Orchard Soil Biology and Mycorrhizal Fungi

Mike Basedow, Dr. Megan Muehlbauer, Jeremy DeLisle, Mario Miranda Sazo, Dr. Deborah Aller, and Dr. Jason Londo

What lives in your orchard soil?

There are likely more species of organisms in a shovel full of orchard soil than exist above ground in the entire Amazon rain forest (NRCS).

Most biological activity takes place in the top 8 to 12 inches in the soil profile. The **rhizosphere**, or rooting zone, is an area of intense microbial activity and is integral to plant and soil relationships.

Plant roots leak energy-rich carbon compounds, sugars and amino and organic acids called **exudates**. Every plant species leaks a unique signature of compounds from their roots. Different microbes are attracted to different exudates. The plants grown play a large role in determining the microbial community in the soil below. Other food sources for soil organisms include leaves and roots. All of these materials are carbon rich containing **organic matter**. Many soil organisms are involved with breaking down this organic matter into smaller compounds that future plants can use, these include:

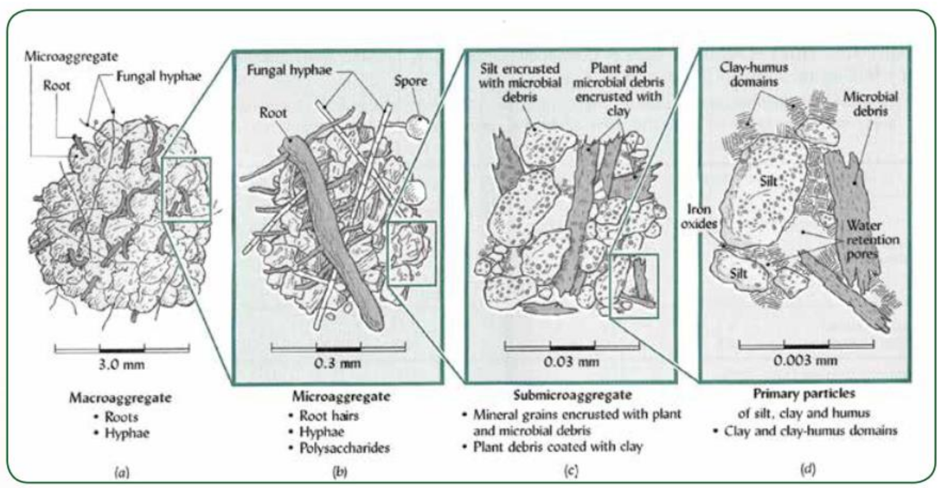
- **Eukaryotes:** This large group includes many fungi, algae, nematodes, earthworms, insects, arthropods, and protozoa that are important in soil ecology. Eukaryotes feed on soil organic matter, soil bacteria, plants, and each other. The larger soil eukaryotes are also "nature's tillers" and help mix the soil and create channels for water and air.
- **General fungi** are important in decomposition, especially organic compounds like lignin. Some of them cause plant diseases, while others live mutualistically with plants. Saprophytic fungi break down organic matter to help produce humus and provide plant-available nutrients.
- **Gram-positive (G+) bacteria** dominate subsurface soils (versus G-). G+ bacteria are widely dispersed in soil and tend to decompose organic material that has been partially decomposed by fungi or G- bacteria.
- **Actinobacteria (formerly Actinomycetes)** are G+ bacteria that are active in decomposition of organic matter and produce geosmin, a compound that generates the "earthy" smell of freshly tilled soils. They break down soil organic matter, including resistant compounds such as cellulose, chitin, and lignin. Actinobacteria can grow deep into the soil, but are sensitive to acidic, low-oxygen, and wet or saturated conditions.

A few benefits of these various soil organisms:

- **Nutrient storage and mineralization:** To consume enough carbon, biota often consume more nitrogen than necessary, creating excess N available to plants. This is part of the important process called mineralization. In mineralization, nitrogen that has been bound to carbon in relatively large molecules (‘organic nitrogen’) is released in ‘mineral’ form as smaller, more soluble, nitrogen containing ions (ammonium and nitrate) that can then be taken up by plants. In some systems, a significant fraction of plants’ nutrient needs can be supplied from the soil when there is enough organic matter and soil biota. **For each percent organic matter increase, you may get an extra 10 pounds of mineralized N from the soil.**

- **Soil aggregates** are built and stabilized by soil biota through the growth of fine roots, fungi, and the soil microbial culture, as well as by the periodic wetting and drying of the soil. Stable soil aggregates are important for maintaining good (crumbly) soil structure or ‘tilth’, enabling adequate air exchange and water infiltration, storage, and drainage. Stable soil aggregation minimizes erosion and flooding. These processes are also critical in sequestering, or stabilizing carbon, in the form of well-decomposed organic materials protected within small pores, and tightly bound to soil mineral particles.

Figure 1. Aggregate size and composition. An active microbial population will build and stabilize soil through production and interaction with adhesive byproducts. Each step (a-d) demonstrates the bonding agents and aggregation of soil as size decreases. Photo from Comprehensive Assessment of Soil Health – The Cornell Framework.



Apple Soil Borne Pathogens: Not all soil dwellers are helpful to our apple trees. Some feed on apple trees directly (nematodes, small mammals) or may cause diseases within the trees (phytophthora root rot).

Symbiotic Organisms: Two other key groups of soil organisms that are not directly involved in decomposition, but are important in soil functioning, are **symbiotic bacteria and mycorrhizal fungi** that associate with plant roots. They include nodule-inducing nitrogen fixing bacteria (rhizobia) and mycorrhizal fungi. They live in close association with plant roots and interact with living plants in a mutually supportive manner.

N fixing bacteria (gram negative bacteria): The nodule-inducing nitrogen fixing bacteria (Rhizobium, Bradyrhizobium, and Sinorhizobium, among others) interact with legumes, such as beans, peas, soybeans, clover, and vetch. The legume roots develop nodules, which house bacterial colonies. Plant tissues provide sugars to the bacteria, while the bacteria convert atmospheric nitrogen to nitrogen that can be used by plants (nitrate and ammonium). Sometimes more nitrogen is 'fixed' than is required by the plant, so the excess is released into the surrounding soil. Some free-living (not plant associated) and associative (close to roots but not in nodules) nitrogen fixation is known to occur in both natural and managed systems. These bacteria dominate surface soils (versus G+) in the rooting zone and breakdown newly added organic matter.

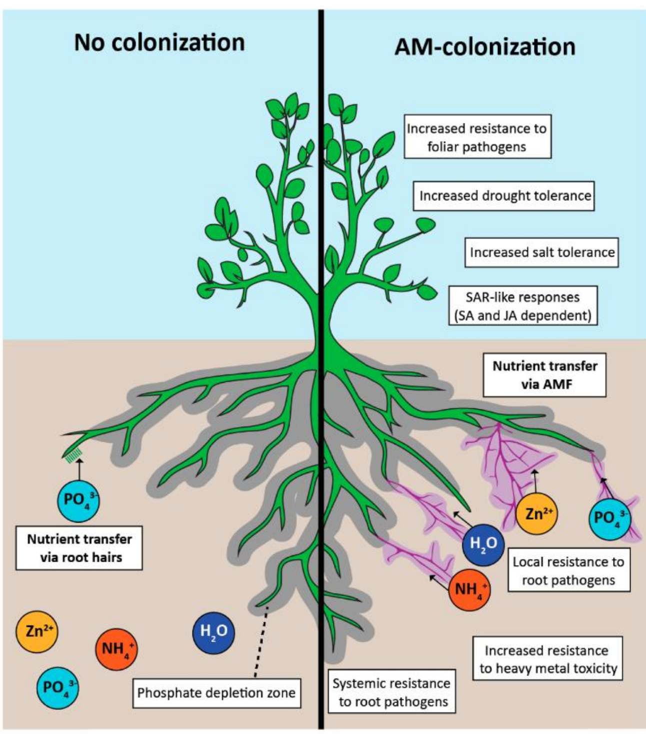
Figure 2. Nodules on alfalfa root fixing atmospheric nitrogen. Photo: T. Råberg, SLU

Mycorrhizal fungi: Most plant roots associate with symbiotic fungi. Over 200,000 species of plants (80% of plant species) associate with some of the roughly 240 species of **arbuscular mycorrhizal fungi (AMF)**. Together with plants, these fungi form joint structures called mycorrhizae (from the Greek words for fungus and root). The plant host provides sugars to the fungus, used for growth and metabolism, in exchange for nutrients. Outside of the root, the fungus grows extensively through the soil profile, and can reach more spaces and absorb more P, N, S, Zn, and Cu (especially phosphorus, which is poorly soluble) than the plant roots alone could.

In addition to providing a nutrient benefit to the plant host, these fungi contribute to both plant and soil health in multiple ways. They can help the plant resist disease and tolerate drought and saline (salty) conditions. The AMF also contribute substantially to the accumulation of soil organic matter and to the formation and stabilization of soil aggregates from their production of glomalin, a protein that protects they hyphae and glues soil particles together.

Figure 3. Mycorrhizal fungi's close association with plant roots form symbiotic relationships. Photo from Comprehensive Assessment of Soil Health – The Cornell Framework.

Figure 4. Positive effects of arbuscular mycorrhizal (AM) colonization. The hyphal network of arbuscular mycorrhizal fungi (AMF) extends beyond the depletion zone (grey), accessing a greater area of soil for phosphate uptake. A mycorrhizal-phosphate depletion zone will also eventually form around AM hyphae (purple). Other nutrients that have enhanced assimilation in AM-roots include nitrogen (ammonium) and zinc. Benefits from colonization include tolerances to many abiotic and biotic stresses through induction of systemic acquired resistance (SAR). Photo credit: Jacott, Murray, and Ridout. 2017. Trade-Offs in Arbuscular Mycorrhizal Symbiosis: Disease Resistance, Growth Responses and Perspectives for Crop Breeding.



Positive effects of AMF - studies on apple specifically:

- Tree Growth – Multiple studies have found AMF have the potential to increase tree growth, as trees inoculated with species of Glomus fungi had greater growth than uninoculated control trees (Reich, 1988; Morin et al. 1994; Forge et al., 2000; Hosseini and Gharaghani, 2015).

- Tree Survival – Berdeni et al. (2018) found mycorrhizal applications decreased the prevalence of nectria canker in apple trees. This disease can kill trees. In addition, an unpublished Pennsylvania demonstration trial found trees treated with MycoApply EndoMaxx had improved tree survival (97.6%) compared to uninoculated trees (88.9%), reducing tree replacement costs by $536 per acre (Racsko, personal communication).

- Drought Tolerance - AMF enhanced drought resistance in crabapple (Huang et al., 2020). A similar study by the same research team found mycorrhizal fungi upregulated drought tolerance genes in apple (Huange et al., 2021)

- Nutrient Use - One greenhouse experiment (Morin et al., 1994) found trees inoculated with mycorrhizae had a higher leaf P concentration than uninoculated trees, despite being grown in a high P soil. The study also found that trees inoculated with the species *Glomus verisforme* had the best nutrient balance. Hosseini and Gharaghani (2015) found plants inoculated with fungi contained more N, P, Ca, Mg, Zn, and Fe compared to control plants. Bokszczanin et al. (2021) found apple trees inoculated with fungi had increased concentrations of N, P, and K in the leaves, and either had no effect or decreased the concentrations of Mg and Ca in the leaves.

SARE Project Objectives

We have established three field sites, alongside greenhouse studies, to compare the potential benefits of inoculating newly planted orchards with four commercial strains of AMF (along with an untreated control), under two fertility management regimes.

We are taking measurements to determine how treatments differ in:
- AMF colonization
- Water use efficiency
- Nutrient uptake
- Tree growth and survival
- Microbial communities within the rhizosphere

Our goal is to help growers determine if any of these four products can provide benefits in these areas, potentially reducing the amount of fertilizer and water that is needed during orchard establishment.

Figure 5. The estimated total microbial biomass of our orchard soils prior to planting. Phospholipid fatty acid (PLFA) is an essential structural component of all microbial cell membranes. The PLFA analysis is used to differentiate microbial groups and estimate the microbial biomass of each group.

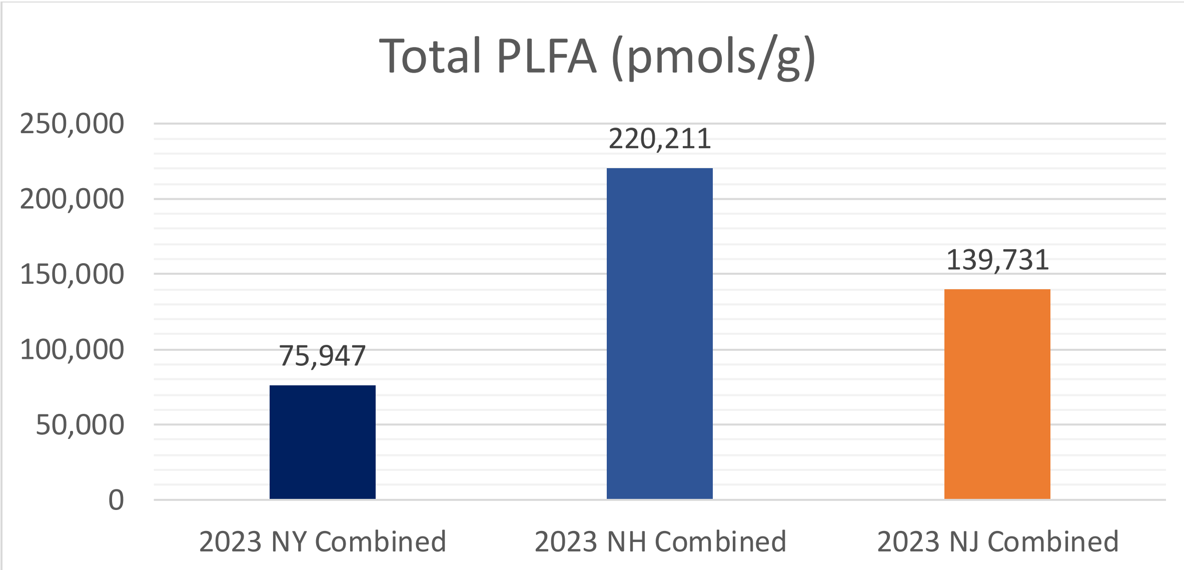
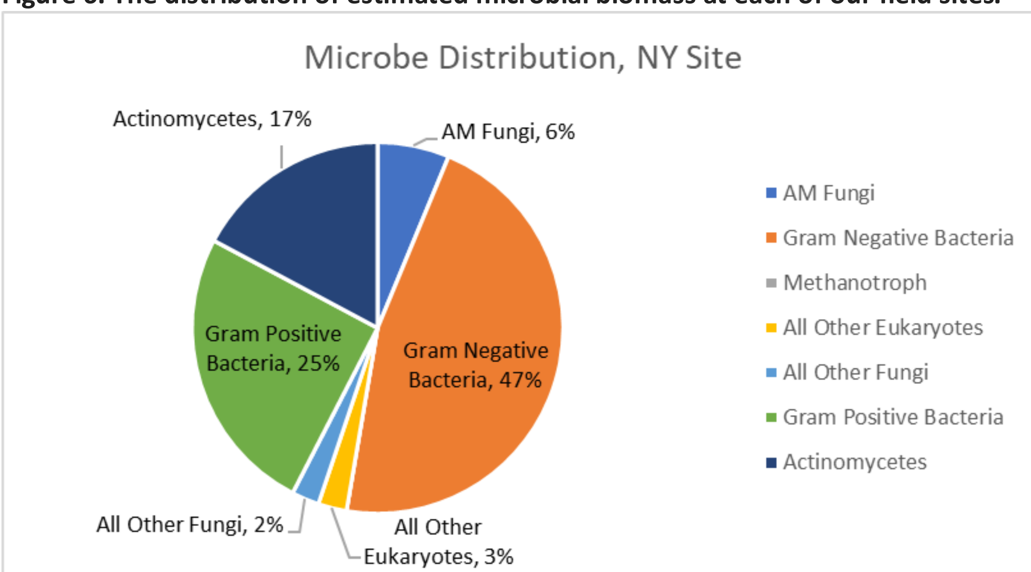
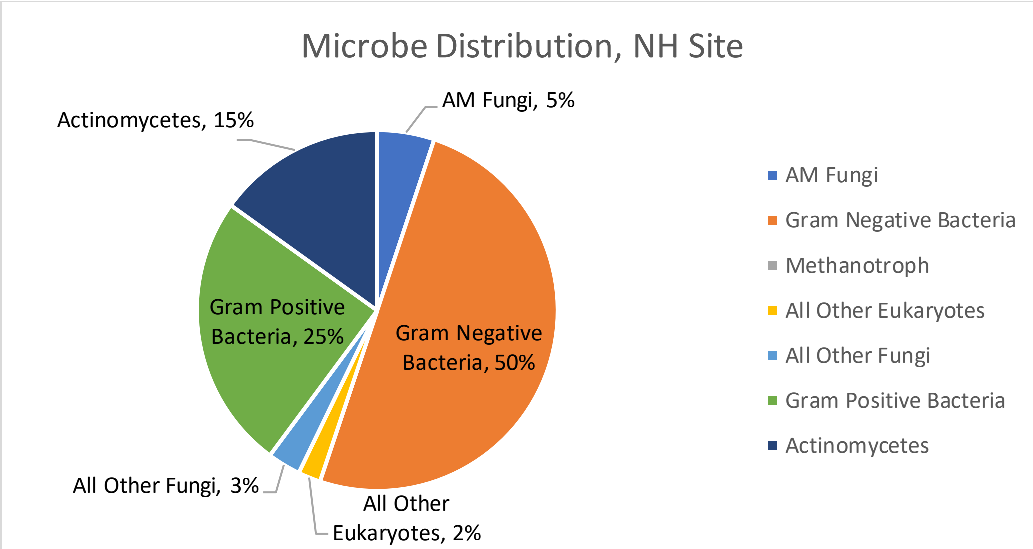


Figure 6. The distribution of estimated microbial biomass at each of our field sites.





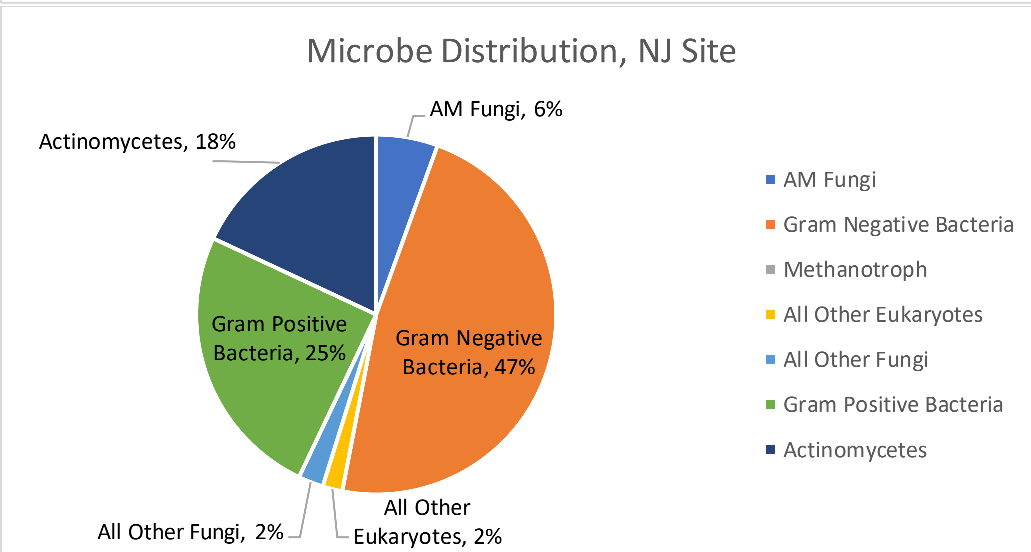


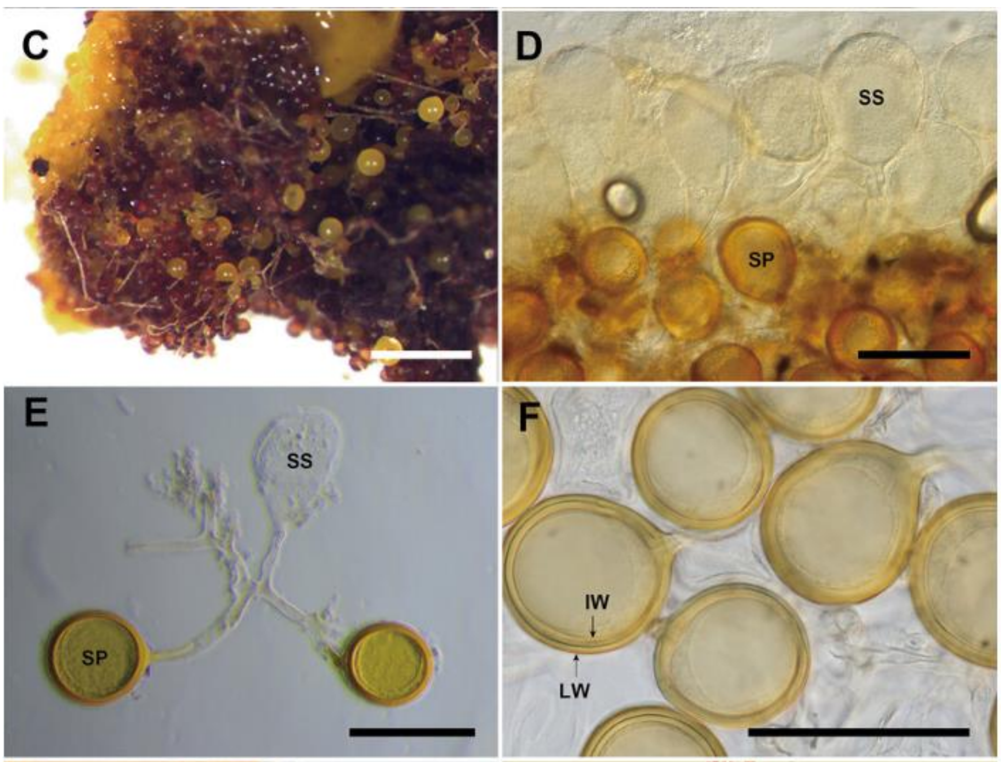
Table 1. A comparison of fungi products being used at our field sites.

Product Comparison					
Product	**Propagules per Tree***	**Rate Per Tree**	**# Trees per Acre**	**Cost per Acre**	**Other Listed Ingredients**
Symvado	1320	1 pouch	1210	435.60	
Mykos Gold	900	3 grams	1210	45.42	
MycoBloom	2160 spores per tree	3 Tablespoons	1210	1269.56	
Promate	2400	2 pouches	1210	1393.92	N,P,K,Ca, Mg, S, Humic acid

Table 2. A comparison of species present within the fungi products being used at our field sites.

Species	Symvado	Mykos Gold	MycoBloom	Promate
Claroideoglomus claroideum	x		x	
Funneliformus mosseae	x		x	
Cetraspora pellucida			x	
Claroideoglomus lamellosum			x	
Acaulospora spinosa			x	
Racocetra fulgida			x	
Entrophospora infrequens			x	
Rhizophagus irregularis	x	x		x
Claroideoglomus etunicatum	x			
Total Species per Product	**4**	**1**	**7**	**1**

Figure 9. R. irregularis spores and swollen structures. R. irregularis is commonly found in commercial AMF mixes. Photo courtesy Clonal spore populations in sporocarps of arbuscular mycorrhizal fungi by Yamato et. Al.



What have we observed so far?

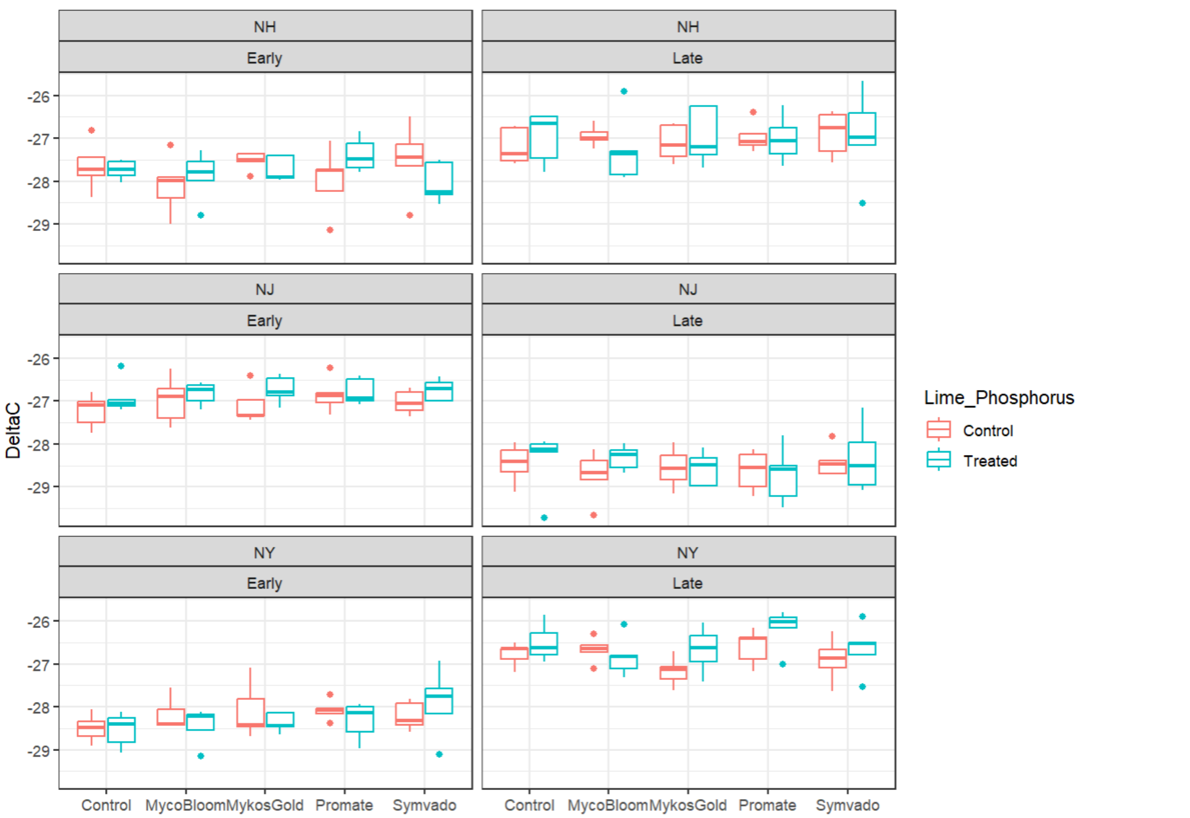
Table 3. Growth at our three field sites by fungi and fertilizer treatment.

2023 Field Trial Trunk Circumference Growth (cm)				
		NY	**NJ**	**NH**
Fungi	Symvado	0.6 B	0.9	1.4
	Mycobloom	0.7 AB	0.9	1.5

	Mykos Gold	0.6 B	0.9	1.5
	Promate	0.9 A	1.0	1.5
	Control	0.7 AB	1.0	1.4
	P-value	0.00382	0.6076	0.87525
Fertility	Low	0.7	1.0	1.5
	High	0.7	0.9	1.4
	P-Value	0.1382	0.2512	0.7481
Fungi x Fertility	**P-Value**	0.6361	0.1098	0.4124

Water Use Efficiency

Figure 10. 2023 Water Use Efficiency Data from our three field sites by timing.



Orchard Root Colonization

Few fungi were found in our 2023 root assessments from this trial. This may have been due to sampling error, as roots may have been too small for adequate colonization.

Our Next Steps:

- Collect additional water use efficiency tests over the next two years.
- Complete leaf nutrient assessments in summers 2024 and 2025. Continued tracking of tree growth and survival.
- Complete a root colonization assessment at each site in fall 2025.
- Collect soil from each treatment for PLFA analysis in 2025.

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