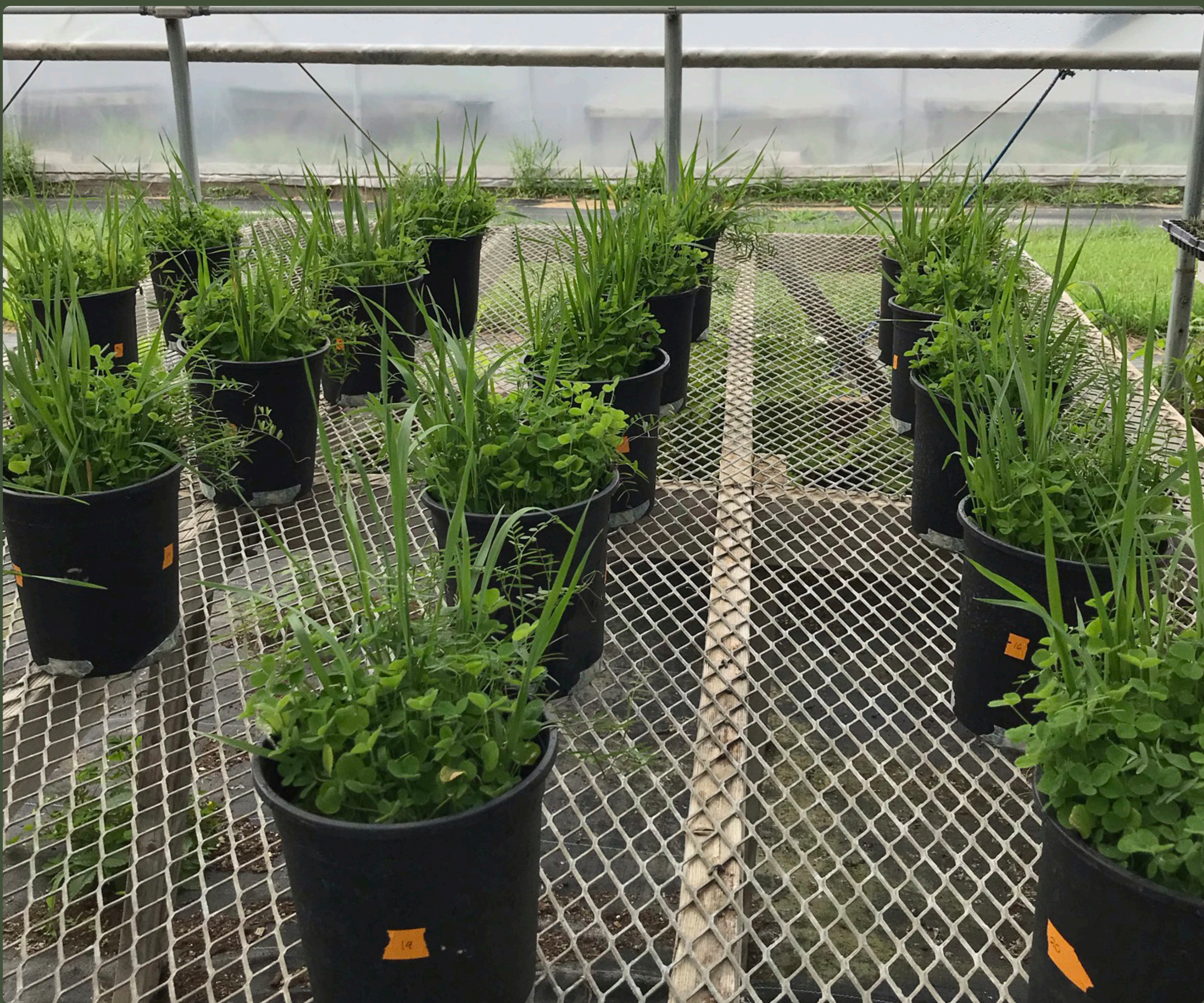
Growing Local Mycorrhizal Inoculum

A GUIDE AND INSIGHTS FROM A FIELD TRIAL



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WHAT ARE MYCORRHIZAE?

Mycorrhizae are the symbiotic associations that form between fungi and plant root systems. There are at least four main types of mycorrhizal fungi, and our work focuses on Aburscular Mycorrhizae Fungi (AMF) because they are the type most essential to agriculture(1). AMF form symbiotic associations with more than 80% of terrestrial plant species, including many agricultural crops(2). Unlike fungi who's fruiting bodies appear above-ground in the form of mushrooms, AMF live their entire life cycle underground as mycelium (the network of root-like threads that make up the fungus, also known as hyphae). To sustain themselves, the hyphae enter and colonize the plant root. Here they perform their symbiotic exchange: the fungi provide mineral nutrients and water to the plant, and receive essential nutrients and carbohydrates in return.

Numerous studies have shown the benefit of a healthy AMF soil community to plant crops(3, 4, 5, 6, 7). The hyphal networks essentially become extensions of the plant's root network, significantly increasing the area of soil the plant can access, and therefore the nutrient and water available to the plant. Without the help of AMF, plant roots can only reach nutrients 1-2mm away, whereas hyphae attached to these roots can extend their reach by 8cm or more(8). Due to this, AMF can be considered a bio-fertilizer, allowing farmers to lower (or eliminate) their chemical fertilizer input, while simultaneously increasing crop yield(4). AMF presence also increases resistance to stressful conditions, such as drought, heat, salinity, and soil pathogens(3,9). Furthermore, AMF improve soil structure and aggregation, drive plant community diversity and productivity, and lower greenhouse gas emissions(10). AMF can be a supportive tool for any farmer aiming to practice organic, regenerative agriculture in order to increase farm resilience in the face of a changing climate.

Our research study and inoculum growth trial took place on unceded Abenaki territory, colonially known as Vermont, in the Champlain Valley. For us, and for many growers working in similar conditions, phosphorus uptake was a key concern. Excess phosphorus, 38% of which is agricultural runoff from farms, contributes to yearly toxic algae blooms in Lake Champlain(11). AMF have demonstrated efficiency at increasing plant's phosphorus uptake(12, 13, 14). Therefore, we understand AMF to be an ally in safeguarding watersheds. We encourage farmers everywhere to protect their waters which are being overwhelmed by the effects of unsustainable farming practices.

Parsley being harvested for data collection at research study field site.

HOW TO PROMOTE AMF ON YOUR FARM.

It may be likely that the soil on your farm already has its own AMF community. However, many common farm practices negatively impact these fungal communities. Tilling breaks up mycelium, and frequent tilling doesn't leave ample time for fungal communities to grow back[15]. Pesticides, fungicides, and herbicides also do damage to AMF communities. Traditional fertilizer use can also be harmful, since excess use of phosphorus discourages AMF growth, inhibiting mutual symbiosis. Whether or not you plan to apply mycorrhizal inoculum on your farm, limiting or eliminating these practices can nurture the microbial soil network, including the AMF community already present in the soil[16]. Other management practices which protect and encourage existing AMF communities include: consciously increasing plant diversity, incorporating polycultures, rotating crops, planting crops that encourage AMF, and utilizing cover cropping[15, 17, 16].

Southern wild field buffer planted with polyculture covercrop at field site at Digger's Mirth Collective Farm in the Intervale, Burlington VT.

It's important to know that some plants don't form associations with AMF, they actually deter them! When trying to enhance AMF presence, don't waste time and resources on doing so for these plants[19, 18, 20]:

Brassicaeceae family- broccoli, brussels sprouts, cauliflower, kale, mustard greens, collards, cabbage, rutabaga
Ericaceae family*- blueberry, cranberry, huckleberry, lingonberry, rhododendron, azalea
Amaranthaceae family- beets, spinach, chard, amaranths, quinoa, lamb's quarters
Others- poppies (Papaveraceae), carnations (Caryophyllaceae)

This is an incomplete list but includes most plants that may be of key concern.

*Instead of AMF, many plants in the Ericaceae family form associations with Ericoid mycorrhiza.

If you're ready to maximize AMF on your farm, inoculation is the way to go. There are two options for inoculating: buying commercial inoculum, or growing your own. Here's a break-down of the pros and cons of each from our experience:

	FARM GROWN	COMMERCIAL
+	• Very cost effective. • Limited labor required once established. • End product is customized to your local environment, so should suit your soil type and existing microbial community well. • Minimal risks associated with introducing a foreign species. • Inoculum will represent a diverse group of AMF, likely with many more species and symbiotic microbes than are present in commercial inoculum[21]. Research indicates higher numbers of AMF species in the soil increase benefits to crops[23].	• Minimal added labor • Time efficient, doesn't require a growing season to prepare
-	• Requires an extra season of planning, time, resources, and space to grow the inoculum. • Success of inoculum will depend on the local AMF community in the soil you apply as a starter and results can't be guaranteed.	• Can be costly • AMF are species specific, so there's no guarantee that the species in commercial inoculum will pair with your crop. • There are typically just a few AMF species in commercial inoculum. 1/3 of market products contain only one species[21]. Having a more robust and diverse AMF community gives much better results[23]. • There are concerns about the impact of introducing foreign fungi to local ecosystems[21]. • Symbiosis between native AMF and the larger microbial soil community is essential to AMF functions which may not be replicated through introduced non-native commercial AMF[23].

Growing your own inoculum is not as intimidating as it may sound. A relatively small amount of labor can come with big rewards. The rest of this guide highlights the process of growing your own on-site inoculum.

GROWING ON-SITE MYCORRHIZAL INOCULUM: 101

Materials Overview

- Plastic pots of uniform volume, cleaned and sterilized with a 30% bleach solution or hydrogen peroxide.
- Growing medium, we use a 1:1 ratio of sand to vermiculite.
- Seeds. We used a ratio of hairy vetch, oats, and crimson clover, to replicate the covercrop mix used in our field trials. You can also use just one plant, grasses are a good option[13].
- Soil inoculum, soil collected from a wild buffer area around your farm.
- Materials to create a modified fertilizer solution (ideally a ratio of .01 parts phosphorous, and .1 parts of all other nutrient concentrations). More detail on this later.
- A pressure cooker, or access to an autoclave.
- Gloves.
- Spray bottle with 70% ethanol solution to sterilize equipment.
- Ideally, access to a soil lab that can check inoculum viability.

Warning! Watch out for soil pathogens
While the purpose of growing mycorrhizal inoculum is to increase the quantity of fungi in the soil, we want to ensure we are only doing this for the beneficial AMF and not any potential soil pathogens, which can include fungi, bacteria, and nematodes.
There are numerous soil pathogen testing options available, from at-home kits to mail-in services. This step is optional, but it's always a good idea to be safe, especially if you're planning on using your inoculum on a large number of crops.

TIMELINE AT A GLANCE

Weeks 0-14: Water 1-2 times daily and fertilize weekly

- Week 0: Harvest soil. Set up pots
- Week 12: Gather root samples to check for colonization, if possible
- Week 14: Stop watering
- Week 15: Cut plant material to soil level
- Week 16: Harvest inoculum. Check viability.
- If viable, transfer and store

UTILIZING A LAB FOR VIABILITY CHECKS: THE HOW AND WHY

Because mycorrhizae cannot be seen with the naked eye, the only way to guarantee your inoculum has enough fungi to be viable is through lab analysis. In the lab, scientists can check the roots of the plants grown in your pots to see approximately what percent of them were colonized by mycorrhizae. They can also extract spores from the medium of your inoculum and quantify those, another important signifier of adequate mycorrhizal presence. Ideally you want a minimum of 60% root colonization and 50 spores for every gram of inoculum.

Unfortunately since this is an emerging field, there are not many labs that offer these services yet, especially in the U.S. We are aware of these that do:

- **INVAM** (The International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi) https://invam.ku.edu/ordering
- **MycoRoots** https://mycoroots.com/services-and-fees/
- **University of Florida Soil Microbiology Extension Program** https://soils.ifas.ufl.edu/soil-microbial-ecology/mycorrhizal-services/soil-biology-tests/

We recognize there are accessibility barriers here, both in the number of labs available and in associated costs. If sending your inoculum to a lab for viability tests is not feasible currently, we still encourage you to try growing your inoculum! Consider testing the viability yourself by setting up a small field trial. Create two plots near each other where the environmental conditions are as similar as possible. Plant the same crop into each plot, applying your inoculum to the crop in one of the plots and leaving the other plot as the control. Let both plots grow, applying the same amount of water and nutrients. Observe any differences you notice. Do some plants seem more robust than others? Did one plot have a higher yield on average? Did plants in one plot grow more quickly? Ideally, you would do at least 3 trials of this in different locations to control for more variables. Even just a simple trial like this can reveal a lot about how well your inoculum may be working.

INOCULATED ⟷ compare CONTROL

GROWING STEPS

1) **Gather your pots of uniform volume.** These can be any size, and the number just depends on how much inoculum you would like to grow. Help with estimating the amount of inoculum required is in the next section, *Utilizing your Inoculum*. If the drainage holes on your pots are relatively large, we recommend covering them with tape on both sides and then poking small holes. You want ample drainage, but don't want the sand medium to leave the pots.

- Clean the pots with soap and water
- Spray and wipe them down with a 30% bleach solution or hydrogen peroxide to ensure there are no airborne microbes or fungal spores that could contaminate your inoculum.
- Let them air dry completely.

GROWING STEPS CONTINUED

2) **Gather your soil inoculum starter.** Find a relatively wild and untouched area of your farm, where perennial herbaceous species known to partner with AMF grow. A wild buffer zone around or between fields is a great option. A helpful chart to reference can be found at https://mycorrhizae.com/mycorrhizal-status-of-plant-families-and-genera/, look in the first column for plants that form endomycorrhizal associations. Once you've found a promising area, you're ready to sample. Using a clean trowel and bucket, sample from the top 10cm, which is where most AMF are. Gather a trowel full every 2 feet apart, along the area you have designated. Mix soil in the bucket gently to create a composite sample. The highest concentration of AMF will be right within and around plant roots, so shake soil off roots as you go to maximize the fungi you gather. You will need 50 grams of soil for each pot.

These are the wild buffers to the north and south of the Diggers' Mirth field site where we sampled soil for our inoculum starter.

3) **Next, prepare your medium.**

- Calculate the amount of medium you will need based on the volume and number of your pots. Pot volume in gallons can be found using this formula: $V=(1/3) \times 3.14 \times H \times (r^2+r \times R+R^2)$, where r is the radius of the bottom of the pot, R is the radius of the top surface of the pot, and H is the height. Multiply your result by the number of pots you have, and then divide that by two to determine how much sand and vermiculite you'll need.
- Mix together calculated amount of sand and vermiculite in equal parts.

Like the pots, your medium will need to be sterilized to ensure there are no pathogens present that could interfere with mycorrhizal association formation. We use an autoclave since we have access to one (autoclaving the medium at 121C ° for 20 minutes), however a pressure cooker will work just as well. If you don't have one, this is the most significant piece of equipment you'll have to invest in or borrow for this process. It is worth it since it guarantees sterilization of your medium.

- Heat the medium at 15PSI (equivalent to 121C °) for 60 minutes, you can do this in clean glass jars or heat tolerant spawn bags in your pressure cooker.
- After sterilization, let the medium cool down completely and then use immediately.

4) Now that your media and soil are ready, it's time to set up your pots!

- Add your sterilized media up to 5cm below the pot tops.
- Add 50 grams of soil inoculum starter to each pot, and gently stir it in with a sterilized tool such as a trowel.
- Add additional sterilized media up to 3cm below the pot tops, and water thoroughly until there is a small amount of drainage through the pot.
- Next, add your seeds. We calculated the number of seeds to use based on the ratio of seeds used per acre when planted as a cover crop, then converting this ratio to the area of the pot surface. Feel free to estimate, making sure the quantity is the same across all the pots. It is crucial to use enough seeds to achieve high germination and plant growth across the entire pot.
- Finally, top off pots with a handful of sterilized medium to cover the seeds.

Above: Luca setting up pots.
Right: pots in greenhouse the day of setup.

5) Place the pots somewhere with full sunlight, ideally a greenhouse. Optimum environmental conditions for mycorrhizal growth are 16 hours of sunlight at 25C °. For the next 3-4 months water the pots once or twice daily (depending on climate, sun exposure, and humidity) to encourage healthy plant growth. Weed as needed, and fertilize weekly (starting the week after setup).

6) Fertilizing: Phosphorus is central in nutrient exchange, so a lack of phosphorus will encourage AMF growth since plants rely heavily on AMF to meet their phosphorus needs. Hence, a low phosphorus fertilizer should be applied to the pots each week. Low phosphorus slow release fertilizers can be applied in small amounts, or you can mix your own to create the optimum ratio of nutrients. Here is our recipe for a modified fertilizer:

Into 2 gallons of water add:

- ***15g North Country Organics 6-0-6***
- ***0.9g epsom salts***
- ***6ml Neptune's Harvest 2-3-1***

Mix thoroughly, and add 240ml (1 cup)* to each pot weekly.

**Our used half gallon pots, you may need to adjust if you use different size pots.*

GROWING STEPS CONTINUED

7) **Maintain pots each week and monitor growth until week 12.** If you found lab access, this is a good time to check plant root AMF colonization levels. Gently harvest a few roots from multiple pots. Put them in sterile bags and submit to the lab to analyze. If most roots are over 60% colonized, continue with next steps. If they're not, consider maintaining plant growth for 2-4 additional weeks before continuing to the next steps.

8) **At week 14,** stop watering and fertilizing the pots. Let them dry out.

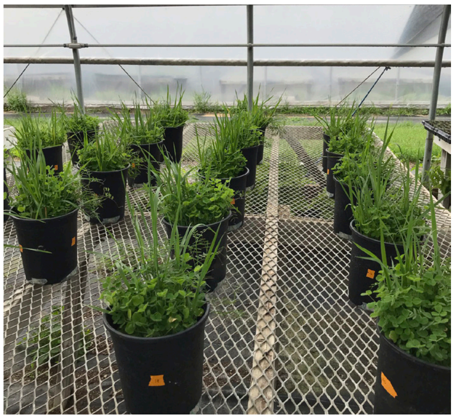
9) **At week 15,** cut the plants at soil level, composting or reusing them as mulch. Continue to let the pots dry out.

10) **At week 16,** gather samples for final viability checks if you have lab access. Again, gently gather some roots from multiple pots (ideally ones you did not gather from before). Submit to lab for root colonization checks. If the lab can conduct spore quantification, gather some medium as well and submit in sterile bags. After that, or if unable to check for viability, continue on to harvesting your inoculum. Dump out the medium onto a clean surface. Cut up the roots into small pieces with sterilized scissors and mix them gently into the medium with a sterilized tool. Ensure there is no above-ground plant material in this, only roots!

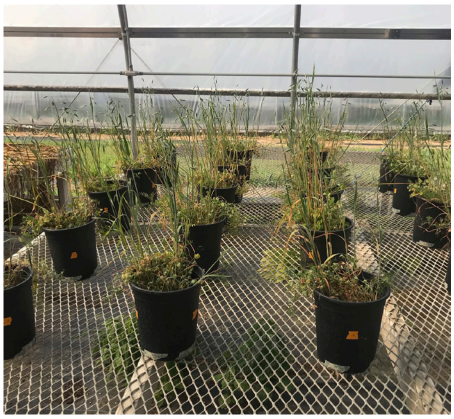
11) **Congratulations, your inoculum is now complete!** If you sent samples to a lab, you're hoping to see average root colonization levels of over 60%, and spore counts of at least 50 spores/gram of soil. This will confirm that your inoculum is viable, meaning that there is enough AMF presence in the mix to effectively inoculate the crops it's introduced to. The inoculum can be used immediately, or stored. If you perform this process in the fall to prepare for the next growing season and temperatures have already dropped sufficiently, inoculum can be left to overwinter in the cold. Alternatively, you can store the inoculum at 4°C, fridge temperature, for up to two years. We stored ours at 4°C, in sterilized bins with holes, covering the holes with breathable tape (see image on page 11).

WEEK 1

WEEK 3



WEEK 6



WEEK 13

UTILIZING YOUR INOCULUM

There are a few options for how to utilize your AMF inoculum. One way is to mix the inoculum into the potting mix you use to germinate seedlings. The recommended ratio of inoculum to potting soil is 1:9 for growing cells 50 cm3 or smaller, and 1:19 for larger cells[(17)]. You can also sprinkle inoculum (about a teaspoon) onto the rootball of the plant, or into the hole you're putting it into, as you transplant plants into the field. Just ensure the soil is well packed before watering so as not to wash away the inoculum. The first option may provide better results because it allows the plants to begin forming relationships with the fungi at the beginning of their growth. Hence by the time they're transplanted into the field their roots will already be colonized by mycorrhizae and nutrient transfer from the soil in the field can begin immediately, while minimizing transplant shock. Either way, you are now successfully implementing your farm-grown local mycorrhizal inoculum. Congratulations!

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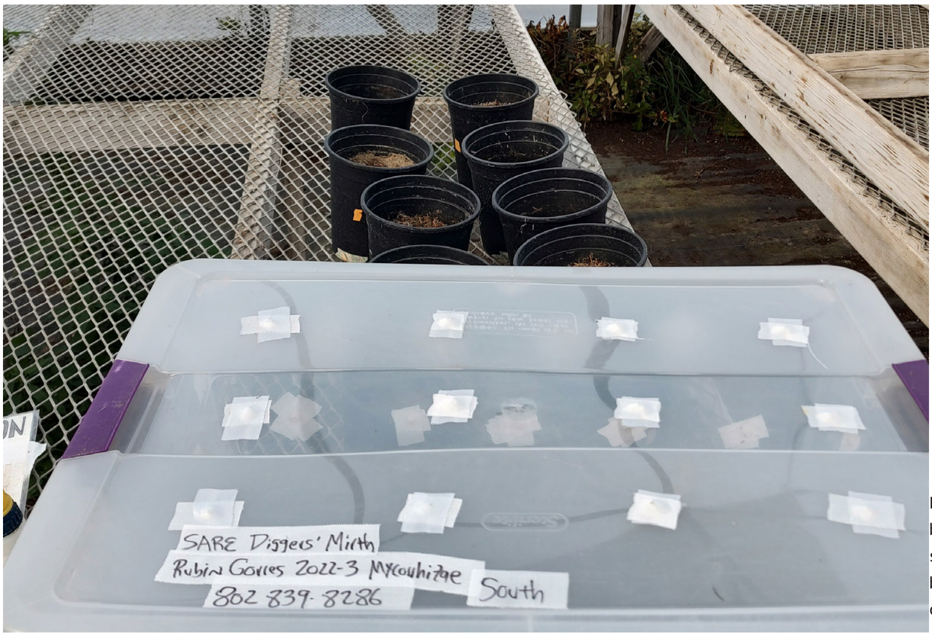

Finished inoculum being transferred into sterile, breathable bins to be placed in a cooler for storage.

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All photos are our own and were taken by either Luca Kolba or Jessica Rubin.