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Francois J. Hartnett
Bard College

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The Fungus Among Us: The Effect of Copper Fungicide on Arbuscular Mycorrhizal Fungi and Implications for Sustainable Agricultural Systems

Senior Project Submitted to
The Division of Social Studies
of Bard College

by
Francois Jasper Hartnett

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1.0 Introduction

On May 4th, 2010, Achim Steiner, who, at the time, was the executive director of the United Nations Environment Programme (UNEP), gave a presentation at the Conference on Climate Change, Agriculture, and Food Security in Nairobi, Kenya (Steiner, 2011). In his presentation, Steiner considered the issue of sustainable development from an agricultural perspective. His interest in agriculture stems from the fact that, to him, agriculture is one of the areas of our global economy that will either determine whether the world can “reinvent” itself in terms of our development pathways and development paradigm over the next two decades or continue down the same pathway, which in the history of modern, industrial agriculture the maximization of production has been the guiding principle for agricultural research, commercialization and policy (Steiner, 2011).

Steiner points out that while we have made tremendous efforts in increasing production, “agricultural productivity…is actually declining.” (Steiner, 2011). In a similar vein, Steiner acknowledges the remarkable ability of modern agriculture to expand production but is appalled by its neglect of mining the very resource it relies on - “...it is a business operation working towards its own demise.” (Steiner, 2011). Not only is our modern agricultural system forcing farmers to mine the very capital they rely on, which is reducing soil fertility and increasing the degradation of ecosystems, it also forces farmers to rely on the continued availability of water and other external inputs such as fertilizer and seeds.

My views are very much in line with those of Achim Steiner. For me, the importance of agriculture also lies in its transformative potential to adapt and cope with our changing world for both environmental and social ends. Although it exists outside the scope of this paper, the social aspects of agriculture are very important to me. These issues are most prominent in the developing world, where industrial agriculture has not yet completely taken over. Despite the failures of our current agricultural paradigm in much of the developing world, including its neglect of smallholder autonomy and production (IFAD, 2013), the loss of local knowledge and practices and the increased reliance on external inputs and the global market, all of which exacerbate the environmental degradation associated with our agricultural paradigm, there is still so much promise to revitalize agricultural communities. I refer to an “agricultural paradigm,” as opposed to “agricultural practices” or “agricultural production” because the issues with agriculture are systemic ones, and need to be addressed from an interdisciplinary perspective. Recognition of the overlap between the social, political, economic and environmental components of agriculture is crucial in order to pursue the systemic changes we need. The capitalist ideologies that are so ingrained within our society have fostered a hostile and dominating attitude towards the natural world and those who labor with it. They have also marginalized small-scale agricultural communities in favor of large-scale production.

From an environmental perspective, the importance of agriculture is impossible to ignore. Agriculture accounts for roughly 14% of global greenhouse gas emissions (IPCC, 2014). Some of the leading emission sources are fertilizer production and use, soil carbon loss from ploughing and methane emissions from livestock. In addition, it has been argued that the conversion of forests to agricultural areas accounts for a similar amount of greenhouse gas emission as agriculture itself (WBCSD, 2008). The runoff of fertilizer and pesticides also leads to eutrophication[1] and water contamination, thereby undermining freshwater and marine ecosystems (WWF, 2017). Finally, agricultural practices have led to wide scale soil degradation

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[1] Eutrophication is a process that results from the excessive accumulation of nutrients in a waterway, which can usually be attributed to agricultural runoff. The abundance of nutrients leads to algal blooms, which reduce water clarity and harm water quality. When these algal blooms die, microbial decomposition drastically depletes dissolved oxygen, creating a hypoxic dead zone lacking sufficient oxygen to support the majority of aquatic life (Chislock et al., 2013).

both in terms of the nutrient content of the soil and the soil microorganisms living within it, the importance of which is just beginning to be realized. Despite the environmental issues that result from our agricultural practices, there is tremendous opportunity within agriculture to combat them. However, it will require a reconstruction of our agricultural paradigm to one that adopts a systems perspective and works with nature rather than against it.

One of the most interesting and important points that Steiner makes in his presentation is that throughout the last few decades the environmental/sustainability movement has been tangled in a complex relationship with the agriculture/food security movement – bound by resources and reality and yet, more often than not, moving in separate directions (Steiner, 2011). Steiner presents the issue of climate change as an opportunity for us to make a transformative change in food security and agriculture, and make agriculture part of the solution as opposed to part of the problem. However, he emphasizes that “we need to change the lens with which we look at the problem, because if you simply follow the same pathway as we have done over the last thirty or forty years you will not produce transformative solutions.” (Steiner, 2011). I would like to draw the connection here between Steiner’s comment and the struggle of the organic movement. The organic movement presents an interesting case study because of what it strived to be in theory versus what it became in practice. Furthermore, the organic movement was the first major movement against the environmental degradation that accompanied agricultural practices, and strived to produce transformative solutions in favor of sustainability. The following section will present a brief history of the organic movement up to the present, ending with the contemporary notion of organic or sustainable agriculture.

1.1 The Organic Movement

Over the course of the 20th century, there was a divergence in attitudes about farming systems. While the majority of farming systems became ever more reliant on mechanization, chemical inputs and pest control, there was a minority movement, originating in Europe that began to formulate an alternative view of farming systems. From the early to mid 1900s, there were a number of key figures who helped develop and spearhead a new agricultural paradigm that was characterized by an emphasis on the importance of soil and maintaining soil fertility (Lockeretz, 2007). This became known as the organic movement, and was a reactionary movement that evolved in parallel to industrial agriculture.

One of the most notorious examples of the historical consequences of our agricultural practices in the U.S. is the 1930s Dust Bowl. The origins of the Dust Bowl can be traced back to 1838 when John Deere and his partner invented a steel plow capable of plowing the prairie's tough soil (Montgomery, 2007). By the mid 1800s, Deere was selling roughly ten thousand plows a year (Montgomery, 2007), and, consequently, capital soon began to replace labor as the limiting factor in agricultural production. Not only would this have implications on how labor was employed on farms but, more importantly, it set the stage for an ecological and humanitarian disaster in the form of large scale soil erosion. Aldo Leopold, who was a conservationist, forester, educator and writer, was one of the first to address the issue of soil loss in the American Midwest. Leopold believed that "the destruction of soil is the most fundamental kind of economic loss that the human race can suffer." (Meine & Knight, 2006). However, he was also concerned with an ecological consideration of the soil, which is evidenced by his formation and promotion of a land ethic. Leopold recognized that because the larger field of agriculture was developing before ecology was born, ecological concepts were slow to be incorporated into agricultural practices (Leopold, 1949). Furthermore, by the very nature of their techniques, the farmer is forced to modify their environment more radically than a forester or wildlife manager. By Leopold’s time there was enough of an incentive for farmers to begin modifying their agricultural management practices. He referred to this as “biotic farming” (Leopold, 1949). In this way, Leopold helped set the stage for the adoption of the organic movement in the U.S.

Lady Eve Balfour, who was a farmer and a leading figure in the organic movement in England in the early part of the 20th century, addressed the issue of soil erosion in her best-selling book, *The Living Soil*. For Lady Balfour, the foundation of society began “within the soil itself” because we are fashioned out of the soil and the products of the soil maintain our existence (Balfour, 1943). Lady Balfour recognized that despite our dependence on the soil for survival, we had come to view it in the same way as early settlers viewed the wilderness – something that needed to be conquered or tamed. She even went so far as to liken man’s conquest of nature to the Nazi conquest of Europe (Balfour, 1943).

Lady Balfour understood that soil erosion is a constant phenomenon, even in fertile soils. It only becomes a problem when the rate of erosion exceeds the rate at which soil biota and natural processes can convert the mineral rock into soil (Balfour, 1943). So while the process of erosion was not new, its acceleration through the use of steel plows was apparent by the mid-1900s. “Probably more soil has been lost since 1914 than in the whole previous history of the world.” (Balfour, 1943).

In addition to the issue of soil erosion, Lady Balfour also recognized the decline of soil fertility. “Soil is a mixture of the disintegrating mineral rock, and humus, with its population of micro-organisms.” (Balfour, 1943). It is these microorganisms and the products that result from their activity, that differentiate soil from subsoil. Lady Balfour believed that our world is dominated by a chemist’s perspective, which maintained that everything is composed of chemicals. This view led us to consider life and death as just a beginning and an ending. Instead, these are just two processes in a continuous cycle of life, along with growth, reproduction and decay (Balfour, 1943). By ignoring this principle, we have failed to conform to the "law of return," which Balfour borrowed from Lord Northbourne, who was another prominent figure in the organic movement. For too long we have considered the plant kingdom and the soil as a "factory" concerned only with converting inorganic material into metabolized material for the benefit of animals and humans (Balfour, 1943). Through the work of Lady Balfour and others like her, we started to realize that to fulfill this function, soils need to be managed properly.

The word "organic" was first used in an agricultural context by Lord Northbourne (Paull, 2014), who was an English agriculturalist. Northbourne coined the term "organic farming" in his book *Look to the Land*, which was published in 1940 and served as a manifesto of organic agriculture (Paull, 2014). Northbourne was writing during the time of the rise of chemical agriculture. With the *Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or other Gases, and of Bacteriological Methods of Warfare*, which was signed in 1925, the wartime chemistry of the first World War was suddenly stopped; however, its power for agriculture was just beginning to be realized. In the interwar years, the chemistry of poisons and the synthetic fixing of nitrogen through the Haber-Bosch process were repurposed for application to agricultural systems (Paull, 2014). This process gave us the ability to manufacture fertilizers by utilizing the abundant nitrogen reserves in the atmosphere, which, at the time, increased crop yields to support a growing population (Smil, 2001). The process was once described as the miracle of "turning air into bread" (Hager, 2008). However, Northbourne saw things differently. In his book, he set up a clash of agricultures – "Organic versus chemical farming" (Northbourne, 1940) – and established an ideological and philosophical foundation for differentiating between the two. A farm that relied on “imported fertility...cannot be self-sufficient nor an organic whole” (Northbourne, 1940).

One of Northbourne’s key contributions to the organic movement was to take Rudolf Steiner’s concept of the “farm as [an] organism” (Steiner, 1958) and develop from it a named practice that constituted a differentiated form of agriculture – organic farming (Paull, 2014). He argued in support of the holistic view that “the soil and the micro-organisms in it together with the plants growing on it form an organic whole” (Northbourne, 1940). Sir Albert Howard, another proponent of the organic movement, was correct in saying that “the first duty of the agriculturalist must always be to understand that he is a part of Nature and cannot escape from his environment. He must therefore obey Nature’s rules” (Howard, 1947).

Both Northbourne and Balfour recognized and emphasized the importance of the soil and the microorganisms within it. Their work formed the basis of the organic movement, which was eventually formalized in standards by the U.S. government. In 1990, the U.S. Congress required that the USDA establish a national standard for products to be considered organic (Savage, 2013). While the USDA wanted to incorporate more science into the organic definition so it would reflect what was safest for both consumers and the environment, the organic community believed the definition should only require that organic farms use only natural inputs (Savage, 2013), despite the fact that this definition is vague and can lead to an abuse of the natural environment if it stands alone. When the National Organic Standards were published in 2002, they reflected the preference of the organic community (Savage, 2013). Because of this, there has been substantial critique of the formalized standards of organic agriculture. Kristine Nichols, former chief scientist at the Rodale Institute, spoke specifically about this. “...I’ve been on these farms where it’s a monoculture of lettuce or spinach and it certainly meets the organic standard, but the dirt that’s being used as a growing medium has no life in it. And yet those people are able to capture those premiums.” (quoted in Greenaway, 2018). Despite this inconsistency in the organic movement, a broader awareness is evolving around the importance of soil health (Greenaway, 2018).

The organic sector has split into two distinct sides. One side is characterized by corporate, industrial agriculture, which is profiting off of the organic standards, while the other consists of farmers and farming institutions that are addressing the issue of soil health within the context of conventional farming systems. Even the USDA is strongly promoting soil health through the Natural Resource Conservation Service (NRCS), whose work consists of providing financial and technical support for farmers and ranchers in the U.S., in order to promote conservation on the ground. An area in which we can see the results of their work is in the growth of sustainable farming practices in the Midwest. Despite the fact that only Roughly 25 percent of land in the U.S. is managed as no-till, and of that, only 6 percent is continuous no –till (ASABE, 2010). Furthermore, these systems utilize a lot of herbicide, which replaces the need to till, and do not use cover-crops so their soil is quite degraded (Mother Earth News, 1984). However, through the efforts of the NRCS over the last decade, a subculture of the no-till movement has formed – promoting a whole range of practices including cover crops, managed grazing and diversification of crops (Greenaway, 2018). These forms of regenerative agriculture and their subsequent benefits, both environmentally and financially, are encouraging farmers to step out of the agriculture mainstream.

While the organic movement failed in its ability to institutionalize sustainable farming practices and ideologies, its pursuit of an alternative and sustainable agricultural system has persisted. Scientists and farmers alike are starting to realize the importance of soil health and what that consists of. In the prewar era, emphasis was put on cover crops and crop rotation in agricultural systems. However, with the rise of chemical agriculture, there was neglect of soil health and, for the remainder of the 20th century, agricultural systems were dominated by bare soils and herbicide application. Recently, there has been a growing recognition of soil ecology and emphasis on managing for soil microorganisms as well. Historically, the lack of emphasis on soil microorganisms did not come from the lack of knowledge about these organisms or their importance, but rather from the fact that the science around these organisms is still relatively new.

As we develop this new agricultural paradigm we need to adopt a systems perspective and start working with the natural world, as a means of replacing our unsustainable inputs with ecosystem services. Of all the soil microorganisms responsible for plant and soil health, mycorrhizae play a key role. My senior thesis is an attempt to engage with the question of sustainable agriculture through a scientific lens. I explore the potential of arbuscular mycorrhizal fungi (AMF), which is a soil-microorganism, in the context of sustainable agriculture, considering its role in terrestrial ecosystems and its implementation in sustainable farming systems. My project also consists of an experiment in which I studied the effect of copper fungicide on AMF. My experiment served as a way to involve myself with the kind of scientific research that is necessary as we look to evolve our agricultural paradigm in ways that are consistent with the natural world.

2.0 Mycorrhizae

Over the course of natural history, fungi have evolved two major nutritional groups. The first consists of saprotrophs or decomposers, which produce enzymes that allow them to utilize the carbon in dead organic matter as their energy source, while the second are characterized as symbionts that gain access to carbon directly from primary producers (Maser et al., 2008). In the case of the fungal symbionts, this symbiosis can take on a several guises. Lichens, which are a symbiosis between certain algae and fungi, represent mutualism (Maser et al., 2008), as do mycorrhiza. A mycorrhiza, from the Greek ‘mykos’ meaning ‘fungus’ and ‘rhiza’ meaning ‘root’ (Allen, 1991), is a mutualistic symbiosis between a host plant and fungus, located in a root or root-like structure. In this symbiotic relationship, energy moves primarily from the plant to the fungus and inorganic resources move from the fungus to the plant (Allen, 1991). All mycorrhizae are composed of an external hyphal matrix and an exchange surface between the plant and the fungus. Fossil records provide evidence of an early appearance of mycorrhizal fungi in plant roots, and it has been proposed that the move from aquatic to terrestrial ecosystems by plants depended in part on their coevolution with mycorrhizae (Allen, 1991; Maser et al., 2008).

The hypha (singular form of hyphae) is the basic body of a fungus, and is comprised of one or more cells surrounded by a cell wall (Celine, 2017). Hyphae are often described as strings or threadlike filaments that increase the surface area of the fungus in the surrounding soil. The mycelium is the vegetative part of the fungus and is comprised of a collection of hyphae in a particular area. Unlike the hyphae, the mycelia can be visible to the naked eye in the form of mold (Celine, 2017).

In a mycorrhizal association, the fungus colonizes the host plant’s root tissue either intracellularly as in endomycorrhizae (which will be explored in detail below) or extracellularly as in ectomycorrhizae (Figure 1).

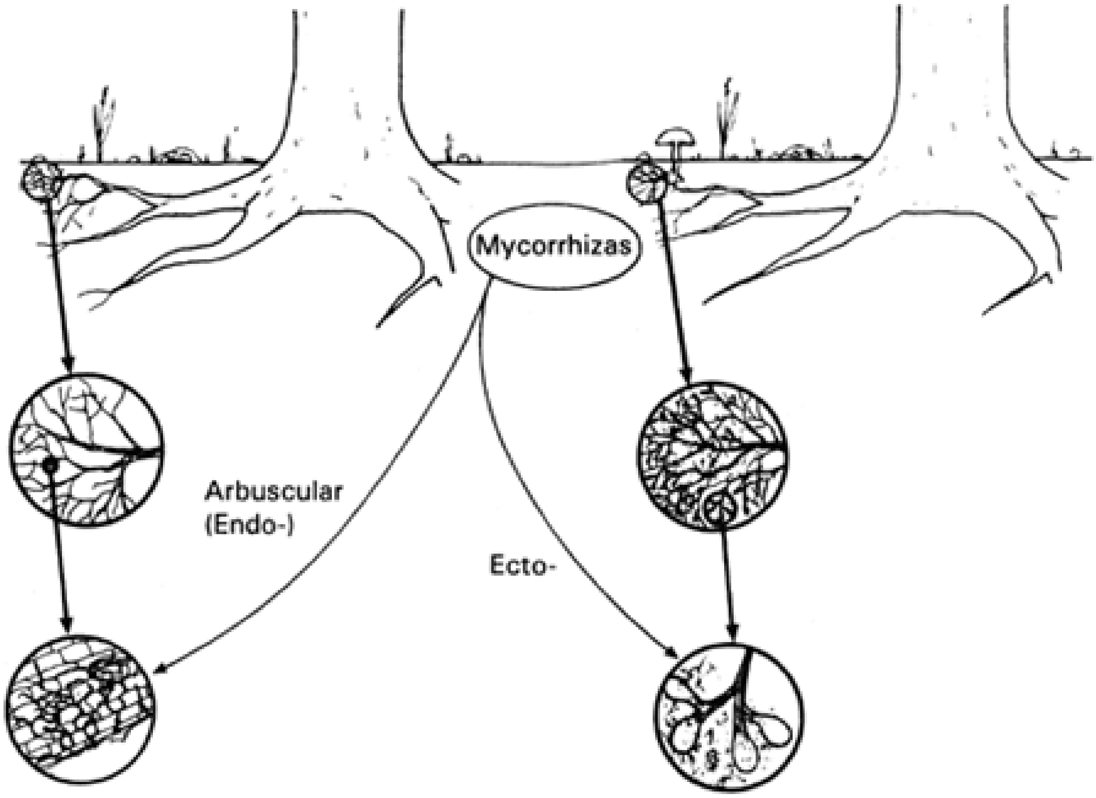

Figure 1. Diagram of endo- and ectomycorrhizae. (Adopted from FAO).

It has been speculated that ectomycorrhizae evolved later than endomycorrhizae, along with the host trees that they now typically colonize, which includes members of the pine, oak, birch and poplar families (Maser et al., 2008). In ectomycorrhizal associations, the fungus usually encloses the surface of the host plant's feeder rootlets with a fungal mantle, and, therefore, the absorption of water and nutrients by the plant is mediated by the enclosing fungus (Maser et al., 2008).

Despite the fact that throughout the 1900s research began to show that intimate biological associations such as mycorrhizae are extremely important and widespread in terrestrial ecosystems, mutualistic symbioses were generally considered to be relatively unimportant in ecological and evolutionary process (Allen, 1991). This view is now known to be false. In fact, this mutualistic symbiosis may be one of the most important biological associations regulating both community and ecosystem functioning (Allen, 1991). In the past few decades, research on mycorrhizae has begun to focus on their use in agricultural systems. Arbuscular mycorrhizal fungi (AMF) are a type of endomycorrhizae that have great potential to be incorporated into sustainable agricultural systems. The following sections will provide a general overview of AMF and then focus on their potential in agricultural systems.

2.1 What are AMF?

AMF are ubiquitous in terrestrial soils and can account for as much as 50% of the microbial biomass found there (Allen, 1991; Bücking & Kafle, 2015; Rodriguez & Sanders, 2015). Over the course of the last 450 million years, they have coevolved with the majority (roughly 70-80%) of terrestrial plants (Smith et al., 2011), and played important roles in the development of plant communities (Van der Heijden & Sanders, 2002). The AM symbiosis plays an important role for the nutrient uptake of numerous plants, including many staple crops such as wheat, corn and soybean (Bücking & Kafle, 2015). AMF have been described as “keystone mutualists” (Rillig, 2004) in terrestrial ecosystems, since primary production in most terrestrial ecosystems is limited by resource availability belowground (Rillig, 2004). The AM symbiosis is a type of endomycorrhizal association, meaning that the hyphae not only grow inside the root of the plant but also penetrate the cell walls of the root and can become enclosed in the cell membrane (Bonfante & Genre, 2010). This association is characterized by the formation of intracellular structures such as arbuscules and vesicles (Rillig, 2004; Verzeaux et al., 2017). Arbuscules are the primary site of nutrient exchange between the plant and the fungus, while vesicles are storage structures for energy, which is stored in the form of neutral lipids (Solaiman & Mickan, 2014).

The extraradical mycelium (ERM) of AMF acts as an extension of the root system for the host plant (Bücking & Kafle, 2015), and performs a variety of functions including formation of spores; formation of runner hyphae, which explore soil and new roots to be colonized; and nutrient uptake (Rillig, 2004). In terms of nutrient uptake, the most important benefit of AMF is the increased uptake of phosphorus (P) and nitrogen (N). However, AMF also increase the uptake of sulfur and manganese, as well as trace elements such as copper and zinc (Bücking & Kafle, 2015). Furthermore, AMF also provide non-nutritional benefits to their host plant such as resistance to several abiotic and biotic stresses including drought, salinity, heavy metals, pathogens and herbivores (Rillig, 2004; Bücking & Kafle, 2015).

2.2 AMF Biology

AMF belong to the fungal phylum Glomeromycota and are obligate biotrophs, which means that they rely exclusively on the carbon supplied from their hosts in order to survive (Rillig, 2004; Solaiman & Mickan, 2014; Bücking & Kafle, 2015). The one exception to the reliance of mycorrhizae on their hosts for survival is the survival of their spores (Maser et al., 2008). Fungal reproductive structures are either sexual or asexual. Fungi produce spores either for genetic recombination (sexual reproduction) or as a means of escaping, both spatially and temporally, environmental stresses. The reproductive structures of the fungus can either be dispersed by wind, water or animals, or they may simply remain dormant in the soil, waiting for opportune environmental conditions (Maser et al., 2008).

The obligate biotrophic nature of AMF and the fact that host plants can suppress their mycorrhizal colonization, especially under conditions of high nutrient supply, has led to the assumption that the host plant is the dominant player in this symbiosis (Bücking & Kafle, 2015).

However, the roughly 450 million years of co-evolution between the plant and the fungus has allowed the fungus to devise strategies that help to control the nutrient transport to its host (Bücking & Kafle, 2015). Bücking and Kafle 2015 describe the symbiosis as a dynamic “biological market,” in which “reciprocal reward mechanisms” ensure there is fair trade between both the partners in the AM symbiosis. These mechanisms, however, can be undermined by ecosystem disturbances (van der Heijdan & Sanders, 2002; Smith et al., 2011; Ryan & Graham, 2002). Further complicating things is the fact that AMF and their plant hosts form a complex network of interactions.

The external hyphal structures of AMF are highly interconnected, which results from hyphae colonizing neighboring plants and the formation of “hyphal fusions” (Verzeaux et al., 2017) between independent mycelia. This interconnectedness allows both partners in the AM symbiosis to choose among multiple trading partners, while also forcing both parties to compete with other partners for nutrient or carbon resources (Bücking & Kafle, 2015). These mycorrhizal networks are important in that they allow for the long distance transport of nutrients, water and stress chemicals between host fungal communities and host plants, including plants of different taxa (Verzeaux et al., 2017) (Figure 2).

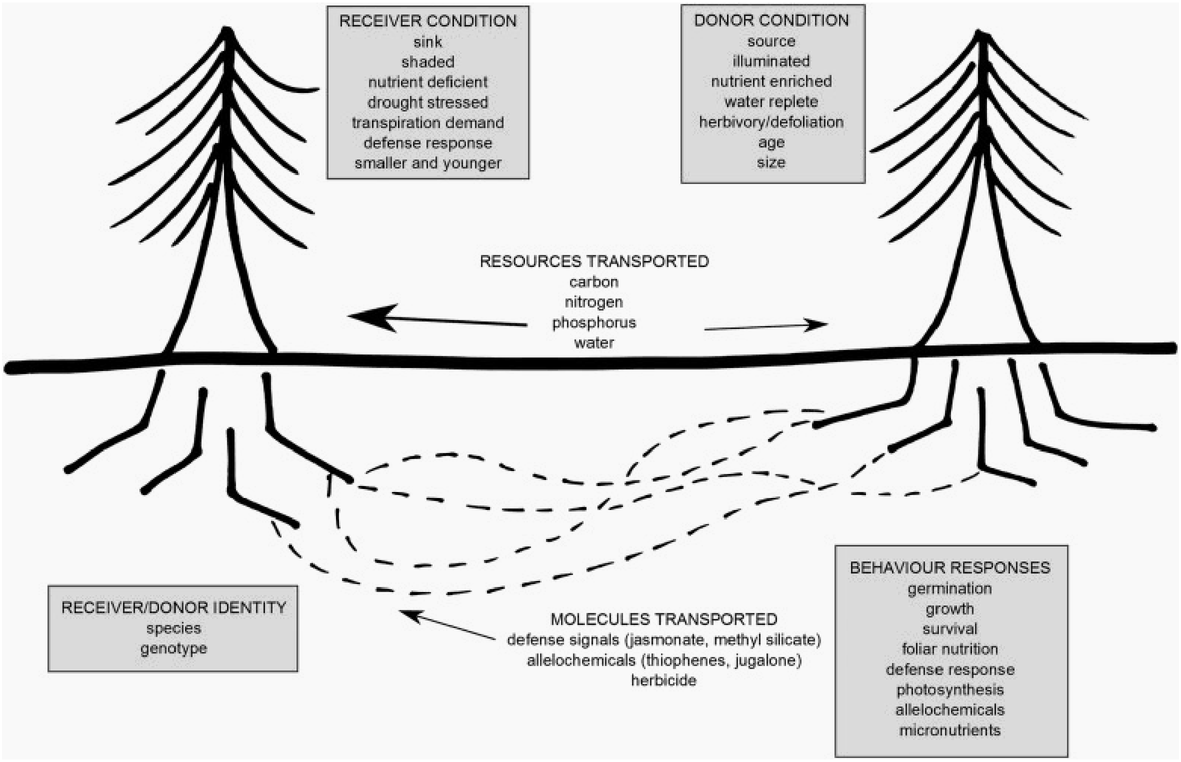

Figure 2. Mycorrhizal networks. (Source: Gorzelak et al. 2015, reproduced with permission of the author).

In addition, mycorrhizal networks have been discussed as potential pathways for the transport of N from donor plants to recipient plants (Bücking & Kafle, 2015). The direct hyphal pathway for resource transfer between plants that is provided by mycorrhizal networks separates valuable resources away from potential disruptions such as competition with soil microbes, fauna, chemical absorption of nutrients to soil particles or physical disturbances of the soil structure (Simard et al., 2012). The transfer of nutrients occurs predominantly by advective mass flow, which is a passive flow that is defined as the concentration of a medium multiplied by the rate at which the medium is flowing (Cushman-Roisin), and is driven by source-sink gradients generated by interplant nutrient differences (Simard et al., 2012).

In exchange for access to nutrients and water, and protection from biotic and abiotic stresses, the host plant transfers between 4% and 22% of its assimilated carbon to the AMF (Bücking & Kafle, 2015). Therefore, AMF can act as an additional carbon sink in the soil.

2.3 Nutrient Benefits of AMF

<u>2.3.1 Mycorrhizal roots expand uptake pathways for nutrients</u>

Plants that are colonized by AMF – utilizing what is called the mycorrhizal uptake pathway (MP) – double the uptake pathways for nutrients relative to roots that are not colonized, using the plant uptake pathway (PP) (Bücking & Kafle, 2015). The PP involves the uptake of nutrients via what are known as high- or low affinity uptake transporters, which reside in the root epidermis or root hairs (Bücking & Kafle, 2015). For nutrients with low soil mobility such as P, uptake via the PP is limited by the development of soil depletion zones (Allen, 1991; van der Heijdan & Sanders, 2002; Bücking & Kafle, 2015; Smith et al., 2011), which form around the root. In contrast, the uptake of nutrients via the MP increases the volume of soil explored due to the hyphae and is performed only by high affinity nutrient transporters located in the ERM (Bücking & Kafle, 2015). From there, nutrients are trans-located along the hyphal network to the intraradical mycelium (IRM), which is located in the root cortex (Bücking & Kafle, 2015) (Figure 2).

Unlike ectomycorrhizal roots, which are enclosed by a fungal sheath and, therefore, restrict the nutrient uptake via the PP, AM roots are structurally unaltered and can thus theoretically utilize both pathways for nutrient uptake (Bücking & Kafle, 2015) though AMF are able to suppress the PP to a varying degree. A strong suppression of the PP will result in a higher mycorrhizal dependency of the host by shifting the ratio between the two pathways to favor the MP (Bücking & Kafle, 2015).

In sum, plants that are colonized by AMF have the potential to utilize two pathways for nutrient uptake, which increases the likelihood of sufficient nutrient uptake. This becomes especially important when considering nutrients that are more difficult to access such as phosphorus.

2.3.2 AMF and P-uptake

Although phosphorus (P) is critical for plant growth and makes up roughly 0.2% of dry weight, it is one of the most difficult nutrients for plants to acquire (Smith et al., 2011). Soils contain a large amount of P that is linked to C-containing compounds to form PO (Plassard & Dell, 2010), which make it difficult for plants to access due to its low solubility and mobility. The majority of the organic phosphorus exists as phosphate esters (Plassard & Dell, 2010), and due to its negative charge, it adheres strongly to the mineral fraction of the soil. To be used by either the plant or the fungus, the phosphate group of PO compounds needs to be released from its ester bond linking it to C (Plassard & Dell, 2010). Therefore, both plants and fungi secrete phosphate enzymes (Plassard & Dell, 2010) that are able to free it up. In addition, there is a low level of freely available orthophosphates (Pi), which are the simplest phosphate compounds (Plassard & Dell, 2010), and are easily absorbed by both plants and fungi.

The difficulty of plant P-uptake is illustrated by the fact that, in spite of high P-levels in soils, farmers apply large amounts of phosphate fertilizers. Consequently, global reserves of phosphate fertilizers are rapidly being depleted (Smith et al., 2011; Rodriguez & Sanders, 2015). Furthermore, P fertilizers are becoming increasingly expensive and their availability is subject to political and economic pressures, which, in turn, are exasperated by the need to increase global food production (Smith et al., 2011). In light of this, we need to do a better job of managing soil P levels. One of the ways in which we can achieve this is through the maintenance of AMF in agricultural soils.

When plants take up Pi through the cells in the root epidermis, including the root hairs (direct pathway), Pi concentrations in the rhizosphere are depleted because replacement cannot keep up with uptake. This is known as the “depletion zone” (Allen, 1991; van der Heijdan & Sanders, 2002; Bücking & Kafle, 2015; Smith et al., 2011). Because plants (as well as fungi) take up P as negatively charged Pi ions, it requires high metabolic energy (Smith et al., 2011). Therefore, plants have evolved a variety of strategies that facilitate Pi-uptake; the most common of which is the AM symbiosis.

Plants that form AM symbioses have two distinct pathways for P-uptake, which involve different cell types, different Pi transporters and access P from different regions and volumes of soil (Smith et al., 2011) (Figure 2).

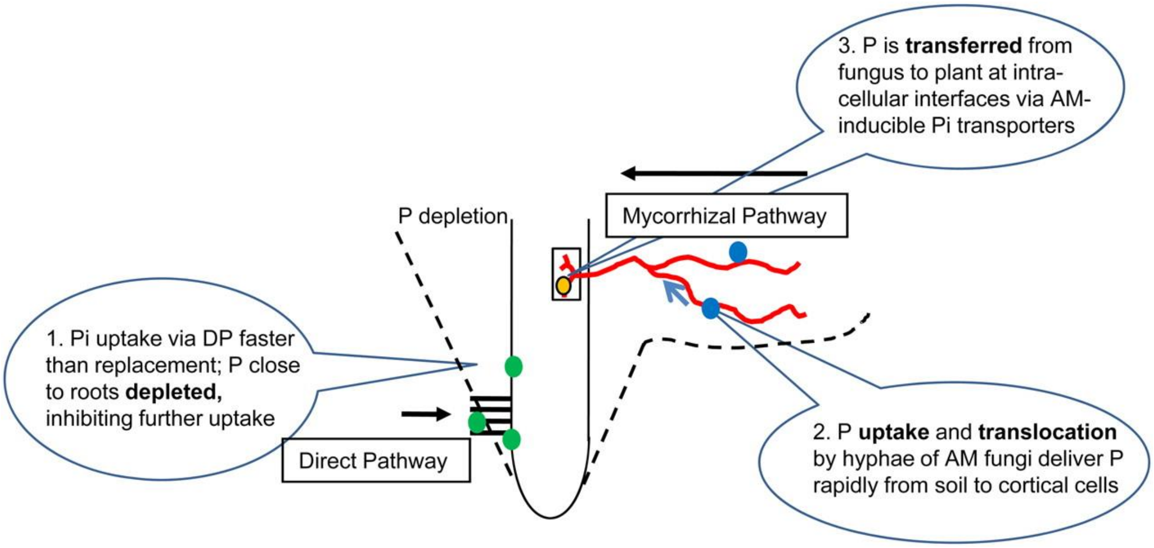

Figure 3. The two pathways of P uptake in an AM root involve different regions of the root, different cell types, and

different Pi transporters. In the direct pathway (DP), Pi is absorbed from the rhizosphere by plant Pi transporters in epidermis and root hairs (green circles) close to the root surface. Uptake is normally faster than replacement by diffusion from the bulk soil, resulting in reduced Pi concentrations (depletion) close to the roots (callout 1). In the mycorrhizal pathway, Pi is taken up into AM fungal hyphae by fungal Pi transporters (blue circles) several centimeters from the root and translocated to intracellular fungal structures (arbuscules and hyphal coils) in root cortical cells (callout 2). Plant Pi transporters, induced in colonized cells (yellow circle), transfer Pi from the interfacial applast to plant cortical cells (callout 3). (Source: Smith et al. 2011, reproduced with permission from the author and editor).

The direct pathway, which involves the plant root, is only able to access Pi in the soil solution that is close to the roots, and involves the expression of genes encoding high-affinity Pi transporters (PiTs). The second pathway, which involves the AM hyphal network in the soil, absorbs Pi via fungal high-affinity PiTs, at distances up to several centimeters from the root zone (Smith et al., 2011), thus extending the depletion zone. Furthermore, the small diameter of fungal hyphae (smaller than that of roots) allows access to narrower soil pores, thereby increasing the soil volume explored. The direct and AM pathways potentially occur independently, and there is a highly variable contribution of P between the two (Smith et al., 2011; Verzeaux et al., 2017). The two pathways operate at the same time but not necessarily in the same region of the root. The direct pathway is most effective immediately behind the root apex; while farther form the root, the AM pathway will contribute to P-uptake (Smith et al., 2011).

In addition to providing a separate and effective pathway through which P is delivered to the cortical cells in the root of the plant, the AM pathway can reduce Pi depletion in the rhizosphere (Smith et al., 2011). The AM pathway is by no means secondary or optional, and is still present whether soil P availability is high or low. High-P fertilization can significantly reduce the percentage of root length colonized by AMF (Ryan & Angus, 2003; Smith et al., 2011); however, this does not imply plant suppression or control of fungal activity. Instead, Smith et al. (2011) found that the reduction of root length colonized is a result of increased root growth, which, consequently, reduces the ratio of colonized to non-colonized root length.

Extremely high P application, however, has been observed to reduce arbuscule development and AM fungal biomass (Ryan & Angus, 2003; Smith et al., 2011).

2.3.3 AMF and N-uptake

Nitrogen (N) fertilizers are currently the most applied nutrient fertilizers utilized in agricultural systems. Since the invention of N fertilizers, we have seen a nearly ten-fold increase in the application of N fertilizers; however, despite the promise of greatly increased yields, there has only been a three-fold increase in yield (Tilman et al., 2002). Therefore, the nitrogen use efficiency (NUE), which is defined as the N contained in the plant divided by the N applied or the percentage of N that is used by the plant, in conventional crop systems has drastically declined (Verzeaux et al., 2017). Roughly half of the N applied to the soil is not used by the crop and is instead taken up by the soil microbiome or is lost through volatilization and leaching (Verzeaux et al., 2017). Consequently it is becoming increasingly important to enhance NUE in agricultural systems for both environmental and economic gains. There are management practices that can reduce the amount of N lost such as improving the synchrony between N application and plant N-demand, and ensuring that soils are not carbon limited. Drinkwater et al. (1998) argue that by maintaining sufficient C-levels in the soil, the excess N could be taken up by the soil microbiome, which would allow the N to be stored and then slowly released to the plant through mineralization. However, despite these management strategies, N losses to the environment are exceedingly high. Therefore, AMF may play a key role in improved N management.

Although the main contribution of AMF to plants in terms of nutrition is through the uptake of P (Smith et al., 2011; Verzeaux et al., 2017), they also contribute to the uptake of N.

While it is clear that AMF transfer N to their host plants through the mycorrhizal network, the contribution of AMF to the N-uptake of their host plant is still under debate. Some research has shown that plants colonized by AMF have a direct benefit of increased N-uptake from the fungus (Verzeaux et al., 2017); however, others have suggested that this is just a consequence of an increased P supply (Bücking & Kafle, 2015). The inorganic N sources nitrate (NO_3^-) and ammonium (NH_4^+) are highly mobile in soils, and therefore, the rhizosphere is less likely to become N depleted. Therefore, it is expected that the external mycorrhizal network does not increase the host plant's access to N in the soil (Bücking & Kafle, 2015). However, it has been suggested that the AMF uptake system has a higher affinity for NH_4^+ than the uptake system of plants, thereby enabling the fungus to take up NH_4^+ from soils with low N concentrations (Bücking & Kafle, 2015). The benefits of N-uptake provided by AMF are further evidenced by the fact that under N starvation, the plant triggers a signal that promotes colonization by AMF (Bücking & Kafle, 2015). The transfer of N from the ERM to the IRM is regulated by the C supply of the host plant, which demonstrates that the host plant is able to regulate fungal gene expression for the uptake and assimilation of N, and can trigger fungal N transport (Bücking & Kafle, 2015).

The AMF hyphal network is of particular importance in intercropping systems that utilize N_2-fixing cover crops, since it has been suggested that the N fixed by these plants can be transferred to neighboring, non-N_2-fixing plants (Verzeaux et al., 2017) through the hyphal network. Because there is a growing interest in intercropping legumes with grain crops in sustainable farming systems, the N-transfer mechanism that is supplied by AMF may be critical in supporting grain production in those models.

By providing an additional pathway for nutrient uptake, AMF can increase the overall uptake and the uptake efficiency of nutrients for their plant hosts. The following section will explore another benefit provided by AMF - their role in soil carbon dynamics.

2.4 AMF and Soil Carbon

2.4.1 Soil Carbon

It has long been known that the soils represent a large carbon reservoir. Of the total carbon that is found in terrestrial ecosystems, 80% is located below ground, in the soil and roots (Ontl & Schulte, 2012; Rillig & Allen, 1999). The soil organic carbon (SOC) pool is an important element of terrestrial ecosystems and is a crucial regulator of C fluxes between the biosphere and the atmosphere (Zhu & Miller, 2003; Solaiman, 2014). Furthermore, the mechanisms that influence SOC storage depend mainly on the net primary production and the distribution of photosynthates between above- and below-ground structures (Solaiman, 2014).

Until relatively recently, however, the extent to which AMF are involved in the sequestration of carbon has been overlooked (Allen, 1991).

2.4.2 The contribution of AMF to soil carbon

A plant’s photosynthetic process is sink constrained. Therefore, the ability of the host plant to deposit carbon in the soil via fungal transactions, increases the sink strength (Zhu & Miller, 2003), and, ultimately, allows for more photosynthetic activity. In other words, plants only have a certain amount of space in which to store the carbon they produce through photosynthesis. By providing an outlet for that carbon, AMF increase the photosynthetic capacity of their hosts. It has been found that roots that have been colonized by AMF receive 4-20% more photosynthate (carbon compounds such as sugars) than non-mycorrhizal roots (Rillig & Allen, 1999). This greater degree of carbon movement to the roots suggests that mycorrhizal plants allow for more phososynthesis.

AMF can contribute to soil carbon storage through the production of extraradical hyphae and a carbon-containing protein called glomalin (Rillig & Allen, 1999; Zhu & Miller, 2003; Rillig, 2004). In a review article, Zhu & Miller 2003 found that soil carbon that is derived from AMF can range from 54 to 900 kg ha^{-1}. While the percent of the root colonized by AMF can be useful for measuring nutrient uptake (Bücking & Kafle, 2015), percent colonization is a less important measure of soil carbon storage (Rillig & Allen, 1999). Instead, extraradical hyphal (ERH) length or biomass, or glomalin turnover may be a better indication (Rillig & Allen, 1999).

The hyphae of AMF represent three pathways for soil carbon sequestration. First, AMF could directly influence soil C sequestration through the growth and turnover of ERH in the rhizosphere and bulk soil, thereby contributing to soil carbon dynamics (Zhu & Miller, 2003; Solaiman, 2014). Staddon et al. (2002), found that ERH turnover averaged five to six days. However, it has been suggested that ERH have slow turnover because soil microarthropods, which are responsible for soil decomposition and mineralization processes, prefer saprophytic hyphae rather than AMF hyphae (Rillig & Allen, 1999).

The second important aspect of the AM fungal carbon-pool is the production of the glycoprotein glomalin by the AM hyphae (Rillig & Allen, 1999; Zhu & Miller, 2003; Rillig, 2004). Glomalin is found in a variety of soils and in quantities of several mg per g of dry soil (Wright & Upadhyaya, 1998). Wright et al. (1996) found that glomalin is only produced in significant amounts by AMF, and therefore, represents a carbon pool that is a direct consequence of AMF. Glomalin is roughly 20-30% carbon (Rillig & Allen, 1999), which, coupled with the recalcitrant nature of the protein (Wright & Upadhyaya, (1996) found that it requires autoclaving for its extraction from the soil), it has been suggested that glomalin may represent a more stable carbon pool (Rillig & Allen, 1999). Through the use of radiocarbon dating, it was found that the residence time of glomalin in the soil is between 6-42 years (Zhu & Miller, 2003). Glomalin also influences soil carbon storage indirectly by contributing to the formation of the hyphal sticky-string-bag, which helps stabilize soil aggregates (Zhu & Miller, 2003).

Third, the ERH is crucial for the formation and maintenance of soil structure, which influences all carbon and nutrient cycling (Rillig, 2004). The ERH contributes to the stabilization of aggregates via hyphal enmeshment and through the production of glomalin (Zhu & Miller, 2003; Rillig, 2004). The hyphal enmeshments can be thought of as a sticky-string-bag, in which the hyphae entangle and enmesh soil particles to form macroaggregate structures (Zhu & Miller, 2003). The physical protection of what would otherwise be labile C inside of macroaggregate structures plays a crucial role in soil C sequestration (Rillig, 2003), because macroaggregates protect carbon from degradation by soil bacteria.

The significance of the contribution of AMF to soil carbon dynamics becomes increasingly apparent when we consider how critical carbon is for sustaining soil health. Carbon is a source of carbohydrates that sustain microbiological life, and also plays an integral role in maintaining soil structure, which increases both water infiltration and water holding capacity, and gas exchange. In addition, the need for carbon sequestration from the atmosphere is becoming ever more important to help mitigate against global warming.

In light of the myriad benefits that AMF provide for our soils and terrestrial ecosystems, it is imperative that our agricultural practices consider how we can protect them in the soil.

3.0 Agriculture and AMF

Globally, industrial agricultural practices have significantly reduced the fertility of soils, making food production reliant on external inputs such as nitrogen and phosphorus fertilizers, which have many environmental consequences. At the same time, the increase in human population is putting increased pressure on agriculture to continuously maximize yields. Consequently, we need to find alternative means by which to provide food, while also reducing our impact on the environment. Sustainable agricultural systems use natural processes to obtain suitable levels of productivity and food quality while decreasing external inputs, in an attempt to lower costs and eliminate environmental pollution (Solaiman & Mickan, 2014). In light of this, greater emphasis is being placed on increasing the utilization of indigenous soil microbes that contribute to soil fertility and increase plant growth and plant protection. AMF are now recognized as essential components of soil ecosystems, and, therefore, research in AMF management in crop nutrition and production can underpin a sustainable agroecosystem (Solaiman & Mickan, 2014). AMF can influence crop growth, nutrition and production, even in phosphorus-rich soils; however, growing crops in less fertile soils could enhance the multiple benefits of AMF in agroecosystems, including decreased nutrient loss (Maser et al., 2008). Therefore, supporting native AM communities and incorporating them into our agricultural management practices will be key as we transition into sustainable farming systems.

Verzeaux et al. (2017) recognize three main requirements for AMF to successfully establish in agricultural soils: 1) the density of AMF propagules within the root zone; 2) chemical communication between plants and AMF hyphae; and 3) diversity of AMF communities within the soil. Key features in this association are the root, the internal fungal structures (arbuscules, vesicles and coils) and the external mycelium (hyphae) in the soil (van der Heijdan & Sanders, 2002; Smith et al., 2011), some of which can act as potential AMF propagules. AMF propagules are the sources of inoculants such as spores, hyphae and colonized root fragments (Verzeaux et al., 2017).

While AMF have been shown to have numerous potential benefits for soil health such as their contribution to soil structure (see Smith et al., 2011; Rodriguez & Sanders, 2015; Verzeaux et al., 2017), their use in agricultural systems is undermined by conventional agricultural practices. Soil disturbances such as tillage and crop rotations can have direct effects on the health and overall functioning of AMF. Furthermore, herbicide has been found to have both a direct and indirect effect on AMF. Most herbicide studies have focused on the herbicide glyphosate (see Druille et al., 2012; Druille et al., 2012), because it is the most widely used herbicide in the world. Lastly, the use of fungicides and soil sterilization also has a negative effect on AMF.

3.1 Importance of soil management for AMF

Soil management is an important aspect of all agricultural systems. Because of the numerous benefits of AMF including increased N and P uptake, protection from biotic and abiotic stresses and carbon sequestration, it is important that agricultural management practices promote native AMF as opposed to harm them. Generally, soil disturbance and the application of pesticides – fungicides in particular – are known to be harmful but currently play a large role in conventional farming systems. This section will review the evidence for the impact of tillage and pesticides, and consider alternative management.

3.2 Tillage

Soil tillage is perhaps the most common agricultural practice throughout history, and has had profound effects on soils (Montgomery, 2007). Although tillage has been shown to reduce both

AMF spore density and hyphal length in temperate and tropical soils, its effects on AMF communities has been varied (Ryan & Sanders, 2002: Jansa et al., 2006). Some AMF communities have been shown to increase under soil tillage, while other species are more abundant under non-tilled soils. Ryan & Graham (2002) reviewed twenty years of research concerning the effects of tillage on AMF in North American soils, which showed that lower P-absorption was associated with tillage. The disruption of the AMF external hyphal network caused by tillage was the main factor contributing to the reduced P-uptake in disturbed soils. The research also showed that the increased yields in no-till systems were only apparent in soils with suboptimal P levels; in soils with higher P availability, such as those typical in the American Midwest, conventional tillage systems were found to have higher yields (Ryan & Graham, 2002).

By disrupting the topsoil, which is where we find the majority of AMF propagules, tillage decreases the density of AMF propagules (Verzeaux et al., 2017). In contrast, no-till systems encourage an increase in propagule density and ultimately better colonization. Furthermore, due to the destruction of the hyphal network, tillage has an effect on the nutrient acquisition of plants and on soil carbon storage. When soils and AMF mycelium are disturbed, glomalin concentrations decrease (Wright & Upadhyaya 1998), which reduces the amount of carbon stored in the soil. Soil carbon can also be removed by soil erosion, therefore, the contribution of AMF to soil aggregate stabilization is important.

3.3 Herbicide

Because of the adverse effects of tillage, farming systems are increasingly moving toward no-till. While this is good for the promotion of AMF, it requires increased use of herbicides for weed control. Therefore, it is appropriate that research investigate whether or not herbicides are negatively impacting AMF. Since the great majority of herbicide tolerant GM crops being used are glyphosate-resistant, the majority of the research concerning the effects of herbicides on AMF has focused on glyphosate. Furthermore, glyphosate is a non-selective herbicide, and is the most commonly used herbicide in the world. However, because Monsanto has developed 2,4-D resistant crops, it is important that further research considers the effect of this herbicide on AMF.

There are two methods for herbicide application - application directly to the soil and application to the plant foliage. Druille et al. (2012) found that spore viability in soils untreated with the herbicide glyphosate were between 5.8- and 7.7-fold higher than in treated soils. Similarly, Druille et al. (2013) found a reduction of 40-46% in spore viability when glyphosate was applied directly to the soil; however, when glyphosate was applied to the plant foliage, spore viability was not different from the control treatment. Both Druille et al. (2012 and 2013) also found similar results for the percent of arbuscules on roots, with a decrease in the number of arbuscules found in plants grown in herbicide treated soil. Druille et al. (2013) found that arbuscules were 20% lower when glyphosate was applied on plant foliage compared to when it was applied to the soil. Furthermore, both papers by Druille et al. (2012 and 2013) found no significant effects of glyphosate on vesicles.

In addition to the direct effect of herbicide on AMF, herbicides can also indirectly affect AMF via their impact on the host plant. By damaging the viability of the host plant, herbicides may negatively affect AMF root colonization and spore and hyphal densities (Druille et al., 2013). Given both the direct and indirect effects of herbicide application on AMF, it is important for farm management systems to weigh the benefits of herbicide application against its costs.

3.4 Fungicide

Fungicides are used in agricultural systems to control for foliar pathogens such as downy mildew and late blight. While most fungicides are not used to target mycorrhizal fungi, their potential affects on these organisms need to be considered. In general, fungicides are applied in a number of ways, each of which needs to be considered in regards to its potential effect on mycorrhizal fungi. The first method is foliar application of the fungicide. This typically does not have an effect on mycorrhizae, which reside on the roots of the plant. Even though some fungicide material may reach the soil, the amount that reaches the root zone is not enough to have any significant, long-term effects, depending on the persistence of the chemical formula. The second method is a soil drench application of the fungicide, which can be detrimental to mycorrhizal fungi; particularly if it is applied before colonization of the root takes place (Plant Health Care, 2009). After root colonization takes place, mycorrhizal fungi tend to be less susceptible to fungicides applied with the soil drenching method because fungicides cannot kill fungal tissue embedded inside the root (Plant Health Care, 2009; Cornell Vegetable Program, 2016). Both foliar and soil applications of fungicides can lead to the accumulation of fungicides in the soil and the root tissue, which, over time, may have a detrimental effect on mycorrhizal fungi. The last method is a fungicidal seed treatment, which has been shown to have minor and inconsistent effects on mycorrhizal fungi (Plant Health Care, 2009). Even though some fungicides have been shown to have a negative effect when applied as a soil drench, the low concentration needed for seed treatment dissipates sufficiently to allow for colonization during early root growth (Plant Health Care, 2009).

Copper fungicide is one of the most commonly used fungicides in the world, and has been used in both conventional and organic farming systems for a long time. Its use as a fungicide can be traced back to the famous “Bordeaux mixture” (Cornell Vegetable Program, 2013; van Zweiten et al. 2007), which was unintentionally discovered in the 1880s. At the time, farmers in the region were using a paste mixture of copper sulfate and lime on the grapes in their vineyards that bordered highways to deter passer-bys from eating them (van Zweiten et al. 2007). However, the French scientist Millardet observed that these grapes were also free of downy mildew, and by 1885 he had completed a series of experiments that confirmed that the mixture controlled downy mildew (van Zweiten et al. 2007).

When used for pathogen control, copper is usually applied in its fixed form (which includes copper sulfate, copper oxide, copper hydroxide, copper oxychloride sulfate and copper ions linked to fatty acids or other organic materials), which lowers its solubility in water (Cornell Vegetable Program 2016). It is usually applied in a spray solution, which is actually a suspension of copper particles, and these particles persist on the plant surfaces after the spray dries (Cornell Vegetable Program, 2016). Each time the plant surface gets wet, the copper deposits gradually release their copper ions, which provides residual protection against plant pathogens. The slow release of copper ions also reduces the risk of phytotoxicity to plant tissues (Cornell Vegetable Program, 2016).

In order to identify the fungicidal effects of copper (Cu) on mycorrhizal fungi, Graham et al. (1980) utilized a pot study to investigate the impact of “basic copper fungicide,” applied to the soil, on the growth of citrus seedlings and on root colonization and hyphal development of the mycorrhizal fungi *Glomus intraradices*. The authors found that the growth of the seedlings inoculated with *G. intraradices* was reduced logarithmically with increasing Cu concentrations. Additionally, regression analysis revealed a negative logarithmic correlation between *G. intraradices* colonization in the root, or hyphal development outside the root and Cu (Graham et al. 1980). Furthermore, there was a significant linear relationship between mycorrhizal colonization or hyphal development and the decrease in both leaf P content and plant growth (Graham et al. 1980). The authors attributed the poor plant growth to P deficiency, prompted by the inhibition of mycorrhizae colonization by Cu. In addition, a study by Giovannetti et al. (2006) showed that the commercial fungicide Ramid 30 Pb, whose active ingredient is copper hydroxide, completely inhibited the germination of spores of *Glomus mosseae*, which is an AMF, even at the minimum doses tested.

The following section will present the experiment I ran, which tested the effect of copper fungicide on AMF using onions (*Allium cepa*) as a model plant in a pot experiment run in a greenhouse. Copper fungicide is widely used in organic agricultural practices. In theory, organic agricultural systems are concerned with the maintenance of soil fertility and the soil microbiota, and while certain organic agricultural practices promote these things, other practices, such as copper fungicide application for disease control comes at a cost to important soil processes.

Copper fungicide is used to control powdery mildew, downy mildew, black spot and rust. Since it is not a broad spectrum fungicide, it is hard to say for sure whether it will have an effect on AMF; however, since it is a fungicide it is likely that there will be an effect. The questions my experiment looked to answer are: 1) does copper fungicide effect AMF? and 2) is there a difference in effect between foliar and soil application of the copper fungicide?

4.0 Experiment

4.1 Methods and Materials

4.1.2 Study site, study materials and growing conditions

The experiment was conducted in a greenhouse located at Montgomery Place (Red Hook, NY 12571), between March 5th and April 1st, 2018. Average temperature in the greenhouse throughout these months was 78.75^0 Fahrenheit. Bulbs of *Allium cepa* (walla walla onions) were obtained from Johnny's Selected Seeds. *Allium cepa* is a herbaceous biennial and is the most widely cultivated taxon in the family Alliaceae (Priyadharsini et al., 2012). Onions are highly mycorrhizal-dependent, and mycorrhizal colonization of onion plants has been shown to improve plant growth and yield under normal and stressed conditions (Priyadharsini et al., 2012). Soil was collected from the fields surrounding the Bard Farm. It was assumed that the soil there would contain a large amount of AMF since all the plants growing there are highly mycorrhizal-dependent. The fungicide used was Bonide Liquid Copper Fungicide Concentrate, which is approved for use in organic farming systems. The main active ingredient is Copper Octanoate. Plants were grown in pots 4" squared x 3.5" tall.

4.1.3 Experimental design and fungicide application

The experiment utilized four different treatments (A, B, C and D) with fifteen replications. For treatment A, onions were grown in field soil, which was assumed to have abundant AMF spore. Soil for treatment B was autoclaved at 121^0 C, for 45 minutes to achieve sterile conditions. Treatment C utilized field soil and was treated with a foliar application of fungicide. Treatment D also utilized field soil but was treated with a soil drench application of fungicide. Both treatments C and D used the same amount of copper fungicide, which was roughly 300 μL or 0.29574 mL; however, while treatment D received all the fungicide at once with the soil drench, Treatment C received the fungicide at three separate times, with one week between applications. For each application, roughly 98 μL or 0.09858 mL was applied to the foliar tissue of the plants.

Copper movement in soils is affected by various factors such as soil composition and pH. In addition, copper forms very tight bonds with organic matter (more so than any other micronutrient) (Römkens et al., 2004), thereby increasing its residence time in soils. Given the characteristic of copper to stay in the soil matrix, it is important to test the effect of copper on various microorganisms such as AMF. Furthermore, by applying it both foliaraly and as a soil drench, my experiment allows me to consider the degree to which copper will be present in the soil and affect the microorganisms there, as a result of how it is applied.

4.2 Measurements

4.2.1 AMF root colonization

Plants were harvested 27 days after planting. Roots were washed with tap water and then the roots from each respective plant were cut into 1-1.5 cm lengths and placed into a cassette. 15 cassettes were collected for each treatment. The roots were then cleared of pigments and residual soil by soaking in a hot 10% KOH solution for 25 minutes. Afterwards, the roots were rinsed with water before soaking in a 2% HCl solution for 30 minutes. This serves to acidify the roots, which is important because the stain is acidic. The roots were then soaked in a Trypan Blue solution for 20 minutes to stain them. Finally, the roots were allowed to soak in a 1:1 glycerol to water solution for a few days, which removes the stain from the non-fungal parts of the roots, thereby allowing the fungal structures to be more easily visible.

Unfortunately, the destaining solution did not effectively remove the stain from the non-fungal parts of the root. As a result, I was not able to use the grid-line intersection method (Zaller et al., 2014) to quantify percent root colonization, and, instead, developed an alternative method. I took 5 roots from each cassette (in some cases I was forced to do less) and cut a 0.5 centimeter piece from each respective root. I then looked at these roots using a dissecting microscope and assigned each root a percent colonization based on the amount of hyphae and arbuscules I saw. I also noted whether or not each root had arbuscules or not. By subsampling independent roots from each plant, I was able to estimate an average colonization for each plant, which, in turn, allowed me to estimate an average for each treatment.

4.2.2 Biomass

After plant harvest, green biomass was separated from dead biomass. Biomass was oven dried for 72 hr at 70^0 C, and dry mass was compared between treatments.

4.2.3 Statistical analysis

Response variables were analyzed using Analysis of Variance (ANOVA).

4.3 Results

The ANOVA test comparing colonization between treatments were not significantly different (F=2.396 ; p=0.077). However, based off of Figure 4, there is a suggestion that there is a negative effect of copper fungicide on AMF (Figure 4). A post-hoc, targeted pairwise comparison (t-test) confirmed this, revealing a statistically significant difference between foliar application of fungicide and soil application of fungicide (F=4.925 ; p=0.034).

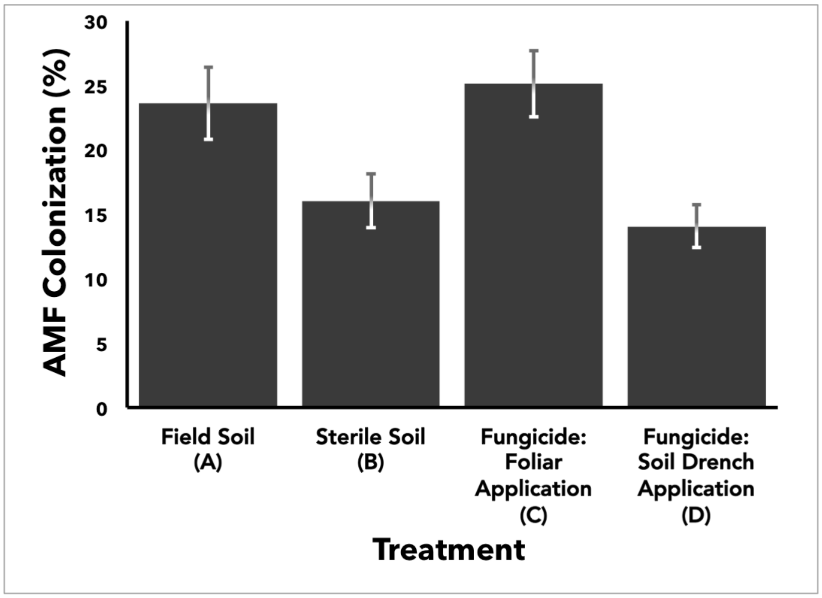

Figure 4 - Effect of Copper Fungicide on AMF Colonization

I also compared total biomass between each treatment (Figure 5), considering each treatment to be like a harvested crop as opposed to 15 individual plants, and, therefore, was unable to do any statistical analysis. Since I was unable to do any statistical analysis, the results should be interpreted as trends in the data as opposed to concrete findings.

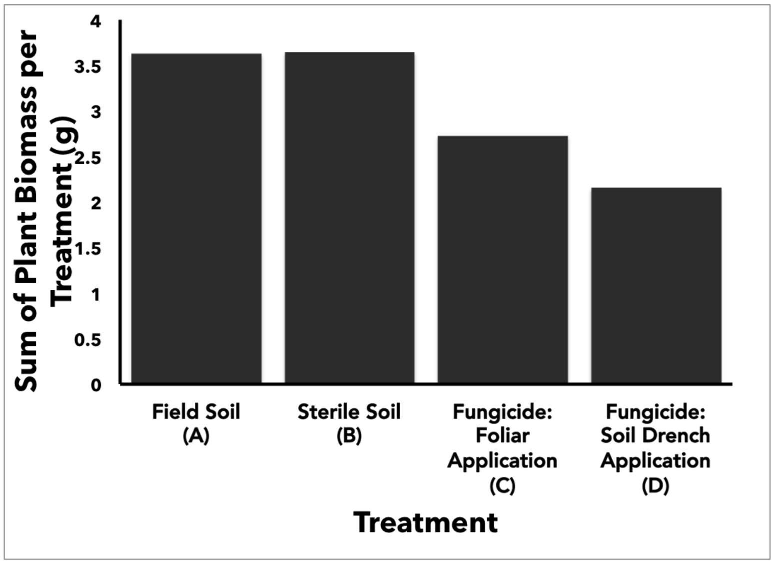

Figure 5 - Effect of Copper Fungicide on Plant Biomass

4.4 Discussion

Given the results of the experiment, I am unable to say for sure that there is a negative effect of copper fungicide on AMF, since my results were not statistically significant. However, the question of whether a fungicide effects a fungus is, perhaps, not that interesting, as one would assume it does. Furthermore, both the Graham et al. (1980) and the Giovannetti et al. (2006) studies showed that copper fungicide has a negative affect on AMF and explained how that was exhibited (see earlier section on fungicide).

The more interesting question my experiment explored was the difference in effect between the two application methods of fungicide – foliar and soil. While the results suggest that foliar application of fungicide does not harm below-ground mutualists, it is important to consider how copper moves within an ecosystem. Copper adheres strongly to soil particles, therefore the copper that is eventually washed off of the foliar tissue of plants can have implications for soil microorganisms. Furthermore, in the last decade or so, there has been a rise in fungicide application (most of which is applied foliarly), which is leading to large-scale soil and water contamination (Israel, 2013). Once the copper particles reach the soil surface, the question arises of how it will come in contact with belowground microorganisms. Since copper adheres so strongly to organic matter, it is hard to assume that the watering of the soil will lead to leaching of copper below-ground; however, since copper runoff into nearby waterways occurs (Römkens et al., 2004), it is safe to assume that some leaches belowground as well. The more obvious offender to copper accumulation in the soil is tillage. Yearly tillage coupled with foliar re-application of fungicide will eventually lead to copper levels in the soil that are similar to direct soil application of fungicide, and will negatively impact below-ground microorganisms such as AMF.

By looking at the difference in total biomass between the treatments, I can make some inferences about the indirect effects of copper fungicide on plant growth, via its effect on AMF. There was roughly a 68% difference in total biomass between treatment A (field soil) and treatment D (fungicide soil drench application), which leads me to believe that the reduction in AMF colonization as a result of the copper fungicide led to less overall plant growth. Since onions respond so well to AMF colonization, any effect on their symbiotic partner should be recognizable in the plant biomass. In contrast, the roughly 33% difference in total biomass between treatment A and treatment C (fungicide foliar application) is not as straightforward, since the pairwise comparison between treatments C and D showed that copper fungicide did not affect AMF colonization when applied foliarly. There is the possibility that the copper fungicide affected other soil microorganisms and thus influenced plant growth, however, more research is needed to investigate that. Lastly, the comparable biomass between treatments A and B (sterile soil) makes sense since autoclaving soil kills the microorganisms in the soil, thereby releasing nutrients.

The results from my experiment also have implications for organic farming systems. Since the copper fungicide I used is certified for organic agriculture, the results further highlight the inconsistencies within organic agriculture. The official mandate that organic farming systems are considered organic as long as they use natural inputs is absurd if these inputs affect the viability of the soil ecosystem. The lack of consideration of ecosystem effects within the organic agriculture community emphasizes the idea that profit-maximization takes precedence over soil health, which not only goes against the ideals of the original proponents of organic agriculture but is also fundamentally unsustainable, both in economic sense and an environmental one.

Eventually the resource in these systems will deteriorate and we will have run out of the capacity to provide substitutes.

Here, I would like to acknowledge some of the potential limitations in collecting and interpreting the data from this experiment. Due to my lack of experience identifying AMF structures on roots, I over-estimated the extent of AMF colonization within each treatment, especially in regards to the presence of arbuscules. This was most pronounced in the sterile soil treatment, in which I found far more AMF colonization than one would expect in sterile soil (mean=16.026), however, there were also irregularities within the rest of the treatment groups. This most likely contributed to the lack of statistical significance I found between each treatment. In addition, because I only collected data once, due to time constraints, I was only able to look at one subsample of all my data, which limited the potential results from my experiment. Lastly, when collecting the plant biomass, I forgot to separate the individual plants biomass within each treatment, and, therefore, was unable to do any statistical analysis to support my claims.

5.0 Conclusion

Over the last 100-1000 years, we have seen a horizontal expansion of agriculture (Steiner, 2011). Techniques such as slash and burn and other technological innovations have allowed us to open up new frontiers for agricultural expansion. However, the paradigm of horizontal expansion of agriculture is deceptive and, ultimately, self-defeating. Furthermore, by abandoning an ecosystem perspective of agroecosystems, we are risking the loss of naturally productive systems. If we are to make the transition to a sustainable systems approach to food production, we need to pay attention to soil ecosystems.

The conversion of what is often referred to as “wasteland” for agricultural production not only destroys ecosystems and reduces biodiversity, since our agricultural systems prioritize a handful of cash-crops, it also undermines ecosystem services that we rely on for water quality maintenance, nutrient and carbon cycling and climate control, among other things. The response from modern, industrial agriculture has been to intensify production on existing lands through the use of hybrid seeds, fertilizers and GMOs. While the intensification of production on existing lands is part of the answer, the current agricultural paradigm is pursuing it in a fundamentally unsustainable way. Instead of continuing our reliance on external inputs, which have all sorts of environmental and social ramifications, we need to focus on changing the structure of our agricultural systems and implementing sustainable agricultural practices. Intercropping systems allow crops to benefit each other as well as the agro-ecosystem as a whole, and have the potential to reduce competition between plants. Natural pest controls can be encouraged by maintaining natural biodiversity – i.e. through the construction of hedgerows. Lastly, by re-incorporating animals on farms, artificial fertilizer can be replaced with natural fertilizer.

In his presentation at the Conference on Climate Change, Agriculture and Food Security, Achim Steiner introduced the idea of a “vertical expansion” and intensification of agricultural systems. The example he presented is that of perennial food crops, which address issues of soil fertility, water retention and, above all, protection of topsoil. Despite the potential of perennial food crops, Steiner expressed concern at the lack of commercial investment and academic research on perennials.

The management of AMF in agricultural systems is another viable way of developing a vertical expansion of agriculture and promoting a more sustainable agricultural system. The symbiotic relationship between plants and mycorrhizae have large-scale ecosystem-wide consequences. The significance of their importance is due to the proclivity of most terrestrial plants to form them (Gorzelak et al., 2015). However, in order to manage for AMF in agricultural systems we will need to engage in further research on how we can encourage these naturally occurring symbiotic relationships in a human-managed environment. Furthermore, it will involve a disassociation from mainstream agricultural practices, which will have many social, economic and political implications.

My thesis is an example of the kind of scientific understanding of the natural world that we will need to accompany our future agricultural paradigm, if we are to make it truly sustainable. However, I do not believe that these efforts alone will be sufficient to address the challenges we face in the context of climate change, both in terms of food security and in general livelihood and continued existence. What we need is to adopt a systems perspective, to borrow Steiner's term, both in our agricultural systems and in the greater framework of our society.

While climate change is perceived as a major threat to agriculture, it is also an opportunity for agriculture (Steiner, 2011). By recognizing the carbon footprint of our current industrial agricultural system, we can begin to see the enormous potential within agricultural to assist in climate change mitigation, both in reducing the emissions from agriculture and its inputs and in its potential to contribute to carbon sequestration. However, these ideas are reliant on incentivizing farmers to make these changes. When we consider the co-benefits and win-wins that are present within agriculture, such as the fact that increased carbon in an agroecosystem benefits the system itself while also reducing the carbon emissions from agriculture, we start to realize the viability of a more sustainable agricultural system and the broader policy context that governs it.

For me, agriculture is one of the avenues through which we can make our transition into the post-carbon, climate apocalypse world we will soon be facing, not only in its ability to deal with some of the issues we are currently facing but also in establishing the kinds of agricultural practices that will become necessary when society as we know it collapses. The question is whether we can recognize and act on this potential before it is too late.

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