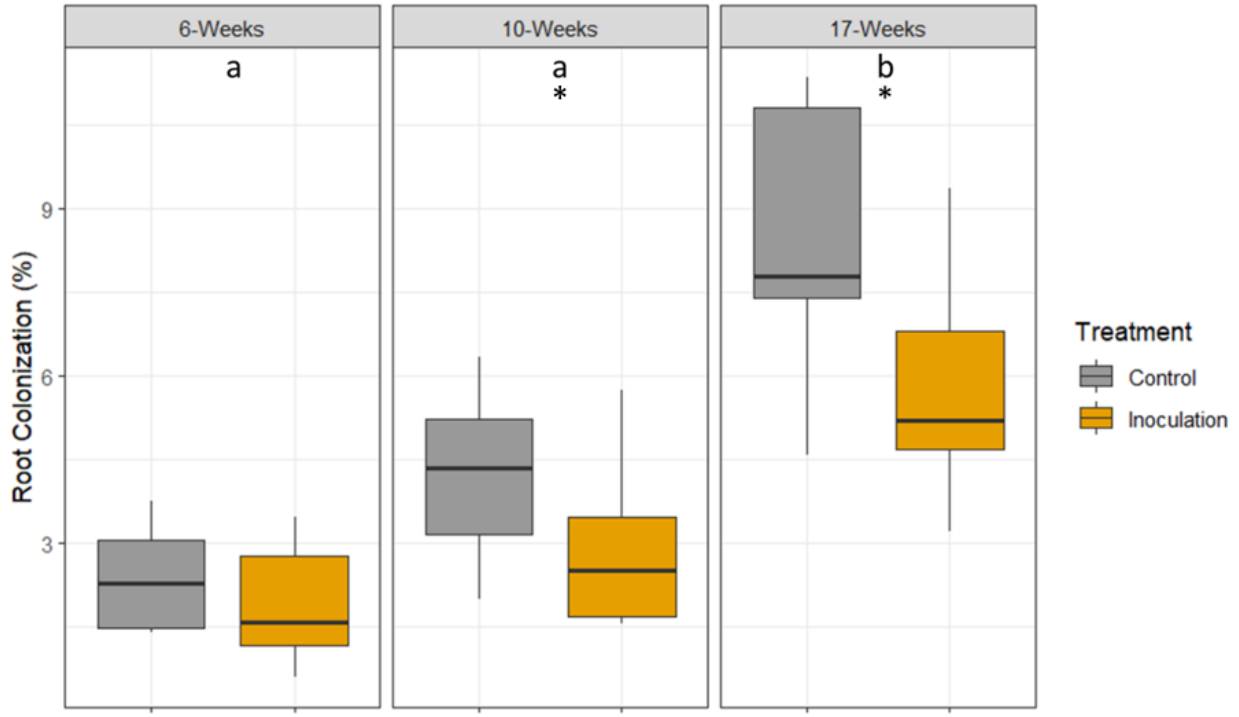

Figure 5. Root colonization throughout the 2021 season (n=3). (*) indicates significant inoculation effects within time points *(P<0.05*), letters indicate differences between time points.

Plant Response to Inoculation Across Management Systems:

Biomass, Yield, and Plant Nutrients (2019-2021):

There was no effect of AMF inoculation on crop biomass and yield in any season. Crop biomass was affected only by season, while yield was affected by both season and management. Biomass was greatest in 2020 with an average of 752.1 kg ha^{-1} and 697.9 kg ha^{-1} more than in 2019 or 2021, respectively (*P <0.0001*, *P <0.0001*; Table 1). However, the same was not true for fruit yield. Yield was greatest in 2019, with an average of 541.3 tonnes ha^{-1} more compared to 2020 (*P <0.0001*) and lowest in 2021, with an average of 255.2 tonnes ha^{-1} less than 2020 (*P*

<0.0001, Table 1). Yield was significantly lower in organically managed (OMT) plots across all seasons with an average of 149.1 tonnes ha^{-1} and 90.9 tonnes ha^{-1} less yield than CMT or LMT plots, respectively (*P <0.0001, P = 0.0001*, Table 1).

	2019			2020			2021		
	CMT	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**
Biomass (kg/ha)	599.3± 104.8^{A}	474.1± 63.4^{A}	472.6± 100.2^{A}	1321.8± 208.5^{B}	1193.1± 185.3^{B}	1287.6± 282.6^{B}	615.6± 219.4^{A}	614.0± 167.0^{A}	479.1± 135.5^{A}
Yield (tonne/ha)	1054.2± 144.0^{b,C}	901.4± 57.8^{b,C}	780.9± 109.9^{a,C}	422.5± 59.0^{b,B}	388.6± 31.8^{b,B}	301.6± 108.4^{a,B}	129.3± 23.2^{b,A}	141.4± 39.1^{b,A}	76.3± 30.6^{a,A}
Plant Nitrogen (%)	4.2± 0.3^{a,A}	4.2± 0.4^{a,A}	4.0±0.5^{b,A}	3.9± 0.2^{a,B}	3.2± 1.6^{a,B}	3.1±0.5^{b,B}	3.7± 0.4^{a,B}	3.8± 0.3^{a,B}	3.3± 0.3^{b,B}
Plant Phosphorus (%)	0.5±0.1^{b}	0.4± 0.0^{cd}	0.4±0.0^{c}	0.4±0.0^{bc}	0.4±0.0^{cd}	0.6±0.0^{a}	0.3±0.0^{e}	0.3±0.0^{e}	0.4±0.0^{d}
Plant Potassium (%)	1.4± 0.2^{abc}	1.2± 0.2^{a}	1.6±0.2^{bc}	1.7±0.2^{c}	1.5± 0.1^{abc}	2.2±0.2^{d}	1.2±0.1^{ab}	1.2±0.2^{a}	1.6± 0.3^{abc}

Table 1. Biomass, yield, plant N, plant P, and plant K means +/- standard deviations. Uppercase letters represent significant differences between seasons while lowercase letters represent significant differences across managements within seasons.

Though unaffected by AMF inoculation, season and management had significant impacts on all plant nutrients measured (Table 1, S1). N, P, K and Cu all varied with both season and management while Zn was only affected by season. Plants in 2019 had an average of 0.56% more plant N than plants from other seasons (2020: *P = 0.0001, 2021: P = 0.0001*, Table 1) in all management systems. Plants from OMT systems generally had an average of 0.42% and 0.49% less plant N than plants from CMT or LMT plots, respectively (*P = 0.0021*, *P = 0.0007*,

Table 1). Plants in 2021 had the least amount of P with an average of 0.12% less and 0.14% less than those in other seasons (2019: *P <0.0001*, 2020: *P<0.0001*, Table 1). However, this difference changed with management. In 2019, plants from CMT plots had an average of 0.08% and 0.06% more P than those from LMT and OMT plots, respectively (*P = 0.0005*, *P = 0.0046*) while in 2020 and 2021 OMT plots had the greatest P content. Organically managed systems (OMT) had an average of 0.14% and 0.18% more P than conventionally managed systems in 2020 (CMT: *P <0.0001* and LMT; *P <0.0001*) and 0.07% more P than conventionally managed systems in 2021 (CMT: *P <0.0001*, LMT: *P <0.0001*, Table 1). Generally, K was significantly enriched in organic plants compared to conventional (CMT/LMT) in each season with an average difference of 0.35% and 0.50%, respectively (CMT: *P < .0001*, LMT: *P <0.0001*). However, in 2019, the difference in K between CMT and OMT plants was non-significant (*P = 0.2276*). Additionally, in the 2020 season, plants had an average of 0.40% and 0.48% more K than those in 2019 or 2021, respectively (*P <0.0001*, *P <0.0001*), except for plants in CMT plots in 2019 and 2020 not showing a significant difference (*P = 0.0506*, Table 1). A general trend of an enrichment of copper in organic plants (OMT) was observed across seasons with an average increase of 6.5 mg kg^{-1} and 5.9 mg kg^{-1} compared to conventional (CMT/LMT) plants (CMT: *P <0.0001*, LMT: *P <0.0001*). 2019 was an exception to this trend as there was no significant difference between plants in OMT and LMT plots (*P = 0.2396*, Table S1). Plants in 2019 had an average of 3.5 mg kg^{-1} and 2.7 mg kg^{-1} less zinc than plants in 2020 and 2021 (*P = 0.0057*, *P = 0.0276*, Table S1).

There was no effect of inoculation on plant nutrients other than plant iron (Table S1). Inoculation significantly increased plant iron in LMT plots in 2020 (Fig. 6). In this year, inoculated LMT plots had an average of 461.67 mg kg^{-1} more plant iron, nearly a two-fold increase, than non-inoculated LMT plots (*P = 0.0236*, Fig. 6). Plant iron varied between seasons. Plants from 2019 had an average of 591.7 mg kg^{-1} and 644.6 mg kg^{-1} more iron than plants from other seasons

(2020: *P <0.0001*, 2021: *P <0.0001*, Fig. 6). However, this difference was not consistent across all systems. There was no significant difference between managements in 2019 while in 2021 plants from CMT plots had 147.7 mg kg^{-1} and 174.2 mg kg^{-1} less iron than plants from LMT and CMT plots, respectively (*P = 0.0248, P = 0.0062*, Fig. 6).

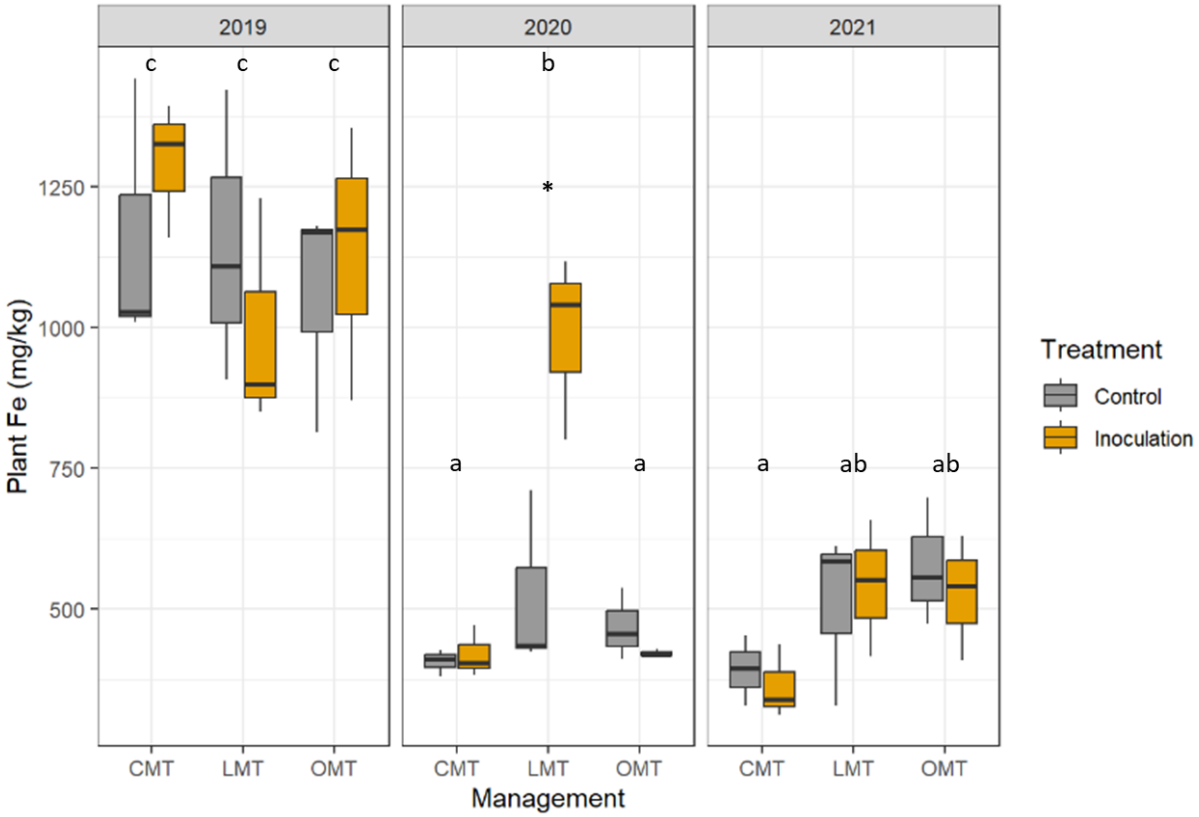

Figure 6. Mean foliar plant iron at anthesis (6-weeks post-transplant). (*) indicates significant inoculation effects within managements *(P<0.05*), letters indicate management effects within seasons.

Fruit Quality and Plant Nutrients (2021):

More extensive measurements of plant nutrients were taken over the length of the 2021 growing season while fruit quality metrics were collected at harvest. There was no significant effect of inoculation on total soluble solids or pH of fruit, two common measures for fruit quality. However, controls plants had significantly increased fruit color compared to inoculated plants in

OMT plots by an average hue angle of 1.5 (*P* = 0.0011, Fig. 7). There was no effect of inoculation on fruit color in either of the other managements (CMT: *P* = 1.0000, LMT: *P* = 0.2383).

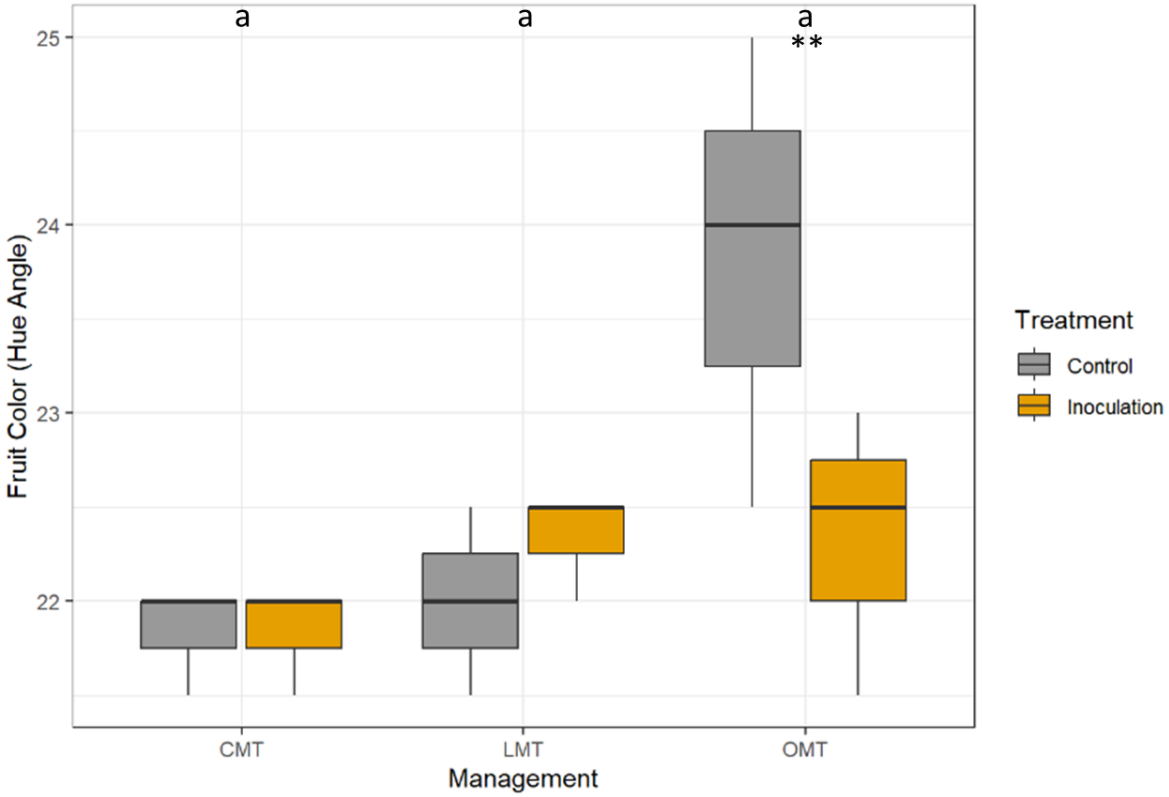

Figure 7. Mean fruit color from marketable fruits measured at harvest (2021; n=3). (**) indicates significant inoculation effects within managements *(P<0.01*), letters indicate management effects.

Sulfur and sodium content in plants were significantly influenced by inoculation. During vegetative growth (6-weeks post-inoculation), inoculation decreased sulfur in LMT plants by an average of 0.07% (*P = 0.0279*) but increased sulfur in OMT plants by an average of 0.14% (*P = 0.0174*; Fig. 8a). There was no effect of inoculation on sulfur content in CMT plants (*P = 0.5100*). There was a moderate correlation (*Pearson* R^2 *= 0.48*, Table S2a) between average soil sulfur and average plant sulfur across managements at this sampling time. Additionally,

sulfur was 0.6% and 0.7% higher, on average, in OMT plants compared to CMT and LMT plants, respectively (*P = 0.0001*, *P <0.0001*; Table S6). At harvest (17-weeks post-inoculation), inoculation significantly increased plant sodium in CMT plants by 140.7 mg kg^{-1} (*P = 0.*0210; Fig. 8b). There was no significant effect on plant sulfur content in the LMT or OMT treatments (*P = 0.1495*, *P = 0.7288*). There was a moderately negative correlation (*Pearson* R^2 *= -0.49*, Table S2c) between average soil sodium and average plant sodium across managements at this sampling time. Plants from plots that received compost (OMT) had 967.8 mg kg^{-1} and 988.8 mg kg^{-1} less sodium than plants from plots that received synthetic fertilizer (CMT and LMT), respectively (*P <0.0001*, *P <0.0001*; Table S6).

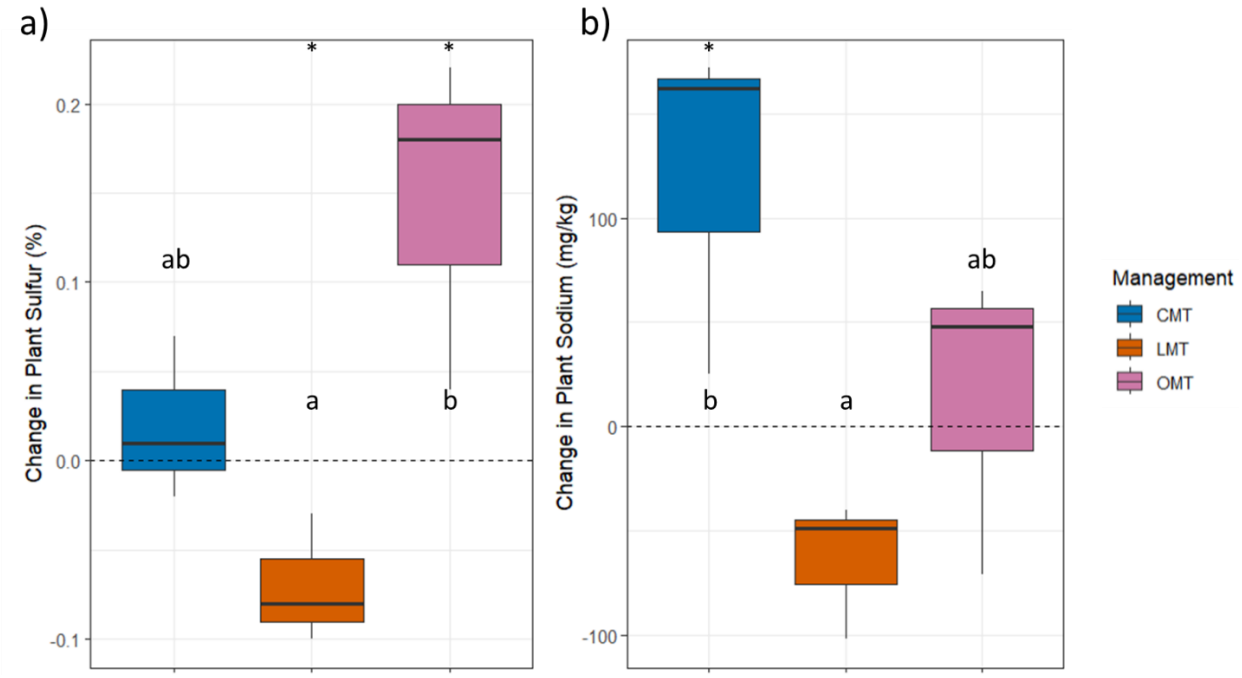

Figure 8. a) The effect of inoculation (Inoculated – Control values) on plant sulfur content during vegetative growth (6-weeks post-inoculation; n=3) and b) the effect of inoculation on plant sodium at 17-weeks post-inoculation (n=3). The dotted line at 0 represents no change associated with inoculation. (*) indicates significant inoculation effects (*P<0.05*), letters indicate significant management effects.

A PCA of all plant variables at 6-weeks post-inoculation found no significant difference when data was grouped by inoculation (*P = 0.941*) but did find the data separated significantly with management (*P = 0.003*, Fig. S1a-b). The same trend was apparent at the other two time points (Fig. S1c-f). As further confirmation, we found no impacts of inoculation on plant N, P, or K, but important system differences emerged. At fruit set (10-weeks post-inoculation), the use of synthetic fertilizers (CMT and LMT) increased plant N by an average of 1.0% and 1.6%, respectively, compared to the use of compost (OMT; *P = 0.0001*, *P <0.0001*; Table S6). At harvest (17-weeks post-inoculation), the significant effect of synthetic fertilizer was only apparent in plots that also received cover crops (LMT). These plants had an average of 0.4% more N than plants that received compost (OMT; *P = 0.0089*). Plants from plots that received compost and cover crops (OMT) had an average of 0.07% at anthesis (6-weeks post-inoculation; *P = 0.0011*), 0.07% at fruit set (10-weeks post-inoculation; *P = 0.0023*), and 0.2% at harvest (17-weeks post-inoculation; *P <0.0001*) more P than plants that received synthetic fertilizer only (CMT; Table S6). At fruit set (10-weeks post-inoculation), plant K was, on average, 0.2% greater in plants from plots that received cover crops and synthetic fertilizer (LMT) compared to plants that did not receive cover crops (CMT; *P = 0.0430*). At harvest (17-weeks post-inoculation), compost application became the determining factor as OMT plots had an average of 0.2% more plant K than both CMT and LMT plots (*P = 0.0451*, *P = 0.0505*; Table S6). At anthesis (6-weeks post-inoculation), plant N had a moderately negative correlation with soil NO_3 concentration (*Pearson R^2: -0.44*) and plant P had a moderately positive correlation with soil P concentration (*Pearson R^2: 0.54*; Table S2a). At fruit set (10-weeks post-inoculation), plant P had a moderate correlation to soil P concentration (*Pearson R^2: 0.61*; Table S2b). At harvest (17-weeks post-inoculation), both plant P and plant K had a strong correlation to soil P concentration (*Pearson R^2: 0.85*) and soil K concentration (*Pearson R^2: 0.75*, respectively (Table S2c). At no time did we find a strongly correlated relationship between root colonization and plant N, P, or K (Table S3-5).

Rhizosphere Response to Inoculation Across Management Systems (2021):

Rhizosphere Nutrients:

Three rhizosphere soil nutrients were significantly affected by inoculation: copper, zinc and boron. At vegetative growth stage (6-weeks post-inoculation), inoculation increased rhizosphere soil copper by 0.19 ppm in CMT plants (*P = 0.0250*), but decreased copper by 0.16 ppm in LMT plants (*P = 0.0465*, Fig. 9a). Inoculation had no effect on organic (OMT) rhizosphere soils (*P = 0.9924*, Fig. 9a). During fruit set (10-weeks post-inoculation), inoculation decreased rhizosphere soil zinc and boron in plants from organically managed systems (OMT) by 0.63 ppm and 0.36 ppm, respectively (*P = 0.0091*, *P = 0.0023*, Fig. 9b-c). At plant maturity (17-weeks post-inoculation), inoculation decreased rhizosphere soil boron and copper by 0.69 ppm and 0.25 ppm in plants growing in organic systems (OMT), respectively (*P <0.0001*, *P = 0.0300*, Fig. 9d-e). There were no significant correlations between rhizosphere nutrients and root colonization (Table S3-5).

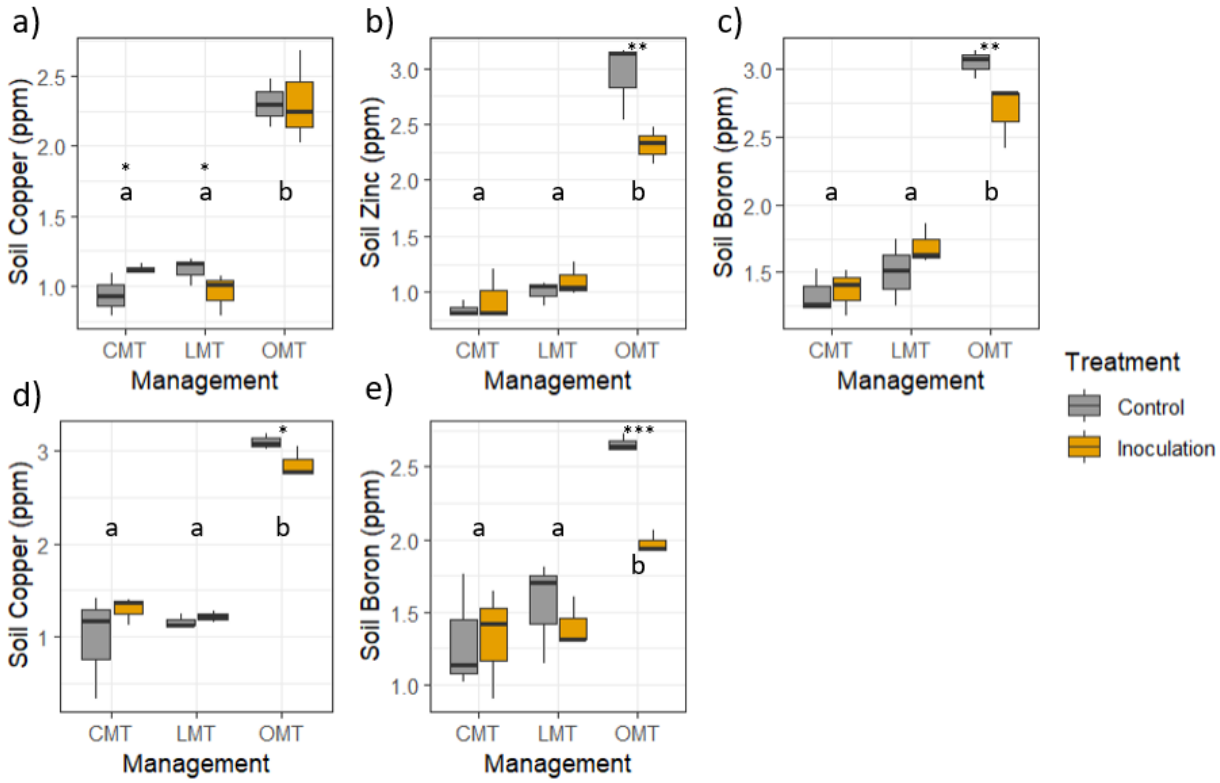

Figure 9. Soil nutrient concentrations at (a) 6-weeks post-inoculation, (b-c) 10-weeks post-inoculation, and (d-e) 17-weeks post-inoculation. (*) indicates significant inoculation effects within managements (*=*P<0.05, **=P<0.01, ***=P<0.001)*, letters indicate management effects.

Nearly all soil nutrient levels in the rhizosphere were influenced by management with the exceptions of ammonium, iron and calcium at all time points, nitrate, sulfur and manganese at 6-weeks post-inoculation and manganese and magnesium at 10-weeks post-inoculation (Table S7). Apart from magnesium, all nutrients were significantly enriched in organically managed plots (OMT; Table S7). During vegetative growth (6-weeks post-inoculation), plants that received compost (OMT) had an average of 32.1 ppm and 51 ppm more P in the rhizosphere than those that did not (CMT and LMT), respectively (*P = 0.0453, P = 0.0066*), and 298.3 ppm more K than those that received cover crops, but not compost (LMT; *P = 0.0174*, Table S7). At fruit set (10-weeks post-inoculation), there was an average of 13.0 ppm and 8.5 ppm less nitrate in the rhizosphere of conventionally managed plants (CMT) compared to the rhizosphere of

those that received cover crops (LMT/OMT; LMT: *P = 0.0072*, OMT: *P = 0.0226*). Similarly, organic plants (OMT) had 45.8 ppm and 48.2 ppm more P (CMT: *P = 0.0003*, LMT: *P = 0.0002*) and 275 ppm and 288 ppm more K (CMT: *P = 0.0079*, LMT: *P = 0.0064*) than conventional systems (CMT/LMT; Table S7). Finally, at harvest (17-weeks post-inoculation), plants growing in plots that did not receive cover crops (CMT) had an average of 7.3 ppm and 6.4 ppm less nitrate in their rhizosphere than those that did (LMT/OMT; LMT: *P = 0.0110*, OMT: *P = 0.0195*) and organic (OMT) plants had an average of 44.3 ppm and 51.3 ppm more rhizosphere P (CMT: *P = 0.0002,* LMT: *P <0.0001*) and 373.5 ppm and 417 ppm rhizosphere K (CMT: *P = 0.0014*, LMT: *P = 0.0008*) than conventional (CMT/LMT) plants (Table S7). The sole exception to the general enrichment of soil nutrients in the rhizosphere of organically grown tomato plants was magnesium. At anthesis (6-weeks post-inoculation), OMT plants had an average of 330.7 ppm less magnesium than CMT plots (*P = 0.0507*) and at plant maturity (17-weeks post-inoculation), OMT plants had an average of 319.7 ppm and 256.7 ppm less magnesium than CMT and LMT plants, respectively (*P = 0.0030*, *P = 0.0089*, Table S7). The significant effect of management was confirmed with a PCA of all soil variables at 6-weeks post-inoculation. There was no significant difference in clusters when data was grouped by inoculation (*P = 0.816*, Fig. S2a), however, when the data was grouped by management, the data separated significantly (*P = 0.006*, Fig. S2b). The same trend was apparent at the other two time points (Fig. S2c-f).

Soil Organic Matter:

At harvest (17-weeks post-inoculation), inoculation significantly increased soil organic matter in the rhizosphere by an average of 0.32% (*P = 0.0076*; Fig. 10a) and carbon:nitrogen (C:N) ratio by 0.46 (*P = 0.0097*; Fig. 10b) across all systems. There was no effect of inoculation on rhizosphere soil TC or TN. Though not a significant difference, inoculation increased rhizosphere microbial biomass by 347.5 ng g^{-1} (*P = 0.3628*; Fig. 10c). Compost applications significantly increased rhizosphere soil organic matter at both fruit set (10-weeks post-

inoculation) and harvest (17-weeks post-inoculation). Organic matter increased by an average of 0.5% at fruit set (*P = 0.0289*) and 0.9% at harvest (*P = 0.0447*) in OMT plants compared to CMT plants (data not shown).

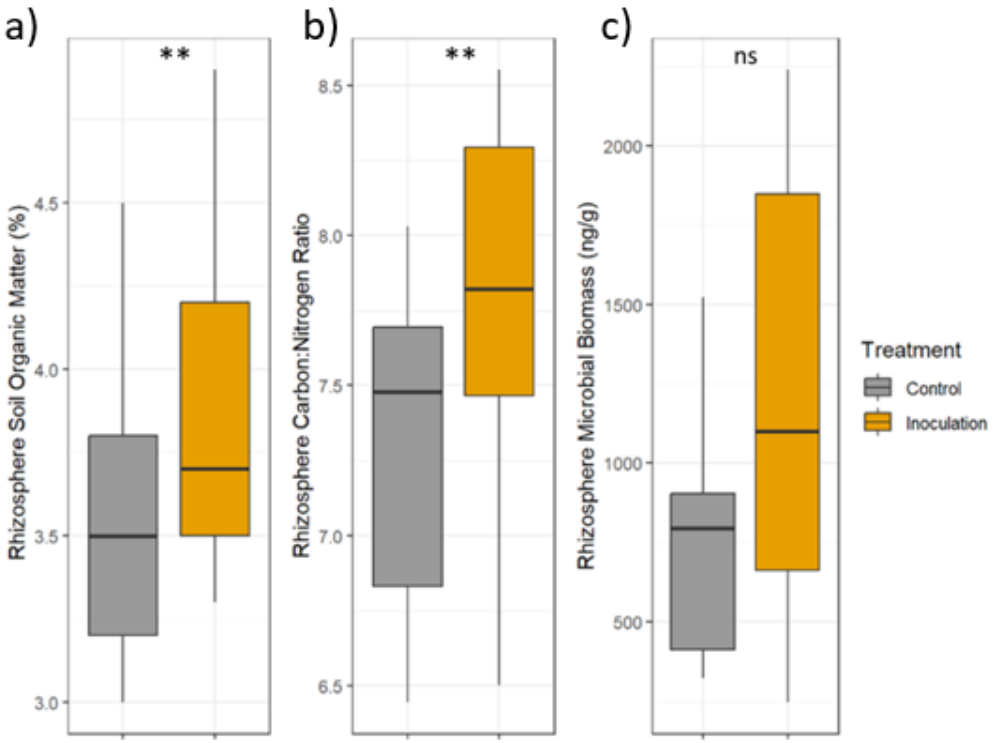

Figure 10. a) Soil organic matter, b) C:N, and c) microbial biomass at harvest (17-weeks post-inoculation, n=3). (**) indicates significant inoculation effect *(P<0.01*).

Bradford-Reactive Soil Protein (BRSP)

There was no effect of inoculation on BRSP at any time point (Fig 11). At 6-weeks post-inoculation, OMT plants had an average of 6.6×10^{-8} mg g^{-1} and 7.2×10^{-8} mg g^{-1} greater BRSP than CMT and LMT plots, respectively (*P = 0.0014*, *P = 0.0016*, Fig. 11a). We observed no effect of management at other time points.

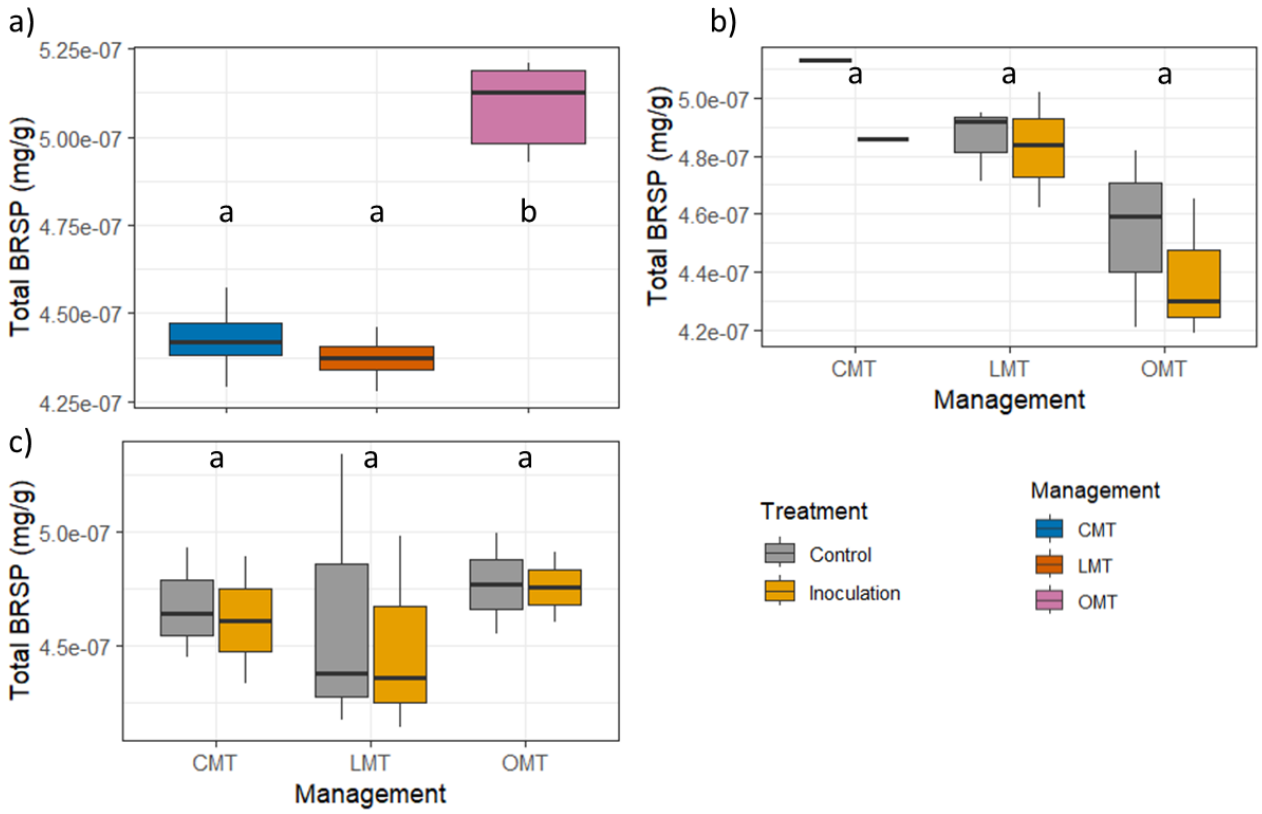

Figure 11. Bradford reactive soil protein in rhizosphere soils at (a) 6-weeks post-inoculation, (b) 10-weeks post-inoculation, and (c) 17-weeks post-inoculation. Letters indicate management effects.

Rhizosphere Microbial Communities

While inoculation did significantly impact several metrics of microbial abundance and diversity throughout the season, there was, notably, no effect on total microbial biomass or on the abundance of AMF in the rhizosphere at any time point (Fig. 12-13). A PCA of all rhizosphere microbial data at 6-weeks post-inoculation found no significant difference when data was clustered by inoculation (*P = 0.774*) or by management (*P = 0.502*, Fig. Sa-b). The same trend was apparent at the other two time points (Fig. Sc-f).

During vegetative growth (6-weeks post-inoculation), inoculation reduced both bacterial and fungal abundance and diversity in only organically managed systems (OMT). On average, the

gram (+):gram (-) bacteria decreased by 0.69 (*P = 0.0109*, Fig. 12), total bacterial biomass was reduced by 193.6 ng g^{-1} (*P = 0.0145*, Fig. 13), actinomycetes were decreased by 3.8% and 46.6 ng g^{-1} (*P = 0.0360*, *P = 0.0106*, Fig. 11, Fig. 13), gram (+) bacteria was reduced by 11.6% and 131.5 ng g^{-1} (*P = 0.0162*, *P = 0.0086*, Fig. 11, Fig. 13), total fungal biomass was 86.8 ng g^{-1} lower, a nearly four-fold decrease (*P = 0.0168*, Fig. 13), and saprophytic fungal biomass was 75.5 ng g^{-1} lower (*P = 0.0178*, Fig. 13).

At fruit set (10-weeks post-inoculation), both inoculation and management shifted microbial diversity. Inoculation reduced the gram (+) to gram (-) bacteria ratio by an average of 0.85 in CMT plots and 0.80 in OMT plots (*P = 0.0010*, *P = 0.0013*), but increased the ratio by an average of 0.63 in LMT plots (*P = 0.0042*, Fig. 12). Inoculation increased gram (-) bacteria by an average of 4.2% (*P = 0.0364*, Fig. 12), increased total fungi by an average of 4.6% (*P = 0.0260*, Fig.12) and increased saprophytes by an average of 4.3% (*P = 0.0210*, Fig. 12*)* across all managements. CMT plots had an average of 11.2% and 20.9% less total bacteria than LMT and OMT plots, respectively (*P = 0.0175*, *P = 0.0020*). Additionally, on average, gram (-) bacteria were 11.7% higher (*P = 0.0176*), total fungi were 6.3% higher (*P = 0.0525*), saprophytes were 5.4% higher (*P = 0.0596*), actinomycetes were 5.3% higher (*P = 0.0122*) and undifferentiated microbes were 27.2% lower (*P = 0.0051*) in organically managed systems (OMT) compared to conventional-fallow systems (CMT).

At 17-weeks post-inoculation, the only significant effect observed was of management on total rhizosphere bacterial biomass (*P = 0.0273*). Organically managed plants showed a trend towards higher bacterial biomass than conventionally managed plots (OMT vs. CMT; 251.8 ng g^{-1}; *P = 0.0578*; Fig. 13).

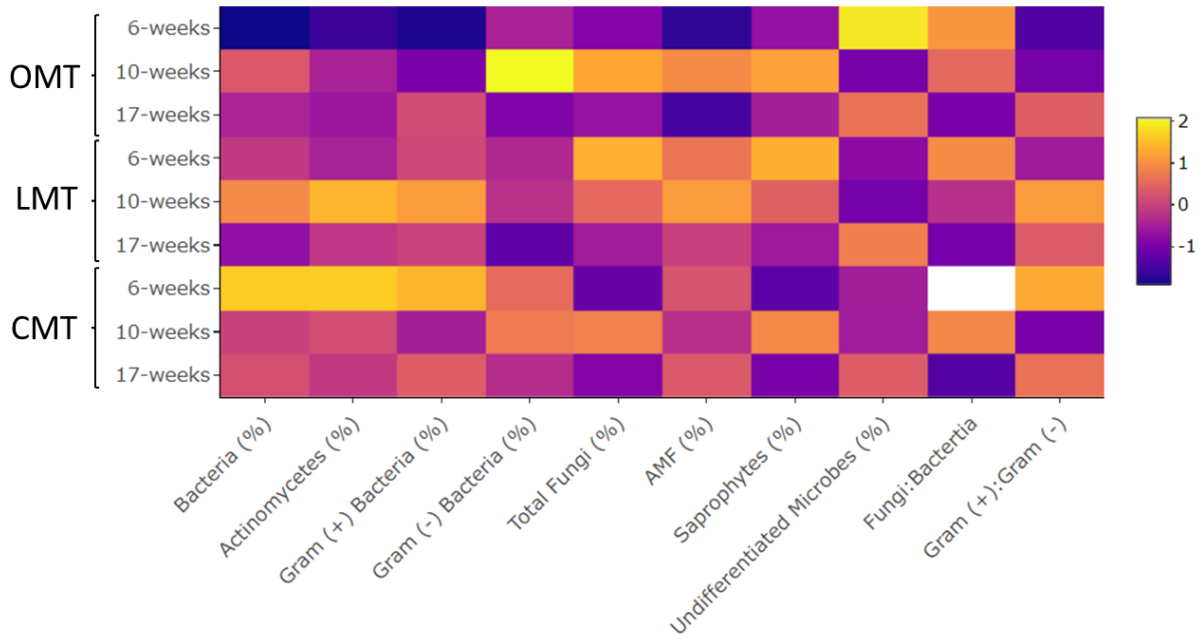

Figure 12. Z scores of normalized mean responses of microbial diversity to inoculation throughout the 2021 season and managements. CMT = Conventional Maize Tomato, LMT = Legume Maize Tomato, OMT = Organic Maize Tomato. Management details are given in Fig. 1. Empty cells represent missing data.

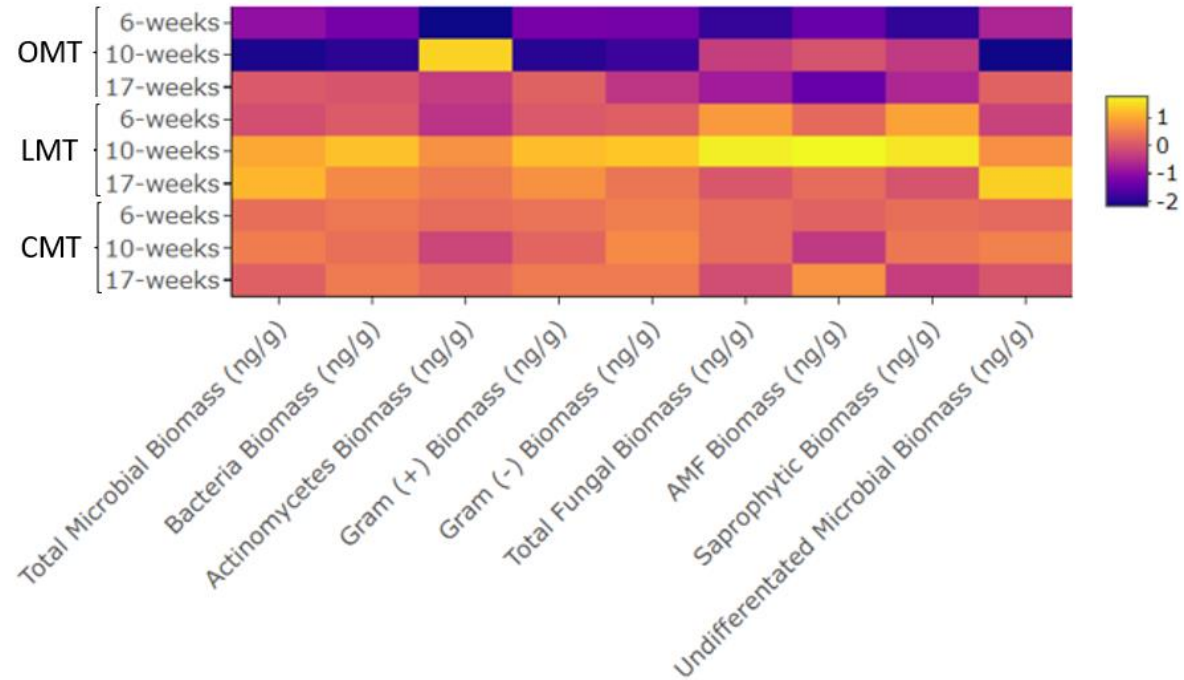

Figure 13. Z scores of normalized mean responses of microbial abundance to inoculation

throughout the 2021 season and managements. CMT = Conventional Maize Tomato, LMT = Legume Maize Tomato, OMT = Organic Maize Tomato. Management details are given in Fig. 1.

Discussion

The goal of this research was to examine the influence of long-term management on the potential benefits of non-native AMF inoculum to processing tomato crops and soil health. We found no consistent significant effect of non-native AMF inoculation on tomato plant growth, yield, or fruit quality over three seasons and across three different management systems. Inoculation impacts on root colonization were inconsistent and we saw a significant drop in colonization across control and inoculated plots from 2019 to subsequent years. No consistent trends were found on the effect of inoculation on rhizosphere soil or plant nutrients throughout the 2021 season. Notably, we observed a significant increase in rhizosphere soil organic matter and C:N ratio with inoculation as well as shifts in the rhizosphere microbial community that were dependent on management and seasonal timing.

Plots that received cover crop and compost had greater aggregate stability, available water holding capacity, total C, active C, SOM accumulation and total N than those that did not, suggesting a more easily navigable habitat to AMF with greater available nutrients and water (Bowles et al., 2017; Fig. 2). As such, we hypothesized that greater colonization would occur in organically managed systems compared to conventional systems. Additionally, conventional management decreased biological activity compared to organic management indicating a less hostile environment to soil microbes in organic soils. Despite this, we saw no effect of management on root colonization in any season (Fig. 6) forcing us to reject our hypothesis that the favorable habitat provided by organic management would enhance the effects of inoculation.

We found inconsistent root colonization rates throughout the study. In 2019, an increase in root colonization with inoculation was observed during vegetative growth, but this effect was not apparent in 2020 or 2021 (Fig. 6). In fact, at fruit set and harvest in 2021, inoculation significantly decreased the root colonization (Fig. 7). Though our 2019 results align with the large body of work that demonstrates the positive effect of AMF inoculation on root colonization (Bender et al., 2019; Lekberg & Koide, 2005; Pellegrino et al., 2015; Zhang et al., 2019), our 2020 and 2021 results contradict these studies and align with other studies that reported no effect (Faye et al., 2013; Monokrousos et al., 2020; Salomon et al., 2022) in root colonization with non-native AMF inoculation. The absence of AMF in our rhizosphere systems was further confirmed by the low amount of BRSP identified in rhizosphere soils (Fig. 10). The inability of our non-native AMF inoculum to increase root colonization leads us to reject our hypothesis that we would observe an increase in root colonization and AMF rhizosphere presence with inoculation.

We observed significantly higher root colonization in both treatments (Control and Inoculation) in 2019 as compared to 2020 and 2021 (Fig. 6). Li et al. (2020) reported an average colonization of 13.6% in the organically managed tomato systems (OMT) at RRSARF. Our 2019 average root colonization rate (16.8%) was on par with this value. However, the average root colonization in 2020 (3.1%) and 2021 (2.2%) were 10.5% and 11.4% lower, respectively. The colonization rates observed in this study are lower than those typically observed in processing tomato studies. Root colonization values as high as 56% in inoculated, greenhouse grown tomatoes (Salomon et al., 2022) and as low 12.3% in non-inoculated, organically field grown tomatoes (Bowles et al., 2016) have been reported with other studies reporting findings in this range ((Cavagnaro et al., 2006; Lazcano et al., 2014). However, our results align with those performed in working agroecosystems. A survey of large-scale Australian processing tomato farms by Cavagnaro and Martin (2011) found low rates of colonization (<15% of root length)

and, in almost half of their samples, no colonization at all. The authors attribute this inhibition of AMF root colonization to soil fumigation practices common in large-scale farming (Cavagnaro & Martin, 2011). Though fumigation was not practiced at RRSARF, there were other large-scale farming practices in place that may have accounted for the low colonization observed in this study.

It is possible we observed the effect of the accidental selection against symbiotic root associations in crop plants. A substantial body of evidence is building to suggest that crop breeding programs focused on reducing pathogenic infections have also resulted in reduced symbiotic capabilities of crops (Porter & Sachs, 2020). Studies in wheat (*Triticum* spp; Hetrick et al., 1992; Zhang et al., 2019) corn (*Zea mays*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*; Zhang et al., 2019) breadfruit (*Artocarpus altilis*; Xing et al., 2012) and sunflower (*Helianthus annus L.*; Turrini *et al.,* 2016) found reduced symbiotic reliance in younger, highly bred lineages. Similar observations have been made in legumes when looking at the ability of younger cultivars to utilize rhizobial populations (Provorov and Tikhonovich, 2003; Kiers *et al*., 2007; Muñoz *et al.,* 2016). It is also possible that the negative effects of fertilization on AMF symbioses have been amplified by breeding programs (Martín-Robles et al., 2018). The symbiotic capabilities of the cultivar used in this study (Heinz 1662), which was patented in 2017, are not known. However, it is possible they are depressed compared to older cultivars or wild types, particularly under fertilization.

Fluopyram, branded as Velum One, was applied to the non-organic systems on July 16, 2019 and on July 14, 2020 at a concentration of 484.8 grams ha^{-1}. Fluopyram is a systemic pyridinyethylbenzamide fungicide that acts by binding to the ubiquinone-binding site of the mitochondrial succinate dehydrogenase, thus inhibiting the fungal respiratory chain complex (Labourdette et al., 2010). Though no reports of fluopyram affecting AMF have been documented, many other fungicides have been demonstrated to have deleterious non-target

effects on AMF (Hernández-Dorrego & Parés, n.d.; Kjøller et al., 2000). Okiobe et al. (2022) observed a significant reduction in AMF root colonization in inoculated greenhouse grown tomatoes when treated with a range of fungicides. Furthermore, recent evidence suggests that fungicides persist in soil years after initial application, perpetuating negative effects on indigenous AMF. In an eight-year grassland study, Pánková et al. (2018) found a general negative trend in the potential for AMF propagules in the soil to establish mycorrhizal colonization in the roots of host plants after fungicide application with effects being observed up to four years after initial application. It is possible that the reduced colonization observed in non-organic systems in 2020 and 2021 is a result from the presence of fungicide residues persisting in soils after the applications in 2019 and 2020. Furthermore, if tilling and transplanting machinery was not properly disinfected after working in non-organic fields, it is feasible that residues may have been transferred to organic fields, resulting in AMF inhibition.

We found no significant effect of non-native AMF inoculation on tomato plant growth, yield, fruit quality, plant macronutrients (NPK) or plant micronutrients reported enriched in previous studies (Cavagnaro, 2008; Cavagnaro et al., 2006; Smith & Read, 2008; Table 1). Similar observations have been reported in previous studies of soybean (Faye et al., 2020) and wheat (Ryan & Angus, 2003; Ryan & Kirkegaard, 2012; Dai et al., 2014;) The average biomass (784.1 kg ha^{-1}) found in this study was slightly lower than that reported in other studies, yet the average yield (149.2 tonnes ha^{-1}) was slightly higher (Hartz & Bottoms, 2009; Patanè et al., 2011; Rodriguez-Ramos et al., 2022). The total soluble solids (5.3˚Bx), fruit color (22.4 Hue angle), fruit pH (4.48) and plant N (3.8%), P (0.4%) and K (1.5%) were on par with that reported in other studies (Barrios-Masias & Jackson, 2014; Cavero et al., 1997; Li et al., 2020; Rodriguez-Ramos et al., 2022). The decrease in fruit color with inoculation observed in organic systems at harvest in 2021 has not been reported before this study. These results contradict a large body of work in processing tomatoes (Bowles et al., 2016; Pasković et al., 2021) and in other crops that have

documented benefits of non-AMF inoculation to plant nutrients and yield (Fagbola et al., n.d.; Gaur & Adholeya, 2002; Lekberg & Koide, 2005; Pellegrino et al., 2012, 2015; Pellegrino & Bedini, 2014).

There were no consistent effects of non-native AMF inoculation on plant nutrient uptake observed throughout this three-year study. In 2019, an increase in plant iron was only observed in plots that received synthetic fertilizer and cover crops (Fig. 3). An increase in plant iron with AMF inoculation has been observed in barley (*Hordeum vulgare*) under elevated soil P conditions (Mohammad et al., 2003). In 2019, rhizosphere P levels averaged 60.3 ppm in CMT plots, 52.5 ppm in LMT plots and 165.5 ppm in OMT plots. The University of California Agriculture and Natural Resources (UCANR) states that a value of over 25 ppm soil P is considered high (Miyao et al., 2019). According to this classification, all our plots would be considered high in P. Therefore, our results differ from Mohammad et al. (2003) suggesting that less P results in greater plant iron uptake. However, this increase in plant iron due to inoculation was not observed in the succeeding seasons. During anthesis in 2021, AMF inoculation increased sulfur in plants that received cover crops and compost, but decreased sulfur in plants that received cover crops and synthetic fertilizer (Fig. 5a). An increase in sulfur has been reported in microcosms of *Agrostis stolonifera* and *Plantago lanceolata* early in plant development (Gahan et al., 2022). This is consistent with our observations as the enrichment in plant sulfur was only apparent during vegetative growth. At harvest in 2021, AMF inoculation resulted in higher plant sodium in conventionally managed plots (Fig. 5b). There is little documented research on the effects of non-native AMF on plant sodium uptake. However, (Evelin et al., 2012) reported a significant reduction in shoot:root ratio of sodium in AMF inoculated *Trigonella foenum-graecum* in saline soils. Though our results contradict this finding, the average electrical conductivity of our soil across all time points was 0.53 mmho cm^{-1} indicating that our soils are not saline. Despite the significant effects of inoculation documented

on plant nutrients, we found no strong correlations between plant nutrients and root colonization. We predicted that increased root colonization would result in increased plant nutrients, yield and improved fruit quality. Though we cannot test for these effects since there was no consistent increase in root colonization attributed to AMF inoculation, the above findings would also lead us to reject that hypothesis.

We observed inconsistent effects of inoculation on rhizosphere soil nutrients in 2021. Nearly all soil nutrients were significantly enriched in organically managed plots apart from ammonium, iron, calcium and magnesium (Table S7). There is little documentation of a link between AMF inoculation and rhizosphere soil nutrient contents. Therefore, we cannot conclude that the effects of inoculation on certain rhizosphere soil nutrients observed in this study are universal or reproducible. Elevated levels of plant copper and zinc due to inoculation have been reported (Cavagnaro et al., 2006; Cavagnaro, 2008; Smith & Read, 2008) ; however, because of the low levels of root colonization it is difficult to conclude that the presence of non-native AMF drove the increases in rhizosphere soil copper, zinc and boron we observed in this study. Additionally, there were no consistent strong correlations between soil nutrients and root colonization. This enrichment of rhizosphere soil nutrients in organically managed plots may have played an additional role in inhibiting non-native AMF colonization as the relationship between high soil P and nitrate and low root colonization is well established (Treseder, 2004).

Failure of non-native AMF inoculants to establish in the soils of agroecosystems has been documented (Faye et al., 2013, 2020; Thomsen et al., 2021; Salomon et al., 2022). Thomsen et al. (2021) found no increase in AMF isolates of inoculated fungus using droplet digital PCR (ddPCR). Our study produced a similar result utilizing a different technique (PLFA). This would seem to suggest that the establishment of our non-native inoculum failed. However, the changes we observed to rhizosphere microbial community composition and SOM suggest the story may not be so simple. While the decrease observed in both bacterial and fungal

abundance and diversity in organically managed systems at 6-weeks post-inoculation may be due to a non-significant increase in undifferentiated microbes in organically managed plots (Fig. 11 - 12), changes in the soil microbiome of organically managed systems due to non-native AMF inoculation have been reported. Non-native AMF inoculation has been associated with shifts in bacterial community composition (Ali et al., 2021). Impacts of non-native AMF inoculations on microbial communities have also been observed in conventional systems (Bender et al., 2019; Hao et al., 2021). This would align with the effects of inoculation we observed at fruit set that impacted all soil management systems. Studies demonstrating changes to the microbial community under AMF inoculation have also been done in natural ecosystems (Monokrousos et al., 2020). The non-effect of inoculation on the rhizosphere soil community at harvest is likely due to the lack of irrigation processing tomatoes receive in the weeks prior to harvest. Without irrigation, most microbes present in the rhizosphere died or desiccated, increasing rhizosphere necromass and, possibly, SOM.

Though the increases in rhizosphere SOM and C:N ratio were marginal relative to the mean of each treatment group, it was an unexpected result given the low root colonization observed in this study. Tautges et al. (2019) reported an average C:N ratio of 10.1 in the upper 15 cm of research plots at RR. It is unlikely we are observing the active generation, reprocessing, reorganization or stabilization of SOM ((Wu et al., 2023) in this study due to the low AMF colonization and low BRSP observed in this study. Instead, another aspect of the inoculum is likely stimulating microbial growth. It is possible that the active inoculum acted as a source of particulate organic matter in the rhizosphere, stimulating the microbial carbon pump (MCP). Recent evidence suggests that much of the stable SOM reported in soils is derived from microbial communities through the decomposition and coalescence of organic compounds into microbial biomass followed by the physical or chemical occlusion of microbial necromass upon their death (Kallenbach et al., 2016). Furthermore, it has been suggested that below ground C

inputs, such as this inoculum, form mineral-stabilized soil C more rapidly and efficiently that above ground inputs (Schmidt et al., 2011; Sokol & Bradford, 2019). In this study, we may have observed the effect of the active AMF inoculum acting as a below ground C input, stimulating microbial communities and resulting in an increase of SOM at the end of the season. It is important to note that Horsch et al. (2023) found a decrease of 15% in SOC in Sudangrass inoculated with Glomeraceae compared to a non-mycorrhizal control, contradicting our results. Further study of this SOM increase is required to elucidate the mechanism behind it.

Conclusion

We did not observe any increases in root colonization due to inoculation in any system refuting our hypothesis that inoculation with non-native AMF would lead to an increase in tomato root colonization and presence of mycorrhizae in the rhizosphere of organically managed systems. Additionally, we did not observe any consistent increases in plant nutrients, yield or fruit quality due to inoculation. Though we did observe some improved soil health indicators due to inoculation such as increased rhizosphere SOM, this was consistent across all managements. Due to the low root colonization, it is unlikely that we observed any effect of successful AMF inoculation. Instead, this may have been the result of adding particulate C into the rhizosphere. This study suggests that this mycorrhizal inoculant is not valuable as an external input into processing tomato fields in California for the goal of improved yields or plant nutrition. However, this inoculum does appear to have value as method of managing for soil health, particularly for increasing soil organic matter. To confirm this finding, greenhouse trials would be required to elucidate the mechanism behind the SOM increase. In addition, longer term trials would also be required to determine the extent to which SOM can be enriched utilizing this and other microbial inoculums. Furthermore, there is still a need to investigate the function of the mycorrhizal networks formed in both monocultures and more diverse systems to mimic healthy, established microbiomes in disturbed farms using non-native inoculums. This study did not investigate the

effect of AMF identity on plant quality and soil health metrics nor on the function of fungal identity as it relates to plant identity. This is another critical area in need of exploration to optimize the use of AMF inoculums in sustainable agriculture.

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Appendix

Supplemental Materials

Table S1. Plant nutrient means ± standard deviations from 2019-2020. Uppercase letters represent significant differences between seasons while lowercase letters represent significant differences across managements within seasons.

	2019			2020			2021		
	CMT	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**
Calcium (%)	3.69± 0.2^{a,B}	3.99± 0.2^{a,B}	4.35± 0.2^{b,B}	3.25± 0.2^{a,A}	3.16± 0.2^{a,A}	3.36± 0.2^{a,A}	3.41± 0.2^{a,AB}	3.30± 0.3^{a,A}	3.36± 0.4^{a,A}
Magnesium (%)	2.46± 0.1^{b,B}	2.68± 0.2^{b,B}	2.34± 0.1^{a,B}	2.24± 0.1^{b,A}	2.22± 0.1^{b,A}	1.93± 0.1^{a,A}	2.49± 0.1^{b,B}	2.49± 0.2^{b,B}	2.12± 0.3^{a,B}
Sulfur (%)	0.66± 0.0^{b,B}	0.69± 0.1^{b,C}	0.87± 0.0^{a,B}	0.86± 0.1^{b,A}	0.88± 0.1^{b,A}	1.58± 0.2^{a,A}	0.87± 0.1^{b,A}	0.77± 0.1^{c,B}	1.42± 0.1^{a,A}
Manganese (mg/kg)	255.17± 33.1^{c,B}	197.33± 14.7^{b,C}	146.00± 9.5^{a,B}	116.33 ±13.8^{b,A}	147.50± 9.1^{c,B}	88.33± 4.0^{a,A}	107.50± 4.9^{ab,A}	114.33± 16.2^{b,A}	94.33± 15.3^{a,A}
Iron (mg/kg)	1225.67 ± 186.9^{a,B}	1069.50 ± 225.3^{a,C}	1093.33 ± 207.6^{a,B}	413.17 ± 33.3^{a,A}	754.83 ± 292.2^{b,B}	445.33 ± 47.7^{a,A}	377.67 ± 59.3^{a,A}	525.33 ± 126.4^{b,A}	551.83 ± 104.4^{b,A}
Copper (mg/kg)	15.67± 2.3^{a,AB}	18.00± 1.3^{ab,B}	19.83± 2.9^{b,A}	13.67± 1.2^{a,A}	15.00± 2.8^{a,A}	24.83± 1.5^{b,B}	17.33± 0.8^{a,B}	15.50± 2.1^{a,AB}	21.50± 1.5^{b,A}
Boron (mg/kg)	112.67± 7.3^{C}	114.83± 3.3^{C}	107.83± 4.8^{C}	96.50± 4.8^{B}	98.50± 6.7^{B}	97.67± 6.4^{B}	90.67± 5.4^{A}	82.83± 4.6^{A}	86.50± 3.7^{A}
Aluminum (mg/kg)	704.00 ± 149.4^{b,A}	601.83 ± 134.3^{b,A}	596.33 ± 170.6^{b,A}	194.67± 22.8^{a,A}	374.67 ± 143.7^{a,B}	219.67± 26.6^{a,A}	190.33 ± 36.9^{a,A}	257.83 ± 57.8^{a,AB}	281.50 ± 50.5^{a,B}
Zinc (mg/kg)	18.67± 2.9^{A}	18.17± 2.3^{A}	19.00± 3.6^{A}	19.33± 2.0^{B}	22.17± 1.5^{B}	24.67± 1.2^{B}	22.33± 4.2^{B}	21.33± 4.3^{B}	20.33± 2.7^{B}
Sodium (mg/kg)	819.83 ± 61.3^{a,A}	1241.17 ± 289.8^{b,B}	757.83 ± 84.6^{a,B}	712.17 ± 90.9^{b,A}	690.67 ± 119.5^{b,A}	327.50± 43.8^{a,A}	1078.33 ± 129.0^{b,B}	1344.00 ± 194.5^{b,B}	640.00 ± 114.2^{a,B}

Table S2. Pearson R^2 for the correlation of a given nutrient in the rhizosphere and foliar tissue at a) 6-weeks post-inoculation, b) 10-weeks post-inoculation, and c) 17-weeks post-inoculation. Values above 0.7 are considered strong correlations. (*) denote correlation of soil ammonium with plant nitrogen and (**) denote correlation of soil nitrate with plant nitrogen.

a)

Plant Nutrient	Correlation with Corresponding Soil Nutrient
Nitrogen	-0.3038*, -0.4349**
Phosphorus	0.5381
Potassium	0.1973
Sulfur	0.4863
Zinc	-0.0163
Iron	0.0349
Manganese	0.1656
Copper	0.8397
Calcium	0.4209
Magnesium	0.5005
Sodium	-0.5853
Boron	0.1324

b)

Plant Nutrient	Correlation with Corresponding Soil Nutrient
Nitrogen	0.4257*, 0.0762**
Phosphorus	0.6110
Potassium	-0.3119
Sulfur	0.7737
Zinc	0.0464
Iron	-0.0458
Manganese	-0.1908
Copper	-0.0765
Calcium	0.3383
Magnesium	0.5229
Sodium	-0.7292
Boron	0.1202

c)

Plant Nutrient	Correlation with Corresponding Soil Nutrient
Nitrogen	0.2430*, 0.1656**
Phosphorus	0.8448
Potassium	0.7539
Sulfur	0.6450
Zinc	0.5314
Iron	0.2525
Manganese	-0.3488
Copper	0.8297
Calcium	-0.1843
Magnesium	0.6224
Sodium	-0.4933
Boron	0.6944

Table S3. Pearson R^2 for the correlation of root colonization with (a) plant and (b) soil nutrients 6-weeks post-inoculation. Values above 0.7 are considered strong correlations.

a)

Plant Nutrient	Correlation with Root Colonization
Nitrogen	0.1260
Phosphorus	-0.0968
Potassium	-0.3450
Sulfur	-0.0454
Zinc	-0.0790
Iron	-0.1320
Manganese	0.2270
Copper	0.0207
Calcium	0.2008
Magnesium	0.2465
Sodium	0.0347
Boron	0.1579

b)

Soil Nutrient	Correlation with Root Colonization
Ammonium	0.5342
Nitrate	0.2813
Phosphorus	0.3522
Potassium	0.4236
Sulfur	0.5246
Zinc	-0.2046
Iron	-0.3500
Manganese	0.2485
Copper	-0.1755
Calcium	0.5639
Magnesium	0.1825
Sodium	-0.1397
Boron	0.1146

Table S4. Pearson R^2 for the correlation of root colonization with (a) plant and (b) soil nutrients 10-weeks post-inoculation. Values above 0.7 are considered strong correlations.

a)

Plant Nutrient	Correlation with Root Colonization
Nitrogen	-0.2784
Phosphorus	0.3703
Potassium	-0.1305
Sulfur	0.3017
Zinc	0.4276
Iron	0.0694
Manganese	-0.0522
Copper	0.1881
Calcium	0.1503
Magnesium	0.2013
Sodium	-0.0859
Boron	0.1229

b)

Soil Nutrient	Correlation with Root Colonization
Ammonium	-0.0751
Nitrate	-0.0870
Phosphorus	0.1322
Potassium	0.0324
Sulfur	0.1483
Zinc	0.0921
Iron	-0.2523
Manganese	-0.4638
Copper	0.0340
Calcium	0.0739
Magnesium	-0.0499
Sodium	0.2381
Boron	0.1824

Table S5. Pearson R^2 for the correlation of root colonization with (a) plant and (b) soil nutrients 17-weeks post-inoculation. Values above 0.7 are considered strong correlations.

a)

Plant Nutrient	Correlation with Root Colonization
Nitrogen	0.0210
Phosphorus	-0.2497
Potassium	-0.2673
Sulfur	-0.2125
Zinc	-0.1360
Iron	0.1573
Manganese	0.2526
Copper	-0.3780
Calcium	0.0781
Magnesium	0.1883
Sodium	0.3040
Boron	-0.2181

b)

Soil Nutrient	Correlation with Root Colonization
Ammonium	0.1356
Nitrate	-0.2702
Phosphorus	-0.3815
Potassium	-0.3282
Sulfur	-0.1922
Zinc	-0.3510
Iron	-0.6009
Manganese	-0.7050
Copper	-0.3739
Calcium	0.0901
Magnesium	0.2388
Sodium	0.1109
Boron	0.0484

Table S6. Plant nutrient means ± standard deviations throughout 2021 season. Letters represent significant differences between managements.

	6-Weeks Post-Inoculation			10-Weeks Post-Inoculation			17-Weeks Post-Inoculation		
	CMT	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**
Nitrogen (%)	3.66±0.4	3.84±0.3	3.31±0.3	2.80±0.3^{b}	3.38±0.2^{c}	1.81±0.0^{a}	1.47±0.1^{ab}	1.74±0.2^{b}	1.31±0.2^{a}
Phosphorus (%)	0.28±0.0^{a}	0.28±0.0^{a}	0.35±0.0^{b}	0.19±0.0^{a}	0.22±0.0^{b}	0.25±0.0^{b}	0.16±0.0^{a}	0.17±0.0^{a}	0.35±0.1^{b}
Potassium (%)	1.21±0.1	1.17±0.2	1.55±0.3	0.88±0.1^{a}	1.11±0.1^{b}	0.90±0.2^{ab}	0.39±0.1^{ab}	0.39±0.1^{ab}	0.56±0.1^{b}
Calcium (%)	3.41±0.2	3.30±0.3	3.36±0.3	2.81±0.2	2.34±0.3	2.72±0.3	4.13±0.3	4.31±0.1	4.33±0.2
Magnesium (%)	2.49±0.1	2.49±0.2	2.12±0.3	1.93±0.2	1.64±0.3	1.71±0.2	2.81±0.3	2.83±0.1	2.45±0.3
Sulfur (%)	0.87±0.1^{a}	0.77±0.1^{a}	1.42±0.1^{b}	0.94±0.1^{b}	0.69±0.1^{a}	1.28±0.1^{c}	1.09±0.2^{a}	0.87±0.1^{a}	1.69±0.1^{b}
Manganese (mg/kg)	107.5 ±4.9	114.33 ±16.2	94.33 ±15.3	81.17 ±10.4	81.50 ±15.1	58.83 ±12.7	117.17 ±15.5^{ab}	128.67 ±8.8^{b}	97.50 ±14.5^{a}
Iron (mg/kg)	377.67 ±59.3	525.33 ±126.4	551.83 ±104.4	282.83 ±72.2	363.83 ±139.7	316.00 ±97.7	713.83 ±253.9	558.17 ±110.7	776.33 ±436.9
Copper (mg/kg)	17.33 ±0.8^{a}	15.50 ±2.1^{a}	21.50 ±1.5^{b}	16.50 ±0.8	15.67 ±2.1	16.00 ±2.5	11.50 ±2.8^{a}	10.17 ±1.6^{a}	16.67 ±1.6^{b}
Boron (mg/kg)	90.67 ±5.4	82.83 ±4.6	86.50 ±3.7	94.33 ±7.9	84.17 ±11.3	96.33 ±8.0	142.67 ±6.0^{a}	146.33 ±10.5^{a}	172.33 ±17.9^{b}
Aluminum (mg/kg)	190.33 ±36.9	257.83 ±57.8	281.50 ±50.5	143.33 ±45.5	181.50 ±67.8	179.83 ±61.6	384.33 ±138.6	310.50 ±60.8	436.67 ±228.4
Zinc (mg/kg)	22.33±4.2	21.33±4.3	20.33±2.7	15.83±3.3	13.50±2.4	15.17±2.9	9.33±2.0^{ab}	6.67±1.0^{a}	10.83±2.1^{b}
Sodium (mg/kg)	1078.33 ±129.0^{b}	1344.00 ±194.5^{b}	640.00 ±114.2^{a}	1666.00 ±284.1^{b}	1304.33 ±275.4^{b}	372.67 ±126.9^{a}	1294.17 ±119.1^{b}	1315.17 ±110.6^{b}	326.33 ±50.3^{a}

Figure S1. PCA plot of plant variables at (a) 6-weeks post-inoculation separated by treatment, (b) 6-weeks post-inoculation separated by management, (c) 10-weeks post-inoculation separated by treatment, (d) 10-weeks post-inoculation separated by management, (e) 17-weeks post-inoculation separated by treatment, and (f) 17-weeks post-inoculation separated by management. 6- and 10-weeks post-inoculation PCA were generated using root colonization, plant nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, manganese, iron, copper, boron, aluminum, zinc, and sodium. 17-weeks post-inoculation PCA was generated using the same plant nutrient variables as the first two time points with the addition of fruit total soluble solids, fruit color, fruit pH, biomass and yield.

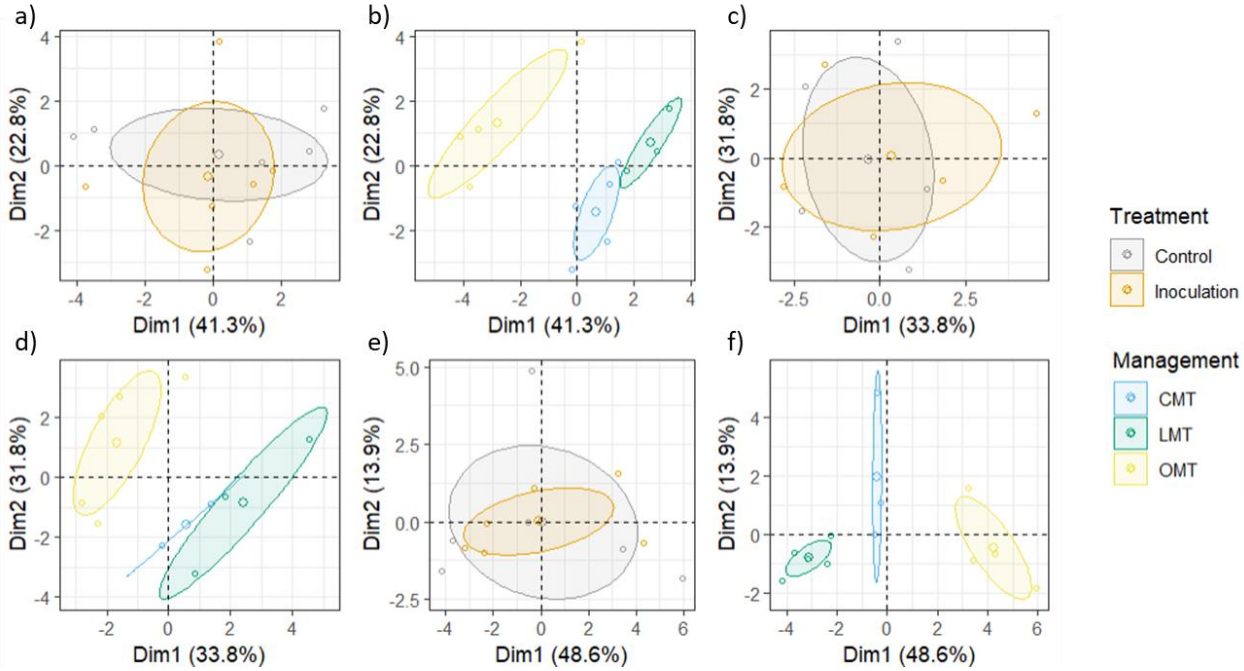

Figure S2. PCA plot of soil variables at (a) 6-weeks post-inoculation separated by treatment, (b) 6-weeks post-inoculation separated by management, (c) 10-weeks post-inoculation separated by treatment, (d) 10-weeks post-inoculation separated by management, (e) 17-weeks post-inoculation separated by treatment, and (f) 17-weeks post-inoculation separated by management. 6- and 10-weeks post-inoculation PCA were generated using soil ammonium, nitrate, phosphorus, potassium, sulfur, zinc, iron, manganese, copper, calcium, magnesium, sodium, boron, cation exchange capacity, Bradford-reactive soil protein, soil organic matter and active carbon. 17-weeks post-inoculation PCA was generated using the same soil nutrient variables as the first two time points with the addition of total nitrogen and total carbon.

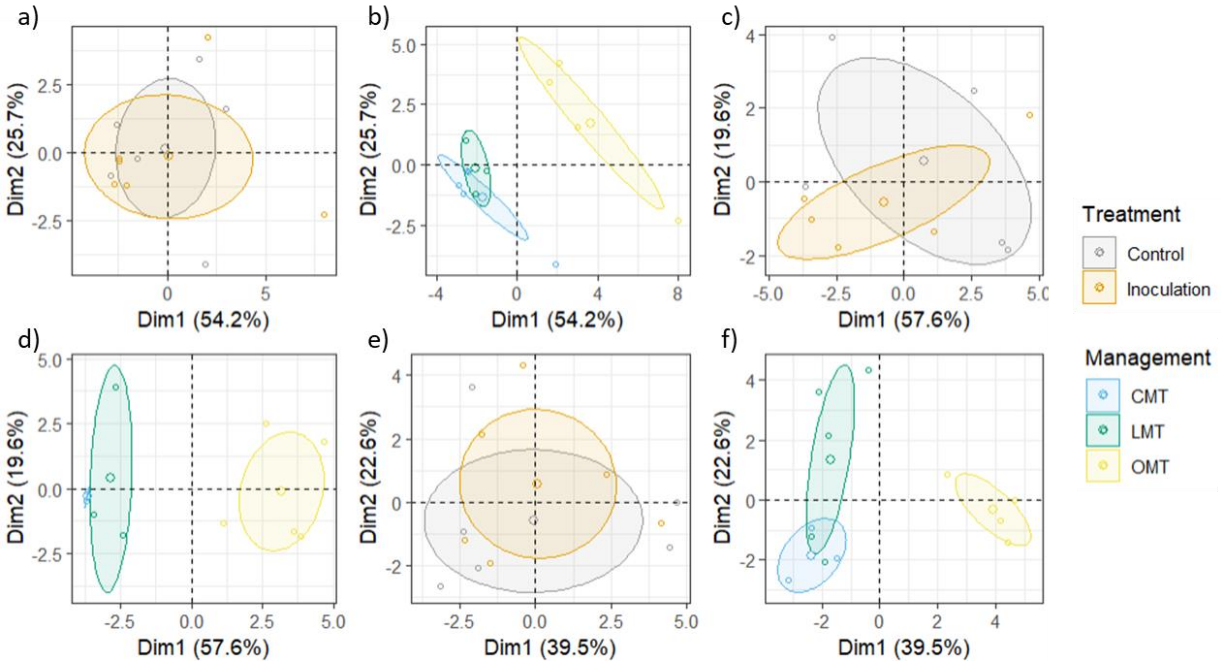

Figure S3. PCA plot of microbial community variables at (a) 6-weeks post-inoculation separated by treatment, (b) 6-weeks post-inoculation separated by management, (c) 10-weeks post-inoculation separated by treatment, (d) 10-weeks post-inoculation separated by management, (e) 17-weeks post-inoculation separated by treatment, and (f) 17-weeks post-inoculation separated by management. 6-,10-, and 17-weeks post-inoculation PCAs were generated using total microbial biomass, diversity index, and abundance of bacteria, actinomycetes, gram (-) bacteria, gram (+) bacteria, fungi, arbuscular mycorrhizae, saprotrophs and undifferentiated biomass as well as fungi:bacteria and gram (+) bacteria:gram (-) bacteria.

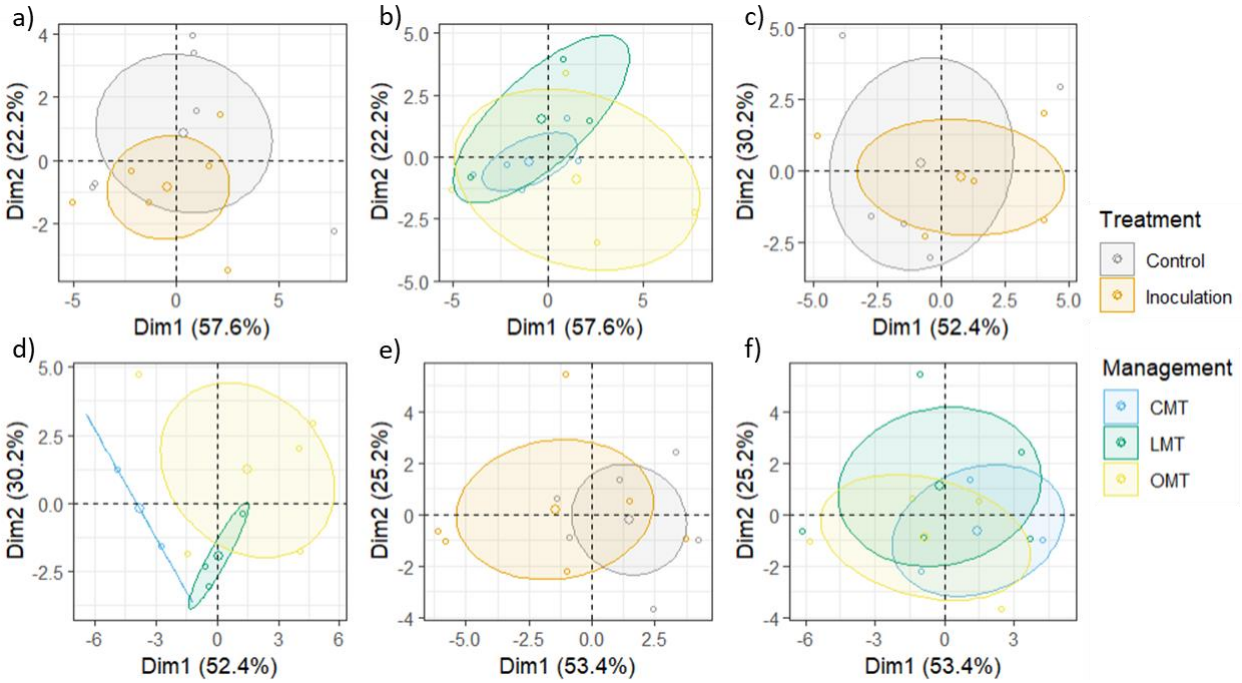

Table S7. Soil nutrient means ± standard deviations throughout 2021 season. Letters represent significant differences between managements.

	6-Weeks Post-Inoculation			10-Weeks Post-Inoculation			17-Weeks Post-Inoculation		
	CMT	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**	**CMT**	**LMT**	**OMT**
Ammonium (ppm)	14.69 ±13.2	10.21 ±2.1	14.80 ±10.7	7.98±2.0	9.39±3.4	6.57±1.8	4.00±1.3	4.53±1.0	3.31±0.7
Nitrate (ppm)	10.37 ±7.6	17.07 ±6.3	23.48 ±13.1	7.79 ±2.6^{a}	20.78 ±8.7^{b}	16.32 ±5.4^{b}	9.03 ±2.2^{a}	16.31 ±2.8^{b}	15.44 ±2.0^{b}
Phosphorus (ppm)	47.85 ±39.4^{a}	27.68 ±2.7^{a}	79.05 ±17.9^{b}	27.87 ±3.8^{a}	25.52 ±10.4^{a}	73.70 ±9.0^{b}	23.10 ±5.9^{a}	16.12 ±2.6^{a}	67.38 ±12.4^{b}
Potassium (ppm)	317.00 ±178.9^{ab}	212.40 ±42.1^{a}	510.67 ±191.5^{b}	228.67 ±24.0^{a}	215.67 ±129.1^{a}	503.67 ±50.1^{b}	200.83 ±48.6^{a}	157.33 ±67.1^{a}	574.33 ±89.6^{b}
Sulfur (ppm)	21.38 ±31.4	11.78 ±4.6	36.72 ±28.7	6.42±1.9	9.77±1.9	41.45 ±16.8	14.02 ±5.6	20.23±8.0	43.65 ±22.8
Zinc (ppm)	1.27±0.3^{a}	1.46±0.2^{a}	2.89±0.5^{b}	0.89±0.2^{a}	1.05±0.1^{a}	2.63±0.4^{b}	0.86±0.4^{a}	0.99±0.1^{a}	3.17±0.2^{b}
Iron (ppm)	19.32±1.6	21.98±2.7	22.57±7.8	13.15±3.3	15.47±7.0	16.46±5.5	12.22±5.8	13.73±3.6	15.63±2.0
Manganese (ppm)	2.85±0.7	7.00±0.9	10.32±7.9	6.38±1.4	5.66±1.0	7.30±1.1	8.38±3.4^{a}	8.98±1.8^{ab}	12.92±1.5^{b}
Copper (ppm)	1.03±0.1^{a}	1.04±0.1^{a}	2.31±0.2^{b}	0.98±0.1^{a}	0.98±0.2^{a}	2.30±0.4^{b}	1.13±0.4^{a}	1.19±0.1^{a}	2.98±0.2^{b}
Calcium (ppm)	2103.50 ±375.1	1869.00 ±208.7	1951.00 ±352.3	1986.67 ±208.6	1759.33 ±180.2	2105.50 ±176.0	1706.50 ±91.6	1634.67 ±49.1	1682.00 ±89.2
Magnesium (ppm)	1613.50 ±101.5	1582.80 ±166.4	1282.83 ±180.9	1713.17 ±210.1	1498.33 ±159.8	1407.67 ±150.0	1438.17 ±82.3^{b}	1375.17 ±68.2^{b}	1118.50 ±90.3^{a}
Sodium (ppm)	69.17 ±12.1^{a}	93.00 ±13.6^{b}	128.33 ±11.5^{c}	76.00 ±13.6^{a}	94.33 ±30.6^{a}	162.67 ±29.2^{b}	72.50 ±29.6^{a}	120.83 ±23.2^{ab}	138.67 ±39.3^{b}
Boron (ppm)	1.50±0.2^{a}	1.39±0.2^{a}	2.12±0.4^{b}	1.35±0.2^{a}	1.60±0.2^{a}	2.87±0.3^{b}	1.31±0.4^{a}	1.48±0.3^{a}	2.32±0.4^{b}