College of Liberal Arts

# Effects of Biochar and Arbuscular Mycorrhizal Fungal Inoculation on the Root Colonization and Growth of Pisum Sativum (Dwarf Snap Peas)

A Thesis in Environmental Science

By

Rebecah Bashant

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**Abstract**

The field of agriculture struggles deeply with the battle between producing food at a high enough rate to feed our ever growing population, while maintaining supports for our struggling environment. Previous research in the field of sustainable agriculture has focused on the use of 0.5%-2% biochar to aid in plant growth and soil amendment, but manufacturers can recommend up to a 10% rate of usage. This study seeks to investigate the effects of 10% biochar, both on its own as well as in conjunction with AMF, on both soil health and plant growth parameters associated with producing higher crop yields. A potted experiment with five treatments and a control were set up, containing different concentrations and combinations of biochar and arbuscular mycorrhizal fungi. The data was analyzed to determine differences in root length, shoot height, biomass, total phosphorus, and colonization of AMF in the roots. Soil parameters, including texture, pH, organic matter, moisture, cation exchange capacity, and available phosphorus were analyzed as well. Significance ($p<0.05$) was shown in soil moisture, soil pH, soil phosphorus, and AMF colonization due to the effects of the treatments. The presence of 10% biochar and AMF showed an increase in AMF colonization and soil phosphorus. The presence of 2% biochar and AMF (T3) had the most effect on the soil pH (7.2). Soil moisture was optimal when treated with only 10% biochar. For farmers, this can mean that these techniques could be explored further for expanded utilization in a farm setting, and can be implemented as per manufacturer instructions for success. Through the furthered use of these amendment and support tactics, steps towards mitigating climate and environmental issues worsened by agricultural practices can be taken, while still providing a high enough yield to financially support farmers, and while producing enough food for the world's population.

This thesis is dedicated to all who are working to increase food production in order to feed our growing world population, while also acknowledging the importance of maintaining a healthy and sustainable relationship with our ecosystems and environment. I would like to thank Grow It Green Urban Farm for the hands on experience throughout my time at Drew, allowing me to understand the importance of alternative farming methods for the environment as well as our communities. I would like to thank Madison Robinson and Jackelyne Esquivel for their work as part of our team for the Drew Summer Science Institute in 2024, where much of this research was carried out. I would also like to thank Dr. Muriel Placet- Kouassi, for providing me with a broader understanding of the world beyond a strictly science standpoint, and Dr. Mary-Ann Pearsall, for furthering my knowledge in both chemistry and geology, for helping me to understand the interconnectedness of scientific fields, and for encouraging me to continue to question why the world works in the ways that it does. I would especially like to thank Dr. Shagufta Gaffar, for introducing me to the incredible field of soil science, for sharing her passion, and for her encouragement, knowledge, and support.

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## Introduction

This work contributes to the overall goal of finding sustainable ways to increase crop yield in agriculture, without resorting to synthetic fertilizers, specifically through exploring biochar and arbuscular mycorrhizal fungi as treatments to enhance soil characteristics that support improved crop growth.

## Agriculture

Agriculture is the practice of growing crops and raising livestock through selection and care of the soil, when these practices are carried out intentionally (McDaniel et al., 2024). The category of practices that fall under the name of agriculture began roughly 12,000 years ago. Prior to this, humans were hunter gatherers. At the end of the most recent Ice Age, the earth saw a shift in landscape and land availability, as the ice sheets retreated, leaving more open space and fertile soil. This shift in land type enabled movement away from the typically nomadic life, to one that was more stationary and required a more stable source of sustenance. A hunter gatherer lifestyle was not as conducive to a larger family size, but once this change took place, a higher population meant that more food was needed. This is a common theme seen throughout history, since this first "revolution", in terms of changes that take place in food technology and the approaches taken towards the production of food. As time went on, agriculture and its practices caused major changes to the landscape of the world. More land was allocated for growing crops, and as a result, community structures shifted to allow for time and resources to farm the land. Societies also saw cultural shifts, as localized farming techniques and native plant varieties emerged and became staples of certain areas of the world. Agriculture also marks the beginnings of the domestication of plants for human use, by actively cultivating plants in certain locations from seed, and caring for them to ensure growth.

**Intensive Agriculture**

Intensive agriculture, also commonly known as industrial or conventional agriculture, is a set of processes that evolved and changed agriculture to make it more profitable and efficient. Rather than focusing on producing enough food for a single community group, intensive agriculture seeks to produce on a level that is global, especially in the wake of shipping developments that allow food from one area of the world to reach the complete opposite side. Intensive agriculture is comprised of many different practices, and it was developed in response to multiple food crises.

One such example of a period of developments in intensive agriculture was the Green Revolution. With a name coined by William S. Gaud of United States Agency for International Development (USAID) in 1968, the beginnings of the Green Revolution can be attributed to Mexico in the 1940s (Cabral et al., 2021). Mexico experienced difficulties with low yields of corn and wheat, leading to issues with meeting the needs of the population. In 1943, the United States' Rockefeller Foundation joined with the Mexican government and created the Mexican Agricultural Program (MAP), focusing mainly on developing hybrid varieties of wheat that produced higher yields, and were more resistant to common diseases. This change allowed for Mexico to go from a country struggling to feed its own population to an influential exporter of wheat (The Editors of Encyclopaedia Britannica, 2009). Slightly after these changes began in Mexico, India began to face similar challenges following their independence in 1947. India was facing droughts and food shortages leading to widespread starvation, and other nations began to encounter similarly devastating food insecurities (The Editors of Encyclopaedia Britannica, 2009).

On a global scale, governments sought to combat these issues, and allocated funding for research into producing high yielding varieties of plants (HYV). High Yielding Varieties, created first through cross breeding and hybridization, and which now can be produced as GMOs, respond extremely well to the application of extremely high levels of synthetic fertilizers and pesticides. These plants, which have been bred to produce a much higher biomass than native varieties of the same plants, require immense amounts of nutrition in order to produce these desired yields. Unlike the native plants, which could experience over nutrition as a form of malnutrition due to this input, the HYVs thrive in this environment. HYVs are hybridized and produced to be able to utilize the high amount of nutrients being provided. Fertilizers provide this high level of nutrient supplement required by these varieties (Chiona, 2017). With the production of HYVs, the increased food production was able to aid in the mitigation of the extreme poverty and malnourishment rates being experienced by large portions of the global population post-war.

This period of growth saw developments that effectively tripled crop production, while only increasing the amount of land used by 30%. This was critical, as the growing population also required more living space. With these positives in mind, there were also some detrimental impacts seen (Cabral et al., 2021). In most areas of the world, certain crops are favored and have been grown since the dawn of agriculture. These specific crops are often deeply intertwined into the functioning of society and the culture of these communities. The processes used have been passed down, and the success of these crops is due to them being grown in the correct climate, as well as generations of knowledge being utilized for their growth. However, with the introduction of high yielding varieties, and their success, many of these native and localized crops were phased out, as their outputs could not compete with the high productivity of the HYV crops. Many of these crops could not handle the high volumes of nutrients added to the soil, and as over nutrition has the same effects as malnutrition, they did not thrive. Additionally, native crops were not capable of being produced at the same level of production as the HYVs, and so they were not prioritized. In addition to the loss of cultural differences, effectively homogenizing much of the food production of the world, a major loss of plant biodiversity also occurred. With the emphasis on the success of monoculture encouraged by the Green Revolution and intensive practices, plant varieties that had been cultivated at a smaller scale were pushed out of production, as they were not grown on as large of a scale. This biodiversity loss was especially seen with the loss of many varieties of indigenous rice (Eliazer, et al., 2019). The native plants were frequently unable to withstand the high amounts of fertilizer and pesticides needed to produce the HYVs, and they were slowly wiped out.

The Green Revolution began to solve a problem, but very quickly spiraled to cause its own host of problems. The balance between acknowledging the major successes of the era, while slowly realizing the harmful implications, became critical as more and more research was carried out in the field. The major increases in agricultural technology had large overarching impacts, both positive and negative.

**Advantages of the Green Revolution**

Intensive agriculture has substantial advantages, aside from those directly associated with the needs that led to the Green Revolution. Certain agricultural outputs such as cash crops and even livestock are in high demand, and this means that high output is required to meet the needs of the population. Intensive practices such as the abundant use of pesticides and fertilizers to prevent pests and boost nutrient uptake allow for small pieces of land to be used to produce lots of food. This creates a very high yield to land use ratio, which is desirable in terms of being able to efficiently produce food. The higher yield and less land being used can also lead to more affordable food prices, as more food that is produced more efficiently decreases costs for farmers, and the costs decrease all the way to the consumer. Since larger crops can be produced with more ease, the entire agricultural process becomes less expensive, leading to lower prices even for healthy and nutritious foods, which otherwise would potentially have higher costs (Sial et al., 2021). Intensive farming practices strive to simplify the agricultural process. On a smaller farm, or in a traditional setting, farmers would need to heavily focus on how different crops can interact and their separate needs. However, with a conventional model, monoculture permitted by intensive agriculture often becomes the standard model, and this means that farmers focus on a single crop, which uses consistent equipment and knowledge to produce. Between different farms, the needs of the population are met at a greater level through dividing the work and streamlining the process. Additionally, a wider variety of successful crops can be produced in this way, because farmers can focus on the success of one or two crops (Sial et al., 2021).

**Limitations of the Green Revolution**

While intensive agriculture included many incredibly important improvements to farming that drastically changed the outlook of our world's population, these positives did not come without challenges and downsides (Figure 1).

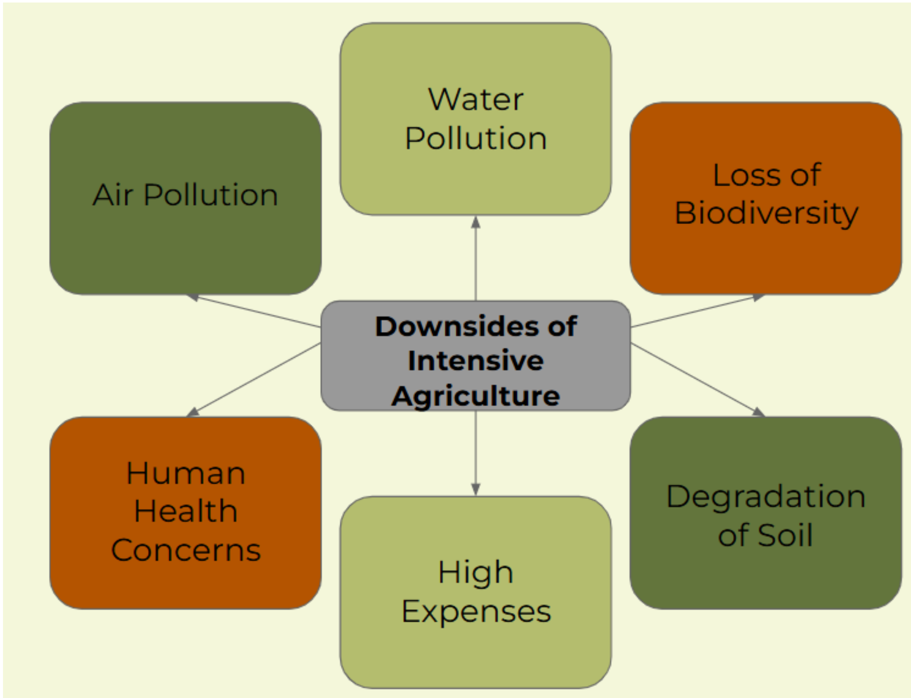



**Figure 1. Downsides of Intensive Agricultural Practices**

Intensive agriculture is a major contributor to the degradation of soil and land in general. When crops are consistently planted in an area with no time in between and no changes in nutrient needs, the soil decreases in levels of organic matter and struggles to maintain a high enough moisture content. This leads to rapid erosion of soil due to water and wind running over the surface. In addition to degrading the land, deforestation and other forms of destruction have become a severe problem, worsened by intensive agriculture. In order to make space to produce large amounts of a single crop, and to produce more food, tree removal and the clearing of forested areas have been utilized as a solution. This leads to habitat loss, fragmentation, and loss of biodiversity, both within native plant and animal species (Sial et al., 2021).

The pollution associated with intensive farming is especially devastating for the planet. When soil cannot absorb and utilize the chemicals meant to support plant growth, rain and other precipitation can cause runoff into nearby waterways, lakes, and ponds (National Oceanic and Atmospheric Administration, 2024). The nutrients are not depleted through this process, and provide unnecessarily high levels of nutrients to these bodies of water, ultimately feeding the algae present. This causes a major boom in algae growth and population. As the growth increases, sunlight is blocked out from reaching beyond the surface of the water, which prevents the other aquatic plants from carrying out photosynthesis. These plants die, which releases abnormal amounts of $CO_2$ into the water. This process, known as eutrophication, creates a feedback loop, as bacteria present in the water aid in the decomposition of these plants, which releases additional $CO_2$. As this occurs, the levels of oxygen within the water become quickly depleted, killing any fish or other organisms that rely on this body of water for life. The impacts of this process can become very far reaching, especially in rivers and streams. Extreme biodiversity loss is a major consequence of this process (National Oceanic and Atmospheric Administration, 2024).

Intensive farming relies heavily on the use of synthetic fertilizers and pesticides to ensure that crops are not impacted by pests or low levels of nutrients within the soil, or at least are able to still produce a high enough yield despite encountering these challenges. Pesticides, herbicides, and insecticides are typically applied through spraying over a broad area of vegetation, and since this is not a targeted approach, food can easily become contaminated and become covered with toxic chemical residue. Beneficial insects may also be killed, and declines in predatory bird populations have led to biodiversity loss, as well as problems with natural pest control systems (Sial et al., 2021). The application of these toxins does not only impact the natural environment; human consumption of and exposure to these substances through over application and non-targeted application causes increased rates of illness and disease (Sial et al., 2021).

**Soil Fertility**

It is important to note, however, that agricultural practices are heavily dependent on the addition of fertilizers in order to produce enough food resources, and the solution cannot be to halt the use of fertilizers. Fertilizers are a substance that is added to soil, with the ultimate goal of increasing the fertility of the soil. The fertility of soil is dependent on the presence of 14 essential nutrients and minerals, with the three most abundant and widely utilized being Nitrogen, Phosphorus, and Potassium, further known as NPK. (Reill, 2019). In most cases, these macro and micronutrients are taken in through the roots of the plant, from within the soil. While these 14 are all naturally occurring in certain quantities, different soils and different climates support different levels. At times, depending on the crop being grown, these nutrients will need to be supplemented by fertilizers, which contain higher levels of what is needed for a specific crop to ensure its growth. Fertilizers can be made synthetically, or from organic matter, and are chosen based on the specific needs of the soil it will be used in. Fertilizers can also be categorized based on which nutrient is most heavily supplemented. Phosphorus fertilizers are any substance that extensively supplements the available phosphorus within soil, for use and uptake by the plants within the soil.

**The Phosphorus Problem in Soil Fertility**

Phosphorus, as one of the three main macronutrients needed for plant growth, is instrumental in the process of agriculture. This element, usually obtained through mining phosphate rock (a non-renewable material) is found naturally within soil, but only about 0.1% of phosphorus in soil is in the form that is available for plant use (Blogger, 2024). The rest of it exists in rocks in the crust and in and the soil, but in an insoluble form which cannot be taken up by plants. This can present major issues when crop yield is considered. Phosphorus is needed within plants for an abundance of purposes. This nutrient is utilized to aid in supporting a higher tolerance to abiotic stressors in plants, such as tolerance to heat, drought, overwatering, as well as overcoming toxins within the soil. It also is critical for cell division, and metabolic processes (Khan et al., 2023). When there is not phosphorus enough in the available form to aid in these processes, we see decreased crop yield, lowered fruit (or whatever portion of the plant is considered edible) production, and harm to the development of both the roots and leafy/stem portions of the plant (Khan et al., 2023).


**Figure 2. The Three-Fold Phosphorus Problem in Soil.** Plants encounter three barriers to achieving uptake of phosphorus.

Phosphorus is necessary for plant growth, and yet there are three main problems that create barriers to allowing plants to access it (Figure 2). The first problem is that typically phosphorus is present in incredibly low amounts within soil. This can vary based on soil type, and water infiltration and retention (Weil & Brady, 2017). Second, out of this already low level, the phosphorus within the soil is typically present as the insoluble form, which is unavailable for plant uptake and use. This means that even if the phosphorus is present, the plants are unable to utilize it unless it becomes soluble. Phosphorus is found in the soluble form as either $H_2PO_4^-$ or $H(PO_4)^{-2}$ (Weil & Brady, 2017). Third, even if the soluble form of phosphorus is added to the soil through a fertilizer, it can quickly be fixed, or turned into an unavailable form. As pH ranges raise or lower, different elements within the soil react more with the phosphorus, making it insoluble, as can be visualized in Figure 3. These insoluble forms can differ depending on the element reacting with the phosphorus to make it unavailable, and can include $Ca_3(PO_4)_2$, $Al(PO_4)$, and $Mg_3(PO_4)$. This presents a problem later on in the soil, as these compounds turn into incredibly insoluble compounds. When fertilizers are applied in such high quantities, this happens at rapid rates, as when fixation occurs, only a small amount of the phosphorus, as low as 10-15%, will actually be taken up by the plants (Weil & Brady, 2017).

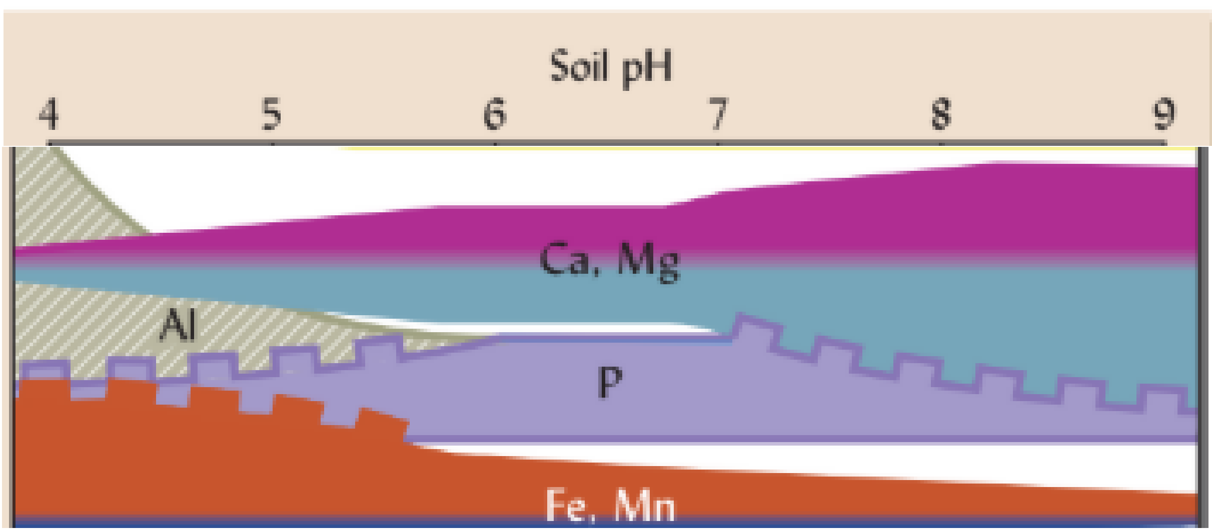



**Figure 3. Phosphorus reacts with other elements to become insoluble at low and high pH values.** (Weil & Brady, 2017) When the pH of a soil is below 6 or above 7, other elements within the soil begin to react with the soluble phosphorus, making it unavailable for uptake by plants. From a fairly neutral pH of 6 to a pH of 7, the phosphorus is able to remain soluble for plant uptake.

While phosphorus is needed for a higher yield, and farmers often experience difficulties achieving the levels needed to meet this goal, it becomes clear that this is a place for research to improve the levels of phosphorus in soil. Due to this discrepancy between the amount of nutrients needed and the actual amount available, current research guides farmers to add substantial amounts of the available form to make up this gap, so that plant productivity is high enough to produce the large amount of food needed (Blogger, 2024). This becomes an issue for various reasons. The amount of fertilizer being applied is not sustainable, as phosphate rock is non-renewable, and the increased use of phosphorus in high quantities for use in phosphorus fertilizers causes the rock to be mined at a faster rate than it is being formed. As a result, the quantities of phosphorus that we have access to are rapidly dwindling, and are expected to run out within the next 300-400 years (Glaser & Lehr, 2019).

While all of these changes were necessary to address the pressing food shortages, the solution created was meant to fix the problem with a short term and immediate result, and longer term issues were not largely considered. As a result, a very effective short term solution was implemented, and over time, it became evident that a longer term fix would be needed in order to maintain food production.

**Sustainable Agriculture**

When the long lasting impacts of intensive agriculture and the Green Revolution are considered, a solution needs to be implemented in order to maintain the high quantity of food required by our population, while mitigating both past damages as well as continued problems.

Sustainable agriculture is a combination of practices used in order to fulfill the present needs of our population, while considering the impacts our choices may have on the future. While goals may differ situationally, overall, The Food and Agriculture Organization of the United Nations has outlined several overarching motivators for integrating sustainable farming practices (FAO, 2023). These goals are outlined in Figure 4. The first of these, and the most pressing, is to ensure that the nutritional needs of people and livestock are able to be fully met. Another goal is to improve the quality of the surrounding environment, whether it is soil, air, or water. Third, is to minimize waste produced, typically through effective use of natural resources and waste cycling. While unrelated to the environment, a fourth goal is being able to sustain farm operations on a financial level, as some sustainable practices can be financially taxing for farmers. This is specifically relevant in terms of small scale farmers who frequently are at the forefront of implementing sustainable practices. A fifth goal is to utilize natural materials and processes to aid in food production when possible, to decrease the amount of work needed. Overall, the main goal is to ensure that the quality and ease of life for both the farmers and the community are put at the forefront of all practices enacted (USDA, 2024). These are large goals, and the concept behind striving to meet them is that the solutions may take longer to enact, but will have lasting impacts. Practices used may also differ depending on the area of the world, geography, climate, and food needs, as well as money available and amount of people able to do work. No matter which of the five goals are prioritized in a scenario, sustainable farming combines traditional practices that have been used since the origins of agriculture with modern scientific improvements in (Alvaro, 2023).


**Figure 4. Goals of Sustainable Agriculture.**

**Positives of Sustainable Agriculture**

Sustainable agriculture has many positive impacts, which allow for its continued development. In many cases, through crop rotation or integrated pest management system, pest and disease cycles can be broken. This cycle is shown in Figure 5 without the intervention provided by sustainable farming. When the heavy use of synthetic pesticides is halted, the cycle of increased resistance by pests is also stopped or slowed, which allows for less invasive and intensive practices to be put into place. Organic farming specifically helps to promote a more nutrient rich and biologically active soil system (Alvaro, 2023). A major issue within agriculture is the rapid erosion of soil, which can be mitigated through no-till farming, because it helps to maintain the structure of the soil. A low or no-till farming system also serves to prevent soil compaction. When soil is compacted, root growth can be stunted, which prevents full plant productivity (Alvaro, 2023). From an economic standpoint, sustainable farming can create cost savings for farmers. Large amounts of synthetic fertilizers and pesticides can be extremely costly, and since sustainable practices steer away from excessive use, there can be a major cost savings in this area. While farmers who rely on one or a few crops to provide their entire income may be severely impacted by fluctuations in the food market, those who employ sustainable techniques and plant a more diversified selection of foods will not be as heavily impacted, if at all (Alvaro, 2023). In a broader view, sustainable agricultural practices can produce food that is considered to be healthier, and safer (Alvaro, 2023). Due to non-precise application of synthetic fertilizers and pesticides, food produced through intensive practices will often be covered in residue, even after being rinsed or washed. This is then consumed, which allows people to be exposed to high levels of harmful substances. Through avoiding the use of synthetic fertilizers and pesticides, this can be avoided, which is a major positive associated with sustainable agriculture (Alvaro, 2023).

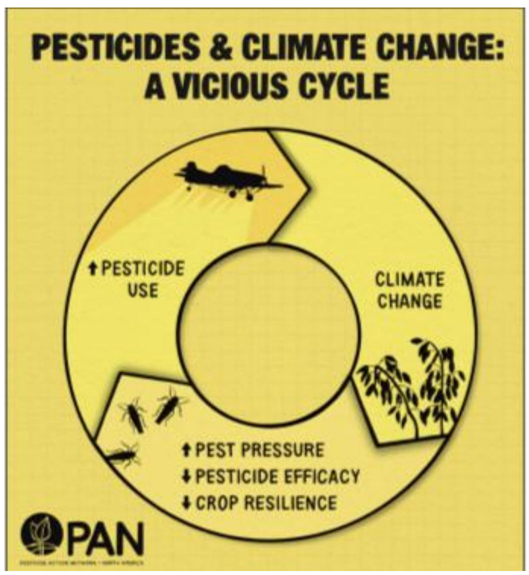



**Figure 5. Pest cycles produced by heavy use of synthetic pesticides.** (Organic Research Centre, 2023)

### Disadvantages of Sustainable Agriculture

While sustainable agriculture succeeds at resolving many present issues associated with farming, there are drawbacks (Figure 6). A major goal of sustainable farming is to implement practices that are able to be sustained long term. However, in order to make the necessary changes, substantial effort and work is required.

While in the long run, costs can be much lower for farmers, the initial costs associated with making transitions from intensive practices to more sustainable can be extremely high. Oftentimes, training is required to learn how to carry out new techniques successfully. In addition to the fees required to be trained, this also means that farmers and other farm employees will need to take the time to learn new techniques, which can put a financial strain on a farm. Training aside, new equipment is frequently needed. While this differs in cost from the large machinery needed for intensive agriculture, the combination of these factors among others can become cost prohibitive for those considering even slight changes to their farming technique. Throughout the transition period, there will be temporary setbacks, which can lead to drops in yield and income (Alvaro, 2023). Unfortunately, sustainable practices do not typically produce crops with the same level of predictability that crops that have been intensively farmed have the capability of producing. These practices are not immediately successful in the way that implementing synthetic fertilizers or pesticides would be. Since they focus on fixing the soil's health and maintaining a healthy system rather than a rapid solution, the results are slower to be achieved, and accumulate over time. Soil and plants cannot immediately adapt to new practices that are focused on amendment rather than putting a bandage on the problem, and may struggle initially. For farmers who do not have the ability to produce lower yields for a period of time, this transition can be very long and difficult (Alvaro, 2023). As previously mentioned, an overarching goal of sustainable agriculture is to be able to require less inputs, and to rely on natural processes to largely run the system. However, human power and time is a large driver of these processes functioning at a rate that successfully provides a large enough yield, which is not the case in intensive situations. Because large machinery and automated systems are largely removed from the farms, people must replace the work being done, and in situations where a farm is fairly small scale or functioning within a small community that relies on heavy food production, this may present challenges (Alvaro, 2023). Regional differences in climate, geography, knowledge, and resources also play a large role in how sustainable agriculture practices are able to be implemented. Certain crops that are only grown in specific areas of the world may have different needs that are unable to be met by a single practice. Some plants may be more sensitive and require more intense care than others, which would require more attention from farmers. Organic materials often used in these practices can be very region-specific, and additionally, more resistant pests impact plants differently depending on many different factors, and they would respond in varying ways to pest management strategies. Since consistency is largely not possible between areas of the world and even from farm to farm, spreading information and knowledge can become increasingly difficult without much trial and error and explicit context about a farm's conditions and needs (Alvaro, 2023).

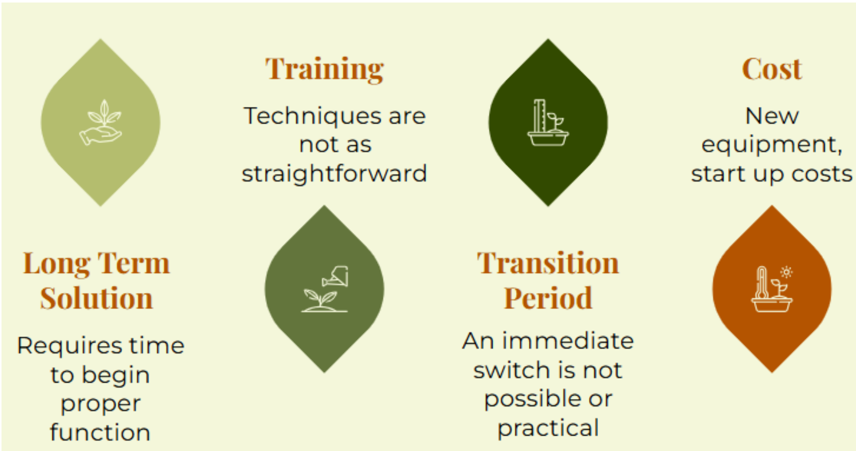



**Figure 6. Challenges associated with implementing sustainable agricultural practices.**

With all of these factors taken into account, the decision of how and where to implement sustainable farming practices becomes very important. Ultimately, the decision of whether or not pursuing sustainable techniques outweighs the positives associated with intensive agriculture is dependent on the goals, and the extent to which sustainable agriculture is able to be carried out. This can vary spatially, depending on climate, terrain, as well as access to resources, both educational and physical. It is understandable that people may want to continue to utilize the intensive practices, since the benefits typically support farmers, and the downsides are often not seen at a farm level. However, the challenges seen can be overcome in order to encourage a combined approach to continuing to support agriculture. The goal is to streamline the processes and expand available information to farmers, so that the negatives discussed prior have lessened impacts on productivity. As more and more research is conducted regarding alternative methods of farming, they will become more accessible and less of a burden to implement.

Overall, it is still possible to produce enough food, if there is an opportunity for a bit of patience and flexibility. This is not a luxury that all farmers can afford, but if those who can make attempts do so, sustainable farming practices will become more and more accessible, and the same will be true for the amount of food produced. With these practices put in place, over time, the soil remains healthier and sees longer lasting impacts without constant input. Healthier soil will produce higher yields, and this is a desirable outcome for farmers, but because it is not an instant benefit, more effort is needed to encourage implementation.

**Practices Associated with Sustainable Agriculture**

In order to meet the goals of sustainable agriculture, a variety of different practices can be employed. Some practices include: cover cropping, integrated pest management solutions, and intercropping, all of which utilize natural processes to focus food production efforts towards higher effectivity. Just like there are different techniques, there are also different categories of sustainable farming that use specific combinations of these practices. Agroforestry, no-till farming, and permaculture are a few examples, but largely, organic farming is perhaps the most extreme and typically is the focus of sustainable agriculture efforts. An example of a sustainable practice used in the place of tilling, called broad forking, is seen in Figure 7. While sustainable agriculture is an overarching category to describe any level of integration of sustainable practices into a more conventional farming situation, the subcategory of organic farming has much more specific parameters.

**Figure 7. Broad forking is used in place of frequent tilling of soil, in order to overturn the soil.**

Organic farming is an all-encompassing process that follows the food produced from the very beginning as a seed, all the way to when and where it is distributed and sold (McEvoy, 2025). The guidelines followed are considered to be strict, and ensure that the food meets certain standards of quality. The use of synthetic fertilizers and synthetic pesticides is completely prohibited, but other methods of pest management and nutrient supplementation are used. The use of genetic modification to improve growth and yield also disqualifies a crop from being labeled as organic

(US EPA, 2018). Overall, these stricter standards strive to enforce sustainable farming practices, but farmers may encounter difficulty with implementing these techniques on a larger scale without immense financial strain.

As discussed prior, sustainable agriculture can effectively function to mitigate many problems caused by intensive agriculture while still producing enough food. Some solutions focus on resolving a whole host of issues on a broader scale, while others are much more targeted and focus on replacing one specific technique associated with intensive agriculture. In farming, the lack of available phosphorus as well as other nutrients remains a dilemma, as we try to step back from large amounts of synthetic fertilizers to provide these nutrients. The soil and land used for agriculture does not contain enough nutrients in an available form, which prevents the high yield that is needed to support the globe's population. Therefore, the solution cannot be to avoid the addition of nutrients through a fertilizer. While synthetic fertilizers provide a large dose of the necessary nutrients, in a way that can easily be used by the plants, the downsides of this method are devastating, and therefore other accessible solutions that align with the goals of sustainable agriculture have been investigated, such as biofertilizers and biochar.

**Biofertilizers**

Biofertilizers are an important part of the systems created with the goals of sustainable agriculture kept in mind.

Biofertilizers are a broad category of products that have been created from biological materials that are not chemically synthesized, and are biodegradable (Saha et al., 2023). Instead of simply providing the nutrients needed for growth through application, microbes contained within biofertilizers function within the soil to increase the available form of NPK (Blogger, 2024). They are categorized based on which type of microbes they contain, as well as what their primary function is within the soil. This includes fixing nitrogen present within the soil, changing phosphorus into its soluble (available) form, and aiding in the synthesis of compound that encourage plant growth, as well as producing phytohormones, which help plants to be able to respond to environmental stressors that may impact growth (Saha et al.,2023). Biofertilizers serve to improve the fertility of soil, to promote the growth of plants, to increase the available nutrients in the soil, and to overall support a higher plant productivity. This occurs when they are applied either within the soil, onto seeds before germination, or through applying to the plant leaves after germination (Barbu, 2021). Biofertilizers function because of the mutualistic relationship created between the microbes and the plant roots.

In addition to being able to support plant growth and productivity, biofertilizers serve as a way to slow down and decrease the environmental impacts caused by the large-scale application of fertilizers (Khan et al., 2023). They function to fix the soil, rather than to require continued application, which is a more sustainable approach in terms of resource utilization. The release of nutrients through the microbes changing the form is slow and steady, rather than the alternative, where large amounts of nutrients are applied directly and are available for uptake immediately. This creates a more long-term solution for the production of plants. Unlike synthetic fertilizers, biofertilizers do not destroy the already existing soil microbiome, and allow for continued support through a healthier and biodiverse soil system rather than continuous application being needed.

**Arbuscular Mycorrhizal Fungi**

Arbuscular Mycorrhizal Fungi (AMF) is a fungus that has been investigated as a microbial biofertilizer. AMF creates a mutualistic relationship with the roots of plants in which they colonize, with the plant providing carbohydrates to the fungus, and the fungus has the ability to facilitate higher quantities of necessary nutrients and water to the plant (Frey-Klett et al., 2011). Unlike other mycorrhizae which can only colonize woody plants, AMF has the capability of colonizing within nearly any plant (Chibuike, 2013). This colonization occurs through the growth of three main structures, which make up the AMF. The growth begins with elongated cell structures called intracellular hyphae, which remains the main structure, and then allows for the formation of vesicles and arbuscules. The arbuscule structure has the appearance of tree branches, while the vesicles are more irregularly shaped and are used as a sort of storage unit in cases of low nutrient exchange (Agarwal A et al., 2011). Vesicles can be seen in Figure 8. The hyphae connect the arbuscules, which are the structure that facilitates the nutrient exchange between the fungus and the plant. A visualization of these structures can be seen in Figure 9. This colonization occurs within the roots of the plants, by penetrating the root cells and creating an extensive network, which categorizes AMF as an endomycorrhizal fungus. This is in opposition to ectomycorrhizal fungi, which colonize in the surrounding area rather that within the cells, as seen in Figure 10 (Marx, 1980). The differentiation between the two is critical, as AMF is incapable of colonizing without the presence of plant roots, which defines the presence as symbiosis. AMF and mycorrhizal fungi in general are naturally occurring within soil, with approximately 80% of terrestrial plants being colonized (Ilyas et al., 2024). This natural quantity is very supportive for native plants and small planted areas, but does not allow for enough colonization to support the high level of growth needed to feed today's population. As a result, substantial research has investigated the process of supplementing the pre-existing AMF to a soil where higher yields are desired.

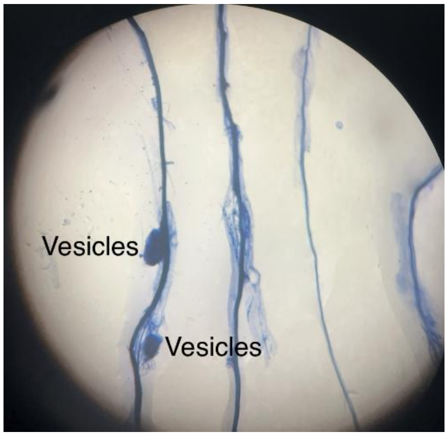



**Figure 8. Arbuscular Mycorrhizal Fungal Colonization within the Roots of *Pisum Sativum*, Vesicles Labeled.**

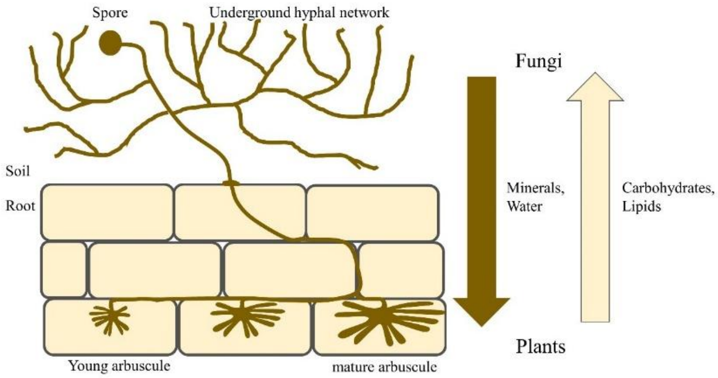



**Figure 9. Structure of Arbuscular Mycorrhizal Fungi.** (Sessoms, 2020)

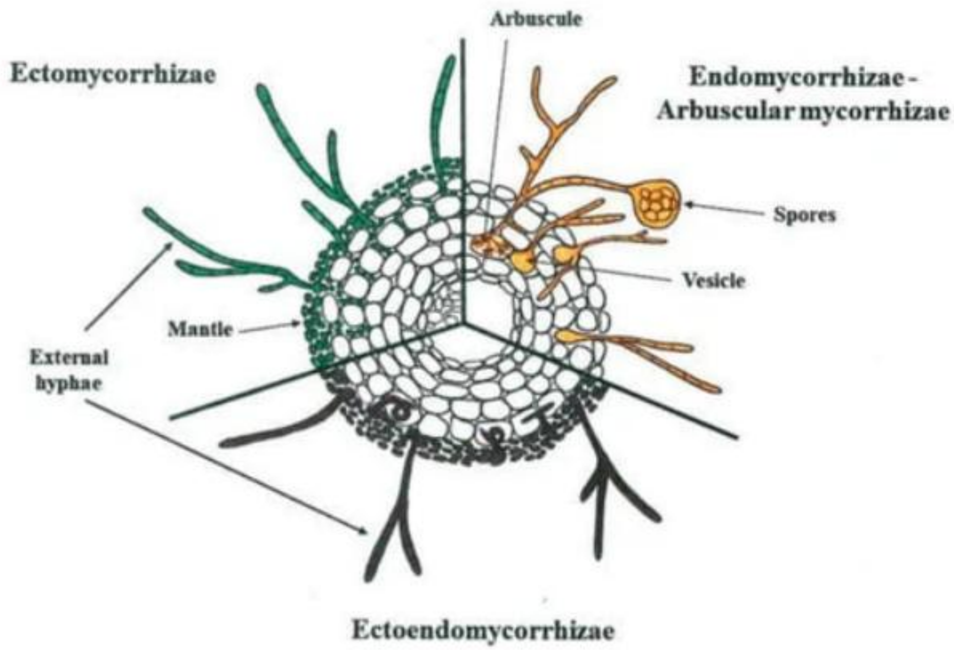



**Figure 10. Visualization of the location of structures in both endo and ecto mycorrhizal colonization of a root**. It is important to note that endomycorrhizal colonization occurs within the root cells, and ectomycorrhizal colonization occurs on the outside of the root. Image from Understanding How Mycorrhizal Works and 10 Ways Your Garden Can Benefit from These, 2025.

In order for additional AMF to be added to a soil, a carrier is required, which is any substance that can support the inoculant. It must be able to hold moisture, be easy to sterilize, be low cost, and it must be a substance that will adhere to the seeds. Most importantly, the carrier needs to ensure that the AMF will survive (Barazetti et al., 2019). Several different carriers containing AMF or any biofertilizer can be created in order to supplement the pre-existing soil AMF quantities. Carriers include liquid formulations, granules, powders, peat, and seed coatings (Mahanty et al., 2016). These formulations can be applied within soil in various different ways, depending on the goals of the farmer and what makes most sense for the way the crop is being planted. When the goal is to colonize the roots of a transplanted plant, granular mycorrhiza can be used. Before planting, the granules are sprinkled onto the roots of the plant, and then it can be placed into the ground. Another similar technique suggests digging a hole, adding the inoculant, and then placing the plant into the hole so that the roots sit directly on top of the inoculant. If established seedlings are being used, the inoculant should be mixed into the soil around the base of the plant, and that the area needs to be covered, as mycorrhizal fungi cannot be exposed to sunlight. If a large area is being planted, the inoculant can be mixed directly into the soil, and then this soil can be used to grow plants. This technique may bring some challenges, as the fungi cannot colonize without an eventual root system, and if it is exposed to sunlight, colonization will not occur, so proper storage of the soil will be required until used (Understanding How Mycorrhizal Works and 10 Ways Your Garden Can Benefit from These, 2025). However, none of these techniques address the most effective method to utilize if the desired planting method is from seed. One method to consider requires making a slurry of the inoculant and water, and then stirring the seeds into this mixture before planting as normal. Another method consists of digging a hole of the proper depth to plant the seeds, pouring the inoculant into the hole, and then placing the seeds in the holes and covering them with soil. Lightly watering the soil after this is important, as to not disrupt the AMF. No matter which application method is chosen, it is most important to ensure that there is direct contact between the AMF and either the seed or the root itself (Racsko, 2019).

**AMF in Soil**

Within soil, AMF has many purposes and benefits. Once the inoculation takes place and the fungus colonizes within the plant roots, it may be able to improve soil quality. When AMF extends hyphae deep into the soil, further than plant roots are able to, these hyphae are able to aid in the formation of micro-aggregates in the soil, and help to uphold the stability of the soil aggregates. Micro-aggregates are soil structures that typically are less than 250μm in size, and are created when minerals, organic matter, and often other biological materials bind together. This occurs due to the breakdown of organic matter within the soil (Neuman, 2017). This process mitigates soil erosion, which can be a major issue in agriculture (Bedini et al., 2009). Through the ability to mitigate soil erosion, movement of phosphorus through and out of the soil is also prevented. AMF produces the glycoprotein called glomalin, which supports the creation and binding of soil aggregates, which as discussed earlier supports a more stable and porous soil structure. A stronger soil structure caused by the presence of AMF prevents the leaching of phosphorus into bodies of water, and also allows more to remain available for plant uptake. AMF also facilitates biochemical changes within the soil that makes phosphorus available for plant use. (Rubin & Görres, 2021).

**AMF on Plant Resiliency**

In cases of stress conditions, plants that have been colonized with AMF have seen drastically improved resiliency, as highlighted in Figure 11. In areas that experience water related stress conditions, plant growth can be inhibited due to lower photosynthesis rates. However, a 2000 study carried out by Quilambo (2000) determined that peanuts (a legume) inoculated with AMF had increases in leaf, root, and shoot growth, and did not experience the typical changes in growth that would be expected under water or phosphorus stress conditions. The hyphae allow for a higher surface area of absorption, which allows for ease in water uptake even when less is present. In addition to improving uptake in certain situations, the structures can also allow for increased defense against the uptake of heavy metals. Heavy metals can be incredibly toxic to plants as well as soil microorganisms, and can harm the soil in situations of long term exposure. However, AMF has shown to be resistant to the uptake of heavy metals, and to have a higher tolerance against their presence (Agarwal et al., 2011. The structures of the mycorrhizae reduce the amount of heavy metals that are able to reach the plant shoots through binding the metals to the hyphae structure rather than continuing to the plant as usual. In this way, plants are supported and can thrive even in environments that are typically hostile for growth (Agarwal et al., 2011). AMF has also been discovered to allow for improved nutrient uptake specifically in drought conditions (Chandrasekaran, 2022).

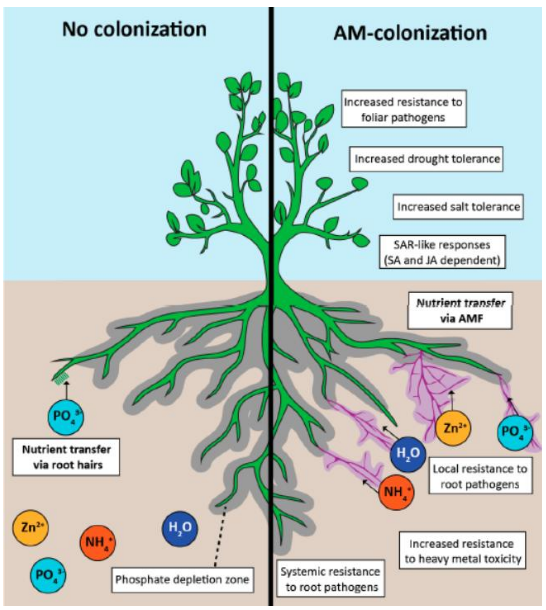



**Figure 11. Effects of AMF colonization on plant responses to environmental stressors as well as the role in nutrient acquisition.** (Sabhya Pathania et al., 2024)

### AMF on Nutrient Uptake

However, AMFs abilities to support the uptake of phosphorus are incredibly important to consider for agricultural improvements, which can be seen in Figure 11. The key role of AMF within its symbiotic relationship is the increase of phosphorus uptake. (Agarwal et al., 2011). Mycorrhizae play a large role in altering the form of phosphorus available in the soil, to allow for successful uptake by plant roots. Mycorrhizae are capable of transporting nutrients such as zinc, iron, and especially nitrogen and phosphorus, further distances than plant roots would be capable of (Rubin & Görres, 2021). Mycorrhizal colonization within plant roots provides numerous benefits. The first is increased absorption from the soil, by the hyphae. The symbiosis between the plants and the AMF increases the surface area available for nutrient acquisition (Agarwal et al., 2011). The arbuscule structure allows for increased translocation of phosphorus into the plants. The vesicles allow for a higher amount of storage of the phosphorus that has been absorbed. Typically, the ions would need to be diffused to the surfaces of the root that are capable of absorbing them, but with AMF colonization, this process is not necessary and uptake occurs much more efficiently. Additionally, this prevents the depletion of nutrients in the zone surrounding the roots, as on a structural level, the hyphae of AMF are able to extend deep into the soil, below the zone that has been depleted of nutrients (Agarwal et al., 2011). Through the secretion of enzymes, unavailable forms of phosphorus can become soluble. In most cases, the inorganic, soluble form of phosphorus is required for uptake by plants, and the arbuscule structure breaks down the organic form, and releases inorganic phosphorus directly into the cytoplasm of the root cells (Agarwal et al., 2011). Ultimately the addition of AMF to a root system through inoculation can increase the early uptake of phosphorus during the growth process, which will improve the potential for a higher crop yield without the need for a phosphorus-specific fertilizer being added. In terms of reforestation, it has been seen that the presence of AMF can increase the survival of seedlings in a forested environment by up to 3 times the typical rate (Agarwal et al., 2011).

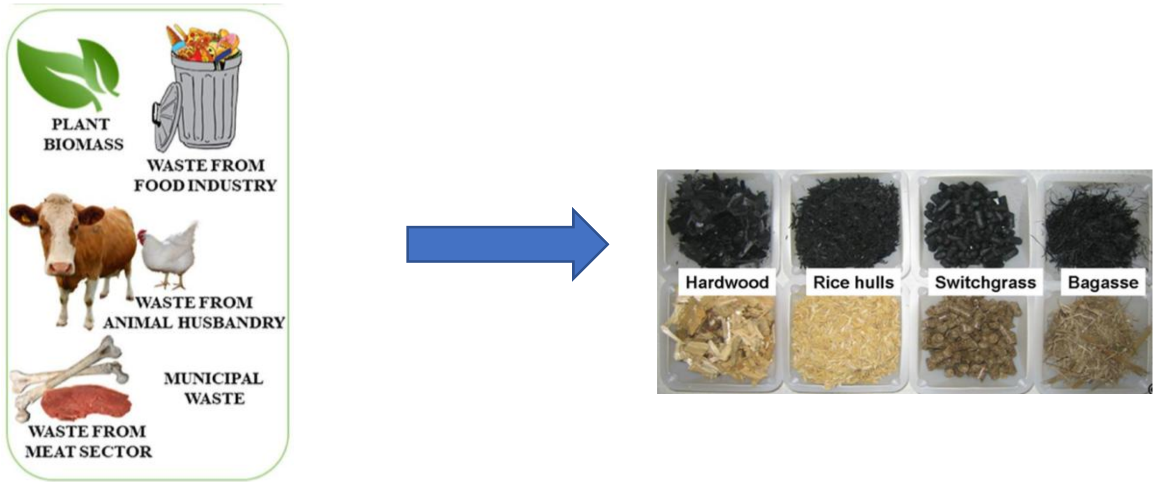
While the addition of AMF into soil has been seen to greatly improve the growth of plants without the need for any artificial amendments or fertilizers, some negative impacts have been seen. Typically, AMF colonization is proportional to the support needed by the plants. If enough phosphorus is present in the soil, then colonization will be lower, as the AMF is not needed (Garrido et al., 2010). In the case that there is an extremely high level of colonization, it is possible that there will be competition for resources between the AMF and the plants, rather than the AMF facilitating enough nutrient and water uptake (Garrido et al., 2010). The high colonization may also interfere with other interactions between soil microorganisms and the plant root (Garrido et al., 2010). Despite the potential for these problems, the positives of inoculating a soil system and roots with AMF as an alternative way to provide the necessary nutrients to plants outweigh the negatives, and resolve the problems associated with phosphorus in soil and plant systems.

**Biochar**

While not a biofertilizer, biochar is a soil amendment and conditioning material that can aid in supporting the goals of sustainable agriculture. It has been investigated in many ways to enhance soil and plant growth.

Biochar is the byproduct of any material that is rich in carbon, formed through the process of pyrolysis. While forming from similar origins, this substance differs from charcoal. As stated, biochar is formed by pyrolysis. The process of pyrolysis is the heating of organic material, to achieve both thermal and chemical decomposition of the biomass, in either the absence of or in extremely low levels of oxygen. While charcoal is also frequently produced through pyrolysis, the two differ in the temperature of production. Biochar typically undergoes pyrolysis temperatures from 600ºC- 1000ºC, whereas charcoal is produced at around 400ºC (WasteX, 2024). This higher temperature used for biochar allows for the biomass to form carbon clusters, giving rise to a structure that makes biochar incredibly resistant to weathering and degradation within the soil, which means that it is able to continue to amend the soil with little to no re-application (Gaffar et al., 2021). This also allows for biochar to have a greater porosity and surface area. Charcoal is a much less stable product, and has a less developed pore structure, leading to a lower surface area (WasteX, 2024). This decrease in stability allows for the carbon to break down much quicker, releasing $CO_2$ into the atmosphere. The intended use differs as well (WasteX, 2024). Charcoal serves a purpose mainly as fuel for heating and cooking, while biochar has agricultural purposes and carbon sequestration purposes. Since charcoal is used as fuel, all of the carbon stored within the structure ultimately is released back into the atmosphere, which differs from biochar, as all carbon stored in biochar remains within the structure (WasteX, 2024).

Biochar can be produced from any carbon rich biomass, frequently the byproducts of agriculture or forestry, such as nut shells, woodchips, tree bark, or straw (Figures 12 and 13). Additionally, industrial byproducts such as paper, and animal waste such as dairy manure can be used (Mylavarapu et al., 2013). These different materials are known as the feedstock that the biochar is produced from, and it is important to know what feedstock you are utilizing. Biochar has been recognized as an incredibly effective method to utilize waste products and even invasive plants that have been removed (Amalina et al., 2022).




**Figures 12 and 13. Biochar feedstocks and final product.** (*Biochar Feedstocks*, 2025; Samoraj et al, 2022)

Biochar's unique characteristics give it the ability to function in many useful ways. Despite the loss of most nutrients present in the organic matter through the process of pyrolysis, many can be maintained in a way that is beneficial for plant use and uptake when biochar is applied into the soil (Khater et al., 2024). Biochar is capable of neutralizing toxins and heavy metals found within soils, which can be incredibly supportive for plant growth (Khater et al., 2024).

**Biochar Characterization**

Biochar has its own characteristics that additionally allow it to be characterized for use. The process of creating biochar varies depending on the intended use. Different changes that occur within the carbon structure due to feedstock choice and pyrolysis temperature allow for properties such as pH, organic matter content, and phosphorus content to differ within different biochars (Alghashm et al., 2018; Khater et al., 2024; Wu et al., 2013). As pyrolysis temperature increases, trends highlighted in a study carried out by Alghashm et al. (2018) indicate the biochar's pH also increases. The feedstock also had an effect on the pH, where a woody biochar produced the lowest pH (6.6), and a leafy feedstock produced the highest (10.4). Organic matter content in biochar is also determined by pyrolysis temperature. An increase was calculated when pyrolysis temperature was increased from 400º C to 800º C, and the highest organic matter content remained in a woody biochar, and the lowest was from a straw feedstock (Wu et al., 2013). An increased pyrolysis temperature additionally increased the available phosphorus content of the biochar, and the highest phosphorus was measured in a woody biochar (Khater et al., 2024). When carbon rich substances undergo pyrolysis at temperatures higher than 700º C, the resulting biochar contains less volatile matter compounds and has a higher surface area, which increases the potential of adsorption of water and nutrients to the particles, as well as improving retention (Azargohar, 2006, 2008; Ippolito et al., 2012). Since both feedstock and temperature can impact the characterization of the biochar, and ultimately its function, these factors must be considered when deciding what biochar to use in terms of agricultural use.

**Benefits from Biochar Application**

Biochar can serve in many ways to support both agricultural systems and our growing environmental needs (Figure 14).

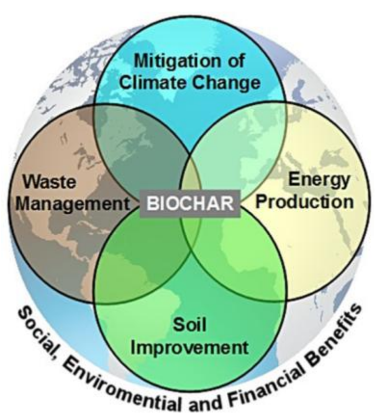



**Figure 14. Benefits from application of biochar**. (BIOCHAR Ancient Origins, Modern Inspirations – Guest Post by Paul Taylor, 2015)

## Carbon Sequestration and Climate Change Mitigation

As mentioned prior, many agricultural practices contribute to increased carbon, as well as other gases, being released into the atmosphere. Biochar's uniquely dense structure allows it to serve as an important resource, considered for climate change mitigation. Soil does serve as a carbon sink, but the carbon is typically not stored in stable forms, and it eventually escapes the soil. This happens even more frequently when practices such as tilling are put in place, which disturb the soil on a regular basis. The addition of biochar into soil increases its ability to function as a sink, as biochars degredation-resistant structure increases the amount of time that carbon remains within the soil (Lehmann et al., 2021). When biochar is made, the carbon stored within the biomass is not released as it would if the material was burned or allowed to decompose naturally (Figure 15). When practices such as allowing past crops to serve as a fertilizer for the soil focus on a waste management and use goal, only approximately 10-20% of the carbon is recycled back into the soil, and it very quickly is released as $CO_2$, meaning that nearly 99% of the carbon is released back into the atmosphere (Woodall, 2023). However, the same material, when turned into biochar, is capable of storing up to 50% of this carbon in a much more stable form (Woodall, 2023). Through using invasive species, biowaste, and other materials that would otherwise be left to decompose and release carbon into the atmosphere as the feedstock, biochar can serve to mitigate climate change through proper waste management as well (Kruger et al., 2009). The two other products produced as biochar is made additionally support climate change mitigation. Bio-oil and syn-gas, the gas and liquid products of pyrolysis, can be used as an energy source. While ultimately this carbon is released back into the atmosphere, a 50% reduction can have far-reaching impacts (Woodall, 2023).

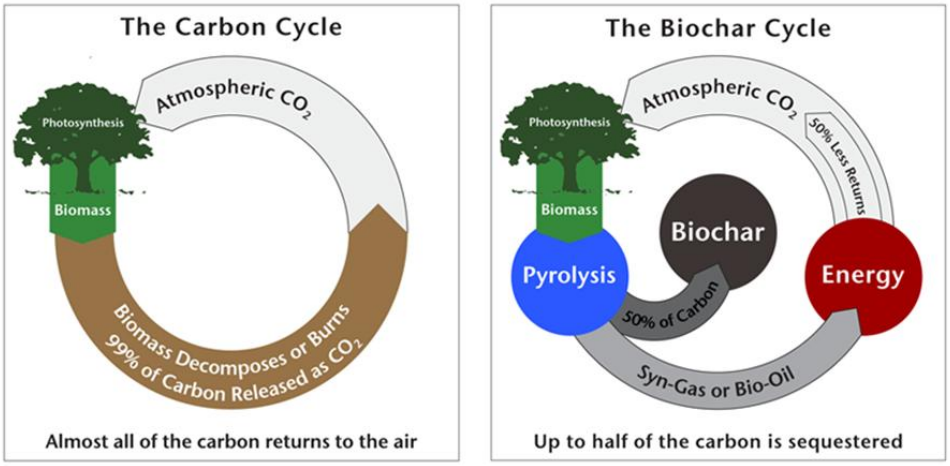



**Figure 15. The typical carbon cycle compared with the biochar cycle.** (Biochar Solutions Inc, 2011)

### Benefits of Biochar on Soil

Biochar aids in improving soil characteristics in multiple ways that ultimately support higher crop yields due to bettered soil conditions. Soil structure is determined by several characteristics, including porosity, bulk density, and particle density. Biochar alters all of these parameters, as well as the texture, porosity, structure, and overall consistency of soils that it is mixed into, which occurs through changing the particle sizes present and quantities, the soil density, pore size, and surface area. Biochar can also act as a binder, to better hold together soil particles, which increases the presence of soil aggregates. This allows for stronger pore structure, and decreases the potential for erosion by water and wind. Biochar can serve to also raise the pH of soil, which may be an important amendment in very acidic soils (Chan et al., 2008). In soils that have been mixed with biochar, there is the potential to increase soil moisture. Through higher surface area, adsorption of water molecules increases, which increases the amount of water available in the soil. Additionally, as biochar increases pore space available, water is able to fill these spaces, increasing soil moisture content and retention. This information specifically regarding moisture can be incredibly important as irrigation is considered in farming, as having a soil that holds more moisture may lessen the stresses related to frequent water supply needs (Scherer, 2017).

**Beneficial Effects of Biochar on Phosphorus Availability in Soil**

As well as improving various other growth characteristics, biochar has been determined to have potential in terms of increasing the availability of phosphorus within soil. Phosphorus effectively leaves a substance at above 700º C, which is why biochar can be an ideal avenue of providing the necessary nutrient, but only when produced at a low temperature. A study carried out by Glaser et. al (2019) indicated that when biochar was produced at a low or mid temperature (between 450º and 600º C), the plant-available phosphorus greatly increased within the soil. Biochar has been shown to increase the cation exchange capacity within soil, which aids in plant access to phosphorus. While feedstock can impact the response, in general, biochar has been shown to increase levels of phosphorus in soil. In acidic soils, a very strong positive effect was seen on the availability of phosphorus, also within the Glaser et al. study (2019). When biochar is produced from a feedstock that is rich in phosphorus, higher levels of phosphorus that is in the plant-available form is able to enter the soil, which indicates that likely, the phosphorus is coming from within the biochar rather than the properties allowing the pre-existing soil phosphorus to become available (Glaser et al., 2019). Overall, biochar has potential as an alternative to synthetic fertilizers as a method for providing phosphorus in the soluble form needed for plant uptake.

**Benefits of Biochar on Plant Growth and Nutrient Acquisition**

Biochar has potential for improving the growth of plants, and increasing the yield of crops. Many effects caused by the addition of biochar into soil also produce changes support plant growth. When soil structure is strengthened, and pore size is increased, compaction is decreased, which allows for better flow of water through the soil column, but also improves the ability of roots to growth through the soil without interference (University of Minnesota, 2018). When plant roots are better able to access depths of soil, there is better availability of air and water, both of which are necessary for growth. Biochar also serves to increase the cation exchange capacity. This parameter, which measures a soil's ability to retain and exchange cations, effects the availability and accessibility of necessary nutrients to the plant, such as phosphorus. With this improved exchange, plant growth has been seen to increase (Agarwal et al., 2022). Through increased ability to provide pore space and retain moisture, biochar supports plants in improved drought tolerance, as more water remains available for longer periods of time within the soil when biochar is present (Agarwal et al., 2022). When plants are more resistant to drought, it becomes easier to grow crops in different regions of the world that may experience different climatic conditions, which allows for higher yields and shared knowledge on a global scale. The same holds true for plants experiencing stress due to high salinity. Biochar has been shown to improve physical attributes such as leaf size and chlorophyll content, as well as photosynthesis, even when under saline stress (Murtaza et al., 2024). On a more practical level, biochar has also been shown to impact the growth of plants. In a study carried out by Murtaza et al. (2024), increases in plant height (29%), and shoot weight (51%), were measured, indicating that biochar is capable of amending and conditioning soil to a capacity that is capable of greatly augmenting crop yields.

### Potential Downsides of Biochar Application

In some cases, biochar may not be the best option to improve soil parameters, rather, it may become a detriment. Biochar has the potential to release hydrocarbons into the soil, and this may initiate the loss of organic matter that exists naturally within the soil (Wardle et al., 2008). In terms of plant growth, in other studies, biochar has been see to not benefit crop yields at all (Schnell et al., 2012), and in other cases, there have been detriments to the overall crop yield caused by the addition of biochar (Ippolito et al., 2012). Despite these potential downsides to the use of biochar in agriculture, it is still an amendment technique that is increasingly researched. Biochar is an expensive product; when purchased from accessible locations such as Lowes and Home Depot, it can cost up to $42 per cubic foot (Pokharel, 2021). While biochar can be sourced in bulk for a slightly lesser cost, adding biochar to soil can become incredibly costly. Most studies have utilized between 0.5% and 2% biochar within the soil, despite manufacturer recommendations for higher rates of application, in attempt to combat the issue of price, and have been met with success at this lower concentration (McIntosh & Hunt, 2025). Overall, further research regarding biochar's impacts on soil quality and plant growth are necessary to determine whether the potentially prohibitive cost to apply over a larger field may be later outweighed by an increased crop yield as a result of the amendment.

### Effects of Biochar and AMF Interactions

Both biochar and AMF have been shown to be effective in supporting higher crop yields due to improved soil health parameters and increased plant growth. In most studies, biochar and AMF have been investigated separately in order to evaluate their potential for effectively enhancing crop yield without synthetic fertilizers or other aids. It has also been found that when used together, different results can be achieved. In some studies, benefits have been observed, while in others, the interactions have shown detrimental effects.

When successful, the two have shown to greatly improve plant growth and soil health parameters, as well as increasing their respective functionalities (Figure 16). Biochar promotes a lower bulk density in the soil, increasing pore space for higher water retention, which provides an environment that is conducive to microbial and fungal growth. Through the ability to provide carbon to microorganisms that aid in the growth of AMF, there is the potential for increased colonization, as biochar has the capability of serving as a substrate for supporting beneficial soil microorganisms such as AMF. The pore spaces provide a habitat for the microorganisms (Mulyadi, 2023). While AMF typically requires a root to allow for colonization, the high surface area and porous structure of biochar provides a space for the hyphae to penetrate, similar to the process carried out within the cells of roots. This can provide an accelerated rate of colonization (Mulyadi & Jiang, 2023). Additionally, when the feedstock of the biochar is high in certain nutrients, the biochar itself can provide nutrients to the soil, namely phosphorus, and then the AMF is capable of absorbing these nutrients, which are then taken up by the plant in the available form (Mulyadi, 2023).

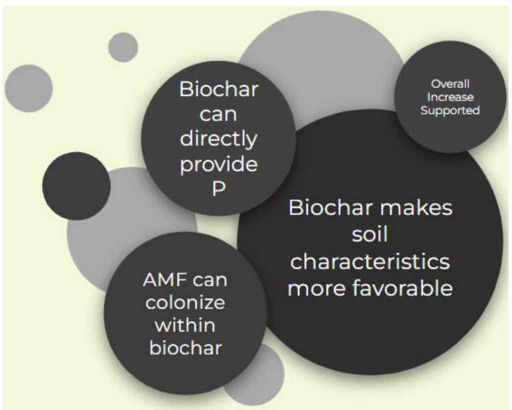



**Figure 16. Effects of Biochar and AMF interactions.**

AMF and biochar effect each other and their functions within soil, but also have the capability of improving plant productivity and nutrient levels. When placed under stressful conditions such as exposure to heavy metals, biochar and AMF have been shown to be able to increase photosynthetic rates and plant biomass, indicating potential for an increased crop yield. In one study examining rice growth, the combined treatments increased plant height, leaf area, and the day post-germination during which flowers appeared, when compared to treatments receiving only biochar or AMF (Mulyadi, 2023). While the same study did determine increased plant growth parameters for biochar and AMF alone, the combined effect was significantly stronger than when used alone (Mulyadi, 2023). In terms of yield, which may be more relevant to farmers, the same study, as well as one prior, also indicated that rice and corn yields increased when grown with a combination of AMF and biochar in varying concentrations (Mulyadi, 2023). Ultimately, when functioning together, phosphorus availability has been shown to increase in the plant-available form. As each tactic is capable of increasing phosphorus when utilized alone, the combination allows for the phosphorus supplied by the biochar (typically from the feedstock prior to pyrolysis) to be either converted into the available form, and then taken up by the plant, or simply by using this supply and having the capability of reaching it through the longer hyphae structure. This combined support allows for two functional alternatives to synthetic fertilizers to be able to collectively improve the uptake and nutrient availability to plants, ultimately increasing plant growth and crop yields (Mulyadi, 2023).

In some cases, it is possible that when AMF colonizes biochar, the degredation of biochar, while typically slow, would experience an increased rate due to the important phosphate-solubizing bacteria (as discussed prior) present on the hyphae of the AMF (Neuberger et al., 2024). This would be detrimental to farmers and those attempting to use biochar to amend the soil, as it is typically relied on to have an incredibly slow rate of degradation and to build up within the soil to increase carbon. However, as the degradation is not microbial decomposition, the rate and effect are not as quick or severe as would occur in an organic material that did not undergo pyrolysis. The structure of the biochar is far too strong to allow for microbial decomposition (Kuzyakov et al., 2014). One study, aimed at determining the effects of biochar on microbial communities, determined that the biochar did not show any significant effect on the colonization of AMF within the roots of the plant (Elzobair et al., 2016). This is very relevant to consider, as a major concern of utilizing the two techniques in tandem is that the AMF will favor colonization within the biochar instead of within the plant root, which would decrease the effects of AMFs ability to facilitate nutrients to the plant. While many studies have been evaluated in order to explore the two methods together, more work needs to be done to consider how the concentration of biochar within soil may impact the location of AMF colonization, and therefore the effectivity of both on plant growth.

## Objectives

I had three main objectives for this study (Figure 17). The first focuses on plant productivity. Through the application of both 2% and 10% biochar, the effects on plant productivity were observed, to determine changes caused by these treatments. The second focuses on the colonization of AMF within the fine roots of the pea plants. Also through the application of 2% and 10% biochar, the effects on root colonization were observed, in order to see the influence of these treatments on potential colonization. The third focuses on the availability of phosphorus to the plants, both in the soil and then within the plant roots and shoots. Several soil and plant parameters were studied to evaluate the effects of the treatments. Overall, the purpose of the study was to evaluate how the growth of dwarf snap peas can be improved through practices that align with a more sustainable approach to agriculture. The goal was to determine how the application of 2% and 10% biochar, as well as AMF, could influence various plant growth parameters that ultimately determine overall plant productivity.

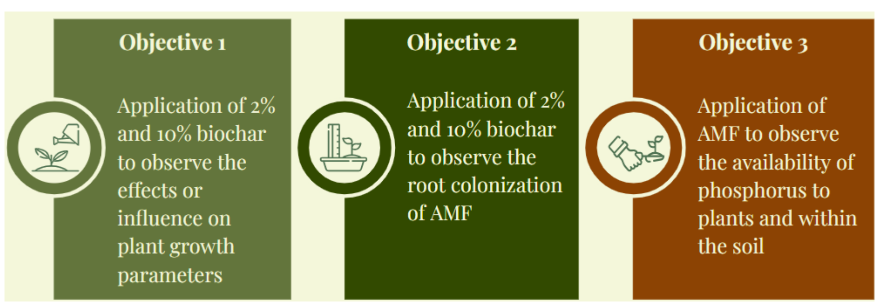



**Figure 17. Objectives of the study.**

**Hypotheses**

From these objectives, I drew three hypotheses (Figure 18). When considering AMF colonization as a result of the treatments, at 2% biochar, I will expect higher root colonization by AMF than determined the in 10% biochar treatments. When phosphorus content is considered, I will expect higher P supply to plants from treatments receiving 2% biochar and AMF application in comparison to the 10% biochar and AMF. When considering an overall holistic view of the results, focusing on parameters that would be the most accessible and useful to broad audiences and farmers, I expect the overall plant productivity (yield, height, dry biomass) will be higher in plants treated with 2% biochar than in those treated with 10% biochar.

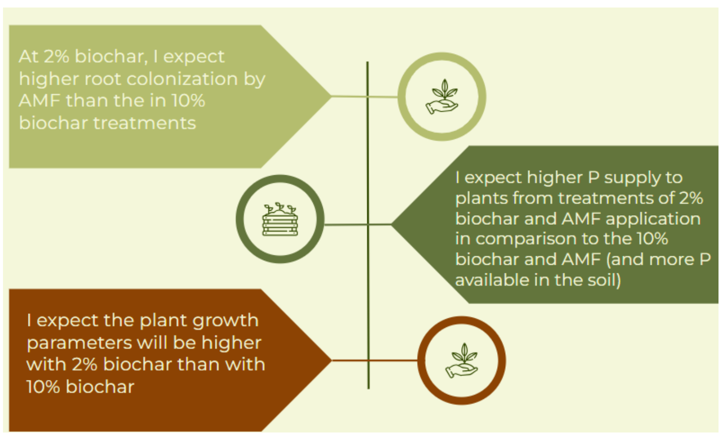



**Figure 18. Hypotheses.**

## Materials and Methods

### *Experimental Design*

This study consisted of five treatments and a control group, and each treatment was replicated five times, for a total of 30 pots. The first group, T0, was the control, which received no compost, biochar, or AMF. The second, T1, received AMF. The third, T2, a mixture of soil and a 2% quantity of biochar. The fourth, T3, received both the AMF as well as the 2% quantity of biochar. The 5th, T4, received a 10% biochar and soil mixture. Finally, the 6th, T5, was treated with AMF and 10% biochar. The treatments are laid out in Table 1.

**Table 1. Treatments**

| Name | Treatment |
|---|---|
| T0 | Control, No Treatment |
| T1 | AMF |
| T2 | 2% Biochar |
| T3 | AMF, 2% Biochar |
| T4 | 10% Biochar |
| T5 | AMF, 10% Biochar |

### Soil Collection

The soil was collected from Grow It Green Urban Farm in Morristown, New Jersey, located near our study site, as can be visualized in Figure 19. This soil was selected specifically because no synthetic fertilizers or pesticides had been utilized to treat this soil. Grow It Green does not use practices associated with intensive agriculture anywhere on their property. Additionally, this soil was taken from a patch at the back of the farm, where no crops were actively being planted or grown (Figure 20). At the time of collection, the patch was overgrown with raspberry bushes, as well as sunchokes, a flowering plant that produces large tubers similar in size and appearance to ginger root. Neither of these organisms had been planted by the farmers at Grow It Green. It is also important to note that soil had been collected from this area a few months prior, and the space was filled in with wood chips and other compostable material in order to replenish the soil. Overall, this soil was chosen because of the guarantee of no added synthetic chemicals, as well as the lack of other intentional amendments that may have altered the study. In order to collect the soil, 32 garbage bags were filled with soil, dug from the same 6ftx10ft patch at a consistent depth into the ground. The top layer of soil was not collected, to avoid additional plant matter and loose stones. Grow it Green is located within 5 miles of the Drew University campus, and so there were no concerns about the soil being used in a location that may not be suited to its needs and typical environment. Some selected properties of the collected soil are presented in Table 2.

**Table 2. Characteristics of Soil Used for Study.**

| Parameters | Values |
|---|---|
| Organic Matter (%) | 17.34 ± 0.55 |
| pH | 7.2 ± 0.04 |
| Sand (%) | 65.42 ± 1.10 |
| Silt (%) | 14.17 ± 1.50 |
| Clay (%) | 20.42 ± 0.42 |
| Moisture (%) | 25.05 ± 1.80 |

Values presented as means with standard error.

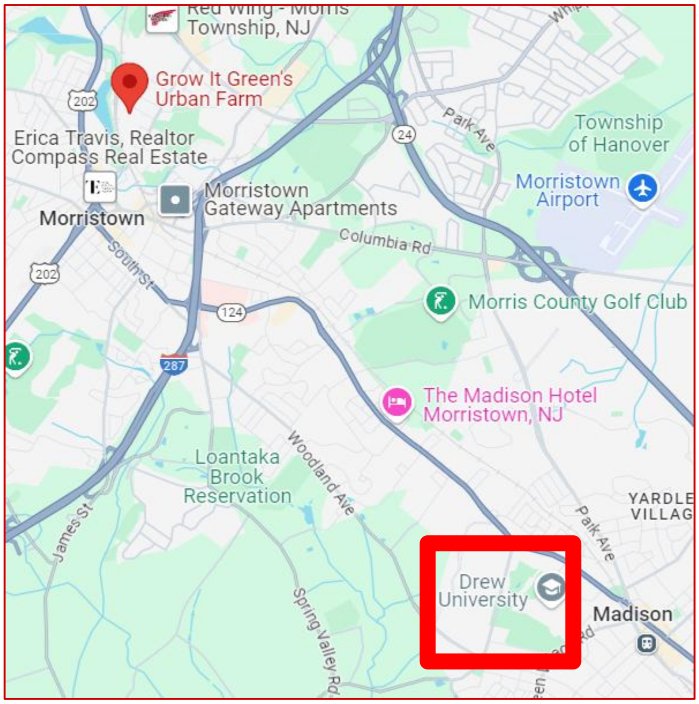



**Figure 19. Location of Grow It Green Urban Farm relative to Drew University Campus.** Red box indicates area of Drew's Campus, in Madison, NJ. Red Pin indicates location of Grow It Green Urban Farm in Morristown, NJ.

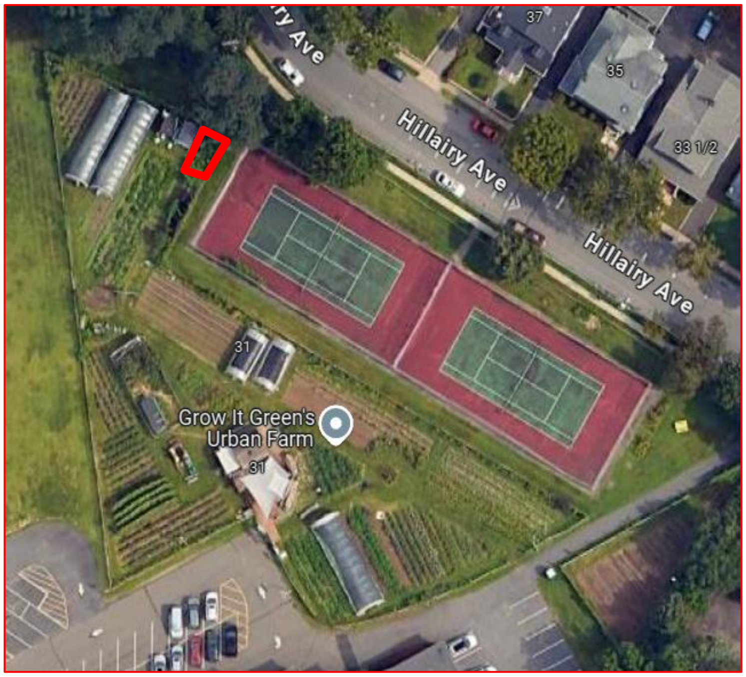



**Figure 20. Site of Soil Collection.** The site where soil was collected at Grow It Green Farm in Morristown, NJ. Indicated by a bold red shape in the upper left corner.

**Setup**

A fenced off area next to the Hall of Sciences building at Drew University was selected for the location of this experiment (Figure 21). During the months of June and July, this plot of land receives equal sun and shade during different times of the day. Additionally, because the plot was fenced off and requires opening a combination lock to enter, deer and other animals that may have impacted the study were effectively removed from the process as potential sources of experimental error. At this level of experimental agriculture, these factors are not being evaluated as factors associated with growth, and so any effects potentially caused by their presence would be considered an error. This location is not covered in any way, and therefore experiences natural rainfall and sunlight. In 2024, the months of June and July experienced weeks of very consistent heavy rainfall followed by weeks of no rainfall. However, the plants were also watered artificially in addition to the rainfall that they received. The chosen plant for this study is Pisum sativum, commonly known as the Dwarf Snap Pea. The ideal growth period for these legumes (as recommended by the packaging) fell within the scope of the study period, which meant that these peas would allow for extensive testing post-growth. Ideally, dwarf snap peas receive 1 inch of water in a 1x1 foot area per week, from planting through termination (Tong, 2022). The pots were watered with approximately 274mL of water, using a spray bottle, three times a week. The spray bottle was used to ensure soil saturation, and to ensure that no soil was displaced by large quantities of water entering the pot at the same time.

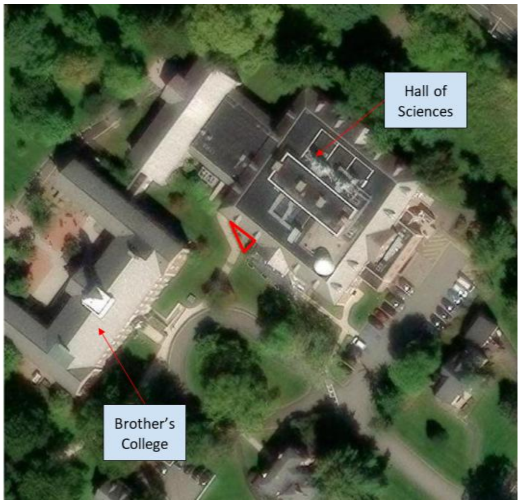



**Figure 21. ArcGIS Satellite Map of the experimental location.** The red triangle located directly next to the Hall of Sciences indicates the location of the testing site. Brother's College and the Hall of Sciences are both labeled as notable landmarks.

Thirty two-gallon (7.57L) plastic pots were used to grow the peas in this experiment. Each pot was filled to the base of the brim (Figure 22) with the soil collected from Grow It Green. From there, each pot received its specified treatments, before the seeds were planted.


**Figure 22. Two-Gallon Plastic Pots, with fill line labeled.**

In order to create the soil mixture used in the treatments receiving biochar, the soil was mixed thoroughly with woody biochar in a separate bin, as can be seen in Figure 23, allowing for a 2% biochar concentration, and the same was done to create a 10% biochar concentration. The Wakefield Premium Organic BioChar was purchased from Amazon, and selected biochar parameters were analyzed, presented in Table 3. In the pots receiving the 2%, 134.8 grams of biochar was thoroughly mixed into the pots of soil. In the pots receiving the 10%, 674 grams of biochar was mixed in the same way into each pot.

**Figure 23. Mixing biochar into soil, 2% and 10% by weight.**

A dairy manure based compost purchased from Amazon was added to each of the treatment pots during setup, excluding the control pots (Figure 24). As per the manufacturer's recommendation, only 25-50% of the total compost amount is added when planting. Due to this, 2.4 grams of compost was added to each pot at the beginning stage. The compost was applied around the rim of the pot, far from the seeds. The compost was lightly mixed under the surface of the soil. This same process was repeated after 21 days to account for the remaining 50% of compost needed.

**Figure 24. Applying compost around the edges of the pot on Day 1 (06/01/2024).**

As indicated above, some of the treatments received an inoculation of AMF. In order to maximize symbiosis, the AMF was placed towards the center of each of these pots, and mixed into the soil in the same location that the seeds were planted (Figure 25). This was done in one application, at the beginning of the experimental period.

**Figure 25. AMF applied to center of pot near seeds.**

In each pot, three seeds were evenly spaced around the center and pressed into the soil approximately two inches deep (Figure 26), and then lightly covered, as per the manufacturer's recommendation (Figure 27). The pots were then immediately watered with 274mL of tap water. While only one plant was analyzed from each pot, three seeds were planted to account for both experimental and natural error and failures. At the point of experimental termination, only one plant was selected for testing from each pot. Multiple seeds are used to minimize loss at the germination stage, and then thinning is done either right after germination, or later on in the study.

**Figure 26. Planting three seeds in each pot.**


**Figure 27. Dwarf Grey Sugar Pod Snow Peas, before planting.** Growing recommendations listed on package.

In order to minimize certain biases associated with this study being set up in a natural setting where external factors had influence over growth and other parameters, a randomized complete block design (RCBD) was created. A RCBD is an experimental design often used in agriculture that is slightly different from an entirely randomized setup; instead the different treatments are considered when the locations are selected. Through this design, a "map" is created which lays out where each pot will be placed (Figure 28, 29). This placement ensures that no row contains two of the same treatment, which minimizes some risk of false conclusions that may occur because of external factors such as sunlight availability.

| | | | | |
|---|---|---|---|---|
| T3R1 | T2R2 | T5R3 | T3R4 | T3R5 |
| T2R1 | T3R2 | T3R3 | T4R4 | T2R5 |
| T5R1 | T4R2 | T4R3 | T1R4 | T1R5 |
| T1R1 | T0R2 | T0R3 | T2R4 | T4R5 |
| T4R1 | T5R2 | T2R3 | T5R4 | T0R5 |
| T0R1 | T1R2 | T1R3 | T0R4 | T5R5 |

**Figure 28. Randomized Complete Block Design layout.**

**Figure 29. Experimental Setup following RCBD.**

## Analysis of Soil Parameters

Methods for selected physical and chemical soil parameters are discussed in this section.

### *Soil Physical Properties*

#### Soil Texture

In order to determine soil texture pre-plant growth, the hydrometer method was used. In this test, a Fisher brand ATSM152H hydrometer was utilized, as seen in Figure 30. Three trials were conducted under the same conditions. 40 grams of soil were mixed with 100ml of sodium hexametaphosphate dispersing solution in each 1000ml graduated cylinder, and then brought up to the full volume with tap water. Parafilm was used to entirely cover the top of each cylinder, and then the tubes were inverted until completely mixed through. Immediately after, the parafilm was removed and the hydrometer was placed inside. Once it stopped bobbing up and down in the water, a timer was started for 40 seconds. The hydrometer depth was measured at this mark, effectively determining the sand content of the soil. A timer was then set for two hours, and the same measurement was repeated at that mark, to determine the clay content of the soil. It is important to note that the cylinders were not moved once they were placed down on the counter after the initial mixing. Silt content was determined based on the proportions of clay and sand determined. Using the soil texture triangle (Figure 31), the overall soil texture name was determined.

**Figure 30. Hydrometer Method for determining soil texture**

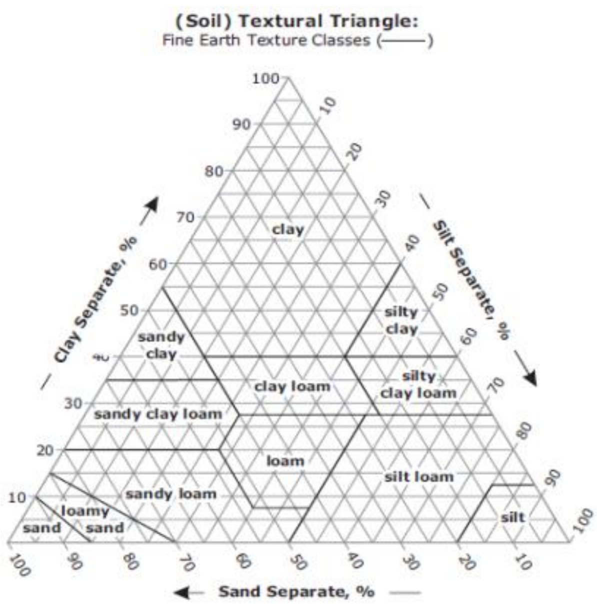



**Figure 31. USDA Soil Texture Triangle.** (USDA, 2024)

## Soil Moisture Content

The soil moisture content is determined through removing all moisture from the soil and examining the weight difference. Three replicates of 5 grams of fresh soil were placed into metal sample cups. These samples were placed into an oven set at 105º C for a 24-hour period undisturbed. After this time, the samples were weighed to determine the difference in weight from moisture loss. The overall moisture content was determined using the following formula:

$$Moisture\ Content\ \% = \frac{mass\ of\ fresh\ soil - mass\ of\ oven\ dry\ soil}{mass\ of\ oven\ dry\ soil} x100$$

***Soil Chemical Properties***

**Soil Organic Matter (OM)**

The organic matter content, repeated after termination, of the soil was measured by using the Loss on Ignition method (LOI). Three samples of four grams of air dried soil were placed into crucible cups. Samples were placed in a muffle furnace set at 500º C for four hours. The samples were left in the oven overnight after the four-hour period, and then weighed. Organic Matter content was calculated using the following formula:

$$OM\ (\%) = \frac{mass\ of\ air\ dry\ soil -\ mass\ of\ oven\ dry\ soil}{mass\ of\ air\ dry\ soil} x100$$

**Soil pH**

Soil pH, which was repeated after termination, was determined using air-dried soil that was not used in the pots to set up the experiment. Soil was mixed in a 1:2 ratio with DI water, using 5g of soil and 10mL of water. The three replicates completed for this test were mixed for two minutes each, and then left to rest for 30 minutes to allow for all large soil particles to settle. A Denver Instruments pH probe was calibrated before the testing began. Between the three replicates, the probe was cleaned with DI water.

**Soil Phosphorus**

***Extraction***

Soil available phosphorus content was determined after the termination of the experiment, through Olsen's sodium bicarbonate colorimetric method (Olsen et. al, 1954), using the soil from each replicate3. In labeled conical flasks, 50 mL of extraction reagent (0.5 M sodium bicarbonate, at pH 8.5) was added to five grams of soil. Using a platform shaker, all samples were thoroughly mixed for 30 minutes. All samples were filtered through Whatman 42 filter paper and funnel, into a clean conical flask. The solution was not clear enough, and so approximately one teaspoon of activated charcoal was added to each flask, thoroughly mixed, and re-filtered using syringe filters (0.45 µm) to achieve a clearer solution. The solution still needed filtering, and so it was left to filter into centrifuge tubes through filter paper. The samples were capped and stored in the refrigerator at 4℃ for analysis.

***Spectrophotometry***

Using a micropipette, further reagents were added. Each sample received five ml of the extract (the filtrate), five mL of a coloring developing reagent, and 15mL of DI water. Each tube was labeled with treatment and replicate code. The coloring agent was made by mixing ascorbic acid, sulfuric acid, ammonium molybdate, and potassium antimony tartrate.  Standard solutions for phosphorus were also prepared in the same manner, with $KH_2PO_4$ (potassium phosphate) solutions ranging in concentration from 0 to 20ppm in $NaHCO_3$. The solution containing 0pm was used as the blank for this experiment, containing sodium bicarbonate, DI, and the coloring reagent. After coming to room temperature for 15 minutes, the samples were individually transferred into cuvettes, and placed separately into the spectrophotometer. Absorption values were recorded at 880nm.

**Biochar Parameters**

The following are the selected biochar parameters analyzed.

**Moisture Content**

Moisture content was measured as a percentage in relation to mass lost after drying at 105°C. Six replicates of 1.0 grams of air dried biochar were measured into crucible cups and placed into a muffle furnace for 24 hours. The samples were then allowed to cool completely, and then weighed. The following equation was used to determine the moisture content of each replicate, and then the mean of the six was calculated:

$$Moisture, \% = [(A - B)/A]\ x\ 100$$

Where, A = grams of air-dried sample used, and

B = grams of sample after drying at 105°C

**Volatile Matter**

After weighing the six replicates to determine moisture content, lids were placed on three and they were put back into the muffle furnace at 950°C for 6 minutes. The furnace was turned off, and the samples were allowed to cool before removal. The weight was then recorded, and volatile matter content was determined using the following equation, as a percentage in relation to the weight lost:

$$Volatile\ matter, \% = [(B - C)/B]\ x\ 100$$

Where, B = grams of sample after drying at 105°C

C = grams of sample after drying at 950°C

**Ash Content**

The remaining three samples that underwent moisture content testing were placed back into the muffle furnace, at 750℃ for six hours. The oven was turned off and the samples were allowed to cool, and then they were weighed. As opposed to measuring weight lost, ash content is determined by weight remaining. The percentage of ash content was determined using the following equation:

$$Ash\ content, \% = (D/B)\ x\ 100$$

Where, B = grams of sample after drying at 105°C

D = grams of residue

**pH**

Biochar pH was determined using a similar process to the soil. Biochar was mixed in a 1:20 ratio with DI water, using 1g of biochar with 20mL of DI water. The two were mixed thoroughly for 2 minutes, and then allowed to settle for 30 minutes. A Denver Instruments pH probe was calibrated before the testing began. Before each of the three replicates, the probe was rinsed with DI to avoid contamination. For each replicate, the pH was recorded and the mean was determined from the three results.

**Table 3. Characteristics of Biochar used for Study**

| Parameter | Measure |
|---|---|
| pH | 8.8 ± 0.04 |
| Moisture (%) | 30.29 ± 0.85 |
| Volatile Matter (%) | 27.82 ± 0.47 |
| Ash Content (%) | 40.37 ± 0.91 |
| Fixed Carbon (%) | 31.81 ± 0.50 |

Values presented as means with standard error. These parameters were measured, but not further analyzed in the results. They will be used for further consideration in future studies.

**Plant and Soil Sample Preparation (Post-growth phase)**

On day 40 of growth, plants and soil were collected for analysis. Each pot was carefully emptied into a clean plastic bin, in order to ensure that no soil was contaminating another sample, and to avoid root breakage. Two quart-sized Ziploc bags of soil were gathered from each pot, for further soil testing. Within each pot, if more than one plant germinated during the duration of the growth period, only one was selected for analysis. The soil was carefully separated from the root system, as much was removed as possible through brushing and shaking, and the plants were set aside and labeled with their treatment and replicate for further testing.

**Plant Parameters**

Methods for selected plant growth parameters are discussed in this section.

**Height and Root length *(above and below ground):***

The height of both the plant roots and shoots were measured after uprooting. After termination, a single cut was made at the interface of the root and shoot, where the soil would have reached during growth, as seen in Figure 32. The root and shoot lengths were then measured separately. The pea stems tend to curl around themselves and in general do not grow in a straight stem in the way that some other plants do. To resolve this problem and accurately measure a shoot height, the stems were stretched lightly along a table until as straight of a line as possible was achieved. A meter stick was utilized to measure the shoot from the base to the finest curled tip to gather a consistent length in centimeters for each. A similar procedure was carried out in order to measure the roots for each replicate. Each plant contained an entire root system made up of thick and fine roots. Root systems were carefully rinsed off using a hose and spray bottle on a gentle setting to remove all soil residue, leaving the roots a pale yellow-white color. The roots were laid out on a clean surface as straight as possible, allowed to air dry for 12-24 hours, and then measured from the top all the way down to the very tip of the fine roots at the bottom, again, for a consistent measuring system for each. Data was collected for each pot's representative pea plant.

**Figure 32. Full De-potted pea plant. Red arrow indicates the distinction between the root system and the shoot.**

**Biomass (Above and Below Ground)**

In order to gather this dry biomass data, each plant shoot and root were placed separately into labeled brown paper bags. These bags were placed into an oven at 70℃ for 72 hours to dry out completely. Once at room temperature, they were weighed while still in the bag. An empty bag was also placed in the oven and then measured to be able to determine the actual weight of each root and shoot.

**Plant Root and Shoot Phosphorus**

Total phosphorus was determined for the plant's shoots and roots. In order to determine the total phosphorus instead of the available form, a digestion was carried out. In a 50 mL beaker, 0.1g of finely ground root tissue was added to 10mL of concentrated nitric acid. The sample was placed onto a hot plate, and digested through boiling for about 15 minutes, until all plant matter was dissolved. The sample was then cooled, and five mL of perchloric acid was added, and placed back on the hot plate. The sample was boiled again until the liquid was colorless. The sample was allowed to completely cool, and then was added to a 100mL volumetric flask and volumed up to the full 100mL. The sample was stored in 100 mL glass bottles, refrigerated at 40ºC, until used for testing. This was carried out for all root and shoot samples in the same manner.

The process for spectrophotometry remained the same as the tests carried out on the soil, by putting individual samples into cuvettes and documenting absorption values at 880nm.

**AMF Within Plant Roots**

Root colonization of AMF was determined through root staining. Before oven drying the roots (Figure 33), 25 fine roots measuring approximately one-inch-long were selected from each plant, as seen in Figure 34. The fine roots are the smallest branches of the root system. They were removed from the system using forceps (Figure 35), and placed into microcentrifuge tubes containing a 10% KOH solution. All samples were placed into an oven at 80℃ for two hours for the purpose of bleaching. Then the roots were rinsed in DI water twice to remove any residual KOH solution.

Each microcentrifuge tube received 0.05% Trypan Blue and lactoglycerol solution. The tubes were further heated at 70℃ for 20 minutes to allow for the staining process. Similarly to before, the roots were rinsed twice with DI water to remove residue and excess dye. To make the slides, each slide was labeled with the treatment and replicate number. The 25 roots were laid out parallel along the slide using forceps, making sure that none touched (Figure 36). Once complete, a drop of lactoglycerol was added to each slide, and was placed into a petri dish with a lid for storage.

**Figure 33. Washed and dried entire root system before fine root separation.**

**Figure 34. Twenty-five selected fine roots.**

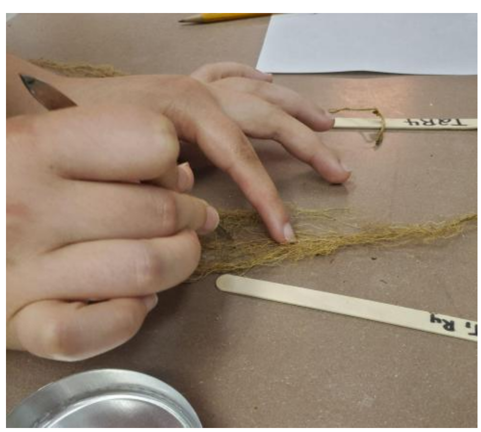


**Figure 35. Forceps being used to select fine roots from the root system.**

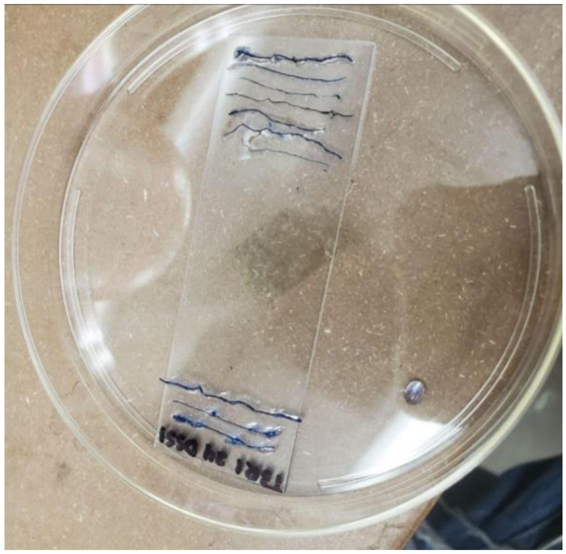


**Figure 36. Trypan Blue dyed fine roots on microscope slide.**

Using a stereo microscope, slides were analyzed one at a time for the presence of one of the three structures indicating AMF colonization, as mentioned within the introduction. The structures were not identified as separate entities, simply if a structure was present, it was marked as such under the specific treatment and replication. AMF colonization was calculated using the following equation:

$$\frac{Number\ of\ Colonized\ Roots}{Total\ Number\ of\ Roots} x\ 100\%$$

**Statistical Analysis**

Data were presented as means with standard errors. Statistical analysis was performed using the statistical analysis system IBM SPSS Statistics 28. One-way Analysis of variance (ANOVA) was carried out for the effects of different treatments on soil physical properties, chemical properties, plant productivity and AMF colonization. Significant differences between each treatment and the control as well as between treatments were also evaluated. Tukey–Kramer post hoc tests were performed to compare mean separation at $p < 0.05$ among treatment. Any differences between the mean values at $p < 0.05$ were considered statistically significant.

**Limitations**

Before analyzing the results of this experimentation, it is important to consider external factors that may have contributed to the outcome aside from the effects of the treatments, and that alters how the results can be viewed. The first, and potentially most influential, was the low germination rates that were experienced by certain treatments. In the pots treated with 10% biochar (T4), only one pot had a plant that germinated. Whether this was because of the effects of the treatment, or due to other factors environmentally, one data point is not enough to form a solid conclusion about the results of a study.

Plants have a recommended period of time that indicates maturity, typically measured in days from germination, to when maturity is reached. In fruit bearing plants, maturity is frequently considered to be when the peak production occurs. For *Pisum sativum* (Dwarf Snap Peas), the recommended growth period is 65 days. However, due to the time constraints of the research period, the plants were removed from the soil for testing 40 days after being planted. While some fruit (peas) were produced, there were not enough to consider this to be growth full maturity, or to evaluate yield. This impacts how certain soil chemical parameters can be changed both by the plants taking up nutrients and growing, and by not having enough time to colonize AMF, and the by not producing a yield. Height, root length, and the distribution of phosphorus found within the different plant parts can also be impacted by being measured at varied points of time within the growth process, as this changes during growth.

Yet another environmentally caused limitation stems from the fact that sun cover was not distributed equally during the growth process. The experimental location was next to a building, that casted a shadow over much of the designated plot for certain portions of the day. However, other portions received direct sunlight for many hours a day, which led to problems with overheating and drying of leaves and stems. These discrepancies between treatments, despite the RCBD, need to be addressed when considering the results. Another environmental factor to consider is rainfall or drought conditions. Both were experienced throughout the course of this experiment. Rain contributes an unknown amount of water to the plants, in addition to the measured water given each week. Soil parameters did impact plant growth from this data.

The following results are compiled and analyzed with these limitations in consideration.

**Results and Discussion**

**Table 4. p-value from One- Way ANOVA**

| Parameter | P- value | Significant or Not? | Notes |
|---|---|---|---|
| Soil Moisture | 0.01 | Yes | Highest moisture content found in treatment receiving 10% biochar (T4) |
| Soil pH | <0.001 | Yes | Lowest pH found in treatment receiving only AMF (T1) |
| Soil OM | 0.39 | No | |
| Soil P | 0.01 | Yes | Highest available phosphorus found in treatment receiving 10% biochar and AMF |
| Shoot Height | 0.90 | No | |
| Root Length | 0.91 | No | |
| Root Mass | 0.34 | No | |
| Shoot Mass | 0.94 | No | |
| Root P | 0.15 | No | |
| Shoot P | 0.66 | No | |
| AMF Colonization | <0.001 | Yes | Significant colonization for all treatments |

This table gives a brief overview of the data that will follow, outlining which parameters measured for soil and plant growth showed statistical significance in comparison with their treatments. Parameters highlighted in green were seen to be statistically significant. The moisture content of the soil, which is a physical parameter, was seen to be significant, with a p-value of 0.01. Chemical parameters of the soil seen to hold statistical significance were pH ($p<0.001$) and phosphorus ($p=0.01$). No significance was seen within the plant growth parameters. AMF colonization was seen to be statistically significant, with a p-value of less than 0.001. Parameters holding significance, as highlighted in green, and others will be discussed in further detail.

**Effects on Soil Parameters**

***Soil Moisture Content***

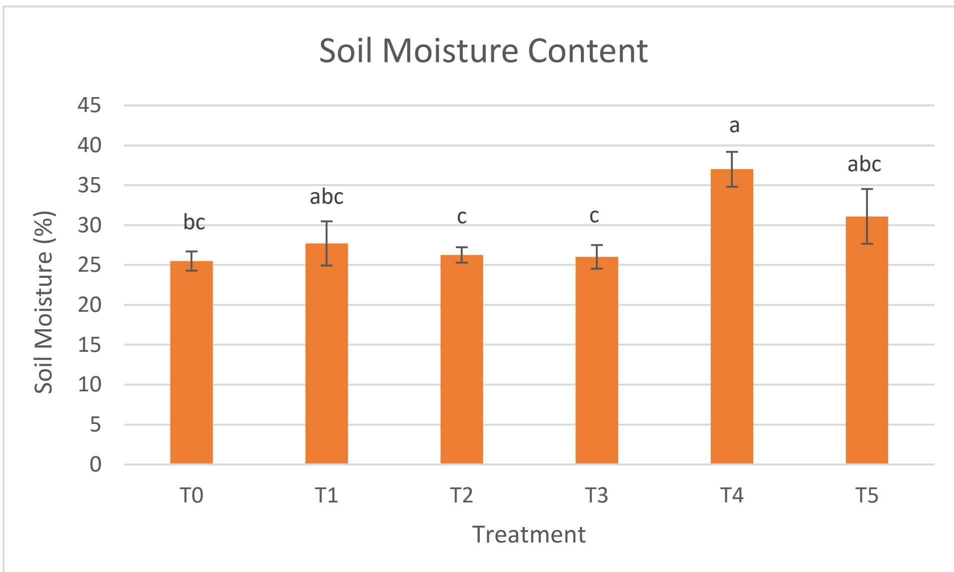



**Figure 37. Effects of different treatments on soil moisture content, as represented by means with standard error.** Treatments are labeled as follows: T0=Control, T1= AMF, T2= 2% biochar, T3= AMF and 2% biochar, T4= 10% biochar, T5= AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$.

As seen in Figure 37, there are significant differences between the control, and between treatments. T4 (10% biochar), which has the highest moisture content (37.00%), is significantly different from the control (T0), as well as from T2 and T3. T2 and T3 only show significant difference in regards to T4. T5 does not show any significant difference to any treatment or the control, and the same holds true for T1.

The two treatments receiving 10% biochar (T4 and T5) did have the two highest moisture contents (37.00% and 31.10%, respectively). The control (T1), had the the lowest moisture content (25.50%). In terms of practical significance, we can see that treatments receiving higher amounts of biochar alter the moisture content of soil when compared with a control.

T4, which had the highest moisture content, received a treatment of 10% biochar. Biochar greatly decreases bulk density of a soil, and increases pore space, which allows for a higher moisture content due to more space being available for water, and higher water retention. A study by Seyedsadr et al. (2022) showed that biochar increases water retention within soil, aligning with the results seen above. Other treatments that received biochar either received it in a lower concentration, or also received AMF at the same time. As T5 is not significantly different from T4, conclusions cannot be made on a statistical level about the influence of the biochar and AMF together, in comparison to just receiving 10% biochar. However, when values are considered, it can be stated that the treatment receiving 10% biochar and no AMF (T4) had a higher moisture content (37.00%) than the combined treatment at 10% biochar (T5) (31.10%). When higher pore space and lower bulk density due to the addition of biochar into soil is considered, an increase in moisture content on a practically significant level can be seen, but this is not consistent when AMF is also present within the treatment. This indicates some level of correlation between the presence of AMF and a lowered moisture content and water retention capability, potentially due to AMF additionally requiring water as a resource (Pauwels et al., 2023).

***Soil pH***

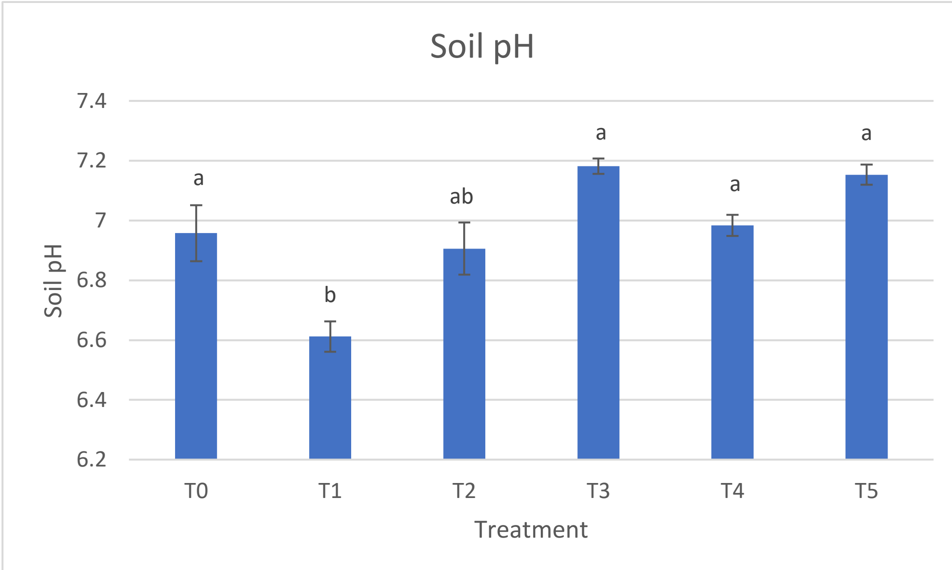



**Figure 38. Effects of treatments on Soil pH, represented as means with standard error.** The treatments can be described as follows: T0=Control, T1= AMF, T2=Fertilizer and 2% biochar, T3= AMF and 2% biochar, T4=10% biochar, T5=AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$.

As seen in Figure 38, T1 (AMF) shows significantly lower pH (6.61) from the control (T0), as well as from T3, T4, and T5. T3 (2% biochar and AMF) shows the highest pH value (7.18). T1, showing the lowest pH (6.61), was treated with only AMF. AMF has the capacity to support a host plant to better function in an acidic soil, but on the contrary, an acidic soil can decrease the potential for AMF colonization within plant roots (Liu et al., 2020). Additionally, AMF releases organic acids into the soil that turn phosphorus into the available form, and these acids decrease the soil's pH. This balance may be the reason why the results are as shown, as the soil was too acidic to foster colonization, and therefore, the acidity remained high. Our soil was very high in organic matter to begin with (17.34%) as the soil came from an organic farm. Organic matter is naturally capable of making soil more acidic through the release of $H^+$ ions during decomposition, and so it can be suggested that the results are largely impacted by the presence of organic matter (Arnall, 2017).

Different plants require different pH levels in order to grow at an optimal rate and for productivity. A neutral range (6-7.5) is typically optimal for most plants, and when the pH of the soil ranges in either direction, whether more alkaline or basic, this can drastically change the nutrients that are available to the plant (Tong, 2022). As soil is more acidic, toxic elements become more readily available, while necessary nutrients such as phosphorus are not. This process occurs as a more acidic soil results in higher fixation of phosphorus by calcium, iron, and aluminum, rendering it unavailable to plants. Likewise, a similar phenomenon occurs with soil that is too alkaline, with fixation occurring due to manganese, zinc, and iron (Preston, 2019). The phosphorus levels found within the soil of T1 (only AMF) were not statistically significant (as will be discussed following Figure 40), but connections can be formed between these levels and the pH of the soil tested from T1. As the pH of a soil increases, phosphorus becomes less prominent in its available form (Cerozi, 2016). This is consistent with the results determined in this study, as the pH of T1 (6.61) is the most acidic of any treatment, while still falling within an optimal growing range, and the phosphorus levels determined for T1 (30.00ppm) are increased when compared to the control (T0) (18.41ppm). This is also supported when the control data is compared, as T0 has a higher pH of 6.96, and T0 has a much lower phosphorus level than those treated, at 18.41ppm. In this case, the prior experimentation suggesting that a higher or lower pH (outside of the neutral range) may cause less available phosphorus within is supported. This becomes relevant for those who may wish to implement either biochar or AMF into their farming practices in order to increase phosphorus availability, because if a solution is put into place that ultimately changes the pH to a level that makes the phosphorus unavailable, the solution is counterproductive.

***Soil Organic Matter***

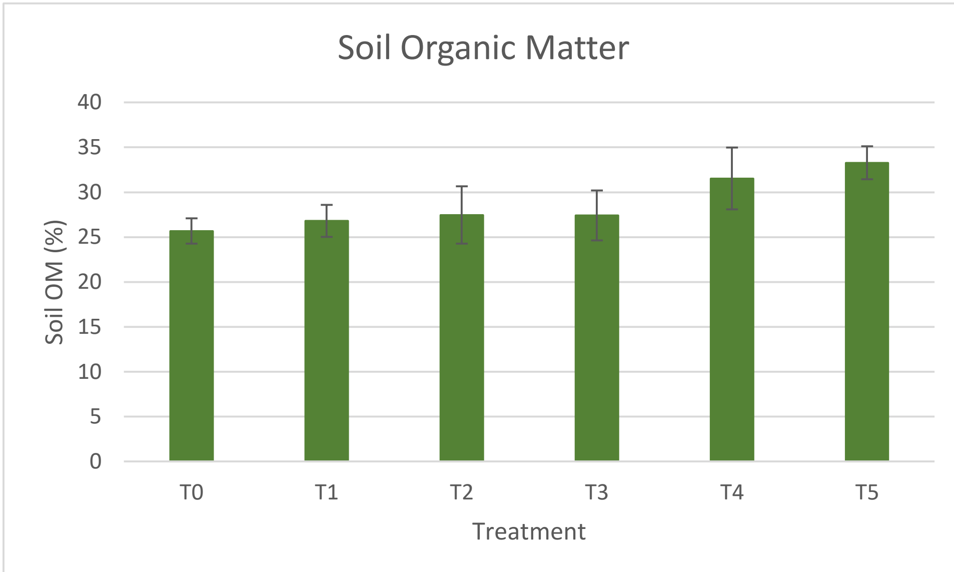



**Figure 39. Effects of treatments on Soil Organic Matter, represented as means with standard errors.** Treatments are labeled as follows: T0=Control, T1=AMF, T2=2% biochar, T3= AMF and 2% biochar, T4=10% biochar, T5= AMF and 10% biochar**.**

There is no significance between any treatments when organic matter content is considered, as shown in Figure 39. The soil, coming from an organic farm, already had a fairly high organic matter content (17.34%), higher than is considered typical for New Jersey soils, which is between 3% and 6% (Fenton et al., 2008). A slight practical difference can be noted between T4 (31.53%) and T5(33.28%), and the other treatments (averaged to 26.84%). These two treatments received higher concentrations of biochar (10%), which may increase the organic matter content within the soil, through preservation of pre-existing organic matter (Ernest et al., 2024).

**Soil Phosphorus**

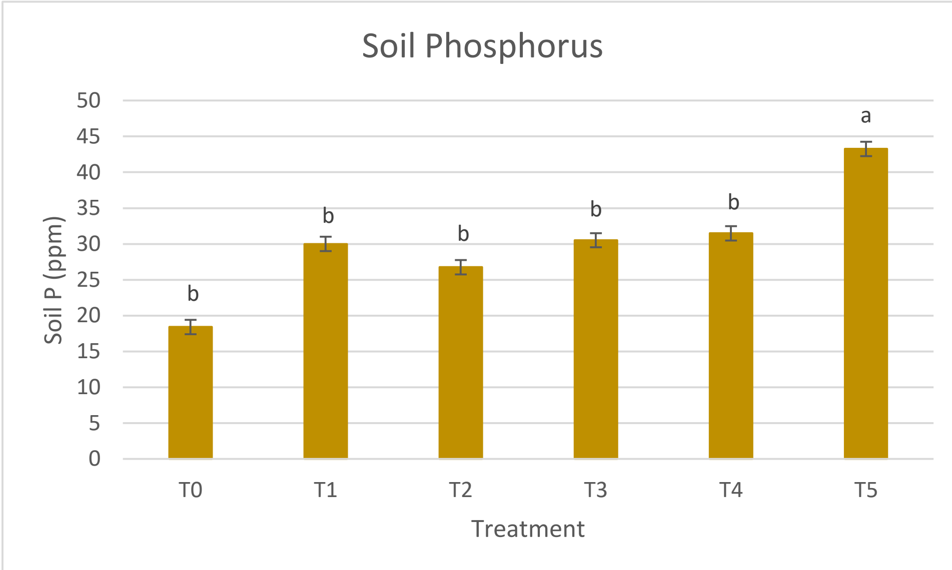



**Figure 40. Effects of Treatments on Available Soil Phosphorus. Data presented as means with standard errors.** The treatments can be described as follows: T0=Control, T1= AMF, T2=2% biochar, T3=AMF and 2% biochar, T4=10% biochar, T5=AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$.

As seen in Figure 40, the soil that received T5 (10% biochar and AMF) shows significantly higher levels of available phosphorus (43.27 ppm) when compared to the control as well as the other treatments. The control shows the lowest phosphorus levels (18.41 ppm). We see fairly consistent results between the other 4 treatments. T5 received AMF as well as 10% biochar. Both biochar and AMF serve as facilitators of phosphorus to the plant (Li & Cai, 2021). From the data as well as this knowledge, we can come to the conclusion that the combination of 10% biochar and AMF allows for more phosphorus to be available for uptake by the plant within the soil. While other treatments received AMF and biochar separately, and T3 received a combination of the two, similar to T5, T3 only received 2% biochar with the AMF. In this case, the higher concentration of biochar allowed for a higher concentration of available phosphorus in the soil.

## Plant Productivity

The effects of the five treatments on different plant productivity parameters were also investigated and are discussed below.

### *Shoot Height and Root Length*

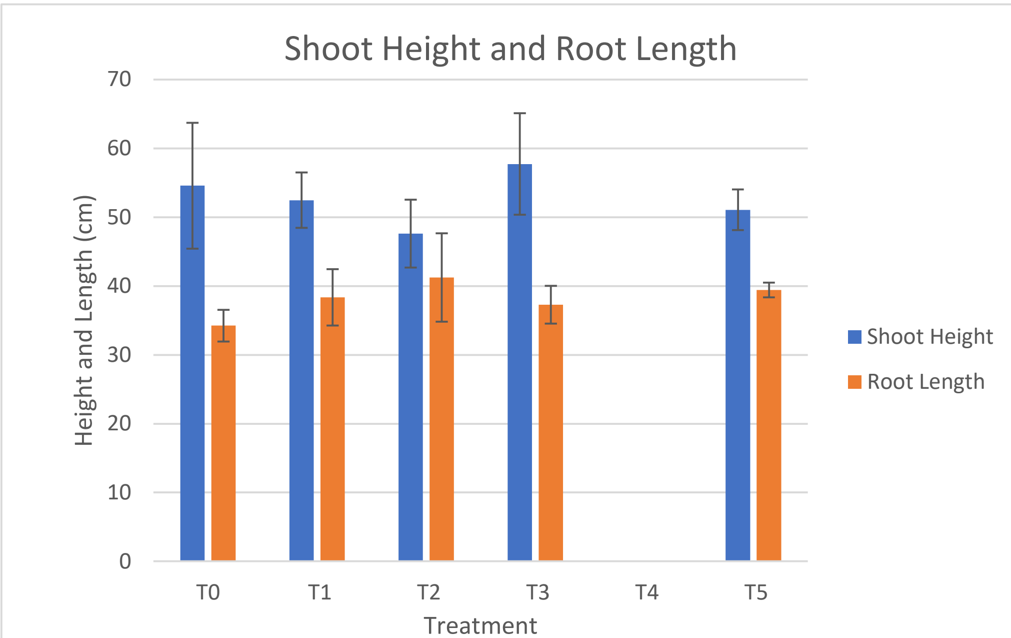



**Figure 41. Effects of Treatments on Shoot Height and Root Length. Data represented by means and standard error.** Treatments are labeled as follows: T0=Control, T1=AMF, T2= 2% biochar, T3= AMF and 2% biochar, T4= 10% biochar, T5= AMF and 10% biochar. No data shown for T4 due to only one replicate producing growth.

As seen in Figure 41, no significant difference occurred between treatments for the shoot height or the root length. This result is consistent with a 2012 study carried out by Jones et al, where the addition of biochar to soil did not increase the plant growth parameters of maize. While no statistical significance was found for the data in Figure 41, we can evaluate practical significance. T3, treated with 2% biochar and AMF, showed the highest average shoot height, at 57.72 cm, and T2 (2% biochar only) showed the highest average root length, at 41.23 cm. When compared with the application of AMF and a higher concentration of biochar (T5), it can be taken as a consideration that a higher biochar concentration may have a negative effect on plant growth parameters, when it is noted that the average shoot height for T5 was 51.07 cm.

**Table 5. Effects of Treatments on Shoot and Root Mass**

| Treatment | Shoot Mass (g) | Root Mass (g) |
|---|---|---|
| T0 | 1.76±0.28 | 0.20± 0.07 |
| T1 | 1.83± 0.60 | 0.31± 0.06 |
| T2 | 1.70± 0.44 | 0.40± 0.11 |
| T3 | 1.55± 0.39 | 0.26± 0.09 |
| T4 | No data | No data |
| T5 | 1.29±0.21 | 0.56± 0.17 |

Values represented as means with standard error. Treatments are labeled as follows: T0=Control, T1=AMF, T2= 2% biochar, T3= AMF and 2% biochar, T4= 10% biochar, T5= AMF and 10% biochar. No data shown for T4 due to only one replicate producing growth.

As seen in Table 5, no significant differences are seen between the mass of the shoots of the plants based due to the effects of the treatments. The same is consistent for the root mass. These results have been seen in a similar study, showing that treatment with biochar did not result in statistically significant changes in plant growth and yield parameters including root and shoot mass (Jones et al., 2012). However, practical significance can follow a trend, indicating that plants treated with a higher level of biochar (10%) when combined with AMF (T5) would produce a lower shoot mass (1.29 g) than those treated with a lower concentration of biochar (2%) and AMF, with a shoot mass of 1.55 g. The opposite trend is shown in root mass, where T5 produced a higher root mass (0.56g) than T4 (0.26). Ultimately, more data is needed in order to form conclusions regarding the effects of treatments on plant growth parameters.

**Table 6. Effects of Treatments on Root and Shoot Phosphorus**

| Treatment | Shoot P (%) | Root P (%) |
|---|---|---|
| T0 | 0.02± 0.01 | 0.38 |
| T1 | 0.05± 0.01 | 0.19± 0.03 |
| T2 | 0.08± 0.01 | 0.16± 0.04 |
| T3 | 0.06± 0.01 | 0.14± 0.03 |
| T4 | No data | No data |
| T5 | 0.07± 0.02 | 0.08± 0.04 |

Values represented as means with standard error. Treatments are labeled as follows: T0=Control, T1=AMF, T2= 2% biochar, T3= AMF and 2% biochar, T4= 10% biochar, T5= AMF and 10% biochar. No data shown for T4 due to only one replicate producing growth.

As seen in Table 6, there is no significance shown between treatments, or with the control. In plants, phosphorus exists within the roots, shoots, and leaves in different percentages throughout the growing process. This process, known as nutrient partitioning, is carried out by plants during growth, allowing different concentrations of certain nutrients to be more present within different parts of the plant during certain phases of growth (Bender et al., 2015). Because the plants did not reach a consistent phase of growth between treatments, we cannot determine the effects of nutrient partitioning on phosphorus levels within the roots and shoots. While there was no statistical significance, individual values can still be considered in a practical sense. In T1 (AMF), the highest root phosphorus content was seen out of all of the treatments (0.19%). The highest phosphorus level was seen in T2 (10% biochar) for the shoots (0.08%). The presence of AMF colonization, which was high (60.00%) in T1, potentially is allowing for the higher root phosphorus in T1, as AMF colonization has been shown to increase uptake of phosphorus into the roots (Li & Cai, 2021).

**<u>AMF Colonization</u>**

The effects of the five treatments on AMF colonization within the roots were determined and are discussed below.

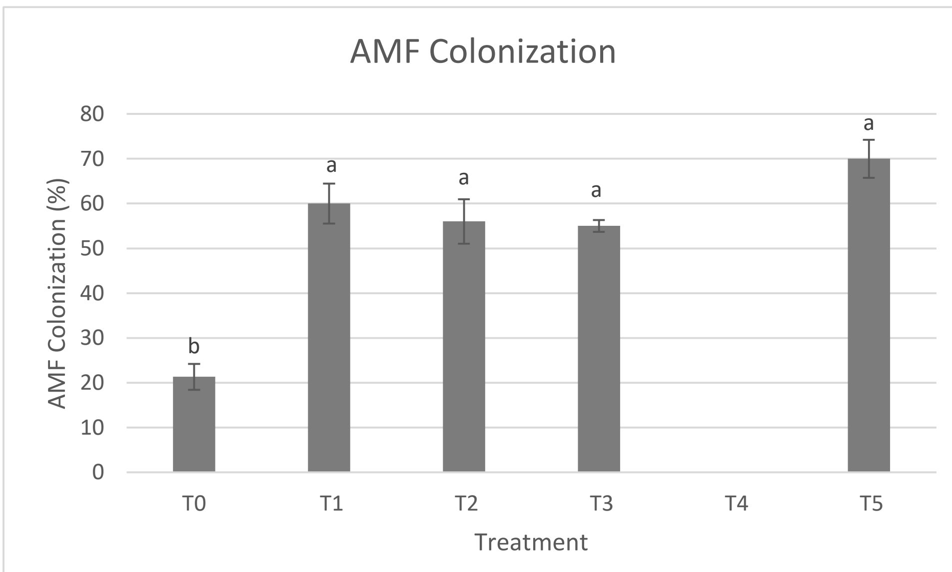



**Figure 42. Effects of Treatments on AMF Colonization in Fine Roots, represented as means with standard error.** Treatments are labeled as follows: T0=Control, T1=AMF, T2= 2% biochar, T3= AMF and 2% biochar, T4= 10% biochar, T5= AMF and 10% biochar. Similar letters indicate not significantly different at $p<0.05$. No data shown for T4 due to only one replicate producing growth.

T5 was significantly different from the control (T0). This indicates that the plants that received T5, which was AMF as well as 10% biochar, had a higher percentage of AMF colonization within the fine roots. It is important to note that T5 contained 10% biochar as well, and the interactions between the higher amount of biochar and AMF are important in terms of potential colonization, as indicated by prior studies such as a 2023 study carried out by Ji et al. In this study, higher root colonization was seen when a high concentration of biochar and AMF were both used as treatments within the study.

As seen in Figure 6, T0 had relatively low colonization of AMF within the fine roots collected. However, an overall upward trend was seen in the other four treatments analyzed. All four treatments significantly differed in percent colonization from the control (T0). T5 showed the highest root colonization (70.00%), but there was not significance between other treatments. However, in terms of practical significance, it can be seen in Figure 6 that all treatments resulted in higher levels of AMF colonization than the control did, indicating potential increases from the use of any treatment.

AMF hyphae are thinner than the roots of plants, and so they are able to uptake more phosphorus from locations that the plants are unable to on their own (Li & Cai, 2021). Through this process, it can be said that a higher level of colonization would allow for more successful and efficient uptake of phosphorus to the plant. Since phosphorus is an essential nutrient for pea growth, growth trends would indicate that a higher AMF colonization percentage in the roots would allow for higher plant productivity.

These results are limited by several factors that occurred throughout the course of this study. The study took place over an active growing period of 40 days from seed to de-potting, which did not allow most plants to reach maturity (65 days is recommended as per the seed packaging). When biochar is present along with AMF, the AMF may colonize within the biochar instead of within the root cells. It is possible that some results seen are due to this phenomenon, rather than the AMF treatment not having an impact on AMF colonization directly. Overall, the treatment of AMF and 10% biochar (T5) produced the highest colonization on a level of practical significance, but all treatments showed higher colonization than the control (T0), indicating that amendments aid in the colonization of AMF.

## Conclusion

Conclusions about the effects of biochar and AMF colonization on soil parameters and plant growth can be determined through considering statistically significant results.

In this study, I have found the use of 10% biochar was able to show the largest increase in soil moisture, and the use of 10% biochar and AMF showed the largest increase in both available soil phosphorus and AMF colonization.

It was determined that in this study that the application of 10% biochar and AMF into soil supported a higher level of AMF colonization than from other treatments. The colonization was the highest in both treatments receiving 10% biochar, indicating that biochar is capable of supporting colonization whether the AMF is already present within the soil, or if more is added as an inoculant. Additionally, the lowest AMF colonization was seen in the control, demonstrating that soil amendments and inoculants do have an effect on the colonization of AMF within the roots of a plant, providing the support needed, even if the AMF is naturally occurring within the soil. The consideration of naturally occurring AMF is relevant because colonization was seen even in treatments that did not receive additional inoculant. Soil moisture content was increased most in both treatments receiving 10% biochar, and showed the lowest moisture in treatments not receiving biochar. It can be concluded that the structure and presence of biochar aids in supporting soil structure and moisture retention, whether AMF is present or not. Consideration of the effects on moisture content can be incredibly important, especially in scenarios where irrigation presents challenges for farmers. Soil phosphorus was lowest when no treatments were given (control), and highest in the treatment (T5) receiving both 10% biochar and AMF. As both of these amendment tactics work to facilitate phosphorus to the plant, it can be concluded from these results that the combination supports facilitation and uptake in a more beneficial way. Soil pH was the highest (most alkaline) in the pots treated with 2% biochar and AMF, and the lowest (most acidic) in the pots treated with only AMF (T1). This measurement is important to consider in terms of phosphorus availability within soil, because very alkaline or very acidic soils are capable of fixing phosphorus into an unavailable form, and so soil pH may have a large effect on plant growth in ways beyond just looking at acidity and alkalinity changing the plant. This can be very important for farmers to consider, as avoiding treatments that alter the pH too much will maintain higher levels of phosphorus within the soil.

This study opens the doors for many future study options, and poses multiple questions that need to be answered. As shown in the data, conclusive results about the effects of 10% biochar and AMF were shown, with increases in both AMF colonization and available soil phosphorus. However, the inconclusive results regarding the plant growth parameters leave questions to be answered about the ultimate impacts of these treatments on crop yield potential. In the future, a study that allowed for full plant maturity to be reached would allow for a full data set with more replicates, allowing for conclusive data, whether supportive of the hypothesis or not. Specifically, if conclusive data was collected about the effects of the treatments on plant root and shoot total phosphorus levels, we would be able to connect the higher level of colonization seen to a higher level of available soil phosphorus, and then determine what this results in when total root and shoot phosphorus is considered. In addition to allowing a longer growth period, implementing more replicates would prevent some of this potential error. If the plants reached maturity in a future study, the results would become more relevant for farmers and those looking to implement these techniques on a larger scale. Ultimately, yield is the parameter of interest for farmers, and this project did not produce a yield in order to examine the effects of the treatments on production in that sense. While success was seen with the 10% biochar treatment, more research needs to be completed utilizing different concentrations of biochar to determine the perfect amount as to not waste resources and funding, but maintain peak plant productivity. In this study, the AMF varieties were not considered within the data to determine what may have been present in the soil before inoculation. This information may change how the study is analyzed, as natural AMF can serve the same purpose as an inoculant, and may change how the data is viewed in terms of how the AMF addition to pots changes the results, or if it is simply preexisting AMF.

These results have very broad reaching environmental impacts if implemented in an agricultural setting. As addressed in the introduction, biochar's carbon sequestration abilities can be incredibly efficient for mitigating one cause of climate change and global warming due to carbon release. If put into practice, the use of biochar can aid in waste management, easing financial burdens associated with waste removal, and support cleaner resource distribution and use, as well as decreasing carbon release into the atmosphere that occurs in typical agricultural practices. Higher uptake of phosphorus already existing within the soil and the ability to naturally alter the form of phosphorus through the use of AMF and biochar will additionally support climate change mitigation and decrease habitat pollution and harm. Through decreased usage of high volumes of synthetic fertilizers to directly provide available phosphorus for uptake and use, toxic runoff of excess phosphorus into rivers, lakes and streams will be also decreased, supporting healthier ecosystems and lowered levels of toxins potentially reaching humans. By researching methods to slow the use of synthetic fertilizers rather than focusing time, money, and energy on researching cleanup methods, resolving climate and environmental related issues caused by agriculture can become much more streamlined and efficient.

While there is much room for improvement and further research to deepen understanding, this study does provide enough context for broader implications. The results demonstrating that 10% biochar specifically shows improvements to soil health parameters when compared to the typical 2% are critical to aiding in the spread of information about the benefits of biochar. When farmers or other people who are tasked with growing crops to feed our population, the question of the best techniques to use are incredibly pressing. The understanding that to a certain extent, specifically at 10% by weight, biochar application in soil is capable of increasing moisture content, pH, and health in general, is crucial for farmers who may be considering making changes. The utilization of waste to create biochar can be incredibly impactful for farms who would like to strive towards more effective waste management, which can be an enticing option. Ultimately, the need for less frequent application of biochar, if repetition is needed, can be a major cost savings when compared to repeated application of high concentrations of synthetic fertilizers. When coupled with the knowledge of other benefits, this technology can become much more accessible to a wider range of farmers. These amendment and growth support techniques be effective on smaller scales where the plants can be properly cared for with lots of human attention. Meticulous care was needed frequently, as we added fertilizer midway through the experimentation, carefully watered the plants three times a week using spray bottles rather than a hose, and pulled weeds. This study was fairly controlled with the exception of being outside and, we did not use sterilized soil, meaning that whatever soil microorganisms had been within the soil prior to experimentation were present to additionally impact results.

Farmers and researchers alike may use this information to consider alternative ways to support crops. If fields struggle with irrigation or erosion, then biochar and AMF may support soil health. While we did not evaluate the erosion that occurs due to factors such as wind and water, we did see changes in moisture and OM, which are both known to indicate the overall health of the soil, and to support other soil parameters that slow the rate of erosion and increase water retention. In a farm setting, these parameters and the associated results would be very practical to understand. In locations where drought or other factors impacting accessibility to resources for irrigation present problems with producing crops, the addition of biochar into the soil at a 10% quantity can substantially support moisture retention, decreasing the need for frequent irrigation.

This study pushed the boundaries of being between a greenhouse potted experiment which is entirely controlled, towards being more of a practical or real life setting, as outside factors such as sun, rain, and things in the soil did have impacts on our plant growth. The results observed from this level of experimentation are more applicable to farmers who may want to begin investigating these techniques, more than if the study was very controlled. This study is a step towards gathering substantial practical data to examine how these treatments may interact with the soil and plants in a field setting. Overall, this study pushes the field of sustainable agriculture to consider combining remediation and plant growth support techniques in order to formulate the best combination that will ensure the highest plant productivity and soil health, in order to continue to support the food needs of the world, as well as the need to protect and support our ecosystems and environment.

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