

It's All about Relationships: AMF-Plant Associations, the Rhizosphere, and Our Changing World

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Abstract

Arbuscular mycorrhizal fungi are obligate biotrophic organisms that facilitate nutrient uptake in exchange for organic carbon as part of a symbiotic association with their host plants. This symbiotic relationship is among the oldest known and has been important for the coevolution of both symbionts. This review explores the relationship between Arbuscular mycorrhizal fungi and their hosts, the role AMF play in the invasive strategies of certain plants, and how severe pressure affects the reciprocal relationship. This review hopes to add to the body of knowledge and to the conversation about these dynamic relationships and the timescale on which they operate, because this information will prove valuable as humanity attempts to manage lands and produce food in an unstable climate. Keywords: arbuscular mycorrhizal fungi, symbiosis, arbuscules, biotrophic, obligate biotrophic symbionts

Introduction

The discovery of AMF (arbuscular mycorrhizal fungi) in the roots of the fossil *Aglaophyton major* dates the symbiotic relationship between AMF and land plants to be more than 400 million years old and supports the idea that this dynamic relationship was key to the rise of terrestrial flora. Some go as far as to propose that this successful co-conquest of land provided the selective pressures that led to the lignification of plant cells, as well as to the development of some chemical defenses (Remy et al., 1994). AMF, classified as mycorrhiza from the Greek 'mycos' fungus, and 'rhyza' root, associate with 70-90% of all land plants, and are obligate biotrophic endosymbionts that provide plants valuable soil nutrients in exchange for organic carbon (Parniske, 2008). The term mycorrhiza, however, is not exclusive to AMF. It also includes a similar type of association that is not as invasive, more commonly known as ECM (ectomycorrhizas) because while the fungus penetrates its symbionts root, it does not penetrate the cell walls of individual root cells as AMF do.

While AMF are a monophyletic group that belong exclusively to the early diverging *Glomeromycota* that form associations with most land plants and are obligate biotrophs, ECM are not monophyletic, they belong to the *Basidiomycota*, *Ascomycota*, or the *Zygomycota* and form associations with roughly 3% of land plants and are not obligate biotrophs (Brundrett, 2002). The ECM-plant association, unlike the AMF-plant association, is facultative. ECM exist and reproduce independently of a symbiont and often produce aboveground sporocarps that, while often edible, can be poisonous. These include various mushrooms, morels, and the tremendously expensive truffles. AMF on the other hand do not reproduce sexually and produce no above ground fruiting structures. The entirety of the AMF life cycle occurs below ground, primarily in the rhizosphere.

The rhizosphere

While exploring these interactions, it is essential to take into consideration that these associations exist within complex systems with many players, all of which are subject to a varying number of pressures, while competing for a limited amount of resources. This below ground arena, which is directly affected by plant root exudates, is also known as the rhizosphere, and it includes biotic and abiotic factors. AMF share this arena with a multitude of organisms, including bacteria, archaea, viruses, nematodes, microarthropods, and oomycetes, as well as with fellow AMF and ECM species. This dynamic interface, because of the composition of its inhabitants, plays a major role in the health, nutrition, and growth of plants in both agro and natural ecosystems (Philippot et al., 2013). It is important to understand that the rhizosphere is the soil community near and directly affected by plant roots. This does not include the bulk soil communities with numbers that often exceed those of the communities in the rhizosphere (Philippot et al., 2013). Existing within vast, extremely complex and diverse communities that consist of

millions of species and billions of individual organisms (Bardgett, 2014), it makes sense that in order to survive and thrive, organisms would form symbiotic associations.

Getting the party started

The initiation of AMF-plant relationships occurs in the organism-divers rhizosphere and involves a series of chemical signals released by both the plant and fungus. Because the primary function of AMF in the AMF-plant relationship is to provide valuable nutrients, plants initiate the communication by releasing chemical signals into the rhizosphere in response to deficits in nutrients, primarily phosphorus and nitrogen (Bouwmeester et al., 2007). Most AMF spores do not require the presence of chemical signals to germinate, although strigolactones have been shown to stimulate spore germination. AMF spores undergo several germination events prior to the detection of chemical signals from a potential host. It is almost as if the fungus scouts for the presence of plant exudates, and then branches in the direction of the exudates found (Parniske, 2008). Strigolactones have been identified as one of the plant exudates that not only induce hyphal branching, but also stimulate the fungus to undergo the changes necessary to begin the symbiotic association (Akiyama et al., 2005; Besser et al., 2006).

Strigolactones were identified over 50 years ago as significant germination stimulants for the parasitic plant genus *Striga*, and it is thought that this genus adventitiously evolved to exploit the signaling mechanism of this ancient association (Parniske, 2005). Unlike the many facultative parasitic plants and like AMF, the plants in the genus *Striga* are obligate biotrophs. Much like parasitic fungi, *Striga* use a haustorium to siphon off water and nutrients from the host plant. “Although many *Striga* and *Orobanchae* species are components of natural vegetations and are appreciated for their colorful flowers, these parasitic plants are particularly known because they can be a nuisance in agriculture, where they can completely destroy harvests” (Bouwmeester et al., 2007). This mechanism by which parasitic plants exploit host-symbiont signaling systems in order to parasitize host plants is merely one example of how the diverse inhabitants of soil ecosystems interact and compete for resources. The AMF-plant strategy has proven to be a successful one, so much so that the disruption of these associations is essential for the success of some invasive species.

Divide and conquer

The success of invasive plant species often depends on their ability to disrupt AMF-plant associations. European invader *Alliaria petiolata* has been shown to suppress the growth of dominant hardwood tree seedlings in northeastern temperate forests (Stinson et al., 2006). *Alliaria petiolata*, also known as garlic mustard, is of the family *Brassicaceae*, a family that is known specifically for its production of plant secondary metabolites known as glucosinolates. These micromolecules are responsible for the plant’s pungent odors. The roots of this invasive species have antifungal properties and phytochemically impede AMF colonization vital to hardwood seedling growth and survival. Thereby suppressing the native species indirectly (Stinson et al., 2006). Given enough time, this suppression of native canopy seedlings has the potential to transform entire landscapes.

This begs the questions, why is *A. petiolata* so aggressive and successful in North American soils and not as aggressive in its native European soils? Is *A. petiolata* more toxic to AMF in North America than in Europe? In a landmark paper, Callaway et al. (2008) addressed these questions, while also pursuing the idea that some invasive species are more successful outside of their home range because they possess biochemicals novel to the ranges they invade. This gives them an advantage because the native AMF species have not coexisted with these invaders over evolutionary time spans, as opposed to the AMF species native to the invader’s home ranges, which have. This idea is also known as the novel weapons hypothesis. Callaway et al. (2008) found that growing *A. petiolata* in soils from four different regions of North America decreased AMF diversity thereby affecting the emergence, growth, and survival of native plant species from the same region, while having no significant effect on the AMF populations in its home ranges of Europe. This suggests that the long coexistence of *A. petiolata*, AMF, and other plant species in its home ranges has led AMF and native plant species to evolve resistance to the biochemicals *A. petiolata* exudes. So, one of the reasons why *A. petiolata* is so successful in North American soils is because it possesses antifungal biochemicals that negatively impact native AMF populations which has devastating impacts on plant species that depend on an AMF symbiont (Callaway et al., 2008).

Wolfe et al. (2008) found similar effects on ECM. *Alliaria petiolata* attacks AMF and ECM vital to the growth and survival of plants in ranges where it is invasive, while other invasive plant species form associations with generalist AMF species present in invaded soils and have the potential to out-compete native plant species. An important factor in the invasive success of *Salidago canadensis* is its ability to form associations with resident generalist or compatible AMF. Studies suggest that *S. canadensis* associates with compatible AMF and competes

against the native species. The success of this relationship has the potential not only to out-compete the native plant species, but also to significantly change the composition of AMF spores in the soil (Zhang et al., 2010). Invasive species often depend on their ability to disrupt existing associations to succeed, but these relationships are subject to current and changing environmental pressures.

Under pressure

Much like the ancient AMF-plant associations, these extant symbionts are under a variation of changing pressures affecting their relationship and ability to adapt to changing conditions. A key factor is the nutrient exchange that takes place inside the plant root cell wall, but outside of the plant's cell membrane with fungal structures called arbuscules (Parniske, 2008). In most cases, both symbionts provide nutrients for the other, but the ability of the symbionts to produce these nutrients is dependent on other pressures, mainly environmental factors.

Many studies have shown that AMF associations are beneficial to plants under water stress, but not many have shown what happens to the relationship when drought becomes terminal and both symbionts go into survival mode. When water is scarce, plants tend to close their stomata and cut off gas exchange to conserve the valuable water keeping them alive. Carbon becomes the limiting factor and the plant is no longer able to conduct photosynthesis. This could be a problem because the plant is no longer producing the organic carbon that it exchanges for increased access to water and phosphorus. It also raises the question, if the plant is unable to exclude its fungal symbiont, could the generally mutualistic relationship become parasitic if water scarcity continues?

In a remarkable study, Kiers et al. (2011) suggests a reciprocal relationship in which both plants and AMF have mechanisms to not only detect and favor symbionts that provide the most nutrients, but also are not enslaved to the other. This makes sense because AMF symbionts often have a vast network of hyphae and are not dependent on one plant. In another study, the AMF association of the grass chalk false-brome was observed under various light conditions. The results of this study suggest that when light is limited, photosynthate becomes the limiting factor and since organic carbon is scarce, the plant selfishly excludes its symbiont (Füzy et al., 2013).

How are plants able to terminate their AMF symbiotic association? Possibly because arbuscules are short-lived structures that are continually being degraded and regenerated (Parniske, 2008), and it has been shown that some plants can degrade arbuscules prematurely if adequate phosphorus is not being provided (Javot et al., 2007). Other studies have shown that the availability of the usable phosphorus in the soil also has a role to play in the formation of these associations (Gosling et al., 2006). Furthermore, this could be used to limit AMF colonization of plants in areas where the environmental conditions would tip the scales towards parasitism (Ryan et al., 2005). Which makes sense because it doesn't benefit the plant to form an association in which it gives up valuable organic carbon for phosphorus that is abundantly available.

Ultimately though, the goal in sustainable agriculture is to minimize the use of synthetic chemicals and encourage/enhance symbiotic relationships with both AMF and the nitrogen fixing bacteria of *Rhizobia*. The dynamics of soil ecosystems and that of the relationships between the organisms that inhabit and influence them has been and continues to be a hotbed for research, especially because of changing environmental conditions.

A change is gonna come

Per a report released by the National Oceanic and Atmospheric Administration, 2014 globally averaged temperatures over land and sea surfaces were the highest ever recorded since they began monitoring them in 1880. This doesn't necessarily mean that temperatures are going up worldwide, but it does mean that in certain areas temperatures are well above normal for extended periods of time, while other areas might be experiencing other changes, such as decreased precipitation or even colder temperatures. Climate change is perhaps the most pressing concern when it comes to global food production and land management strategies because of the diverse consequences of climate change in different regions of the world. Changes in temperatures along with rising atmospheric CO₂ levels and decreased access to water have the potential to adversely affect rhizosphere residents (Philippot et al., 2013). It will be interesting to see how the diverse life that exists within the rhizosphere is affected by climate change. After all, soil microorganisms have a greater capacity to adapt in contrast to their above ground symbionts.

More importantly, due to the multitude of organisms that exist within the earth's soil, it is possible that within that diversity exist organisms that are better suited for warmer or colder conditions (Philippot et al., 2013). In an interesting study Gavito and Aguilar (2012) isolated AMF from three regions that differ in climate (Finland, Denmark, Spain) to test if AMF from colder climates have an advantage growing in colder temperatures when compared to those from warmer climates and vice versa. They found that this was not the case for the AMF species

they isolated and studied. They found that all AMF isolates reduced development, particularly that of the external mycelium at 6°C and 12°C, regardless of their native climate, but all had comparable increases in development with increasing soil temperatures. Ultimately this suggests “AMF have a narrow window to develop in cold regions of the world where temperatures below 15C prevail, which ought to be exploited, especially in agroecosystems where management is aimed at improving crop performance and soil quality” (Gavito & Aguilar, 2012).

In a different study, Zoppellari et al. (2013) observed drought tolerance in *Zea mays*, which was inoculated with a consortium of AMF and rhizospheric bacteria, isolated under osmotic stress selective pressures. They found that plants inoculated with this consortium showed a higher tolerance to water deficiency and suggested that the use of AMF and bacteria selected for drought tolerance could be a suitable practice to improve crop performances in environments with low water availability.

While these two studies focus on drought tolerance and response to changing temperatures, Drigo et al. (2013) focused on the long-term effects of increased atmospheric CO₂ levels on rhizosphere carbon flow and microbial community dynamics. The study was conducted over the course of three years and among other things found that increased CO₂ over long periods of time increased the capacity of AMF to act as a carbon sink. Also, increases in atmospheric CO₂ led to significant changes in AMF community composition, similar in a sense to *Salidago canadensis*. These pressures are of a completely different nature and essentially select for AMF species capable of thriving under the conditions provided. Change in atmospheric CO₂ of course has broader and more severe impacts to rhizospheric interactions:

These changes will impact the downward flow of energy and interactions between plants and rhizosphere inhabitants. These responses will influence not only the soil community composition and biodiversity but also organic matter decomposition dynamics and resultant patterns of nutrient cycling with unpredictable consequences for numerous ecosystem properties such as plant metabolism, plant competition, direction and rate of succession, above and belowground community composition, ecosystem net primary production, and agricultural yields (Drigo et al., 2013).

Discussion

The Greek philosopher Heraclitus stated, “The only thing that is constant is change.” Climate change is happening and understanding its potential effects on the rhizosphere and its inhabitants will be essential for mitigating and possibly reversing its effects. The AMF-plant symbiotic relationship is one between equals and has endured over 400 million years. We human beings on average live about 70 years, which is nothing compared to the evolutionary time spans that these rhizospheric relationships have had to develop. Time is important to understand because human actions have the potential to significantly change the dynamics of these below ground relationships, which directly affects the above ground flora. Take for example the introduction of *Alliaria petiolata* to North America in the 1800s. The effects of this are currently being felt and will be felt for generations to come. Callaway et al. (2008) suggests that by transporting organisms far past their historical dispersal limitations, humans may have mixed species that do not share common evolutionary trajectories. This anthropogenic change has the potential to alter the landscape of North America for ages to come. Climate change, on the other hand, has the potential to transform landscapes around the globe and is one of the greatest challenges facing this generation.

This review was compiled around the driving idea that symbiosis in its most dynamic forms is one of the main drivers of evolution, and it highlights outstanding contributions to humanity’s collective knowledge about life in one of its most specific forms. More importantly, it is an ode to symbiosis. It is an ode to the driving force that led a fungal organism, with its controlled chemical might and diverse survival strategies, to specialize to the point of depending entirely on another organism to live and propagate. Symbiosis has led to a successful, important relationship, the importance of which ranges from agriculture to the global carbon cycle. It is important to further research and understand AMF-plant associations, particularly the role they play in the carbon cycle with regards to climate change. Further understanding of these symbiotic rhizospheric associations is essential for the development of sustainable agricultural systems, as well as proactive land management strategies.

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