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Mycoheterotrophy: An Introduction

Vincent S.F.T. Merckx

> The snow plant (*Sarcodes sanguinea*) is more admired by tourists than any other in California. It is red, fleshy and watery and looks like a gigantic asparagus shoot. Soon after the snow is off the round it rises through the dead needles and humus in the pine and fir woods like a bright glowing pillar of fire. In a week or so it grows to a height of eight or twelve inches with a diameter of an inch and a half or two inches; then its long fringed bracts curl aside, allowing the twenty- or twenty-five-lobed, bell-shaped flowers to open and look straight out from the axis. It is said to grow up through the snow; on the contrary, it always waits until the ground is warm, though with other early flowers it is occasionally buried or half-buried for a day or two by spring storms. The entire plant—flowers, bracts, stem, scales, and roots—is fiery red. Its color could appeal to one's blood. Nevertheless, it is a singularly cold and unsympathetic plant. Everybody admires it as a wonderful curiosity, but nobody loves it as lilies, violets, roses, daisies are loved. Without fragrance, it stands beneath the pines and firs lonely and silent, as if unacquainted with any other plant in the world; never moving in the wildest storms; rigid as if lifeless, though covered with beautiful rosy flowers.
>
> John Muir (1912)

1.1 Motivation

"Mycoheterotrophy" describes a plant's ability to obtain carbon from fungi. Many plants are capable of mycoheterotrophy; some closely related others are not. Plants with the ability for mycoheterotrophy ("mycoheterotrophic plants"), particularly those that completely depend on fungal carbon during their entire life cycle ("fully mycoheterotrophic plants" see definitions below), have attracted the attention of biologists for centuries. Studies on their unconventional mode of life have led to novel perspectives in ecology and evolution. Since the term mycoheterotrophy (as "myco-heterotrophy") was coined by Jonathan Leake in 1994, scientific research on the topic has increased considerably (Fig. 1.1). Leake's groundbreaking review on mycoheterotrophic plants unfortunately preceded several technological advances that revolutionized the field, and only few review papers have appeared since. Most notable is the *New Phytologist* Tansley review by Martin Bidartondo (2005) and several relevant chapters in *Mycorrhizal Symbiosis* by Sally Smith and David Read (2008). However, these works mainly focus on the ecological and physiological aspects of mycoheterotrophy, and to date, a single volume has not been dedicated to all aspects of mycoheterotrophy. I hope that this book's multidisciplinary approach in discussing mycoheterotrophy will appeal to scientists and students who wish to understand the biology of mycoheterotrophic plants and that this overview will contribute to an enduring interest in these extraordinary plants.

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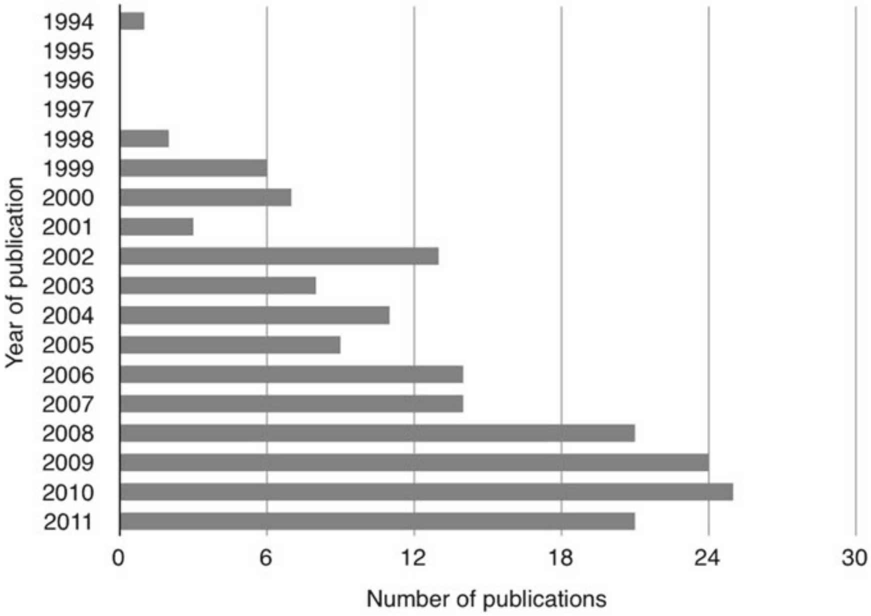

Fig. 1.1 Number of papers published each year since 1994 that are labeled with the topic "myco-hetero*" or "mycohetero*" in Thomson Reuters' Web of Science (as of January 2012)

1.2 Mycorrhizal Symbiosis

1.2.1 Mycorrhizas

Mycoheterotrophy cannot be discussed or appreciated without a basic understanding of the mycorrhizal symbiosis. The term mycorrhiza is derived from the Greek words for "fungus" and "root" and implies the association of specialized soil fungi ("mycorrhizal fungi") with plant roots. In general, this association is mutualistic because both partners benefit: mycorrhizal fungi improve the nutrient uptake of their host plants, and in return they receive photosynthetically fixed carbon that is essential for growth and reproduction of the fungi. The mycorrhizal association is probably the most important symbiosis in nature and plays an essential role in the maintenance of most terrestrial ecosystems such as grasslands and forests. Over 90% of all plant species form mycorrhizas, including most crops. Despite the literal meaning "fungus roots," mycorrhizas also occur in "primitive" plants that do not have true roots such as liverworts and hornworts. Moreover, fossil evidence indicates that the earliest land plants were also associated with hyphal fungi, and it is now generally accepted that the colonization of land by plants relied on a symbiotic association with fungi.

The main diagnostic criteria for the types of mycorrhizas formed in nature are the identity of the fungi engaged in the symbiosis and the morphology at the symbiotic interface of plant and fungus. The two dominant types of mycorrhizas are the arbuscular mycorrhizas (AM) that involve nearly all members of the Glomeromycota and a wide variety of host plants, and the ectomycorrhizas (EM) that involve some members of the Basidiomycota and Ascomycota and several woody plants.

For a comprehensive synthesis on the subject of mycorrhizas, I strongly recommend the book *Mycorrhizal Symbiosis* by Sally Smith and David Read (2008). Here I provide a basic overview of a few aspects of the mycorrhizal symbiosis that are essential in the context of mycoheterotrophy.

1.2.2 Arbuscular Mycorrhizas

Arbuscular mycorrhizas are the most common mycorrhizal type. They are formed by fungi of the phylum Glomeromycota and the majority of land plant species. The arbuscular mycorrhizal symbiosis is extremely ancient, and AM fungi were probably important in the colonization of land by plants, although some evidence suggests that some members of the Mucoromycotina predate the Glomeromycota as the earliest mycorrhizal fungi (Bidartondo et al. 2011). The name "arbuscular" is derived from the characteristic treelike structures, the arbuscules, which occur within the cortical cells of many plant roots and also some mycothalli colonized by AM fungi (Smith and Read 2008). Arbuscular mycorrhizal fungi are obligate symbionts: they cannot survive as free-living organisms. They are also puzzling organisms for taxonomists because they are asexual, multinucleate, and difficult to cultivate, and therefore, species diagnosis and identification remains under debate. However, under the current morphological and molecular concepts, there are no more than 300 species of AM fungi known (Öpik et al. 2010; Krüger et al. 2011). This number strongly contrasts with the number of potential host plant species: perhaps 80–90% of all land plant species are able to form arbuscular mycorrhizas. This illustrates the potential for promiscuity in the arbuscular mycorrhizal symbiosis. Indeed, studies show that an arbuscular mycorrhizal plant typically associates simultaneously with multiple AM fungi, and an AM fungus often associates simultaneously with multiple plants (Giovannetti et al. 2004). This allows for the formation of mycorrhizal networks, linking plants of the same or different species by a shared mycorrhizal fungus. These arbuscular mycorrhizal networks are essential for the existence of mycoheterotrophy because they allow for physiological continuity between an autotrophic plant, its arbuscular mycorrhizal fungus, and a mycoheterotroph (Chap. 5; Fig. 1.2). Mycoheterotrophic interactions through AM fungi occur in lycophytes, ferns, and angiosperms. Over 230 species of fully mycoheterotrophic angiosperms are dependent on AM fungi.

1.2.3 Ectomycorrhizas

Trees in the families Pinaceae, Fagaceae, Dipterocarpaceae, Myrtaceae, and Fabaceae found in both temperate and tropical forests associate with hundreds of ectomycorrhizal fungus species of Basidiomycota and Ascomycota (Bonfante and Genre 2010). Therefore, EM fungi are essential components of the world's forests. The ectomycorrhizal habit has evolved multiple times independently in the evolution of land plants (Bruns and Shefferson 2004). And while the EM symbiosis is considerably younger than the AM symbiosis, fossil, biogeographical, and molecular clock data suggests that EM associations have a long history (Le Page et al. 1997; Moyersoen 2006; Hibbett and Matheny 2009). Over 7,000 species of fungi are known to form ectomycorrhizas, most of them belong to the Basidiomycota, but the actual EM fungi diversity may be considerably higher (Smith and Read 2008; Tedersoo et al. 2010). An EM root is characterized by the presence of three structural components: a mantle of fungal tissue which encloses the root, a labyrinthine inward growth of fungal hyphae between the epidermal and cortical cells (Hartig net), and an outwardly growing system of hyphal elements which extends into the soil (extraradical of external mycelium) (Smith and Read 2008). There may be considerable variation in morphology and development of these structural elements. In general, the EM symbiosis is considered to show a low level of specificity, and it has been demonstrated that an individual tree may have 15 or more different fungal EM partners simultaneously (Saari et al. 2005). Analogous with the AM symbiosis, this allows for the formation of common mycorrhizal networks, linking plants by a shared ectomycorrhizal fungus. Besides ectomycorrhizas, Ascomycota and Basidiomycota are known to form other types of mycorrhizal structures, often categorized as ectendo-, ericoid, arbutoid, and orchid mycorrhizas. However, the type of mycorrhiza formed can be influenced by the identity of both plant and fungus: it is known that the same fungus can form different types of mycorrhiza depending on

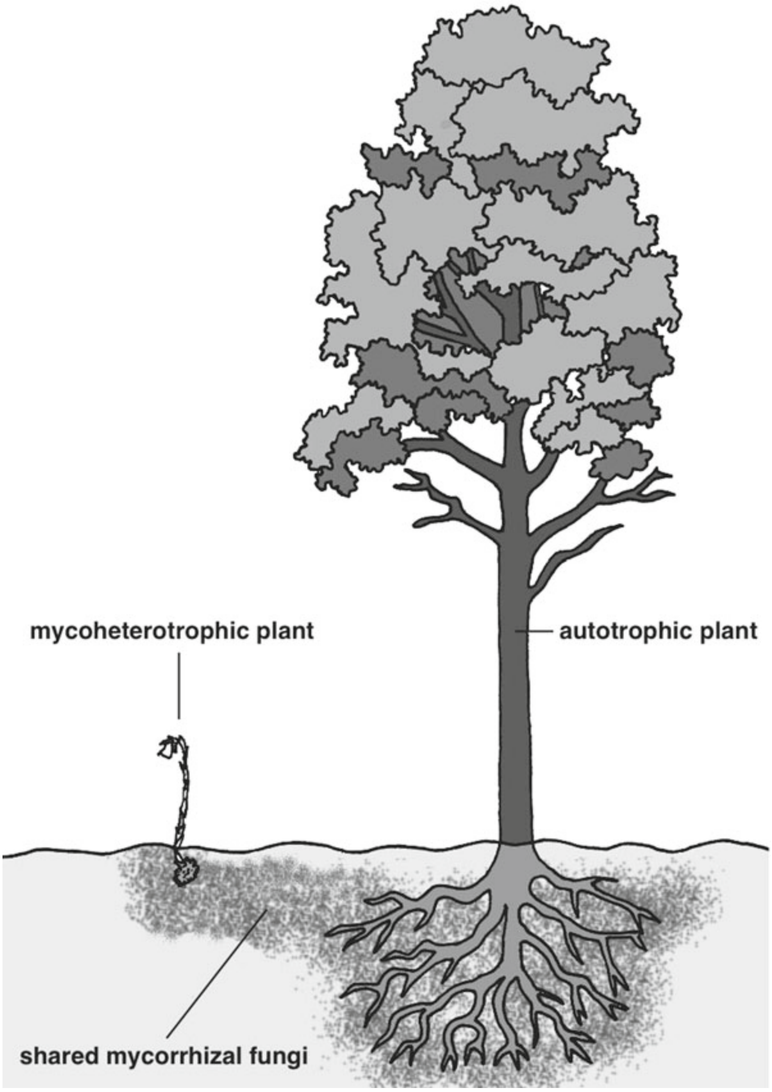

Fig. 1.2 Many fully mycoheterotrophic plants obtain carbon from surrounding autotrophic plants through shared mycorrhizal fungi

the identity of the plant associate. The mycorrhizas involved in the mycoheterotrophic interactions of Monotropoideae, Pyroleae (both Ericaceae), and Orchidaceae have been classified as monotropoid, arbutoid, and orchid mycorrhizas, respectively. Yet, with the exception of mycorrhizal associations between orchids and saprotrophic fungi (SAP), it has been demonstrated that in all of these mycoheterotrophic interactions, the fungi involved simultaneously form ectomycorrhizas with surrounding green plants (to establish the tripartite interaction necessary to sustain the mycoheterotrophic interaction). Therefore, here we group all these interactions as ectomycorrhizas. Mycoheterotrophic interactions through EM fungi occur in liverworts and angiosperms. One liverwort species and at least 48 species of angiosperms (all in Orchidaceae and Ericaceae) are fully dependent on EM fungi for their entire life cycle, although in some cases EM and SAP fungi co-colonize the roots of fully mycoheterotrophic plants (Table 7.1).

1.2.4 Mycorrhizal Associations with Saprotrophic Fungi

The Orchidaceae is one of the largest and most diverse plant families and contains an estimated 22,000 species (Stevens 2012). Almost all orchids produce extremely small seeds ("dust seeds") that contain very few reserves. Consequently they are dependent on carbon and other nutrients provided by mycorrhizal fungi during their early developmental stages ("symbiotic germination") and can be classified as initial mycoheterotrophs. The fungi produce intracellular coils in the embryos of seedlings and in the rhizomes or roots of adult plants (Smith and Read 2008). Fungi isolated from the seedlings of orchids were initially classified under the Basidiomycota genus *Rhizoctonia* (e.g., Bernard 1899; Burgeff 1909). However, molecular phylogenetic analyses demonstrate that rhizoctonia-forming fungi are polyphyletic and phylogenetically spread over the Basidiomycota orders Ceratobasidiales, Tulasnellales, and Sebacinales (Taylor et al. 2002). Rhizoctonia-forming fungi isolated from orchids have been shown to be saprotrophic and are thus able to obtain carbon and other nutrients from decaying matter (Smith and Read 2008). Associations with these SAP fungi are found in all major orchid lineages including the first-diverging Apostasioideae (Kristiansen et al. 2004; Yukawa et al. 2009). An initial dependency on rhizoctonia-forming fungi is thus likely the ancestral state of Orchidaceae, and it has been hypothesized that the unique ability of orchids to recruit free-living SAP fungi into novel mycorrhizas may have led to dramatic expansion of their potential habitat and has triggered their radiation (Ogura-Tsukita et al. 2009). During orchid evolution, many mycoheterotrophic lineages have switched to an association with EM fungi (Chap. 5). However, compared to the total number of species in Orchidaceae, EM orchids represent only a small minority of species. All other orchid species are mycoheterotrophic on SAP fungi at least during their initial development, and therefore, next to AM and EM fungi, SAP fungi are the third source of mycoheterotrophy in plants.

1.2.5 Mutualism–Parasitism Continuum of the Mycorrhizal Symbiosis

Typically the mycorrhizal symbiosis is a mutualistic interaction: both the plant and the mycorrhizal fungus benefit from the association. In a simple model this interaction can be classified as a "win-win" situation. However, evolutionary theory suggests that mutualisms are best viewed as "reciprocal exploitations" that nonetheless provide net benefits to each partner (Herre et al. 1999). This view stresses the disruptive potential of conflicts of interests among the partners and advances the prediction that mutualisms are vulnerable to exploitation (Bronstein 2001; Sachs and Simms 2006). It also emphasizes the dynamic nature of symbiotic interactions: the outcome may vary considerably depending on the context. Thus rather than classifying symbiotic interactions into distinct categories (e.g., mutualism, parasitism, commensalism), they should be viewed as dynamic points along a continuum (Bronstein 1994).

The mycorrhizal symbiosis can be envisioned as such a continuum, in which the mutualistic plant–fungus interaction is the midpoint, and exploitation of a plant by a mycorrhizal fungus, or vice versa, are the endpoints (Egger and Hibbett 2004; Fig. 1.3). Costs and benefits of the mycorrhizal interaction are difficult to determine and may be context dependent (Johnson et al. 1997). However, there is considerable circumstantial evidence for the existence of exploitative strategies by mycorrhizal fungi (e.g., Klironomos 2003; Reynolds et al. 2005; Bever et al. 2009). Mycoheterotrophy is an exploitative strategy that represents the other end of the plant–fungus mycorrhizal continuum, in which the plant exploits its associated mycorrhizal fungi to obtain carbon and other nutrients (Fig. 1.3). The continuum between mycorrhizal mutualism and mycoheterotrophy is dynamic, and shifts along the continuum can occur at a developmental, ecological, or evolutionary timescale (Chaps. 5 and 8). For example, a plant may rely on mycoheterotrophy at the initial stage of its life cycle but develop into an autotrophic mature plant

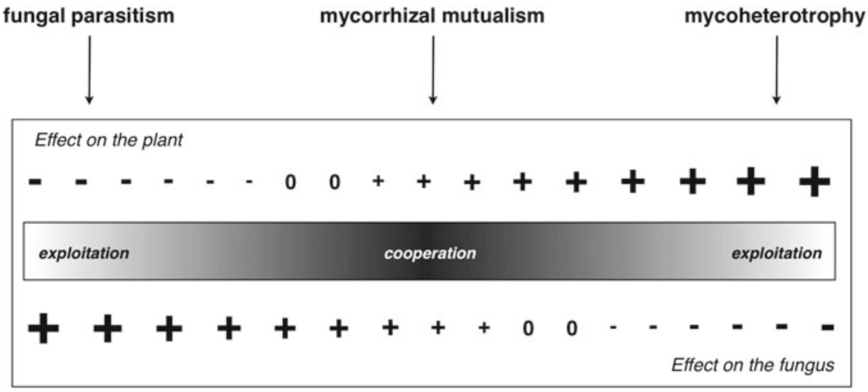

Fig. 1.3 Representation of the symbiotic exploitation–cooperation continuum of the mycorrhizal symbiosis based mutual effect of the interaction on the plant and fungal partners. Three potential outcomes of the interaction (fungal parasitism, mycorrhizal mutualism, and mycoheterotrophy) are indicated above. Figure adapted from Bronstein (1994) and Egger and Hibbett (2004)

(initial mycoheterotrophy) thus shifting from one point in the symbiotic continuum to another during its development. Some plants are able to combine autotrophy and mycoheterotrophy at maturity ("partial mycoheterotrophy," see further). However, where partial mycoheterotrophy can be placed between mycorrhizal mutualism and mycoheterotrophy depends on the context. It is possible that a partially mycoheterotrophic plant exploits its associated mycorrhizal fungi to the same extent as a fully mycoheterotrophic species. But theoretically, a partially mycoheterotrophic plant that acquires a significant amount of its carbon through photosynthesis will demand less carbon from its mycorrhizal fungi than a full mycoheterotrophic plant with a similar net carbon requirement. Stable isotope signatures of partially mycoheterotrophic plant species have revealed intraspecific variation of carbon uptake from fungi depending on light availability (Gebauer 2005; Zimmer et al. 2007; Preiss et al. 2010; Chap. 8). Thus it seems that partially mycoheterotrophic plants do not always exploit their mycorrhizal fungi fully. This suggests that, at least for some plant species, there is a positive correlation between the amount of carbon gained through mycorrhizal fungi and the degree of exploitation of their mycorrhizal fungi (Fig. 1.4).

1.3 A Short History of Research on Mycoheterotrophy

It would require a separate book to provide a detailed historical overview of the pioneering work of early naturalists that has provided the foundations of our understanding of mycoheterotrophy. Therefore, I have limited myself to a rather personal account, highlighting a few of the turning points that have revolutionized the field, with an emphasis on more recent developments. More comprehensive overviews of the early research on mycoheterotrophic plants are provided by Rayner (1927) and more recently by Leake (1994) and Bidartondo (2005).

The story of scientific research on mycoheterotrophy is tightly linked with advances in technology and methodology and begins in the nineteenth century. A major controversy in the 1840s marks the start of a scientific journey that still continues today: naturalists debated over the question of whether *Hypopitys monotropa*[1] (Ericaceae) is parasitic on the roots of beech trees

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[1] Based on recent genetic evidence, we place *Monotropa hypopitys* its own genus, *Hypopitys*, with the single species *Hypopitys monotropa* (see Chap. 2).

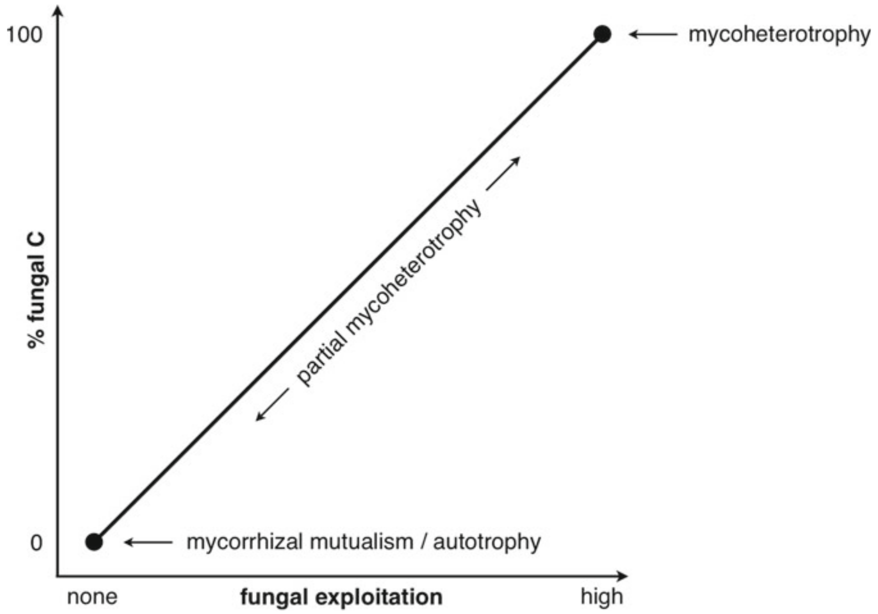

Fig. 1.4 Hypothetical model showing plant dependence on fungal carbon (C) as a function of the amount of fungal exploitation (*solid line*). When a mycorrhizal plant is fully autotrophic, it does not exploit its mycorrhizal fungi for carbon. On the other hand, when a plant completely depends on fungal carbon, fungal exploitation is high. Consequently, increased levels of partial mycoheterotrophy may relate to increased levels of fungal exploitation

or not. In the heated discussion that followed, it became clear—mainly through microscope observations—that the hairy fibers that link the roots of *Hypopitys* to those of surrounding trees are not parasitic plant haustoria but fungi (Rylands 1842). Around the same time, the first description of fungal infections in the roots of "saprophytic" orchids appeared (Reissek 1847; Schacht 1854; Prillieux 1856; Reinke 1873; Drude 1873). Pfeffer (1877) suggested that the observed fungus also penetrated the soil and brought nutrients to the orchid. An important next step was taken by Franz Kamienski, whose detailed investigation resulted in a breakthrough paper in which he postulates that *H. monotropa* lives on a fungus that is connected to tree roots and provides the first unambiguous description of a mycorrhiza (Kamienski 1882). To his merit, he explicitly discusses his observations in the context of the then recent definition of symbiosis, of which both antagonistic and mutualistic examples were known (de Bary 1879). But Kamienski writes: "It is not necessary to prove that those two forms of symbiosis are but extremes and that between them are to be found an infinite number of intermediary forms […]," and he concludes that the symbiosis between *Hypopitys* and its fungus is an "example of the most striking of the 'mutualistic' union of two vegetative organisms" (Kamienski 1882 *fide* Berch et al. 2005). Daniel MacDougal went on to suggest that many achlorophyllous plants gain "complex substances" by a symbiotic association with root-colonizing fungi, a concept he described as "symbiotic saprophytism" (MacDougal 1899). Francke (1934) observed fungal hyphae release their contents into root cells of *H. monotropa* and successfully grew *Hypopitys* seedlings from seeds by inoculating them with the mycorrhizal fungus extracted from mature plants.

The rich tradition of microscopy work on fungus–plant interactions of mycoheterotrophic plants is still continued today, now aided by powerful electron microscopes (e.g., Imhof 1999; Massicotte et al. 2005; Domínguez et al. 2009). Nevertheless, many aspects of the physiology of the mycoheterotrophic fungus interaction still remain unclear (see Chap. 4).

In the 1950s, research on mycoheterotrophy entered a new era with the experimental fieldwork of Erik Björkman (1960). He observed that physical separation of *H. monotropa* from tree roots resulted in a reduced growth of the former, and he was able culture a fungus from *Hypopitys* roots that formed ectomycorrhizas with pine roots. More importantly, he used radioactive-labeled isotopes to reveal that more carbon and phosphorus are transported from *Picea* to *Hypopitys* than to any other neighboring plant. This was the first "direct" demonstration of a fully mycoheterotrophic plant being energetically dependent on surrounding trees through shared mycorrhizal fungi. Radioactive tracer experiments have later been repeated to study mycoheterotrophic interactions, although for obvious reasons mostly in vitro rather than in natural habitats (McKendrick et al. 2000a; Bidartondo et al. 2003). Recently a series of radioactive-labeling experiments were used to show that the green orchid *Corallorhiza trifida* actually derives most of its carbon through mycoheterotrophy (Cameron et al. 2008, 2009).

The development of DNA sequencing and amplification techniques in the 1970s and 1980s revolutionized the field of biology. Ecologists were quick to adapt these new techniques for the identification of mycorrhizal fungi whose characterization had been hampered by their undifferentiated morphologies and difficulties in culturing them (e.g., Simon et al. 1993). Among the first to identify the fungi in the roots of fully mycoheterotrophic plants with molecular methods were Ken Cullings and colleagues, who sequenced the ectomycorrhizal fungi associated with the roots of monotropes (Cullings et al. 1996). This work was further elaborated by Kretzer et al. (2000), Bidartondo et al. (2000), and Bidartondo and Bruns (2001, 2002). Almost simultaneously, the first reports on the molecular identification of fully mycoheterotrophic orchids were published (Taylor and Bruns 1997; McKendrick et al. 2000b, 2002), followed by molecular studies on mycoheterotrophic plants living on arbuscular mycorrhizal fungi (Yamato 2001; Bidartondo et al. 2002). These and subsequent studies confirmed that most full mycoheterotrophs obtain carbon from surrounding green plants, through shared ectomycorrhizal or arbuscular mycorrhizal fungi. It was already known for a long time that some fully mycoheterotrophic orchids associate with litter- and wood-decaying ("saprotrophic") fungi (e.g., Kusano 1911; Hamada 1939), and these fungi were first identified with molecular methods by Yamato et al. (2005), Ogura-Tsujita and Yukawa (2008), Ogura-Tsujita et al. (2009), and Martos et al. (2009). The molecular identification of the fungi associated with fully mycoheterotrophic plants revealed an important and novel aspect of the mycoheterotrophic interaction: while autotrophic plants typically associate with multiple distantly related fungi and a mycorrhizal fungus often associates simultaneously with distantly related plants, fully mycoheterotrophic plants frequently show high specificity toward narrow lineages of fungi (Chap. 7).

The development of DNA sequencing tools also caused a revolution in the unraveling of the evolutionary relationships of plants (e.g., Chase et al. 1993). Due to high sequence divergences, the evolutionary relationships of mycoheterotrophic plants were often difficult to infer, yet despite this difficult start, phylogenetic studies based on DNA data soon proved to be extremely valuable for resolving lasting problems about the taxonomic position of "difficult" mycoheterotrophic plant lineages (e.g., Molvray et al. 2000). In many cases, this led to dramatic changes in our understanding of mycoheterotrophic plant relationships (Caddick et al. 2000, 2002; Cameron et al. 2003). Subsequently, phylogenetic hypotheses based on DNA data were successfully used to study divergence times and biogeographical scenarios of mycoheterotrophic plant lineages (e.g., Merckx et al. 2008).

Achlorophyllous mycoheterotrophic plants offer excellent opportunities to study genome evolution. But while the first complete plastid genome of a holoparasitic plant (*Epifagus virginiana*) was published in 1990 by Claude dePamphilis and Jeffrey Palmer (dePamphilis and Palmer 1990), it took until 2008 before the first mycoheterotrophic plant genome was sequenced (Wickett et al. 2008). The recent development of high-throughput sequencing methods offers promising new opportunities to study genome evolution and has already resulted in the complete chloroplast genomes of the fully mycoheterotrophic orchids *Rhizanthella gardneri* (Delannoy et al. 2011) and *Neottia nidus-avis* (Logacheva et al. 2011).

In the early 2000s, another approach helped the study of mycoheterotrophy. It was already established that nitrogen (N) and carbon (C) in fungi are isotopically distinct from N and C of accompanying vegetation (Gebauer and Dietrich 1993; Gleixner et al. 1993); thus, the stable isotope's natural abundances of N and C presented tools to study nutrient fluxes between fungi and mycoheterotrophic plants in their natural habitats. The first studies confirmed that achlorophyllous mycoheterotrophic plants are isotopically different from the surrounding green vegetation (Gebauer and Meyer 2003; Trudell et al. 2003). Gebauer and Meyer (2003) also discovered that the carbon stable isotope values of several green orchid species in their study fell between those of autotrophic and achlorophyllous mycoheterotrophic plants and consequently produced the first evidence of partial mycoheterotrophy. More evidence for partial mycoheterotrophy through ectomycorrhizal fungi in temperate orchids was gathered by Bidartondo et al. (2004), Julou et al. (2005), and Abadie et al. (2006). Using stable isotope measurements, partial mycoheterotrophy was soon after discovered in Ericaceae as well (Tedersoo et al. 2007; Zimmer et al. 2007), and stable isotope analyses were subsequently adapted to study full mycoheterotrophic plants living on saprotrophic fungi (Ogura-Tsujita et al. 2009) and arbuscular mycorrhizal fungi (Merckx et al. 2010; Courty et al. 2011) (Chap. 8).

Recently, additional poorly known aspects of mycoheterotrophic plants became the focus of investigations, including population genetics (Taylor et al. 2004; Klooster and Culley 2010; Beatty and Provan 2011; Dowie et al. 2011) and reproductive biology (Klooster and Culley 2009; Hentrich et al. 2010). However, despite the long history of research and the increasing interest in mycoheterotrophic plants, many facets of this extraordinary plant–fungus interaction remain unresolved. More research is awaited on identifying the drivers behind fungal specificity, chemical signaling in the interaction, specific pathways of metabolite transport, evolution of plastid genomes, and morphological and physiological convergences between different types of mycoheterotrophy and their genetic background. Thanks to technological advances, there is no doubt that many of these questions will be successfully answered in the coming years.

1.4 Concepts and Terminology

Nearly all plants are autotrophs: they convert carbon dioxide into organic compounds, especially sugars, using the energy from sunlight in a process known as photosynthesis. Achlorophyllous mycoheterotrophic plants have lost the ability to perform photosynthesis and are able to obtain carbon through a symbiotic association with fungi. The majority of fully mycoheterotrophic plant species associate with fungi that are mycorrhizal with surrounding autotrophic plants (either arbuscular mycorrhizal or ectomycorrhizal fungi). Typically, mycorrhizal fungi receive carbon from autotrophic plants in exchange for water and soil minerals. The fungal carbon on which these mycoheterotrophs rely thus ultimately comes from autotrophic plants (Fig. 1.2). At least a few species of mycoheterotrophic orchids are known to associate with saprotrophic fungi, which obtain carbon from dead or decaying organic matter. Mycoheterotrophy occurs in most major groups of land plants, including liverworts, lycophytes, ferns, angiosperms, and perhaps gymnosperms as well (Fig. 1.5). There are about 23,000 species of land plants that rely on a mycoheterotrophic interaction at some stage in their life cycle. Most are orchids that rely on fungal carbon during the early stages of their development. At least 514 species of angiosperms and a single liverwort species entirely depend on fungal carbon during their complete life cycle ("full mycoheterotrophs"; see definitions below). Fully mycoheterotrophic plants have at least 46 independent origins in land plant evolution (see Chap. 5).

Because it was assumed that they lived directly on soil organic matter, mycoheterotrophic plants

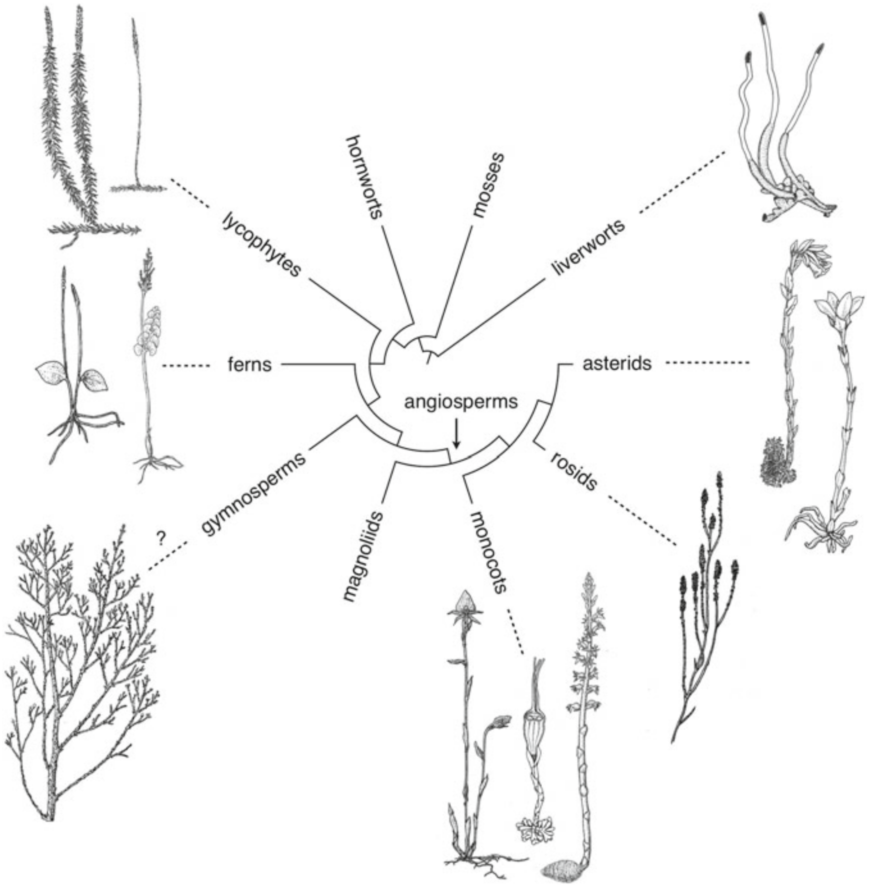

Fig. 1.5 Simplified land plant phylogeny. For each land plant group that contains mycoheterotrophic species, a few examples are shown (not drawn to scale). For lycophytes and ferns, autotrophic sporophytes are shown instead of the mycoheterotrophic gametophytes. For clarity, a few angiosperm lineages were omitted; see Fig. 5.4 for a complete angiosperm tree

were long regarded as "saprophytes" (a term which still appears in new literature from time to time). However, since the pioneering work of nineteenth-century naturalists, evidence from experimental, physiological, and molecular studies has continuously demonstrated that mycoheterotrophs depend on an association with fungi. In contrast, there is no evidence to date that direct carbon transfer from dead organic material to plants exists. "The myth of saprophytism" (Leake 2005) is thereby shattered, and we can advise strongly against the use of the term "saprophyte," as this misrepresents the mode of life of these remarkable plants.

The persistence of the incorrect term "saprophyte" highlighting the need for a consistent terminology based on clear definitions is necessary for meaningful discussion of mycoheterotrophic plants. Unfortunately, a unifying terminology has never been established, and in the current literature, different terms for the same phenomena compete with each other. I made an attempt to create a simple and consistent terminology that we will use throughout this book and that hopefully will be adapted by other authors as well. The first phenomenon we need to define is mycoheterotrophy itself:

"*Mycoheterotrophy*" *is the ability of a plant to obtain carbon from fungi*.

An important aspect of this definition is that it is solely based on the plant's ability to obtain

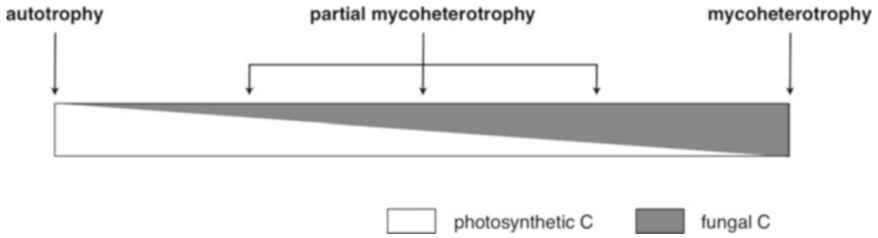

Fig. 1.6 Schematic representation of the trophic strategies autotrophy, mycoheterotrophy, and partial mycoheterotrophy based on the carbon (C) source a plant uses

carbon from root-associated fungi. It is likely that the metabolite fluxes of a mycoheterotrophic plant–fungus interaction differ from a normal mutualistic (mycorrhizal) plant–fungus interaction for other nutrients than carbon as well. For example, stable isotope analyses have shown that many mycoheterotrophs are distinctly enriched in ^{15}N compared to autotrophic plants growing at the same localities. However, ^{15}N enrichment is not a universal feature for mycoheterotrophic plants, and the extent of ^{15}N enrichment is not always linearly related to the extent of heterotrophic carbon gain (Leake and Cameron 2010).

Mycoheterotrophy is sometimes termed "epiparasitism" as well (e.g., Björkman 1960). This term stresses the fact that carbon can be received from green plants through a common mycorrhizal association but excludes dependence on saprotrophic fungi. Also, it remains unknown whether mycoheterotrophy has measurable costs to the green plants that supply carbon to the mycorrhizal fungi targeted by mycoheterotrophic plants. Therefore, "mycoheterotrophy" should be preferred over "epiparasitism."

Mycoheterotrophy is a trophic strategy that contrasts with autotrophy.[2] However, some plant species have been shown to be able to simultaneously combine autotrophy and mycoheterotrophy:

"*Partial mycoheterotrophy*" *is the ability of a plant to obtain carbon simultaneously through autotrophy and mycoheterotrophy.*

Thus autotrophy and mycoheterotrophy are the extreme ends of a continuum of trophic strategies that can occur in plants (Fig. 1.6). All intermediate strategies between autotrophy and mycoheterotrophy are designated as partial mycoheterotrophy, even though the relative amount of carbon received through either autotrophy or mycoheterotrophy may differ considerably. Partial mycoheterotrophy has also been termed "mixotrophy" (e.g., Selosse and Roy 2009), but we prefer "partial mycoheterotrophy" because "mixotrophy" is already used in a more general and different context (e.g., bacteria (Eiler 2006), protists (Thingstad et al. 1996), and sea anemones (Bachar et al. 2007)).

Autotrophy, mycoheterotrophy, and partial mycoheterotrophy are different strategies used by plants to obtain complex organic compounds (carbohydrates). It is now known that some plants can change their trophic strategy during their development. For example, the initial developmental stage of all orchids is a nonphotosynthetic protocorm that relies on mycoheterotrophy (Alexander and Hadley 1985; Leake 1994; Rasmussen 1995; Rasmussen and Whigham 1998). Nevertheless, most orchids lose their dependence on fungi as a source of carbon and develop into mature plants that solely rely on autotrophy. In addition, there is evidence that some plant species show plasticity in trophic strategy in relation to the environmental conditions in which they are growing. For example, research by Katja Preiss and colleagues has demonstrated that the orchid species *Cephalanthera damasonium* and *C. rubra* strongly supplement their carbon gain through photosynthesis by organic carbon from fungal partners under low-light conditions but become almost completely autotrophic when they are

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[2] In a broad sense, autotrophy includes both phototrophy, in which light is used as an energy source (photosynthesis), and lithotrophy (or chemoautotrophy), in which inorganic compounds are oxidized (chemosynthesis). In the context of plants, autotrophy is restricted to phototrophy.

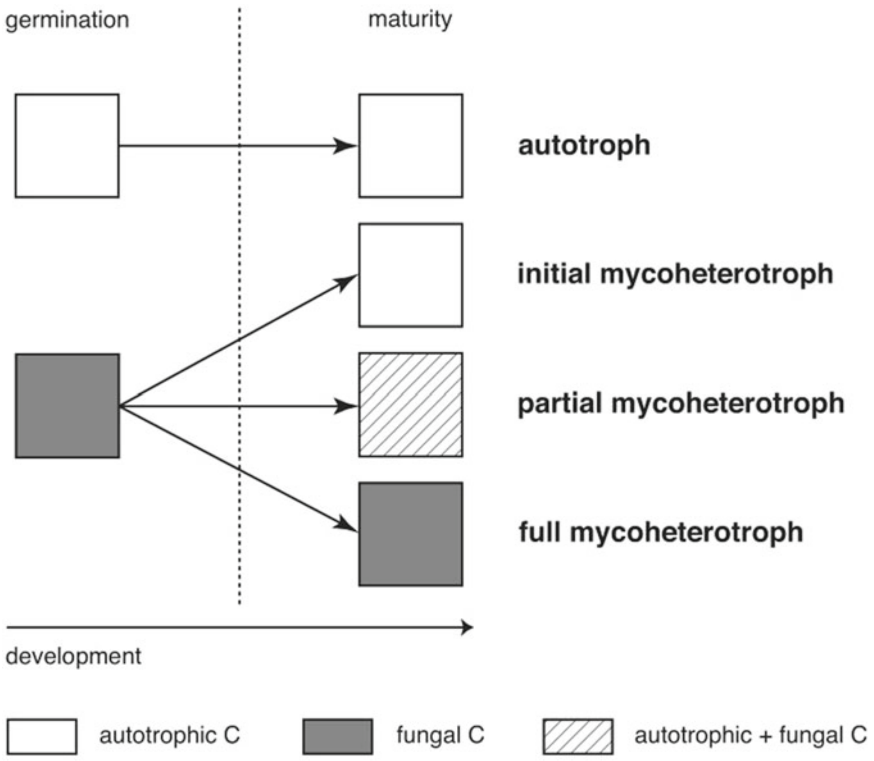

Fig. 1.7 Schematic representation of the different types of mycoheterotrophic plants known, based on life stage and their primary source of carbon (C)

exposed to sufficiently high irradiances (Preiss et al. 2010).

This trophic plasticity hampers the assignment of a plant species to a particular trophic category. Yet, for a comprehensive discussion of mycoheterotrophy, the designation of plant species according to their trophic capabilities is required. Therefore, I propose the following trophic categories, in which the developmental context plays an important role (Fig. 1.7).

A "fully mycoheterotrophic" plant ("full mycoheterotroph") solely depends on fungal carbon during its entire life cycle.

A fully mycoheterotrophic plant thus derives all of its carbon from fungi and does not need a functional photosynthetic apparatus. Plants that lack visible traces of chlorophyll and do not have a direct connection with autotrophic plants—and thus are not holoparasites—are putative fully mycoheterotrophic plants. Over 500 species of land plants fall into this category, the vast majority being angiosperms (Chap. 2).

An "initially mycoheterotrophic" plant ("initial mycoheterotroph") is fully dependent on associated fungi for its carbon supply during the early stages of development.

An initially mycoheterotrophic plant species relies on mycoheterotrophy in the beginning of its life cycle. In a broad sense, all full mycoheterotrophs are initial mycoheterotrophs as well, but we propose to use the term particularly for species that depend on autotrophy or partial mycoheterotrophy at maturity. Hence all orchids, except those that are fully mycoheterotrophic, are initial mycoheterotrophs (>20,000 spp.). Species of Pyroleae (Ericaceae) are probably also initial mycoheterotrophs (Smith and Read 2008; Eriksson and Kainulainen 2011). Other plant species that produce small dustlike seeds with limited nutritional reserves (e.g., Rubiaceae, Buddlejaceae, Gesneriaceae) are putative initial mycoheterotrophs as well (Eriksson and Kainulainen 2011). In their early development, the sporophytes of lower plants are "temporary parasitic on the gametophyte" (Leake et al. 2008). If those gametophytes depend on mycoheterotrophy, the sporophytes of these species (Chap. 2) can be considered initial mycoheterotrophs as well.

A "partially mycoheterotrophic" plant ("partial mycoheterotroph") combines autotrophy and mycoheterotrophy to obtain carbon during at least one stage of its life cycle.

By definition, partial mycoheterotrophic plants have retained a functional photosynthetic apparatus. Because partial mycoheterotrophs retain chlorophyll, their trophic life strategy can only be detected by examining their physiologies (Chap. 8). Partial mycoheterotrophy has been shown to exist in a few species of Orchidaceae, Gentianaceae, and Ericaceae but may be present in other plants families as well. Green plant species that are closely related to fully mycoheterotrophic species or can survive in extreme low-light conditions (e.g., forest understory habitats) are prime candidates for undiscovered partial mycoheterotrophy. The dependence on fungal carbon can greatly differ between different partially mycoheterotrophic species (Gebauer and Meyer 2003) and between specimens of the same species that grow in different light conditions (Preiss et al. 2010). Even seasonal fluctuations occur within plant populations (Hynson et al. 2011).

Note that the terminology above is not necessarily mutually exclusive: all species of Orchidaceae are initial mycoheterotrophs, and they can develop into either autotrophic, partially mycoheterotrophic, or fully mycoheterotrophic mature plants. In the latter case, however, the term "full mycoheterotroph" is preferred.

1.5 Parasitic Plants

Fully mycoheterotrophic plants are often confused with parasitic plants. However, parasitic plants are a distinct category of achlorophyllous plants. These include holoparasitic plants, which obtain carbon from autotrophic plants through a direct physical attachment. The enigmatic *Rafflesia arnoldii* (Rafflesiaceae), which produces the biggest flower in the world, is one such example (Nais 2001). There are about 390 species of holoparasitic plants. In addition, about 4,100 eudicot plant species are hemiparasites: they have retained the ability to photosynthesize, but they primarily absorb water and nutrients from their host plants (Heide-Jørgensen 2008). The parasitic mode of life has evolved at least 11 times independently in the evolution of eudicots (Barkman et al. 2007). A single gymnosperm (*Parasitaxus usta*) may be a holoparasite as well, although there are indications that a fungus is involved in the interaction, and therefore, the species is sometimes regarded as a mycoheterotroph (see Chap. 2).

Mycoheterotrophy and holoparasitism represent distinct evolutionary pathways toward heterotrophy in plants. Yet there is often a striking morphological convergence between mycoheterotrophs and holoparasites: in general, both have highly reduced leaves, contain little or no chlorophyll, and produce prodigious numbers of seeds that cannot establish in absence of a host (Cameron and Leake 2007). Therefore, it is not surprising that Linnaeus initially considered *H. monotropa* to be a species of *Orobanche* (Leake 1994)!

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