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Stealing or living off crumbs: Exploitation of the mycorrhizal network by mycoheterotrophic plants

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Abstract

Mycorrhizal networks, though often unappreciated, play a crucial role in ecosystems by connecting over 90% of plant species worldwide, facilitating a symbiotic exchange of resources. Fungal symbionts provide water and soil nutrients for plants in exchange for photosynthesized sugars. However, some plants, known as mycoheterotrophs (MH), exploit this system by deriving both carbon and other nutrients from fungi, reversing the typical bidirectional flow. MH plants can be initial, partial, or fully mycoheterotrophic, with varying dependence on fungal carbon throughout their lifecycle.

Despite the evolutionary occurrence of mycoheterotrophy in various plant taxa, the mechanisms behind this resource acquisition and its evolutionary drivers remain poorly understood. Recent research suggests that full MH plants might have evolved to exploit the "take now, pay later" strategy, allowing them to extend their dependency on fungal carbon without reciprocation. Alternatively, the sanctions imposed by fungi to prevent exploitation of trade may be ineffective, enabling MH plants to persist unnoticed. Another hypothesis posits that MH plants are "living off crumbs" without impacting fungal fitness by being small and having minimal carbon requirements, thus making sanctions unnecessary.

Understanding the paradox of mycoheterotrophy—how these plants exploit fungal partners without apparent reciprocation—is essential. Future research should focus on the fitness consequences of mycoheterotrophy on fungi and autotrophic plants, and the exact mechanisms of resource exchange. This knowledge could enhance our understanding of parasitism, mutualism, and evolutionary strategies in plant-fungal interactions.

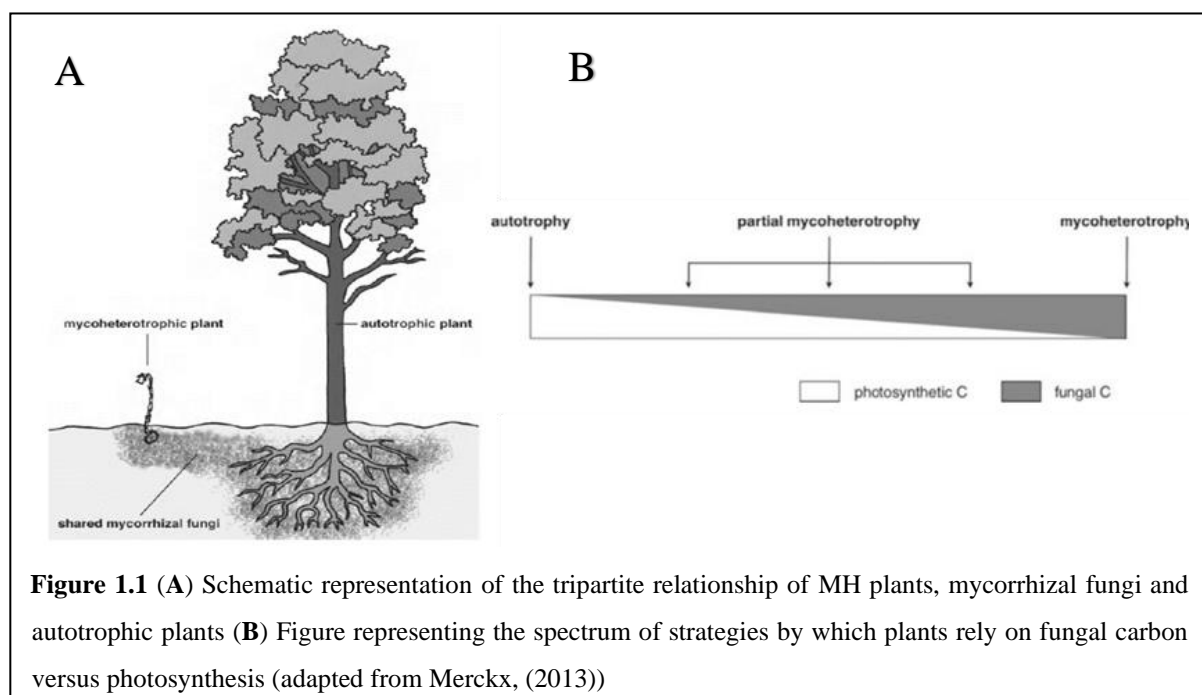
Introduction

When looking at most ecosystems, although hidden, the mycorrhizal network connecting plants cannot be ignored. Over 90% of plant species form connections with mycorrhizal fungi and occur throughout the world (van der Heijden et al., 2015). The purpose of this interaction between fungi and plants is to trade resources in a symbiotic manner (Smith and Read, 2008). In this network, most fungi are connected to multiple plant species and plants also interact with different fungi, including both ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) species (Giovannetti et al., 2004; Saari et al., 2005). The trade in this promiscuous network has been compared to the market economy, where individuals monitor the amount of resources they receive and adjust their export accordingly (Wyatt et al., 2014).

The purpose of this symbiotic interaction is to help overcome the most common constraint on plant growth worldwide which is nutrient limitation (Du et al., 2020). The mycorrhizal network serves as an extension of the root system which can improve the water and nutrient uptake of plants severalfold (Jakobsen et al., 1992). In exchange for these resources, plants trade photosynthesized carbon which usually does not limit their growth and send it to the fungi in the form of glucose and other sugars (Shachar-Hill et al., 1995). Thus, it can be said that mycorrhizal fungi are heterotrophic and rely on this trade as their main carbon source.

Besides capturing carbon-dioxide via photosynthesis, some plants have found a way to get their carbon from elsewhere. Unlike autotrophic (AT) plants, mycoheterotrophic (MH) plants not only get water and soil nutrients from fungal partners but also carbon (Smith and Read, 2008). This means that the usual resource flow changes from a bidirectional trade to a unidirectional flow from fungus to MH plants. Ultimately, these plants take the photosynthesized carbon from the fungus originating from another AT plant (see Fig. 1.1).

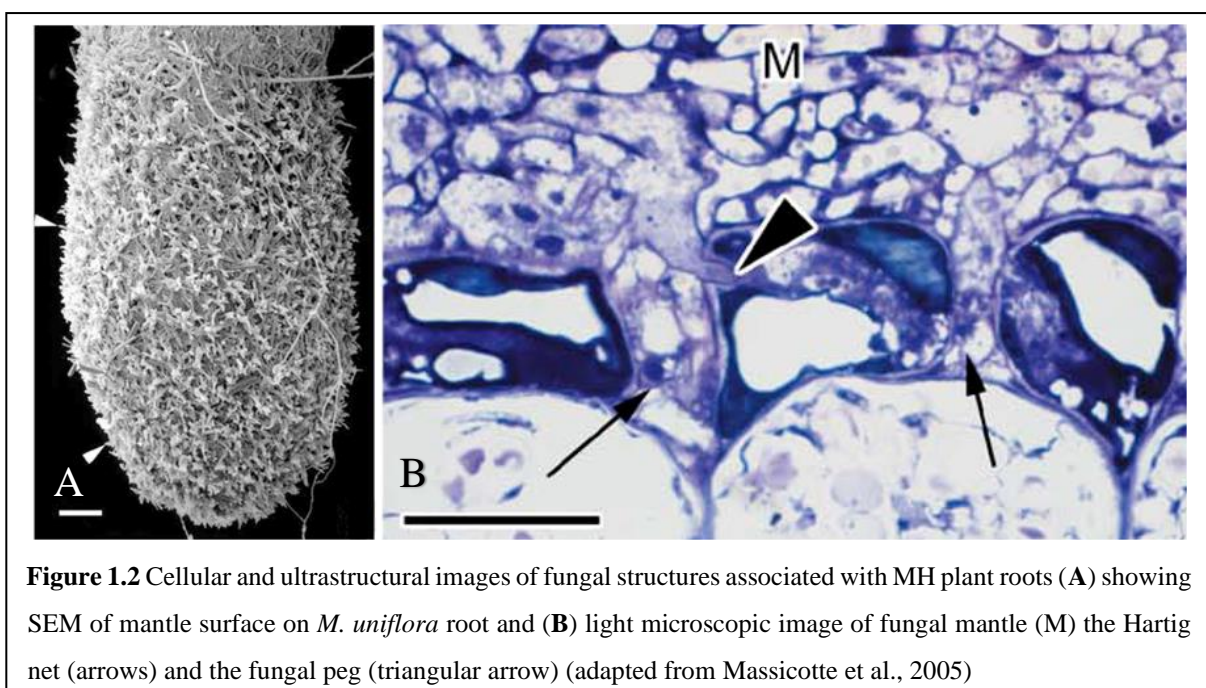
Most MH plants, for example green orchids, rely on this fungal carbon source at the beginning of their life cycle, and only get such support during early development. These initially mycoheterotrophic plants turn into fully autotrophic individuals and switch back to the usual bidirectional trade of resources with their fungal partners (Gebauer and Meyer, 2003). In many other species labelled as partial mycoheterotrophic however, this tendency to rely on fungal carbon continues throughout their life cycle. Depending on the season or on environmental conditions they can adjust their carbon uptake from the mycorrhiza (Giesemann et al., 2021).



Partial MH plants are still chlorophyllous and have the capacity to produce their own carbon through photosynthesis. Lastly, there exist fully mycoheterotrophic plants, which have lost their ability to photosynthesize and entirely rely on the mycorrhizal network as a carbon source throughout their lifecycle.

The exact way how MH plants get established in the tripartite relationship and connect themselves to the mycorrhizal network is not yet fully understood. It has been argued that for a seed from a mycoheterotrophic plant to start germinating, it needs to sense unique chemical cues coming from an already present mycorrhizal fungus (Bruns and Read, 2000). In most cases, MH plants are more specialized compared to autotrophic plants in terms of which fungi they associate with (Zhao et al., 2021). If these plants try to rely on an unsuitable fungal partner, they will not develop into mature plants or will not even germinate (McKendrick et al., 2000). Once a connection is made, the fungus creates a mantle around the seed and later on colonises the plant roots (Leake et al., 2004). From here on out, the plant accommodates its fungal partner in its roots and rhizome and acquires resources from it.

The mechanism of this resource acquisition is still not clearly identified on cellular and tissue levels. What is generally seen in MH plants, however, is that the fungal colonisation pattern of the roots are rather similar even in unrelated taxa. This overarching trend is that as the fungus colonizes the root tissues via straight hyphae, it eventually reaches a layer in the parenchyma, where coiled up hyphae form and reside continuously. Moreover, hyphae also pass to neighbouring layers where they lose their coiled form, and they get digested, possibly increasing the carbon and nutrient gain of the MH plant (Imhof et al., 2020). The Hartig net,



which is the net of hyphae extending into the root tissue has multiple functions, but is generally considered the structure necessary for resource trade in the mycorrhizal symbiosis (Smith and Read, 2008). From this Hartig net emerge so-called “fungal pegs” (see Fig. 1.2), which have been speculated to be the site of nutrient uptake in MH plants, but it has not been empirically tested (Massicotte et al., 2005). Fungal pegs structurally resemble plant cells specialized for short distance transport of solutes which support the same idea (Gunning and Pate, 1969).

To achieve a constant inflow of resources from the fungus to the roots, the MH plant must maintain its sink strength and be able to draw nutrients from the hyphae. It is poorly understood how this is sustained on a mechanistic level. The existing speculations suggest that these plants have to create a concentration gradient which would help in creating a draw-down effect (Finlay and Read, 1986). By converting carbon to sucrose, MH plants could potentially make the carbon unavailable for the fungus, since many species lack the necessary invertase needed to utilize sucrose (Parrent et al., 2009). Because the exact mechanism of resource transfer is not known, it is difficult to explain how MH plants seem to be able to exploit their fungal partners and maintain a unidirectional inflow of carbon and nutrients without giving anything back. For this reason, it is important to make an inventory of the fitness consequences of mycoheterotrophy for this tripartite interaction between the autotrophic plants, mycorrhizal fungi and mycoheterotrophic plants.

The Fitness Consequences of Mycoheterotrophy

Mycoheterotrophy has evolved at least 46 times independently in liverworts, lycophytes, ferns, gymnosperms and flowering plants (Merckx, 2013) and these plants occur almost everywhere around the globe. But despite this diversity, these unrelated taxa often show convergent evolution towards a specific morphology. By examining these common evolutionary trends, we can gain a deeper understanding of their adaptations and uncover how these plants persist with their unique lifestyle.

The majority of fully MH plants are discovered in dense tropical forests in the shaded understory, where most AT plants struggle to get established (Leake, 1994). Partially MH plants show similar trends but can also be found in open areas, for example bogs (Mathews et al., 2009). Another overarching trend is that most species seem to prefer moist environments with consistent rainfall and minimal seasonal variation (Linder, 2001).

When it comes to morphology, the most noticeable characteristic of MH plants is that they are very small and have a low biomass. This is mostly due to a loss or reduction of aerial features.

The shoots are often very thin and fragile (see Figure 2.1) not showing visible signs of secondary thickening and the leaves are either completely absent or reduced in full mycoheterotrophs (see Figure 2.1) (Leake, 1994). Moreover, the seeds of these plants are usually very simple and have reduced in size but are mostly produced in greater quantities than AT plant seeds (Arditti and Ghani, 2000; Maas and Ruyters, 1986). These adaptations suggest that a simplified blueprint is necessary for these plants to decrease their carbon requirements and allow them to survive solely from fungal carbon.

Since MH plants need to rely on their fungal partners for all their resources, it is not surprising that their subterranean morphology also shows convergent evolutionary trends, although there is a greater variety of characteristics in this case. Generally, the roots of these plants look very different from AT plant roots, being short and thick and often growing in a star-like pattern (see Figure 2.2) (Leake, 1994). Lacking root hairs, the resource uptake is ensured by having thick enough clumps that can house the mycorrhizal fungi inside (see Figure 2.2). As mentioned previously, there is a common trend in the fungal colonization pattern of these

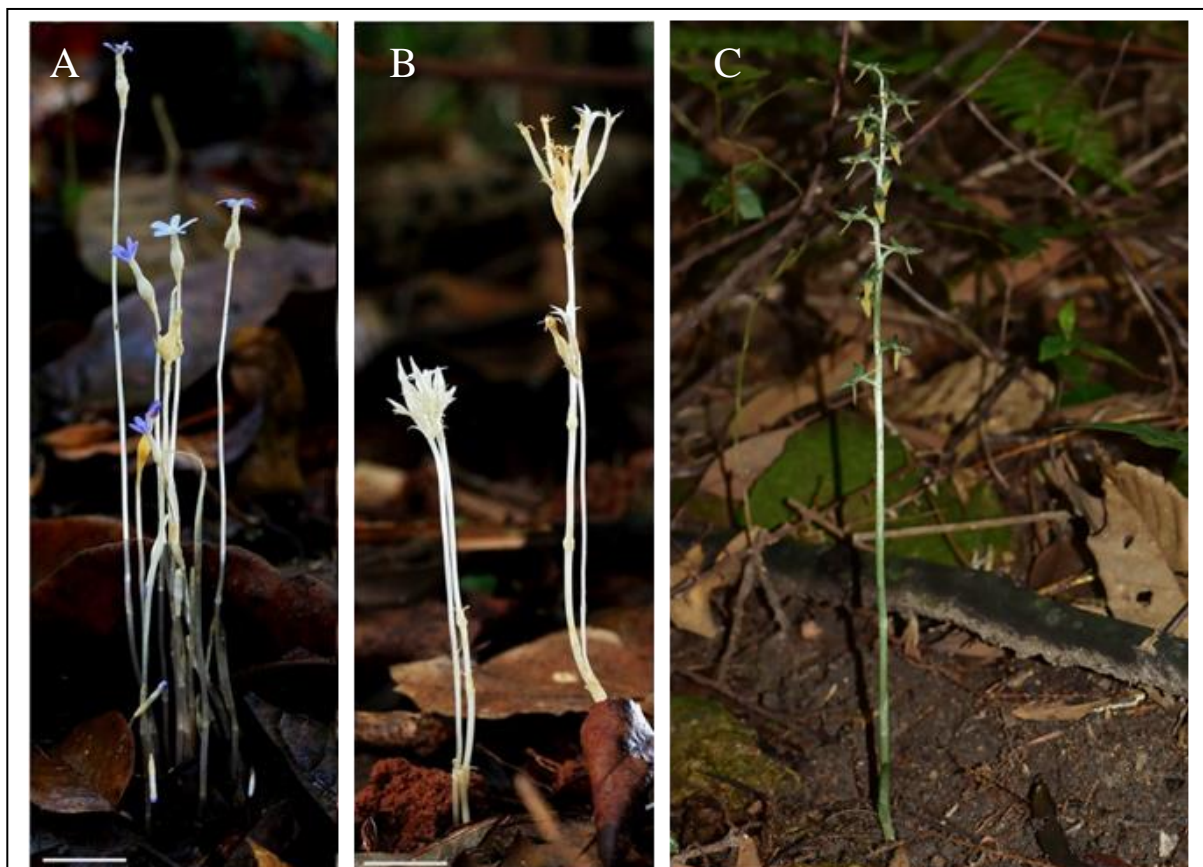
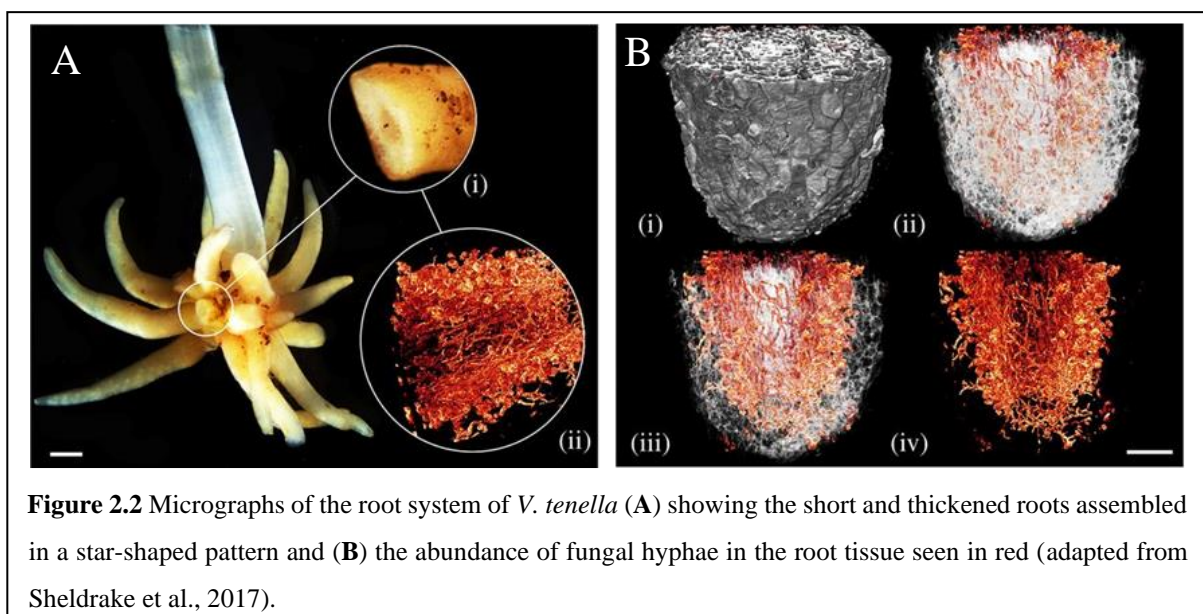


Figure 2.1 Images showing the convergent evolutionary trends in above-ground morphology in MH plants. Slender stems seen on (A) *Voyria tenella*, (B) *V. corymbosa* plants in a lowland tropical forest in Panama (adapted from Sheldrake et al., 2017) and (C) reduced leaves on a *P. guangdongensis* plant growing in a shady forest understory (adapted from Li et al., 2022).

thickened roots, where some tissue layers can accommodate the fungal hyphae continuously while in other layers they get digested by the plant (Imhof et al., 2020). Since such characteristics can be observed in a wide array of taxa, it can be speculated that these changes in morphology helped in the increase of nutrient uptake efficiency.

Lastly, there seems to emerge a common trend in the ecology of these plants, namely that they have a higher specificity for fungal partners than AT relatives (Taylor and Bruns, 1997). This could either indicate that they are selecting the most optimal fungal hosts to exploit based on how much resources they can obtain, or that more and more fungi reject these plants, and their range of available hosts decrease to the “naïve” species.

Based on an evolutionary ecology perspective, we could make the assumption that MH plants found an empty niche and started adapting to fill that niche. But what were the evolutionary drivers that lead to the invention of mycoheterotrophy? The most obvious answer outlined earlier is light availability. Since mycoheterotrophy allows these plants to obtain a portion or all of their carbon without photosynthesis, they can thrive in darker environments (Leake, 1994). They are able to outsource the task to surrounding AT plants capturing carbon at the canopy level and they can harvest that carbon through mycorrhizal fungi in the dark understory. This has been demonstrated in partially mycoheterotrophic orchids, where higher irradiance levels correlate with a greater reliance on mycoheterotrophy (Preiss et al., 2010). By occupying dark niches in the understory, MH plants can avoid competition by AT plants which would be more efficient at capturing carbon via photosynthesis under different conditions (Bidartondo, 2005).



Another hypothesis for a possible driver of mycoheterotrophy is the easier establishment in nitrogen poor environments (Read and Perez-Moreno, 2003). Alternatively, avoiding desiccation by growing within leaf litter might also drive plants towards mycoheterotrophy (Leake et al., 2008). These two drivers, although possible, cannot be applied for all cases and are just minor factors in the evolution of MH plants. Because these factors do not necessarily lead to mycoheterotrophy light availability is the best candidate for the main evolutionary driver.

To fully understand how this unusual and possibly parasitic interaction between MH plants and mycorrhizal fungi is possible, we first need to look at how mutualism is maintained between AT plants and their fungal symbionts. Explaining the stability of mutualistic relationships has always been a difficult subject, considering that organisms have selfish interests and are expected to cheat whenever the opportunity arises. Nonetheless, we see that the symbiosis between mycorrhizal fungi and AT plants are still maintained. To highlight the fact that selfishness plays an important role, this symbiosis is often described as reciprocal exploitation (Herre et al., 1999). It is also generally accepted that the occurrence of cheating, or its attempt, in mutualistic relationships is ever-present (Yu, 2001). Theoretically, these mutualistic relationships should break down because they are costly for both partners for two reasons. First, at the time of initial association the resource sharing abilities of the other partner cannot be assessed and second, due to the presence of cheating individuals (Kiers and Heijden, 2006). In view of these factors, it has been hypothesized that spatial structuring, namely that both plant and fungal partner are limited in dispersal, can stabilize these relationships. If there is a luxury resource exchange where a surplus resource is traded for a resource that is in high demand, it can also help in stabilizing mutualism. Lastly, if an organism can control who it associates with and also reward good partners and punish cheaters, it can lead to stability. Kiers and Heijden, (2006) argue that these conditions are likely to be met in case of mycorrhizal fungi and AT plants. Having established that normal plant-mycorrhiza interactions are stable and mutualistic or at least reciprocally exploitative, we need to examine the case of MH plants.

From the tripartite relationship of AT plants, mycorrhizal fungi and MH plants, the autotrophs should be looked at first. Since they do not interact directly with MH plants, the potential extra fitness costs are difficult to quantify in autotrophs. The ultimate source of carbon for MH plants is the photosynthesized sugars that originate from the surrounding AT plants, which would indicate that the plants lose some of their carbon to MH plants. However, no research has been done on whether the presence of MH plants increases the carbon expenditure of autotrophic

plants or not, so it is possible that there are no direct fitness consequences for them (Merckx, 2013). Even if expenditure increases, research by Corrêa et al. (2012) showed that carbon remains in excess, and an increase in export to fungal partners does not affect plant growth. Because MH plants also take nutrients from the mycorrhizal fungi, it is also possible that less nutrients can reach the AT plant partners subsequently, which can be an indirect fitness consequence. However, due to the size difference of several magnitudes between AT plants and MH plants, there is likely no detectable change in nutrient availability. Thus, AT plants are not affected.

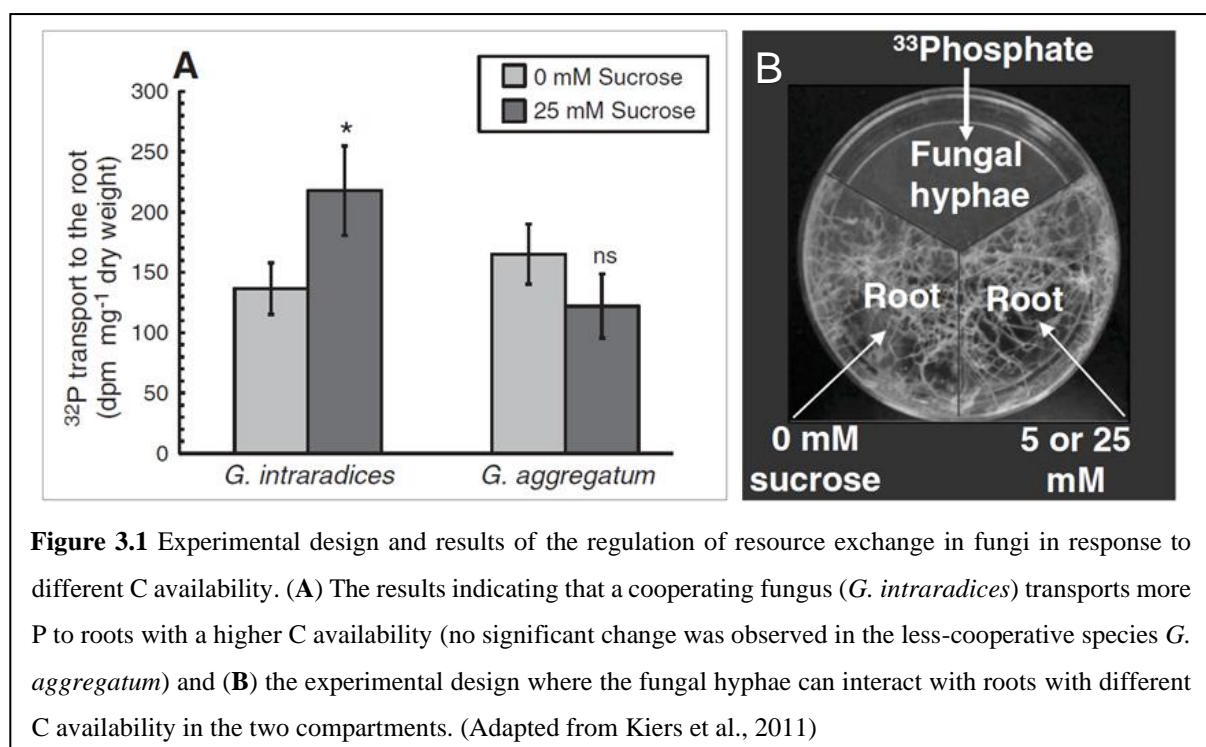
Shifting the focus to the mycorrhizal fungi which are in direct contact with MH plants, the fitness consequences might be more apparent. When it comes to initial and partial mycoheterotrophy, it has been suggested that a “take now, pay later” strategy is at play (Field et al., 2015). This means that an initial help received from fungi is later paid back when plants start relying more on autotrophy and start supplying their partners with photosynthesized sugars. This has been empirically shown by measuring C flux in initial MH orchids (Cameron et al., 2008). It was confirmed that there is a net plant-to-fungus C flux, indicating that throughout their lifecycle, the plants repay the initial help they received during early development. In partial MH plants the same thing can hold true, but the process is not life stage dependent, but based on environmental factors. The main paradox arises when we consider mycorrhizal fungi that associate with full MH plants. By definition, full MH plants rely on fungal C for their entire life cycle, and thus never give back carbon to the fungi. Presently, there has not been any evidence of reciprocity of any form (Merckx, 2013). Since fungi do not seem to benefit from associating with full MH plants, they only have negative fitness consequences, whereby they lose carbon and nutrients.

There is no consensus on whether MH plants can be rightfully called parasites, however all current evidence suggests that they are exploiters of the mycorrhizal fungi (Bidartondo, 2005; Egger and Hibbett, 2004). The case of MH plants might be best described as a third-party parasitism, where the pair-wise mutualism between fungi and AT plants are invaded by exploiter mycoheterotrophs. Some spatial models suggest that mutualism is resilient to such exploiters, although the exploiter populations are mostly unstable (Bronstein et al., 2003; Wilson et al., 2003). However, it was also argued that if the competition between the mutualist and the exploiter (AT and MH plants) is not severe, and if the exploiter is a specialist, the population is more likely to remain stable. The case of yucca moths supports this. Yucca plants and yucca moths are in an obligate mutualistic relationship where the yucca moth pollinates

the plant's flowers and in turn its offspring oviposited in the flowers can eat some of the seeds. This was seen as a pair-wise mutualism, but eventually additional species of moths have been observed which lay their eggs in fertilized flowers without pollinating and thus exploit the plants and rely on the pollinators too (Pellmyr et al., 1996). Thus, just like MH plants, these third-party parasites can also persist.

Getting away with stealing or living off crumbs?

It has already been established that for the mutualistic relationship between AT plants and mycorrhizal fungi to persist, the reciprocity of the on-going trade needs to be ensured. That is to say, mycorrhizal fungi must be able to differentiate between plants and supply nutrients to each according to how much carbon they receive (Hart et al., 2013). The in-vitro experiments of Kiers et al. (2011) with arbuscular mycorrhizal (AM) fungi support this notion. Their results indicate that there is an enforcement of cooperation both from the fungal partner and the plant host. An environment in a petri-dish was created where the hyphae of the AM fungus *Glomus intraradices* could interact with two separate root systems from the plant *Medicago truncatula* (see Figure 3.1). One of these root compartments was given a 25 mM sucrose supply and the other one was not given anything. It was successfully shown that the fungus transported more P to the root with higher C availability (see Figure 3.1). Although there are still knowledge gaps about how this trade is monitored on a molecular level, it is suggested that there must be regulatory checkpoints at the interface between fungi and plants which have a control over transport systems (Garcia et al., 2015). Such regulatory element could be the P transporter PT4



and the monosaccharide transporter MST2, which have been shown to be co-regulated (Helber et al., 2011). This supports the idea that there is a strict regulation of resource exchange at the plant-fungus interface. Having such direct control over how much nutrients are traded could help mycorrhizal fungi to avoid cheaters like MH plants. No research has been done to see whether this monitoring is done at the interface between MH plants and fungi which makes their relationship difficult to understand. Mycoheterotrophy is still widespread, and they seem to avoid punishment from the fungi they exploit. To disentangle this paradox, the evolutionary history of mycoheterotrophy needs to be explored.

Over the past decades, the advancements in phylogenetic tools allowed researchers to shed light on how mycoheterotrophy has evolved. Now, there is a consensus that MH plants first evolved as initial mycoheterotrophs, followed by partial mycoheterotrophs and finally full mycoheterotrophs (see Figure 3.2) (Selosse and Roy, 2009). As previously mentioned, the evolutionary driver towards mycoheterotrophy might have been light availability. Genomic studies have shown that the molecular machinery for normal mycorrhizal symbiosis was retained in MH orchids which might suggest that this machinery was modified to allow exploitation (Miura et al., 2018).

It is argued that the most crucial step happened when the “take now, pay later” strategy got exploited. This system made initial and maybe even partial mycoheterotrophy mutualistic but full MH plants have learnt to cheat this by averting payback and exploiting fungi without ever sending resources back (Bidartondo, 2005; Motomura et al., 2010). Recent findings provide

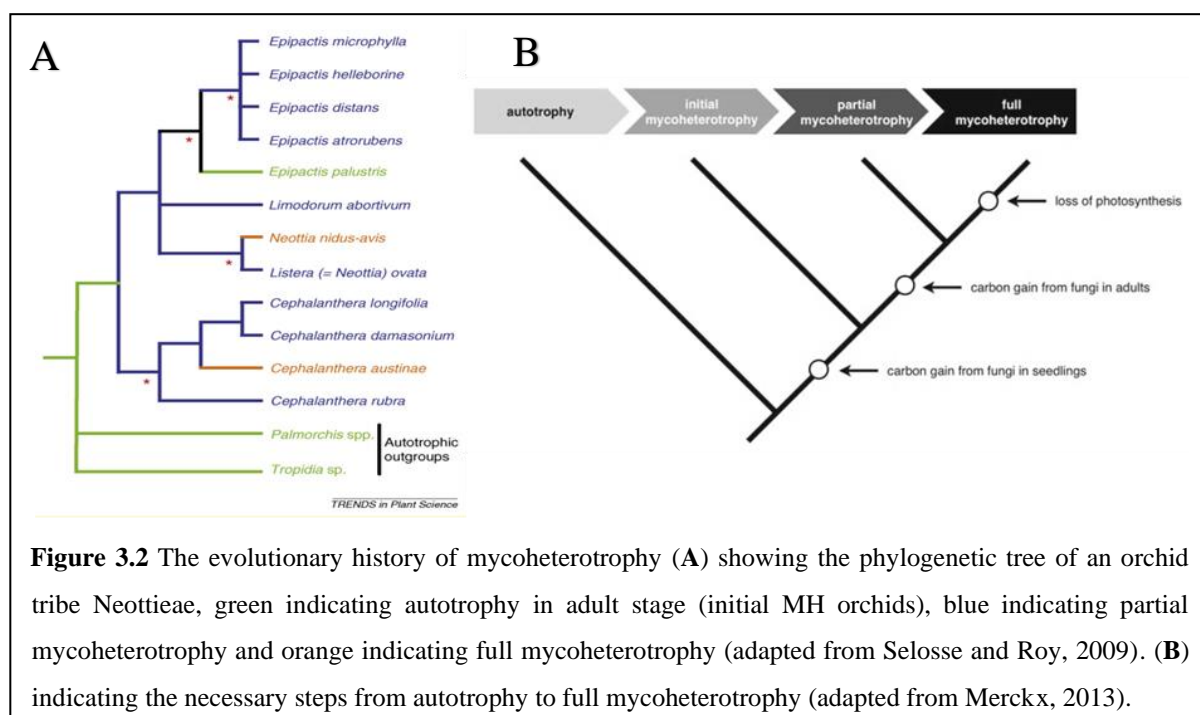


Figure 3.2 The evolutionary history of mycoheterotrophy (A) showing the phylogenetic tree of an orchid tribe Neottieae, green indicating autotrophy in adult stage (initial MH orchids), blue indicating partial mycoheterotrophy and orange indicating full mycoheterotrophy (adapted from Selosse and Roy, 2009). (B) indicating the necessary steps from autotrophy to full mycoheterotrophy (adapted from Merckx, 2013).

important supporting evidence for this. In initial MH orchids, the trehalase enzyme necessary to process fungal trehalose is expressed during the protocorm (germinating stage), but in full MH species this enzyme is upregulated to hijack trehalose from fungi (Li et al., 2022). By staying in the protocorm stage for longer and avoiding the normal shift from mycoheterotrophy to autotrophy these orchids have learnt how to continue exploiting fungi. It can be argued that the “take now, pay later” strategy can only work for the fungi if they are able to track where initial MH plants are in their lifecycle and only provide carbon during early development. By remaining in an early stage of development, MH plants could have tricked this system and continued to exploit fungal partners. This is further supported by evidence revealing that some MH plants spend almost their entire life cycle underground and only sprout for reproductive purposes (Shefferson et al., 2011). In light of these findings, it can be hypothesized that MH plants fool the fungi by remaining in their germinating stage and thus cheating the “take now, pay later” system that fungi are adjusted to.

An alternative explanation for how MH plants can persist in the exploitation of mycorrhizal fungi is that the sanctions deployed by fungi might not always be effective in preventing MH plants from cheating. As explained earlier, there seem to be controlling mechanisms that help fungi regulate how much resources they want to give other plants (Kiers et al., 2011). However, some findings indicate that in a more diverse community of plants and fungi, some individuals may be able to “hide in the crowd” and persist (Hart et al., 2013). Although it has not yet been shown for fungi, some studies suggest that a variation in the ability of hosts to discriminate between partners could also play an important role and may allow cheaters to stick around (Grman, 2012). Additionally, discrimination usually does not mean the complete absence of cheaters and some plants still allow low-quality or costly fungal partners to colonize (Hart et al., 2013). A similar phenomenon might be happening with fungi, where they may not fully cut ties with MH plants, and some would still interact with them. If mycorrhizal fungi indeed differ in their ability to discriminate between plants or in their ability to sanction effectively, that provides an answer to the specialization seen in MH plants. As mentioned previously, unlike AT plants, many MH plants are specialists in terms of what groups of fungi they associate with. It has been hypothesized that this pattern emerges because fungi increasingly “deny” MH plants and their specialized partners are the ones still available for exploitation (Bidartondo, 2005; Bruns et al., 2002; Yagame et al., 2016).

Since there is currently no empirical evidence that MH plants have a significant negative impact on their fungal partners’ fitness, it is possible that there was no need for the evolution of

sanctions (Frederickson, 2013). Most MH plants have undergone convergent evolution becoming very small and losing unnecessary features, thus minimizing the amount of carbon they need to take from fungal partners. This would indicate that these plants did not need to trick fungi, but instead have evolved to live on a small amount of carbon and other nutrients without causing significant losses for fungi. It is also suggested that partner discrimination and sanctions might be methods that bring more costs than benefits, meaning that a low level of exploitation might be tolerated by fungi (Frederickson, 2013).

Conclusion

The field directly working with mycoheterotrophic plants is still in its early stage and there are many unknowns regarding their ecology, physiology and evolution. The aim of this paper was to shed light on the unusual strategy used by MH plants and highlight its paradoxical nature. All current evidence suggest that these plants are third-party exploiters of the common mycorrhizal symbiosis between fungi and autotrophic plants. Given that fungi have been shown to exert control on resource trade with plants, it is difficult to comprehend how MH plants can steal carbon and other nutrients without providing any fitness benefits for their fungal partners. With the expanding understanding of mycoheterotrophy, three hypotheses are beginning to take shape. Firstly, MH plants might have been able to cheat the “take now, pay later” system by extending their early development stages and thus relying on fungal help for longer without any payback. Their developmental patterns and gene expression levels both support this idea. Another possibility is that sanctions deployed by mycorrhizal fungi are not effective enough to completely prevent exploitation by MH plants and thus they are able to “hide in the crowd”. The difference in discrimination abilities in fungi favour this hypothesis. Lastly, it could be that instead of stealing from fungi by tricking them, MH plants could just be “living off the crumbs” and their presence does not influence the fitness of fungi in any way. Most MH plants are very small and have optimized carbon usage, which could allow them to remain relatively harmless and make sanctions unnecessary.

Currently, the exact fitness consequences of mycoheterotrophy are unknown for each member of the tripartite relationship and the mechanism behind the resource acquisition and the maintenance of fungal connections are also unclear. More research is needed to get a clear answer to the biggest question of mycoheterotrophy. Although difficult, laboratory or field experiments should be conducted to directly assess the fitness consequences of mycoheterotrophy on fungal partners and other AT plants. To prove that there is indeed no reciprocity of any kind, the exact site of resource exchange should be examined. This might

shed light on any potential pay back mechanisms that might have been missed so far and they might also help to discover potential ways in which the fungi are tricked. Research on mycoheterotrophy should be encouraged, because almost 10% of described plants show deploy this strategy. Moreover, knowledge on their exploitative behaviour might aid research on parasitism and the maintenance of mutualism, potentially producing novel evolutionary theories.

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