Interactions between plants and fungi: costs and benefits of having a mixotrophic or mycoheterotrophic life style

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Cover: Four Swedish plant species. From left: *Monotropa hypopitys* (Ericaceae); *Neottia nidus-avis* (Orchidaceae); *Chimaphila umbellata* and *Pyrola rotundifolia* (Ericaceae). The first two species are strictly mycoheterotrophic, while the last two are putatively mixotrophic. Photos by Veronika A. Johansson.

Summary

Mycorrhiza is one of the most important symbiotic relationships and is based on an exchange of resources where the fungi supply plants with mineral nutrition in return for plant assimilates. Some plants, so called mycoheterotrophs, lack chlorophyll and hence the capacity to photosynthesize. Instead many of them act as epiparasites on mycorrhizal fungi and receive all their required nutrition from their fungal hosts. There is also several green plants, called mixotrophs, which are thought to obtain their carbon both through photosynthesis and via fungal links, but are at least during their initial development fully mycoheterotrophic.

It has proven hard to study the nutritional exchange between these plants and their associate mycorrhizal fungi, even though stable isotope analysis has proven to be a useful tool when studying this relationship. It is also unclear how mixo-and mycoheterotrophic plants may influence species diversity, plant communities and ecosystems. This essay aims to discuss the costs and benefits of having a mixo- or mycoheterotrophic life strategy. The main focus will be on the nutritional exchange between heterotrophic plants and their fungal hosts and the methods used to study these relationships.

Sammanfattning

Mykorrhiza är en av de viktigaste symbiotiska förhållanden och bygger på ett utbyte av resurser där svampen levererar mineraler till sin värdväxt och får i sin tur organiska föreningar från växten. Vissa växter, så kallade mykoheterotrofer, saknar klorofyll och därmed förmågan att fotosyntetisera. Istället parasiterar de på mykorrhizasvampar och får på så sätt all nödvändig näring. Det finns också flera grönbladiga växter, så kallade mixotrofer, som tros få sin kol från både fotosyntesen och genom att parasitera på mykorrhizasvampar.

Det har visat sig vara svårt att studera näringsutbytet mellan dessa växter och deras värdsvampar även om användandet av stabila isotoper på senare tiden blivit ett viktigt instrument. Det finns även lite kunskap om hur/om mykoheterotrofa växter kan påverka biodiversiteten, växtsamhällen och ekosystemprocesser. Denna uppsats syftar till att diskutera kostnader och fördelar med att ha en mixo- eller mykoheterotrofisk livsstrategi. Tyngdpunkten kommer att ligga på det näringsmässiga utbytet mellan heterotrofa växter och deras värdsvampar och de metoder som används för att studera dessa relationer.

Introduction

Plants interact with fungi in multiple ways, forming a range of interactions from mutually beneficial (mutualistic) to detrimental for one part (parasitic). One of the most widespread and important symbiotic relationship is the formation of mycorrhiza. Approximately 80% of all terrestrial plants are associated with mycorrhizal fungi (Smith and Read 2008). This symbiosis is based on an exchange of resources where the fungi supply plants with mineral nutrition and increased area for water uptake, and in return the fungi receive plant assimilates. This relationship is especially important in habitats with limiting sources of nutrition. A single plant can associate simultaneously with multiple fungi and a single fungus can associate simultaneously with multiple plants, resulting in complex networks. Different types of mycorrhiza are classified on the basis of their fungal connections. Plants associating with fungi from the Glomeromycota mainly form arbuscular mycorrhiza (AM), while those associating with Ascomycetes and Basidiomycetes form a variety of mycorrhizas, like ectomycorrhiza (ECM), arbutoid (ARB), ericoid or orchid mycorrhiza. Associations with arbuscular mycorrhiza are most diverse in tropical regions and grasslands, while ectomycorrhizal fungi are dominant and diverse in temperate forests (Smith and Read 2008).

In natural ecosystems plants can obtain as much as 80% of their requirements of nitrogen and up to 90% of phosphorous from mycorrhizal fungi (van der Heijden et al. 1998). It has also been shown that mycorrhizal fungi can provide the host plants with resistance to stress, drought (Auge 2001) and in some cases to soil pathogens (Sikes et al. 2009). Mycorrhizal connections also play a large role in plant communities by facilitating and influencing seedling recruitment (e.g. McKendrick et al. 2000a, McKendrick et al. 2002, Leake et al. 2004) and by altering plant-plant interactions.

However, not all interactions between plants and mycorrhizal fungi are symbiotic. The cost-benefit relationship between plants and mycorrhizal fungi can vary depending on e.g. limiting resources, environmental factors or life strategies of different plants or fungi, and the responses to these factors can range from positive to neutral to negative depending on the species. Some plants, so called mycoheterotrophs, lack chlorophyll and hence the capacity to photosynthesize. Instead many of them act as epiparasites on mycorrhizal fungi and receive all their required nutrition from their fungal hosts (Björkman 1960, Leake 1994), which allows them to colonize deeply shaded forest habitats. This tripartite relationship implies that the mycoheterotrophic (MH) plants indirectly parasitize on neighbouring autotrophic plants (mainly trees) through specialized mycorrhizal connections (Fig. 1; Leake 1994). There are more than 400 species of known MH plants in 11 plant families, most of which have a tropical distribution, but there is also those with a strictly temporal distribution, like members of the Ericaceae and some species of orchids (for further reading, see Leake 1994). The most well studied MH plant is *Monotropa hypopitys* (Ericaceae; Leake 2004, Bidartondo 2005), but there is also a significant amount of studies made on achlorophyllous orchids (e.g. Taylor and Bruns 1997, McKendrick et al. 2000b, Barrett et al. 2010). Furthermore, there are several other green plants, called mixotrophs (MX), which are thought to obtain their carbon both through photosynthesis and via fungal links. Far less is known about these plants and the extent to which they exploit their fungal hosts. However, all MH and most MX plants produce so called dust seeds with minimal reserves and are thus putatively initially mycoheterotrophic (for further reading see Eriksson and Kainulainen, in press). Mixotrophy in green plants, using mycorrhizal fungi for carbon gain, was first discovered in orchids (Gebauer and Meyer 2003, Bidartondo et al. 2004, Selosse et al. 2004) and more recently amongst pyroloids (Ericaceae; Tedersoo et al. 2007, Zimmer et al. 2007) and in the genera *Bartonia* and *Obolaria* (Gentianaceae; Cameron and Bolin 2010).

The two key tools that have contributed to the discovery and advances made in the study of MX (and MH) plants are stable isotopes and molecular methods used to identify the fungal hosts. By using stable isotopes it is possible to trace the source of specific nutrients in food webs and to study fluxes in ecosystem processes (Dawson et al. 2002). Fractionation against heavy isotopes is common in biochemical processes and therefore it leads to an accumulation of the heavier isotopes in higher trophic levels. This means that organisms usually have similar isotopic signatures (especially ^{13}C) to their food source (“you are what you eat”). Studies of MH and MX plants (henceforth referred to as heterotrophic plants) show that MH plants have an isotopic signature more similar to mycorrhizal fungi, while MX plants most often are placed in a range between MH and autotrophic plants (e.g. Trudell et al. 2003, Julou et al. 2005, Tedersoo et al. 2007).

The overall aim of this essay is to discuss costs and benefits of having a mixo- or mycoheterotrophic life strategy. The main focus will be on the nutritional exchange between heterotrophic plants (MX plants in particular) and their fungal hosts as well as the methods used to study these relationships. I will furthermore discuss how mycorrhizal parasites can influence species diversity, plant communities and ecosystems. In order to prepare the reader for these discussions, I will start by summarizing the fundamental mechanisms behind the transfer of nutrients between heterotrophic plants and their hosts.

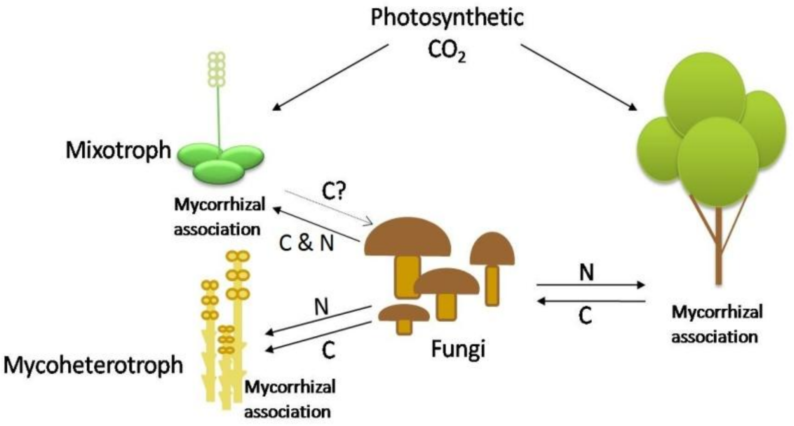

Figure 1. A schematic representation showing carbon and nitrogen exchange in a tripartite relationship between an autotrophic plant, mycorrhizal fungi and a mixo-and mycoheterotrophic plant (modified from Selosse and Roy 2009).

Physiological ecology of mixotrophic and mycoheterotrophic plants

Sources of carbon and nitrogen

It has proven hard to study the nutritional exchange between heterotrophic plants and their associate mycorrhizal fungi. Studies have so far failed to determine the compounds from which mycoheterotrophic plants obtain their carbon (C) and nitrogen (N), the physiological mechanisms behind the transfer and whether these nutrients are processed differently compared with autotrophic plants sharing the same fungus. It is generally agreed that the reduced root systems of MH plants ought to make them insufficient at direct nutrient uptake, and to date, there is no anatomical evidence to support that the relationship between mycorrhizal fungi and mixotrophs would differ from that of mycoheterotrophs (Rasmussen and Rasmussen 2009). Two basic pathways have been suggested to be involved for carbon transfer from fungi to plants: (i) the turnover of fungal biomass, where the breakdown of intracellular hyphea provides the plants with carbon (and nitrogen); and (ii) the transfer of fungal compounds through membranes at the fungal-plant interface (Bidartondo 2005). The first pathway could demonstrate how members of the Orchidaceae possibly gain their nutrition, since they have a vast intracellular colonization followed by hyphal break down while the latter pathway is more applicable to e.g. Monotropoideae where intracellular penetration is less common. Even though the digestion model (i) explains the similarity in ^{13}C found between heterotrophic plants and mycorrhizal fungi it does not explain the fact that ^{15}N values of MH plants differs from those of the associated fungi (Trudell et al. 2003). This model has further been criticized by Leake (1994), who suggests that although plants may obtain some carbohydrates by intracellular break down of hyphea, this would be an inadequate source of carbon.

Mixotrophy in algal communities has been known for quite some time, and just as with the algae, plant mixotrophy may not at all times be based on C demand. Instead C may be a side-product hitchhiking along with phosphorous (P) and N. If this is true we might expect soil mineral availability to be a regulating factor for mixotrophic plants dependence on fungal C (Selosse and Roy 2009). Given that both heterotrophic and the majority of autotrophic plants presumably receive N from mycorrhizal associations, there must be physiologically different pathways to how this occurs, since these different groups of plants have varying isotopic content. A possible explanation to this might be that heterotrophic plants mainly gain organic N compounds (e.g. amino acids) from their fungal hosts, while autotrophic plants are supplied with mineral N compounds (NO_3^- or NH_4^+; Gebauer and Meyer 2003). It is also possible that some of the C obtained by the plants could originate as part of amino acids, such as glutamine or glutamate, which are transported as a nitrogen source from the fungi to the plant (van der Heijden and Horton 2009). This possible linked transport may be intriguing, but the varying results and the general lack of correlation between ^{13}C and ^{15}N abundances suggests that C and N are obtained through different biochemical pathways (Selosse and Roy 2009).

To avoid back flow of nutrients from the plant to the fungus the plants may be dependent on synthesizing compounds that are unavailable to the fungi or produce storage cells that are spatially separated from infecting fungi (Bidartondo 2005). Orchids and other species with tuberous roots and rhizomes have a broad cortex in which starch is stored, where zones of infection may be spatially separate from zones of storage, as seen in *Neottia nidus-avis* (Orchidaceae). This may be achieved by means of fungitoxins (Leake 1994). The unusual storage sugars, gentianose and gentiobiose, which can only found in the roots and rhizomes of members of *Gentiana* spp., may be types of storing carbohydrates which are unavailable to the fungal symbionts (Leake 1994).

To furthermore complicate the matter of N acquisition, there is also an unknown number of plants that harbour endophytes that can fix N. Endophytes are endosymbionts, usually a bacterium or fungus, which lives within plants without causing disease. Studies have suggested that, in addition to being involved in nutrient cycling of heterotrophic plants, they might also be important in germination and recruitment processes (Tedersoo et al. 2007, Zimmer et al. 2007, Vincenot et al. 2008). Even though most plants associate with endophytes, the significance and physiological role of this relationship is poorly studied and remains unresolved.

Stable isotope analysis

The majority of biologically important elements occur as two or more stable isotopes, with one being more abundant than the other (s). Fractionation of the isotopes by different physiological and biological processes leads to a small but measurable concentration difference in biological materials and substances which can provide insights about natural fluxes among organisms and between organisms and their abiotic environment. Carbon has two stable isotopes, ^{12}C and ^{13}C, and one radioactive isotope, ^{14}C. Carbon stable isotopes are fractionated primarily by photosynthesis and can be a useful tool for e.g. measuring photosynthetic activity, and how favourable the environment is for plants of interest. Nitrogen has two stable isotopes, ^{14}N, and ^{15}N which are often used to trace sources of nutrients and are frequently linked to agricultural activities like the effects of fertilization. Together, C and N isotopes can be utilized to study heterotrophic plants specificity towards their fungal hosts as well as to measure the degree of mycoheterotrophy.

The first definitive evidence of fungus to plant carbon transfer was provided by means of radioactive labelling experiments (Björkman 1960, McKendrick et al. 2000a), where ectomycorrhizal autotrophic plants have been exposed to$^{14}CO_2$ to demonstrate the movement of C to surrounding MH plants via connecting mycorrhizal fungi. Double labelled [^{13}C-^{15}N] glycine has also been used to evaluate the direction of C and N transfers between autotrophic, MX and MH plants via the fungal connections (Cameron et al. 2006, Bougoure et al. 2010). Most studies to date have, however, focused on the natural abundance of C and N isotope ratios. This method gives a time-integrated view of the C and N assimilated throughout the period when the plant tissue was synthesised in contrast to radioactive labelling techniques (Dawson et al. 2002). To measure the range of fungal dependence for heterotrophic nutrient acquisition researchers have used $\delta^{13}C$ and $\delta^{15}N$ values in MX and MH plants and compared them with adjacent autotrophic plants and fruit bodies of mycorrhizal fungi. The isotope analysis method generally used, measures the total abundances of ^{13}C and ^{15}N in dried and ground samples with an online continuous flow CN analyser coupled with an isotope ratio mass spectrometer (Ohlsson and Wallmark 1999). Isotope abundances are expressed in delta (δ) values, where $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the molar ratio of heavy to light isotope of the samples and the respective standard (i.e. $^{13}C/^{12}C$ or $^{15}N/^{14}N$). By using linear two-source isotope-mixing models (Gebauer and Meyer 2003) it is then possible to estimate the fungal dependency and compare different heterotrophic plant species.

Carbon and nitrogen signatures of mycoheterotrophic and mixotrophic plants

All Pyroloid species investigated to date show that these plants N demand is supplied by their fungal associates. Investigations of fungi-derived C have, however, shown varying results and appear to be site specific and possibly influenced by light availability. Tedersoo et al. (2007) showed in their study the first quantitative evidence for mixotrophy in Pyroleae (Ericaceae). The results showed that *Orthilia secunda*, *Pyrola chlorantha*, *Pyrola rotundifolia* and *Chimaphila umbellata* acquired between 10.3% and 67.5% of their C from fungi. The high levels of N and ^{15}N found, also corroborated the species dependence on fungal nutrition. Similar results, regarding the N acquisition of pyroloid species, were also detected by Zimmer et al. (2007) and Hynson et al. (2009b). But unlike Tedersoo´s (2007) study, they found no significant difference in $\delta^{13}C$ content when comparing the putatively mixotrophic species with the autotrophic reference species. The only exception to this was one site of *O. secunda* (Zimmer et al. 2007), which was found to acquire 28±12% of the C from a fungal source. It should however be mentioned that this particular site was a very dark beech forest. Furthermore, the increasing number of studies within the subject has shown that the ^{15}N enrichment in plant tissue is not a general feature of MX and MH plants. This may be because different taxonomic and functional groups of fungi vary in their ^{15}N enrichment (Gebauer and Taylor 1999, Leake and Cameron 2010) which will be discussed further below. For example, mixotrophic orchids and pyroloids have been shown to sometimes contain higher levels of ^{15}N than fully MH plants (Hynson et al. 2009b).

Studies of *Goodyera* species have shown that these orchids, once they develop green leaves, seem to become fully autotrophic rather than follow any trends towards mixotrophy (Hynson et al. 2009a), even though their habitat preferences are similar (shaded boreal forests), or often the same as other mixotrophic species, e.g. *P. chlorantha*. In counter to other studies of mixotrophic plants they seem to be depleted in ^{13}C compared to surrounding autotrophic plants. These results are congruent with Cameron et al. (2006) which showed in vitro that about 2% of the photoassimilated $^{14}CO_2$ labeled carbon were transferred from seedlings of *Goodyera repens* (Orchidaceae) to the connected fungi. Another similar study, also by

Cameron et al. (2008), showed that the net transfer of C from *G. repens* to the fungal host was five times greater than the C transfer from fungi to plant. The depleted $\delta^{13}C$ values found in the *Goodyera* species could also be a result of the isotope food chain models, where the source of the nutrient is left depleted in the heavier isotope compared to its sink (Fry 2006). Depleted ^{13}C values has also been found in *Aceras, Orchis* and *Ophrys* species (Orchidaceae), which may suggest a flux of organic C from these plant species to their associate fungi (Liebel et al. 2010). These plants were, however, all enriched in ^{15}N which suggests a possible mixotrophic strategy where organic nutrients are gained from the fungi.

It is possible that there is a continuous natural variation in the range to which a plant species is mixotrophic and there may be multiple plant species that remains to be discovered as mixotrophic. But the intraspecific variation found in isotopic content suggests that the varying results of isotope accumulation could be a result of different light availability in the investigated habitats, since mixotrophic species most likely take advantage of their fungal associates differently depending on their capacity to photosynthesise. Multiple studies have suggested light level to be negatively correlated to fungal C dependency (e.g. Gebauer and Meyer 2003, Bidartondo et al. 2004, Abadie et al. 2006, Zimmer et al. 2007, Preiss et al. 2010, Liebel et al. 2010). The variations in isotope accumulation could also be explained by the fungal taxonomy and C source of the fungi involved (Bidartondo et al. 2004, Julou et al. 2005, Tedersoo et al. 2007) or by spatial and temporal differences of different habitats (Taylor et al. 2003). For example, saprotrophic and litter decaying fungi are generally more enriched in ^{13}C than ECM fungi since lignocelluloses, and thus wood have higher $\delta^{13}C$ than other plant materials (Taylor et al. 2003, Martos et al. 2009), and would consequently lead to higher values of heavier isotopes in the plant material as well. There is however an overlap between ECM fungi and saprotrophic fungi where, in particular, the $\delta^{15}N$ values of some ground growing saprotrophic fungi has been found to be more similar (higher) to ECM fungi. Furthermore, a small number of ECM fungi can also be placed in the saprotroph group due to either high levels of $\delta^{13}C$ or low levels of $\delta^{15}N$ (Taylor et al. 2003). Because of this it might be essential to identify the specific fungi/fungus involved in tripartite associations to fully understand the complete cost- benefit relationship.

Limitations and considerations concerning the use of stable isotope analysis

There are multiple environmental factors to consider when sampling for isotope analysis to avoid isotope misrepresentation. For example, light availability, precipitation, temperature, evaporation events etc can affect the photosynthetic rate of plants, the soil respiration and the isotope composition of the surrounding atmosphere (e.g. Ekblad and Högberg 2001). Sampling should hence take place at some distance above ground and preferably at the same distance for all samples, to avoid isotope distortion from CO_2 released from soil respiration. It is also preferable to sample during similar weather conditions and time of day. These marks should also be taken into account when sampling for comparable autotrophic baseline reference species. Martos et al. (2009) used recently fallen leaves collected from the ground as reference, due to the lack of understory reference plants. Leaves from tree canopies are, however, more enriched in ^{13}C compared to understory plants and are therefore not suitable to use for this kind of analysis (Liebel et al. 2010). The choice of mycoheterotrophic 'end-species' may also be as important as the choice of autotrophic reference plants when studying MX plants since the isotopic signatures of obligate mycoheterotrophic plants can vary and hence affect the estimated levels of putative mycoheterotrophy in investigated MX plants (Hynson et al. 2009b). It may also be important to separate different reference species into functional groups, depending on e.g. their mycorrhizal hosts or their capacity to fix N_2 from the atmosphere. The reference species should furthermore grow within close vicinity to the heterotroph, to make sure that environmental conditions are similar (for standardized sampling protocol, see Gebauer and Meyer 2003).

A major limitation to the analysis of isotope content of mixotrophic species is that they only establish the contribution of fungal C to biomass (anabolism) and does not take into account catabolic reactions. Additional investigation will thus be needed to fully understand C metabolism in mixotrophs (Selosse and Roy 2009). Furthermore, the fact that ^{15}N enrichment is not a general feature of MX and MH plants and the lack of a coherent pattern between ^{13}C and ^{15}N content has lead to critique against the use of linear two-source isotope-mixing models when estimating the heterotrophic N gain of MX plants. Further assessment of this model, based on both ^{13}C and ^{15}N enrichment, suggests that it can only provide an estimate of the level of heterotrophy and not an absolute value (Leake and Cameron 2010).

Moreover, the challenge of designing a suitable method to study the putative in situ carbon transfer from mixotroph to the associate fungi still remains. The in vitro studies by Cameron et al. (2006, 2008) of plant-to-fungi transferred C are the only studies to date that tries to resolve this matter (as mentioned above). But whether if these results can reflect the in situ C transfer is uncertain (Rasmussen and Rasmussen 2009).

Costs and benefits of a tripartite relationship

Mycorrhizas are in general considered to be mutualisms where both plant and fungal symbionts benefit from the bilateral exchange of resources. However, this is not always the case, and there seems to be a continuum of responses where a range in the net fitness cost to the plant or to its fungal hosts is to be expected. The complete cost- benefit relationship of mycorrhizal ‘cheaters’, like MH and MX plants is principally unknown. Since multiple evolutionary pathways has lead to the development of this convergent trait (Leake 1994), the likely scenario is that the heterotrophic plants benefit from this relationship, but whether or how this affects the fungal hosts or the associated autotrophs remains in large parts unstudied. If there is a cost to the fungus and its autotrophic partner, this goes against the suggestion that photosynthetic plants may force sanctions against less beneficial fungal symbionts (Bidartondo 2005). However, this relationship is often referred to as an ‘unholy alliance’, since the autotrophic plants involved are not able to select against the mycorrhizal parasites.

Mycorrhizal fungal networks provide a wide range of services to plants and ecosystems. The most important benefit is probably nutrient acquisition, followed by the facilitation of seedling establishment. For mycoheterotrophic and mixotrophic plants this may be particularly important, since fungal connections are essential for germination and seedling development and may act as a bottleneck on recruitment (Bidartondo and Read 2008). But mycorrhizal networks may have varying effects on seedling establishment. Even in a single type of mycorrhiza there may be varying outcomes ranging from mutualistic to parasitic relationships and this may change over the life cycles of a single plant-fungus partnership (van der Heijden and Horton 2009), likely depending on varying environmental conditions (Smith and Read 2008). For example, when the nutrient balance of a mycorrhizal association is disturbed, fungi may behave in a weakly pathogenic manner (Smith and Read 2008), and in habitats with limiting light conditions heterotrophic plants may, to a larger extent, use their fungal hosts for carbon gain. Mycorrhizal connections may also lead to alterations in for example a plants morphology, growth rate or phenology, which could complicate a cost-benefit analysis. The highly reduced root systems seen in most heterotrophic plants clearly suggest that the absorptive functions are mainly performed by their fungal partners. These reduced roots may be insufficient at direct nutrient uptake but on the other hand they might compensate for the C possibly lost when developing roots compared to e.g. non-mycorrhizal plants (Johnson et al. 1997). There may also be a temporal difference of costs and benefits for both heterotrophic plants and mycorrhizal fungi. The plants may benefit during one year, or one part of the year and have larger net cost at other occasions that instead are beneficial to the fungi.

Dijk et al. (1997) suggested a tradeoff between lifespan and vitality of heterotrophic plants, where long lived orchid species were suggested to associate with more dominant and aggressive fungal species, supposedly leading to larger seedling mortality, but greater and more long-term success as adults. Short-lived species were suggested to associate with less aggressive fungi, leading to a more successful recruitment but lesser competitive abilities as adults. But this theory remains to be proven. An interesting question, which could be approached by using stable isotope analysis, is whether established seedlings of mixotrophic plants derive more, equal or lower benefits from their fungal hosts than coexisting older and larger plants of the same species. Cameron et al. (2006, 2008) provide the first evidence of an initially mycoheterotrophic plant that upon becoming photosynthetic starts returning C back to its mycorrhizal fungi. Further studies of the same genera suggest that seedlings of *Goodyera oblongifolia* (Orchidaceae) are more dependent on fungal connections during earlier stages of seedling development (Hynson et al. 2009a).

Most MH plants have proven to be extremely specific towards their fungal associates, even though the fungi remain generalists (e.g. Bidartondo and Bruns 2001, Bidartondo 2007, Roy et al. 2009). The cost of this specificity may be that it prevents them from host jumps, which most likely limits their distribution (Bidartondo 2005). What effect this has on the fungi remains unknown. Studies made on *M. hypopitys* (Ericaceae) have however shown that the plant appear to stimulate the growth of both their mycorrhizal fungus and their associated autotroph roots (Bidartondo et al. 2000), which could be beneficial for all involved parties and thus perhaps compensate for the C loss. Studies have on the other hand revealed that fungi supporting orchids have a lower fruiting body production compared with related unparasitized fungal species, which may suggest that the fungal fitness is reduced by orchid associations (Jones and Smith 2004). This could also imply that the fungus switches to vegetative growth rather than reproduce sexually, which could benefit the mycoheterotrophic plants since it could slow down coevolutionary fungal responses. The fungus might therefore be less inclined to avoid parasitism if the MH seeds are colonized by the same fungal individual as their parents (Bidartondo 2005). The study by Bidartondo and Bruns (2005) shows that seeds of *M. hypopitys* germinate best when subjected to a fungal species of the *Russula* genera, specific for the maternal plant. Furthermore they show that germination also can be induced by closely related fungi from the same genera, however, less successfully. Whether this is due to a possible cost-benefit relationship concerning the nutrient acquisition, morphological differences or if it is a result of the putative ‘arms race’ between heterotrophic plants and host fungi is uncertain.

In habitats with natural high soil fertility or fertilized soils there is often no benefit to plants associated with mycorrhizal fungi. Instead growth can be reduced due to the fungal carbon demand, and if nitrogen is not a limiting factor, the transfer of ^{15}N between fungi and heterotroph would not be as prominent compared to a habitat with less soil nitrogen (Smith and Read 2008). This pattern was found in the putatively MX plant, *Pyrola asarifolia* (Ericaceae), in a study of a natural edaphic gradient, where inorganic N resources increased with site productivity (Kranabetter and MacKenzie 2010). The plant had a negative correlation in foliar ^{15}N with soil N, ranging from +8 ‰ on the poorest sites to -1‰ on the richest sites. Kranabetter and MacKenzie (2010) furthermore, noted a general decline in understory ECM, ericoid and arbutoid plant communities on the more productive sites. However, most AM species responded in contrast positively towards increased levels of productivity, suggesting different adaptations for exploiting soil resources.

Impacts on plant communities and ecosystems

Parasites are thought to be potential ‘keystone species’ in plant communities. By generally reducing recourses from dominant species they make it possible for other species to coexist, leading to increased species diversity. But whether MH or MX plants could fit in to this role remains uncertain. Do heterotrophic plants naturally occur at locations which already possess a high fungal diversity or do they promote fungal and endosymbiont diversity by parasitizing on more common species? Even though some heterotrophic plants, in temporal communities, are specialised on relatively common genera of fungi like e.g. *Suillus, Tricholoma* or *Sebacina* (e.g. Tedersoo et al. 2007, McKendrick et al. 2002, Liebel et al. 2010), it is unclear if these fungi are dominant species and if they have an effect on species diversity or nutrient allocation. It is also unknown to what extent MH and MX plants affect the fungal diversity and productivity in a community. Some manipulation studies have observed that plant productivity, diversity and nutrient acquisition increased with increasing fungal diversity (e.g. van der Heijden et al. 1998, Jonsson et al. 2001) while other studies found no effects (van der Heijden et al. 2006). Results from studies (e.g. Tedersoo et al. 2007) show that there is a substantial flow of carbon to MX and MH plants from the surrounding trees. This suggests that understory plants may influence the dynamics and composition of forest communities (Tedersoo et al. 2007). It is however, unclear to what extent this actually affect the trees, but since pyroloids are more common than orchids and also wintergreen, they might have a greater impact on forest communities. Furthermore, to date, no studies have been performed to investigate if mycorrhizal networks formed by MX or MH plants can promote seedling establishment of neighbouring plants. Since some MH plants have been shown to induce growth in roots of associated autotrophic plants (trees) it may be possible that they can assist in the seedling establishment of these species.

Fungal communities are in general very patchy, and the structure of heterotrophic plant populations would be expected to reflect the distribution of the host fungi or the past distribution if fungal switching or losses of hosts are involved. Studies have shown that germination in some cases are more successful in close proximity to adult plants (e.g. Batty et al. 2001, McKendrick et al. 2002), but unrelated in others (McKendrick et al. 2000b). This could suggest that some MX or MH plants retain their fungal hosts while others in the latter case switch to different hosts, or that soil properties like pH, moisture etc, changes into becoming more unfavourable at some distance from the main population. But few species have been studied which makes it hard to know the ecological significance or draw generalizing conclusions concerning the fungal switching. But if fungal switching is involved in heterotrophic plant life histories, this could complicate the considerations for conserving these species.

It is hard to study plant-mycorrhiza interactions in the field because most plants are colonized by multiple fungal species (networks), each with its own cost benefit interaction. However, this also emphasizes the importance to study plant-fungi interactions since the loss of species or community turnover could have major impacts on ecosystem services and biodiversity. The extreme fungal specificity seen in mycoheterotrophic plants also gives a good example of the importance of mycorrhizal biodiversity for the preservation of plant communities and ecosystem function (Leake 2004). Single mycorrhizal species or even subspecies can be of crucial importance for the success of these species.

Anthropogenic influences

Climate change and increased levels of greenhouse gases is an ongoing process and important field of research which could what's more be important to consider when discussing cost-benefit relationships between plants and fungi. It has been suggested that elevated levels of atmospheric CO_2 should alter mycorrhizal function by increasing the benefits regarding nutrient uptake and at the same time by increasing the plants photosynthetic rates decrease the net C costs for mycorrhizal host plants (Oneill 1994). Studies involving AM fungi have, however, failed to confirm this theory (Johnson et al. 2003, Johnson et al. 2005). Instead they found that increased CO_2 enrichment generally reduced the benefits of AM fungi, but also that it varied depending on the plant and fungal taxa. It is hard to know if raised CO_2 levels could have an effect on MX or MH plants, but presuming that associated fungi is disfavoured by CO_2 enrichment, it should most likely also affect mycorrhizal parasites. Since many MX plants have few or no stomata (Leake 1994) and hence a naturally low photosynthetic rate and MH plants no photosynthesis at all, the direct effects of CO_2 enrichment may be low or non-existent.

Furthermore, the use of industrial fertilizers has increased exponentially over the last decades (Vitousek 1994) and is most likely also leading to alterations in the cost-benefit relationship between mycorrhizal fungi and plants. When limiting recourses are increasing in availability this could change a balanced mutualistic relationship and also possibly turning them into parasitic associations (Johnson et al. 1997). This may be especially important for grasslands and agricultural areas, but since many plantations and forest nowadays are being fertilized and also with nutrient leakage from agricultural areas as well as atmospheric deposition into forests, this might be an increasing problem for forest habitats and understory plants. Pyroloids, Monotropoids and orchids are known to be sensitive to anthropogenic N deposition, and it is also well documented that increasing levels of N is associated with decreases and loss of fungal diversity. This could possibly result in the loss of key fungal symbionts and in turn contribute to the loss of these plant species. The extreme specificity seen, particularly in MH plants, also gives a good example of the importance of mycorrhizal biodiversity for maintaining plant diversity and ecosystem functioning. Because of these inherent traits MH plants have been suggested to act as excellent indicators of undisturbed forests (e.g. Moola and Vasseur 2004). Many of the heterotrophic plant species are currently decreasing in number, which is most likely due to logging and preservation of old- grown forests are hence essential for conserving these species.

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