The Physiological Ecology of Mycoheterotrophy

8

Nicole A. Hynson, Thomas P. Madsen, Marc-André Selosse, Iris K.U. Adam, Yuki Ogura-Tsujita, Melanie Roy, and Gerhard Gebauer

8.1 Introduction

Mycoheterotrophic (MH) plants have been centerpieces for the study of the physiological aspects of the mycorrhizal symbiosis for over 150 years. A.B. Frank who in 1885 coined the term "mycorrhiza" conducted research on the anatomy and physiology of the fully MH species *Hypopitys monotropa* (syn. *Monotropa hypopitys*) (Frank 1885). Due to the charismatic, often highly derived, appearance of many MH plants, it is no surprise that they have captured the interest of researchers throughout the centuries. These plants continue to draw attention from various realms of science because they consistently require that our fundamental understanding of many ecological, evolutionary, and physiological theories be expanded upon. Both the ecology and evolution of mycoheterotrophy are tightly coupled to plant–fungal interactions or ecophysiology, and this subject is the focus of this chapter. From an ecological perspective, MH plants that associate with mycorrhizal fungi represent the best-known examples of mycorrhizal networks, where unrelated plants transfer elemental compounds via shared fungal symbionts (Chap. 1; Simard and Durall 2004; Selosse et al. 2006). From an evolutionary perspective, fully MH plants that associate with mycorrhizal fungi are the primary example of one extreme in the mycorrhizal continuum, ranging from plants giving carbon (C), to plants receiving C from their fungal symbionts (Chap. 1).

Within the plant kingdom the MH strategy has arisen numerous times throughout evolutionary history and involves not only mycorrhizal fungi, but saprotrophic (SAP) fungi as well. The physiology of mycoheterotrophs and their mycorrhizal fungi represent the only clear example of a complete reversal in the normal flow of nutrients in the mycorrhizal symbiosis, where instead of

N.A. Hynson (✉)
Department of Botany, University of Hawaii at Manoa, Honolulu, HI 96822, USA
e-mail: nhynson@hawaii.edu

T.P. Madsen
Department of Integrative Biology, University of California, Berkeley, Berkeley, CA 94720, USA

M.-A. Selosse
Centre d'Ecologie Fonctionnelle et Evolutive (CEFE-CNRS), Montpellier, France

I.K.U. Adam • G. Gebauer
Laboratory of Isotope Biogeochemistry, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, 95440, Bayreuth, Germany

Y. Ogura-Tsujita
Department of Chemical and Biological Sciences, Japan Women's University, 2-8-1 Mejirodai, Tokyo, 112-8681, Japan

M. Roy
Centre d'Ecologie Fonctionnelle et Evolutive (CEFE-CNRS), Montpellier, France

Laboratoire Evolution et Diversité Biologique (UMR5174 EDB), Université de Toulouse, Toulouse, France

V.S.F.T. Merckx (ed.), *Mycoheterotrophy: The Biology of Plants Living on Fungi*,
DOI 10.1007/978-1-4614-5209-6_8,

plant-derived C being traded for nutrients acquired by fungi (Kiers et al. 2011; Selosse and Rousset 2011), both C and other nutrients have a net unidirectional flow from fungus to plant. Furthermore, fully MH plants that depend on SAP fungi are the only known examples of plants whose primary C source is derived solely from complex dead organic substrates. Their existence reopens the important, but debated question of reentry of organic C into plants via mycorrhizal interactions (Baldrian 2009; Selosse et al. 2010). Sometimes it is these exceptions to conventional biological systems that provide the greatest insights into ecosystem function as a whole, and we approach here the ecophysiology of mycoheterotrophy with this broader context in mind.

The purpose of this chapter is to provide a practical and theoretical framework for the study of MH plants' ecophysiology. We will highlight the findings from recent studies that have provided inroads into unraveling the curious functioning of MH plants. Of all the chapters in this book, this chapter will most likely be the quickest to become outdated, because at the time of writing, the field of MH plant ecophysiology is in rapid development. Techniques for high-throughput sample analysis especially for elemental and isotope studies are gaining momentum, as are new techniques for studying source–sink relationships between plants, fungi, and the environment in situ. However, with the application of new methodologies, it is important to remember that within the specific field of MH research, the questions being asked have remained fundamentally the same for over 200 years (Rayner 1927 and references therein). These questions include, who are the players in MH associations? What are the plant and fungal trade-offs for MH interactions? What factors, environmental or otherwise, select for mycoheterotrophy? And more specifically, what forms of C and other nutrients are transferred from fungi to mycoheterotrophs? To date, the majority of research on mycoheterotrophy has addressed the first question and we now have a substantial amount of information on the functional and phylogenetic diversity of fungi that associate with mycoheterotrophs (see Chaps. 5, 6, and 7 for more detail).

A frequent pattern among most MH species is specificity for particular fungal hosts. However, these fungi have a wide phylogenetic breadth within the kingdom Fungi. This indicates that rather than phylogenetic conservatism, it is either physiological or evolutionary pressures that select the fungal hosts for mycoheterotrophs (Hynson and Bruns 2010). To date, the most in-depth research has been focused on fully MH orchids and other taxa that are dependent on mycorrhizal fungi, particularly ectomycorrhizal (EM) fungi, to meet their nutrient demands. Although some fully MH species subsist on compounds derived from SAP fungi, they appear to occur only among the orchids (Selosse et al. 2010). However, the vast majority of fully MH species associate with arbuscular mycorrhizal (AM) fungi in the tropics, but due to a bias of field research to temperate regions, much of what we understand about the ecophysiology of mycoheterotrophy comes from temperate species.

What are the potential ecophysiological determinants of mycoheterotrophy? The physiological dependency of mycoheterotrophs on fungi starts with the earliest stages of seedling development when there are chemical cues between seed and fungus that trigger germination (Bruns and Read 2000). However, germination also seems to be the tightest bottleneck for MH plant survival (Bidartondo and Read 2008; Eriksson and Kainulainen 2011; Tesitelova et al. 2012; Chap. 5). This is because the seeds of many MH species will only germinate in the presence of fungi that are closely related to those associated with adult plants, but upon reaching maturity, no plants have been found with the "wrong" fungal host. However, we cannot rule out the possibility that there are rare instances of individuals surviving with alternative hosts, and that this may lead to permanent host switching, explaining jumps from one fungal partner to another.

At the early developmental stages, securing a source of C and other nutrients is of paramount importance to the plant. It has been argued that for C and other nutrient transfer to occur from fungus to MH plant, the plant must create a concentration gradient that has a draw-down or sink effect on the plant-fungal network (Finlay and

Read 1986). This gradient could be created by the rapid transformation of compounds received from the fungus by the mycoheterotroph into forms that are unavailable for fungal use, or by storage apart from the plant–fungal interface. This has been somewhat demonstrated in the mutualistic interactions of mycorrhizal fungi with autotrophic host plants where plant carbohydrates derived from photosynthesis are converted by fungi into trehalose and polyols that are largely unavailable for plant uptake (Smith and Read 2008). The primary example of fungal assimilated C compounds being converted to plant carbohydrates by a mycoheterotroph is from a study by Smith (1966). In her laboratory experiments two rhizoctonia fungi first colonized the MH seedlings of the orchid *Dactylorhiza purpurella*, then fungi were fed ^{14}C labeled sucrose, which they transformed into trehalose, a portion of which was transferred to the orchid seedlings which transformed it into glucose, fructose, and sucrose (Box 8.1). Interestingly, invertases are absent from at least some EM fungi (Martin and Selosse 2008; Parrent et al. 2009) and AM partners (Tisserant et al. 2012), so that sucrose may well be unavailable for fungal use. Similar to

Box 8.1 Radioisotope and Stable Isotope Labeling

The use of naturally rare radioactive isotopes (e.g., ^{14}C, ^{32}P, or ^{35}S) has a long history in biochemistry and biology. Many physiological matter pathways of living organisms were elucidated using radioisotopes as tracers. For example, Melvin Calvin and colleagues discovered in the late 1940s and 1950s the photosynthetic C reduction cycle of plants and mapped the complete route that C travels during photosynthesis, starting from its absorption as CO_2 to its conversion into sugars and other organic compounds by using ^{14}C as a tracer. In 1961 Calvin was awarded the Nobel Prize in Chemistry for his discovery. Nuclear weapon tests in the 1950s and 1960s were responsible for a doubling of the natural ^{14}C concentration in the atmosphere (Levin and Kromer 1997). This increase in ^{14}C concentration in the atmosphere has been used in ecological studies as a "natural" tracer to investigate turnover rates of organic C pools and the age of soil organic C stores (e.g., Trumbore 2000). Later on rare stable isotopes (e.g., ^{13}C, ^{15}N, or ^{18}O) were added to the suite of tracers in field and laboratory experiments on matter fluxes and transformations between ecosystem pools. Isotopes, i.e., atoms belonging to the same element and possessing equal numbers of protons and electrons, but different numbers of neutrons, are considered as ideally suited tracers because of their almost identical chemical and physical properties. In a typical tracer or isotope labeling experiment a known and usually small amount of a naturally rare isotope in a defined chemical form (e.g., $^{13}CO_2$) is added to a source pool (e.g., atmosphere of a growth chamber containing $^{12}CO_2$) and then recovered transformed in a sink pool (e.g., tissue of a photosynthetic plant) after a known amount of time. The total amount of an element (e.g., ^{13}C tracer and ^{12}C from the growth chamber atmosphere) that moved from the labeled source pool (A) to the sink pool (B) can be calculated knowing the atom% excess of pool A (I_A), and the mass (P_B) and atom% excess of pool B (I_B) at the end of the experiment (from Stark 2000):

$$M_{AB} = (P_B \times I_B) / I_A$$

Dividing of M_{AB} by the time of the experiment gives the flux rate from A to B.

Isotope labeling experiments are based on the following assumptions:

- Added isotope and natural isotope behave identically.
- The source pool is uniformly labeled, and this labeling remains constant over the duration of the experiment.
- Addition of the tracer does not change flux rates.
- No labeling gets lost from the sink pool.

(continued)

Box 8.1 (continued)

These assumptions are not always entirely valid. For example, due to fractionation effects isotopes behave very similarly, but not entirely equal (see Box 8.2). However, isotope fractionations can be ignored in labeling experiments as long as isotope enrichments well above natural isotope abundance are used.

Radioisotopes in source and sink pools can easily be quantified using scintillation counting (e.g., Schimel 1993). In addition autoradiography is a long known and elegant technique to visualize the radioisotope distribution in a sink pool (e.g., Schimel 1993). The use of stable isotopes in tracer studies requires more sophisticated analytical techniques (e.g., isotope ratio mass spectrometry, see Box 8.2); however, they bear no health or environmental hazards and therefore can easily be used in tracer experiments on field level. Furthermore, stable isotopes provide access to trace elements for which no natural radioisotopes exist, e.g., nitrogen and oxygen. For further details on the theory and application of radioisotopes and stable isotopes as tracers we refer readers to Schimel (1993).

autotrophic plant-to-plant C transfer via shared mycorrhizal fungi, in MH plants, the demand or sink strength could be life stage dependent, as well as seasonally and environmentally variable (Lerat et al. 2002; Simard and Durall 2004).

The recent revelation of cryptic or partial mycoheterotrophy in green plants has highlighted the variation in plants' dependency on fungi to meet their C demands. Partial mycoheterotrophs are green plants that appear to be fully autotrophic, but meet some portion of their C demands via fungi in a mixotrophic nutrition that will be explored in detail in this chapter. Since these findings, partial mycoheterotrophy has been proposed as a potential evolutionary pathway to full mycoheterotrophy. However, the underlying determinants, geographical and phylogenetic extent of partial mycoheterotrophy are unknown and currently an area of active research. Ecophysiological methods such as radioactive and stable isotope probing, measuring plant assimilatory and respiratory responses to environmental gradients such as light availability, and natural abundance stable isotope analysis are all critical tools for the study of mycoheterotrophy and their applications are discussed in detail in this chapter. Methodological limitations and considerations for the study of physiological ecology of mycoheterotrophy will also be outlined.

The final section of this chapter will address areas for future research and draw attention to the gaps in our current knowledge of MH ecophysiology. This field is ripe for the undertaking by new methods and researchers. In the years to come, many of the current limitations to studying mycoheterotrophs, and plant–fungal interactions in general, will become obsolete as we continue to develop new quantitative and noninvasive methods to study these systems in situ or perhaps even ex situ (Yagame et al. 2012). What is critical now is that robust theoretical frameworks be established a priori that will engage researchers from across fields and provide a sound foundation for the interpretation of forthcoming data on MH plants. In future research, MH plants will continue to be model systems for the study of the ecophysiology of plant–fungal interactions. This is due to their complete dependency on often a sole fungus to meet their nutrient demands, the fact that they are phylogenetically and geographically widespread, and many represent a profound modification of the most common and abundant mutualism on earth, the mycorrhizal symbiosis.

8.2 From Mutualism to Parasitism: Deconstructing the Continuum of Plant–Fungal Interactions

8.2.1 Mycorrhizal Networks

Since the definitive experiments of Erik Björkman in the 1960s where the transfer of a ^{14}C label applied to the phloem of pines was traced to

surrounding individuals of the fully MH species *Hypopitys monotropa*, researchers continue to test for the presence and extent of mycorrhizal networks in nature. In its simplest form, a mycorrhizal network consists of two plant individuals of the same species connected via a shared mycorrhizal fungus. Due to the diffusivity of the mycorrhizal symbiosis and the large size of fungal individuals in some species (some genetic individuals can cover several m^2; Douhan et al. 2011) it is almost certain that these connections exist in nature, but to what extent they actually link different plants and act as conduits for C and mineral nutrient exchange between plants is the subject of much debate.

Fully MH plants that associate with mycorrhizal fungi provide the best examples of C transfer from unrelated plants via shared fungi. Because this tripartite network of autotrophic host plants, mycorrhizal fungus, and mycoheterotroph involves only unidirectional C and mineral nutrient flow to the mycoheterotroph, it is often referred to as an epiparasitism rather than a mutualism. However, the impact of mycoheterotrophy on partners' fitness (especially on the autotrophic host plant) is currently unknown. The evidence for mycorrhizal networks where there is transfer of C or other nutrients among plants that engage in a mutualism with their mycorrhizal fungi is less clear-cut. The first quantitative ex situ laboratory studies to show significant transfer of C between autotrophic plants via shared AM or EM fungi were conducted by Francis and Read in 1984 and Finlay and Read in 1986. Since then, numerous field manipulation and laboratory experiments have taken place to test the significance of mycorrhizal networks in plant establishment, survival, and below-ground resource sharing. These studies have had mixed results, leading some researchers to question the overall importance of mycorrhizal networks (Fitter et al. 1998; Robinson and Fitter 1999; Wu et al. 2001; Pfeffer et al. 2004). However, there is mounting evidence that (1) mycorrhizal networks are common in nature (Simard and Durall 2004; Selosse et al. 2006), (2) there is the potential for bidirectional C, nitrogen (N), and phosphorus (P) movement between plants (Lerat et al. 2002; Teste et al. 2009), and (3) depending on environmental factors such as light availability the sink strength of "receiver" plants in mycorrhizal networks can increase (Finlay and Read 1986; Simard et al. 1997). All these factors could have profound effects on interplant competition, plant and fungal diversity, and community dynamics (Simard and Durall 2004; Selosse et al. 2006). Recent field studies of mycorrhizal networks have focused on their role in seedling establishment (Nara 2006; Teste et al. 2009), survival (McGuire 2007), and growth (Booth 2004). These studies provided strong support that mycorrhizal networks can be critically important in early forest succession stages and tree recruitment. However, they suffer from similar limitations such as the difficulties in assessing the physical presence of fungal connections between plants, measuring long-term net C flow from donor to receiver plants, and the contributions of mycorrhizal networks to plant fitness over temporal and life stage gradients. Future efforts in the study of mycorrhizal networks should be focused in these areas as well as gaining a better understanding of the environmental plasticity of mycorrhizal networks; if they are controlled by plants and/or fungi; if the payoffs of networking are stronger selective forces than the benefits of competition; and finally, whether plants receiving benefits from networking are true "cheaters," providing no reciprocity, or if they somehow compensate for what they receive.

It is becoming undoubtedly clear that the mycorrhizal symbiosis is far less static than it was historically thought to be. Furthermore, where a particular plant or fungus falls along the continuum of mutualistic to parasitic in relation to its symbiotic partner(s) appears to be potentially life-stage, environmentally, and community driven. Fully MH plants may provide one exception to this plasticity due to their absolute dependency on fungi. Thus, quantifying complete C budgets for mycoheterotrophs over the course of their lifecycles, as well as the fitness costs to their fungi (and perhaps autotrophic host plants) will provide much needed constraints for modeling plant–fungal interactions.

8.2.2 Determination of Full Mycoheterotrophy

8.2.2.1 Evidence Based on Radioisotope and Stable Isotope Labeling

In 1881 it was hypothesized that *Hypopitys monotropa* shares a symbiotic fungus with neighboring forest trees and is nourished by these trees through a common mycelial network (Kamienski 1881). However, at that time this hypothesis was not widely accepted. It took almost 80 years until Kamienski's hypothesis was for the first time experimentally confirmed based on radioisotope labeling experiments. It was Björkman (1960) who demonstrated in field experiments that ^{14}C-labeled glucose and ^{32}P-labeled phosphate injected into the phloem of spruce and pine trees were translocated within 5 days to adjacent *Hypopitys monotropa* plants. Other neighboring understory plants, like *Vaccinium myrtillus*, *V. vitis-idaea*, and *Calluna vulgaris*, remained unlabeled (for details on isotope labeling see Box 8.1). Björkman (1960) furthermore demonstrated that a trenching of *H. monotropa* plants from adjacent tree roots by metal sheets severely reduced their development. He concluded that these observations confirm the existence of hyphal connections between *H. monotropa* and neighboring trees and indicate a selective C and P transfer from the trees to *H. monotropa* plants through shared fungal hyphae.

It took another 40 years until further substantial evidence on a selective C transfer from trees to an MH plant through linked fungal mycelia was successfully documented—again using radiocarbon as a tracer. In microcosm experiments McKendrick et al. (2000) fed shoots of *Betula pendula* and *Salix repens* plants growing in association with the leafless orchid *Corallorhiza trifida* with $^{14}CO_2$ and traced the movement of the isotope by a combination of digital autoradiography and scintillation counting. Direct C transfer assimilated by both of the autotrophs to *Corallorhiza* plants occurred only in those cases where plants had already been connected to a shared mycorrhizal fungus. *C. trifida* seedlings introduced to the microcosms as controls immediately before isotope labeling and thus lacking these hyphal connections failed to assimilate significant C amounts. McKendrick et al. (2000) furthermore documented that *C. trifida* plants linked to *B. pendula* and *S. repens* through mycorrhizal hyphae gained 6–14% in biomass during the 25–28 weeks period of the microcosm experiment, while *C. trifida* plants growing in microcosms with *Pinus sylvestris* failed to develop hyphal links and lost 13% of their weight over the same period. Nearly at the same time, fungal ribosomal DNA also provided evidence that the same fungal individuals occurred in the roots of surrounding trees and of the MH orchids *Cephalanthera austinae* (Taylor and Bruns 1997) and *Neottia nidus-avis* (Selosse et al. 2002), supporting a link to trees by individual fungal mycelia. In another microcosm labeling experiment it was shown that a ^{14}C label provided as CO_2 to *Betula pendula* seedlings was transferred to the non-photosynthetic liverwort *Aneura* (*Cryptothallus*) *mirabilis* through a shared mycorrhizal fungus (Bidartondo et al. 2003). In this case a *Tulasnella* sp. was identified to form simultaneously an EM association with trees and a connection with *Aneura mirabilis*.

Also using the microcosm approach, but stable isotope labels (^{13}C and ^{15}N) instead of radioactive isotopes, Bougoure et al. (2010) investigated the tripartite matter exchange between the fully subterranean orchid *Rhizanthella gardneri*, a mycorrhizal fungus from the genus *Ceratobasidium* and the photosynthetic shrub *Melaleuca scalena*. They demonstrated that up to 5% of the C applied as $^{13}CO_2$ to the autotrophic shrub was transferred to *R. gardneri*. *Rhizanthella gardneri* also gained 6% of the C and 22% of the N fed as [^{13}C-^{15}N] glycine to the soil through the mycorrhizal fungus. The non-stoichiometric C and N transfer from the glycine source through the fungus to *R. gardneri* is explained by fungal glycine transformation and respiratory ^{13}C-loss (see also Taylor et al. 2004).

Isotope labeling experiments are not only used to trace matter fluxes between ecosystem compartments, but also to document a lack of matter fluxes or reduced fluxes, for example due to missing or reduced metabolic activity. Cameron et al. (2009) provide an example for this kind of tracer application. They compared the potential for CO_2 assimilation by the MH orchid *Neottia nidus-avis*

to that of the leafless but chlorophyll containing orchid *Corallorhiza trifida*. CO_2 assimilation of these two orchid species was further compared to the leafy and green (chlorophyllous) orchid *Cephalanthera damasonium* and to *Fagus sylvatica* seedlings using ^{13}C isotope tracers in the field. The $^{13}CO_2$ assimilation rates decreased in the order *Fagus*>*Cephalanthera*>>*Corrallorhiza*>*Neottia*. These results indicated that the photosynthetic capacity of the *Corallorhiza trifida* individuals on the day of this experiment was closer to the fully MH *Neottia nidus-avis* than to the autotrophic *Fagus sylvatica* or the apparently partially MH (PMH) *Cephalanthera damasonium* (for further details on this tracer experiment see Sect. 8.5.1).

8.2.2.2 Evidence Based on Natural Abundance ^{13}C and ^{15}N

Independent investigations by Gebauer and Meyer (2003) and Trudell et al. (2003) discovered a considerable enrichment of heavy C and N isotopes in the tissues of fully MH orchids and monotropoids (Ericaceae) in comparison to surrounding autotrophic plants (for further details on the stable isotope natural abundance approach see Box 8.2). This enrichment in ^{13}C in the investigated fully MH plants was explained by these species tapping into alternative C sources to atmospheric CO_2 utilized by autotrophic plants in photosynthesis. Fully MH plants enrichment in ^{15}N was thought to be due to these plants receiving compounds enriched in ^{15}N compared

Box 8.2 Stable Isotope Natural Abundance

Most elements of biological interest are composed of two or more stable and/or nonstable (radioactive) isotopes. Isotopes of an element have by definition identical numbers of protons and electrons. However, they are distinguished by the number of neutrons in their nucleus and therefore have different atomic mass units. Identical numbers of protons and electrons are responsible for the mostly equal chemical and physical properties of isotopes. Nonetheless, the difference in the number of neutrons causes slightly different symmetries of atom nuclei and the electron sheath of isotopes. These differences in atomic properties cause thermodynamic isotope effects, i.e., the equilibrium constants of isotopes are slightly different, and kinetic isotope effects, i.e., the speed constants of isotopes in (bio)chemical reactions are slightly different. Due to these isotope effects, the isotopic composition of ecosystem compartments changes in predictable ways as elements cycle through the biosphere. Geochemists exploit changes in the isotopic composition of various biospherical pools to understand principles of the global element cycles. During the last two decades biologists have also become increasingly aware of the potential information that can be gained from the analysis of (mostly stable) isotope natural abundance variation. The use of stable isotope natural abundance analysis provides a nondisruptive method for studying ecosystem fluxes that complements the long established use of stable and radioactive isotopes in tracer experiments (see Box 8.1). However, the adoption of stable isotope analysis has been relatively slow to the field of biology due to: (1) Limited access to isotope ratio mass spectrometers (IRMS) which require specific knowledge of sophisticated analytical techniques to operate and are quite expensive. Also, many biologists do not have sufficient backgrounds in analytical chemistry to successfully run this equipment or interpret the resulting data. (2) The framework for advances in many fields of biology, and specifically in ecophysiology, are based on manipulative experiments, i.e., mostly short-term manipulations of organisms or environmental conditions and analysis of responses to these manipulations. Exploiting information from stable isotope natural abundances, however, provides new perspectives based on an essentially different conceptual framework.

(continued)

Box 8.2 (continued)

Gaining information of biological relevance from variations in stable isotope natural abundance does not necessarily require experimental manipulation. The "experiments" are all in situ processes in nature. Stable isotope natural abundance of various ecosystem compartments integrates these processes and associated matter fluxes (sources) over time. A typical example of process information based on stable isotope natural abundance is the isotopic distinction between plants following the C3 and C4 pathways of photosynthesis. Due to the different isotope fractionations by the two enzymes involved in primary CO_2 fixation: Rubisco in C3 plants and PEP carboxylase in C4 plants, C3 and C4 plants have distinct stable carbon isotope signatures. Conversely, typical source information is the origin of compounds in a product pool, e.g., the total N pool of a legume acquired from soil-borne N compounds taken up by the roots versus N gained through symbiotic fixation of isotopically distinguished atmospheric N_2 in root nodules. Thus, analysis of stable isotope natural abundance in various ecosystem compartments provides process and source information, and care must be taken to avoid a mix up of these two kinds of information.

The two elements of major interest in this chapter, carbon (C) and nitrogen (N), are both composed of two stable isotopes: ^{12}C and ^{13}C, or ^{14}N and ^{15}N, respectively. In addition, C also has a natural very rare radioactive isotope: ^{14}C. In analogy to most other elements of biological interest, the light stable isotopes of C and N (^{12}C and ^{14}N) are much more abundant than the heavy ones (^{13}C and ^{15}N). While ^{12}C contributes about 98.89 atom% to the terrestrial C pool, ^{13}C contributes only about 1.11 atom%. The relative abundance of ^{15}N is even lower. This isotope contributes only about 0.37 atom% to the terrestrial N pool, thus leaving 99.63 atom% to the isotope ^{14}N. The variation in relative abundance of heavy isotopes between ecosystem compartments driven by isotope fractionation due to thermodynamic or kinetic isotope effects is rather low. For C and N the natural variation in relative abundance of the respective stable isotopes in nature ranges at a maximum by 0.1 atom% or 0.02 atom%, respectively. Highly precise detection of such small variations in stable isotope natural abundance requires sophisticated analytical techniques. Specifically designed IRMS measuring the frequency ratios of stable isotope pairs of the respective elements in relation to defined reference gases (in our case CO_2 or N_2, respectively) coupled online to different types of gas sample preparation devices like elemental analyzers, pyrolysis units, and gas chromatographs are at present most commonly used to fulfill these requirements. Due to the fact that the analysis of stable isotope natural abundances is based on abundance ratios, in a sample of unknown isotope abundances, its isotope ratios are measured and then related to stable isotope abundance ratios of a defined standard. The so-called δ notation is commonly used to express isotopic compositions:

$$\delta x = (R_{sample} / R_{standard} - 1) \times 1{,}000[‰]$$

where x in our case is either ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. International standard reference material for C stable isotope abundances is PDB (limestone from a fossil belemnite of the PeeDee strata in South Carolina) with a $^{13}C/^{12}C$ ratio of 0.0112372 and for N stable isotope abundances is N_2 gas in the atmosphere with a $^{15}N/^{14}N$ ratio of 0.0036765. By subtracting 1 from the $R_{sample}/R_{standard}$ ratio and subsequent multiplying by 1,000 the δ value provides information about the deviation in the respective heavy isotope abundance between sample and standard in ‰. For example, a δ value of +1,000‰ is equivalent to a twice as high heavy isotope abundance in a sample than in the respective standard.

For further details on principles of the stable isotope natural abundance technique and on the exponentially increasing spectrum of applications in plant ecophysiology and

(continued)

Box 8.2 (continued)

ecology in general we refer readers to reviews by Dawson et al. (2002) and Fry (2006). Three aspects out of the huge range of stable isotope natural abundance applications that are of specific importance for the study of mycoheterotrophy are: (1) the principles of how environmental factors affect carbon isotope signatures of plants with C3 photosynthesis (Farquhar et al. 1989), (2) the concept of an isotopic shift along food chains (DeNiro and Epstein 1978, 1981), and (3) the finding of a significant enrichment in heavy C (Gleixner et al. 1993) and N isotopes (Gebauer and Dietrich 1993) in fungal fruit bodies in comparison to plant tissues.

to surrounding autotrophic plants that share the same mycorrhizal fungi. Owing to MH plants' obligate association with various functional groups of fungi (see Chap. 7), and based on the findings from earlier isotope labeling studies, fungi were proposed as the most likely alternative C and N source of fully MH plants. As further support for this, many fungi were already known to be enriched in the heavy isotopes ^{13}C and ^{15}N in comparison to autotrophic plants from the same habitat due to their specific physiology and access to C and N sources also enriched in heavy isotopes (see review by Mayor et al. 2009). Following the food chain concept (Fry 2006) the C and N isotope signatures of MH plants should be similar to, or even more enriched in heavy isotopes than in their fungal source (Trudell et al. 2003). Moreover, the relative enrichment in heavy isotopes in fungi is not a uniform feature, but is specific to different functional and taxonomic fungal groups. For example, fungi forming EM associations tend to be more enriched in heavy C and N isotopes than the majority of SAP fungi (Kohzu et al. 1999; Taylor et al.; 2003; Mayor et al. 2009). In contrast, AM fungi tend to be more depleted in ^{15}N than EM fungi, and are apparently not enriched in ^{13}C compared to their autotrophic host plants (Courty et al. 2011 and references therein). The relative ^{13}C enrichment in EM fungi is related to the gain of ^{13}C-enriched carbohydrates from photosynthetic plant partners (Gleixner et al. 1993). Among EM fungi, species associated with overstory trees are more enriched in ^{13}C than species associated with understory trees (Högberg et al. 1999), and species capable of decomposing recalcitrant soil organic compounds are more enriched in ^{15}N than species with a preference for inorganic N compounds (Gebauer and Taylor 1999). For SAP fungi their respective C and N source (wood, leaf litter, humus, etc.) determines their isotope signature (Gebauer and Taylor 1999; Kohzu et al. 1999). Thus, according to the isotope food chain concept, the differing patterns in the isotope signatures of the various functional groups of fungi is expected to be mirrored by MH plants associated with them. In the following section we provide a comparative overview of our current knowledge on C and N isotope natural abundance in fully MH plants associated with EM, wood- and litter-decomposer SAP, and AM fungi and discuss their ecophysiological implications.

8.3 Isotopic Patterns in Full Mycoheterotrophs Based on Fungal Host

8.3.1 Fully Mycoheterotrophic Plants Associated with Ectomycorrhizal Fungi

C and N stable isotope natural abundance data of MH plants associated with ectomycorrhizal fungi (EM-MH plants) are already available for a considerable number of species collected in a broad range of habitats of wide geographic distribution (Fig. 8.1). These data include field collections from Europe (Gebauer and Meyer 2003; Bidartondo et al. 2004; Julou et al. 2005; Abadie et al. 2006; Tedersoo et al. 2007; Zimmer et al. 2007, 2008; Liebel et al. 2010; Liebel and Gebauer 2011), North America (Trudell et al. 2003; Zimmer et al. 2007; Hynson et al. 2009b),

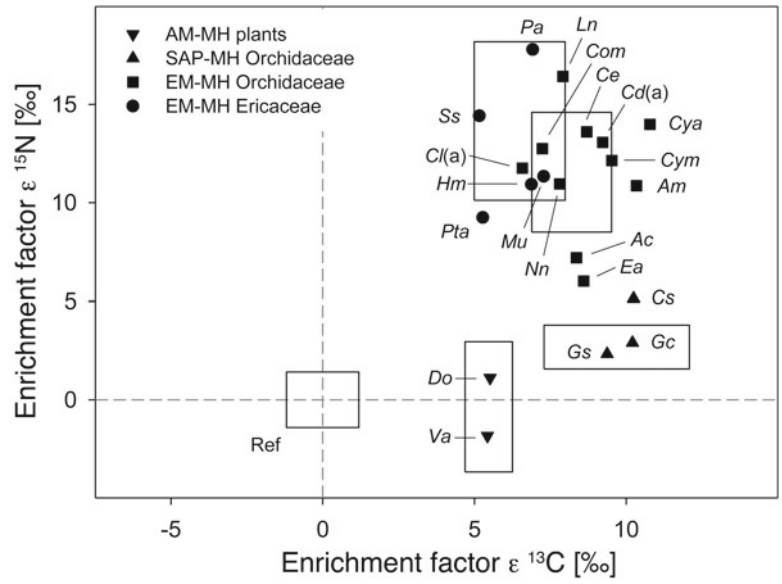

Fig. 8.1 Mean enrichment factors (ε, see Box 8.3) for ^{13}C and ^{15}N of fully mycoheterotrophic (MH) plants associated with fungi forming ectomycorrhizas (EM-MH Orchidaceae and EM-MH Ericaceae), with saprotrophic wood-decomposer fungi (SAP-MH Orchidaceae) and with fungi forming arbuscular mycorrhizas (AM-MH plants). The *boxes* represent one SD of the mean ε values for the four significantly distinguished groups of MH plants and for the photosynthetic reference plants (Ref, $n=659$) collected together with each of the mycoheterotrophs. Abbreviations of the respective species and numbers of replicates (n) as following: *Ac*=*Aphyllorchis caudata* ($n=3$); *Am*=*A. montana* ($n=4$); *Cd*(*a*)=*Cephalanthera damasonium albino* ($n=10$); *Ce*=*C. exigua* ($n=5$); *Cl*(*a*)=*C. longifolia albino* ($n=9$); *Com*=*Corallorhiza maculata* ($n=10$); *Cs*=*Cyrtosia septentrionalis* ($n=1$); *Cya*=*Cymbidium aberrans* ($n=3$); *Cym*=*C. macrorhizon* ($n=6$); *Do*=*Dictyostega orobanchoides* ($n=8$); *Ea*=*Epipogium aphyllum* ($n=5$); *Gc*=*Gastrodia confusa* ($n=5$); *Gs*=*G. similis* ($n=10$); *Hm*=*Hypopitys monotropa* ($n=23$); *Ln*=*Lecanorchis nigricans* ($n=3$); *Mu*=*Monotropa uniflora* ($n=8$); *Nn*=*Neottia nidus-avis* ($n=36$); *Pa*=*Pyrola aphylla* ($n=39$); *Pta*=*Pterospora andromedea* ($n=10$); *Ss*=*Sarcodes sanguinea* ($n=15$); *Va*=*Voyria aphylla* ($n=8$). Data compiled from Gebauer and Meyer (2003), Bidartondo et al. (2004), Julou et al. (2005), Abadie et al. (2006), Tedersoo et al. (2007), Zimmer et al. (2007, 2008), Hynson et al. (2009b), Martos et al. (2009), Ogura-Tsujita et al. (2009), Roy et al. (2009a), Liebel et al. (2010), Merckx et al. (2010), Motomura et al. (2010) and Liebel and Gebauer (2011)

and Asia (Ogura-Tsujita et al. 2009; Roy et al. 2009a; Motomura et al. 2010) covering habitats from boreal coniferous forests, deciduous or mixed temperate forests, evergreen forests in Mediterranean climates to tropical forests. All of these habitats are forests at least partially composed of tree species known to form EM—an essential prerequisite for a tripartite matter flux between trees, EM fungi, and EM-MH plants. The C and N stable isotope natural abundance data of EM-MH plants currently available from the literature mostly also contains stable isotope natural abundance data of accompanying autotrophic plants. Using these autotrophic plants as references for C gains independent of fungi, site-independent differences between autotrophic "reference" and MH "target" species can be calculated. These calculations allow for comparisons of isotope enrichment between species across broad geographic regions (enrichment factors ε, see Box 8.3). Figure 8.1 compiles the entire currently available C and N stable isotope natural abundance data for EM-MH plants suited to calculate enrichment factors. The data set is composed of 11 orchid species belonging to five different tribes within the subfamily Epidendroideae and five species of Ericaceae belonging to three different tribes within the subfamily Monotropoideae. Among this data set are EM-MH species that have served as model organisms to elucidate matter fluxes between trees, EM fungi, and mycoheterotrophs (like

Box 8.3 Normalizing Stable Isotope Natural Abundance Data: The ε Approach

As detailed in Box 8.2, the isotopic composition of an ecosystem compartment (e.g., the leaf of a chlorophyllous, photosynthetically active plant) is driven by the isotope signature of its C source (CO_2 in the atmosphere) and by the isotope fractionation during the process of assimilation, for example CO_2 uptake through the stomata and enzymatic reduction in photosynthesis. Thus, with known C isotope signature of CO_2 in the atmosphere and known isotope fractionation during CO_2 assimilation the C isotope signature of a green plant leaf following the C3 pathway of photosynthesis can be predicted as following:

$$\delta^{13}C_{leaf} = \delta^{13}C_{air} - a - (b - a)c_i / c_a$$

with *a* as the fractionation caused by the slower gas phase diffusion of $^{13}CO_2$ relative to $^{12}CO_2$ (4.4‰), *b* as the fractionation caused by discrimination of the enzyme Rubisco against $^{13}CO_2$ (27‰), c_a as the atmospheric CO_2 concentration and c_i as the leaf internal CO_2 concentration (Farquhar et al. 1989).

While *a*, *b,* and c_a might be considered as fairly constant, neither $\delta^{13}C_{air}$ nor c_i is constant in nature. For example, CO_2 assimilated by the leaf of a plant from a temperate forest understory is composed of CO_2 from the free atmosphere (current $\delta^{13}C \approx -8$‰) and CO_2 from soil respiration ($\delta^{13}C \approx -25$‰) with nonconstant mixing ratios. The leaf internal CO_2 concentration c_i depends on all of those environmental parameters that affect stomata regulation and CO_2 assimilation (light climate, atmospheric water vapor pressure deficit, soil water status, leaf temperature, nutrient supply, etc.) and can thus be highly variable over time and in space.

To make realistic comparisons of stable isotope values between autotrophic and mycoheterotrophic (MH) plants from different habitats it is essential to have either the variables mentioned above remain constant (which is almost impossible under field conditions) or to normalize δ values for site-specific environmental stochasticity. For the study of MH plants this normalization is done by analyzing the isotope signatures of target plants (e.g., putative MH species) together with reference plants (a selection of chlorophyllous C3 plants of different life forms and taxonomic groups that are associated with different types of mycorrhizas and thus representing the spectrum of plants living together with the target plants) growing in close spatial proximity and thus experiencing over time the same microenvironmental conditions as the target plants (for further details of the sampling concept see Gebauer and Meyer 2003). This approach allows calculating (1) the variability of isotope signatures of reference plants and (2) differences between reference and target plants, and (3) testing of these differences for their statistical significance. Following this approach, the conventional δ values (see Box 8.2) are converted into differences. Stable isotope nomenclature uses the enrichment factor ε to describe the difference in relative stable isotope abundance of a product and its substrate (Högberg 1997). To calculate ε the mean δ value per element of the reference plants from a single sampling plot is subtracted from the individual δ values of each plant collected at the same plot (irrespective of whether target or reference plant) as follows:

$$\varepsilon_{Sx} = \delta_{Sx} - \delta_{Refx}[\%]$$

with *S* as a single value of a sample from a reference or target plant, *x* as a sampling plot within a certain study site, and Ref as the mean value of all reference plants (Preiss and Gebauer 2008). Only in cases from the literature where target and reference plants of a study site were not collected plot-wise do we use a simplified site-wise instead of plot-wise ε value calculation. This simplified approach is accompanied by a loss in resolution (Preiss and Gebauer 2008). Irrespective of these approaches the mean ε value of all reference plants at a certain study site is always 0‰, with an associated standard deviation that represents the small variance in the differing physiologies of autotrophic plants. Thus, converting site-dependent δ values into site-independent ε values opens the option of meta-analyses over sites and the presentation of multiple datasets within a single graph.

Hypopitys monotropa: Björkman 1960) or to identify the specificity towards certain EM fungi (like *Corallorhiza maculata* and *Neottia nidus-avis*: Taylor and Bruns 1997; McKendrick et al. 2002; Selosse et al. 2002). Also among this data set are, however, also species that were just recently identified as EM-MH species based on isotope abundance analyses (like *Pyrola aphylla*, the only known fully EM-MH species among the tribe Pyroleae: Zimmer et al. 2007; Hynson et al. 2009b), vegetative albino forms of usually chlorophyllous orchid species (*Cephalanthera damasonium* and *C. longifolia*: Julou et al. 2005; Abadie et al. 2006; see Sects. 8.4.1 and 8.4.3 for further information on albino phenotypes), and MH species with taxonomically close relatives associated with functional types of fungi other than EM fungi (like the relatives of *Epipogium aphyllum*: Roy et al. 2009b; Liebel and Gebauer 2011). Furthermore, the data set contains species strictly specialized on narrow EM fungal strains (like *Corallorhiza maculata*, *Neottia nidus-avis,* and *Hypopitys monotropa*: Taylor and Bruns 1997; McKendrick et al. 2002; Bidartondo and Bruns 2005) and species associated with a fairly constant set of EM fungi (like *Epipogium aphyllum* and the albino variants of *Cephalanthera damasonium* and *C. longifolia*: Julou et al. 2005; Abadie et al. 2006; Roy et al. 2009b; Liebel and Gebauer 2011) or even with multiple EM fungi (like *Pyrola aphylla*, *Aphyllorchis caudata,* and *A. montana*: Hynson and Bruns 2009; Roy et al. 2009a).

Irrespective of their wide geographical distribution, phylogenetic breadth within the plant kingdom, their occurrence in different forest types, and their varying degree of EM fungal specificity, what all of these EM-MH species have in common is that they are significantly enriched in the heavy C and N isotopes compared to neighboring reference plants. However, the enrichment factors for both C and N are not constant between MH species, presumably due to variations in the isotope signature of their respective fungal associates. The mean enrichment ranges from 5.3 ± 1.2(SD)‰ (*Sarcodes sanguinea*, Ericaceae) to 10.8 ± 0.6(SD)‰ (*Cymbidium aberrans*, Orchidaceae) for ^{13}C and from 6.0 ± 2.1‰ (*Epipogium aphyllum*, Orchidaceae) to 17.8 ± 2.7‰ (*Pyrola aphylla*, Ericaceae) for ^{15}N. Thus, the variation between EM-MH species in heavy isotope enrichment is greater for N than for C and probably reflects the broader variation in fungal N isotope abundance than fungal—and specifically EM fungal—C isotope abundance (Mayor et al. 2009). Current data furthermore indicate a difference in the heavy isotope enrichment of EM-MH species belonging to the Orchidaceae and to the Ericaceae (Fig. 8.1). The investigated 11 EM-MH species belonging to the Orchidaceae are significantly (Mann–Whitney *U*-test: $U = 1731.0$, $p < 0.001$) more enriched in ^{13}C (mean $\varepsilon^{13}C = 8.2 \pm 1.3$‰, $n = 94$) than the five EM-MH species belonging to the Ericaceae (mean $\varepsilon^{13}C = 6.5 \pm 1.5$‰, $n = 95$), and the EM-MH Orchidaceae (mean $\varepsilon^{15}N = 11.6 \pm 3.1$‰, $n = 94$) are significantly ($U = 2941.5$, $p < 0.001$) less enriched in ^{15}N than the EM-MH Ericaceae (mean $\varepsilon^{15}N = 14.2 \pm 4.0$‰, $n = 95$). The significant difference in C and N stable isotope enrichment between the investigated EM-MH representatives of the two plant families becomes obvious from this meta-analysis thanks to a continuously increasing number of data points available from the literature. Though yet to be confirmed, these differences between orchids and ericaceous MH plants may also relate to the evolution of different mechanisms for MH nutrition in these two families.

8.3.2 Fully Mycoheterotrophic Plants Associated with Saprotrophic Fungi

Besides the tripartite associations with trees and EM fungi, some fully MH orchids associate with free-living wood-decomposer or litter decaying fungi. Interestingly, all of these fully MH plants are orchids and belong mostly to the subfamily Epidendroideae, similar to many EM-MH Orchidaceae. Such saprotrophic-MH (SAP-MH) orchids are typically found in the litter-rich forest floor or beside decomposed woody materials such as decayed tree trunks, stumps, logs, and pruned branches. In 1911, Kusano reported on an association of the MH orchid *Gastrodia elata* with the normally plant pathogenic wood-decomposing fungus *Armillaria*, and Hamada (1939) also found

this fungus associating with the MH orchid *Cyrtosia sepentrionalis*. Subsequently, various *Armillaria* species have been isolated and identified from these orchids (Terashita and Chuman 1987, 1989; Cha and Igarashi 1995, 1996; Matsushita et al. 1996; Terashima et al. 1998; Ota et al. 2000; Kikuchi et al. 2008a, b; Sekizaki et al. 2008). In the world's largest MH climbing orchid, *Erythrorchis ochobiensis*, an association with the SAP *Erythromyces crocicreas* (Basidiomycota) was reported by Hamada and Nakamura (1963) and, later, a wide range of wood-decomposing fungi, such as *Lentinula edodes* and *Pleurotus ostreatus*, were shown to induce seed germination and plantlet formation in symbiotic culture (Umata 1995, 1997, 1998a, b, 1999). The MH orchid *Epipogium roseum*, the sister species of the EM-MH orchid *Epipogium aphyllum*, has been shown to associate with fungi from the SAP family Psathyrellaceae (Basidiomycota), including *Coprinellus disseminatus* (Yamato et al. 2005; Yagame et al. 2008a). Notable is that *Epipogium roseum* (Yagame et al. 2007) can be cultivated for its whole lifecycle with wood-decomposer fungi under laboratory conditions. Such instances of in vitro cultivation are also known for other SAP-MH orchids (Burgeff 1932, 1936; Umata et al. 2007), and *Gastrodia elata* is now routinely cultivated symbiotically with *Armillaria* spp. for commercial use of its tubers (Chou 1974; Sung et al. 1995; Xu and Guo 2000). In addition to these reports, several other SAP decomposer fungal genera such as *Fomes*, *Marasmius*, *Xerotus*, *Campanella,* and *Gymnopus* have been reported to be associated with MH *Gastrodia* species, its related MH genus *Didymoplexis,* and some species of the MH genera *Galeola* and *Erythrorchis* (Burgeff 1932; Campbell 1962, 1964; Dearnaley 2006; Dearnaley and Bougoure 2010). Although information about fungal specificity of SAP-MH orchids is still rather scarce, recent reports showed a wide range of specificities, from a strict association between *Eulophia zollingeri* and the *Psathyrella candolleana* species group (Ogura-Tsujita and Yukawa 2008), to an association with a constant set of fungal partners, like *Gastrodia confusa* and representatives of the Mycenaceae (Ogura-Tsujita et al. 2009), to a broad spectrum of associated fungi in the case of *Wullschlaegelia aphylla* or *Gastrodia similis* and multiple Basidiomycetes related to the genera *Mycena*, *Marasmius*, *Psathyrella,* and *Resinicium* (Martos et al. 2009). Apparently different lineages within Psathyrellaceae and Mycenaceae have been repetitively targeted by independent MH orchid lineages (Selosse et al. 2010).

Associations between decomposer SAP fungi and fully MH plants as well as laboratory cultivation experiments provide a hint that fully MH plants also can cover their C and N demand through associations with these fungi in nature. As with EM-MH plants, stable isotope natural abundance analysis is a powerful tool to elucidate the nutritional sources of MH plants associated with decomposer fungi. The still very small stable isotope natural abundance data set available for SAP-MH orchids and accompanying reference plants indicates a significant enrichment in ^{13}C and ^{15}N due to C and N gain from the fungal source, but with a different pattern than found for EM-MH plants (Fig. 8.1). The so far investigated SAP-MH orchids (*Gastrodia confusa* from a wet-temperate bamboo forest in Japan and associated with several species of wood-decomposer *Mycena* fungi (Ogura-Tsujita et al. 2009), *Gastrodia similis* from tropical rainforests and secondary forests in La Réunion and associated with the wood-decomposer *Resinicium* (Martos et al. 2009), and one individual of *Cyrtosia septentrionalis* collected in a warm-temperate evergreen broadleaf forest in Japan (Motomura et al. 2010)) indicate a ^{13}C enrichment (mean $\varepsilon^{13}C = 9.7 \pm 2.4‰$, $n = 16$) that is significantly higher than EM-MH (vs. EM-MH orchids $U = 456, p = 0.012$; vs. EM-MH Ericaceae $U = 212$, $p < 0.001$) and a ^{15}N enrichment (mean $\varepsilon^{15}N = 2.7 \pm 1.1‰$, $n = 16$) that is significantly lower than found for EM-MH orchids ($U = 2$, $p < 0.001$) and EM-MH Ericaceae ($U = 0$, $p < 0.001$). Though not suited for the ε approach due to the absence of true reference plant data, the C and N isotope signature of one other MH orchid species associated with wood-decomposer fungi (*Gastrodia sesamoides* from open woodland in Queensland, Australia; (Dearnaley and Bougoure 2010) also points towards a considerably high ^{13}C enrichment and a ^{15}N enrichment lower than found for EM-MH plants. The most

likely reason for the different pattern of C and N isotope natural abundance between SAP-MH plants and EM-MH plants is a difference in the isotope signature of the C and N sources utilized by their respective fungal hosts. These few investigated SAP-MH plants are consistently associated with wood-decomposer fungi, and wood is known to be enriched in ^{13}C and depleted in ^{15}N (Gebauer and Schulze 1991; Gebauer and Dietrich 1993; Gebauer and Taylor 1999). Presently, stable isotope natural abundance data are available only for one SAP-MH orchid associated with litter decaying fungi (*Wullschlaegelia aphylla* from a tropical rainforest in Guadeloupe; Martos et al. 2009). These data point towards lower ^{13}C enrichment in SAP-MH orchids associated with litter decaying fungi than in SAP-MH orchids with wood-decomposer fungi. Undoubtedly more data on stable isotope signatures of SAP-MH orchids (especially associated to litter decaying fungi) and accompanying reference plants are required to confirm these preliminary conclusions. Also, the geographic distribution of SAP-MH orchids and their apparent preference for wood-decomposers among the SAP fungi require further investigation. At present we only can conclude that SAP-MH orchids obviously prefer humid climate conditions under which wood or litter decomposition rates are high.

8.3.3 Fully Mycoheterotrophic Plants Associated with Arbuscular Mycorrhizal Fungi

The AM symbiosis is one of the oldest plant symbioses on earth, estimated to be at least 400 million years old (Smith and Read 2008). It is therefore not surprising that this lineage of fungi has been infiltrated by the cheating strategy of fully MH plants on many more independent occasions than EM or SAP fungi. The majority of fully MH plants grow in tropical forests dominated by photosynthetic plants that associate with AM fungi. For this reason it was assumed in analogy to the EM-MH plants that among these fully MH plants in AM dominated forests an association with AM fungi occurs. Based on fungal DNA analysis from the mycorrhizal roots of several fully MH plants, five *Voyria,* and one *Voyriella* species (Gentianaceae) from tropical forests in French Guiana and *Arachnitis uniflora* (Corsiaceae) from three subantarctic forest sites in Argentina, and neighboring green plants, Bidartondo et al. (2002) showed that these plants indeed associate with AM fungi and display a fungal host specificity similar to many EM-MH plants. This finding suggested that AM fungi mediate a C transfer between autotrophic AM plants and AM-MH plants. Recent stable isotope natural abundance data confirm this suggestion (Fig. 8.1). Though phylogenetically distant, the fully MH plants *Voyria aphylla* (Gentianaceae) and *Dictyostega orobanchoides* (Burmanniaceae) also collected in a tropical forest in French Guiana are both significantly enriched in the heavy isotope ^{13}C in comparison to neighboring photosynthetic plants (Merckx et al. 2010). The relative ^{13}C enrichment of these AM-MH plants (mean $\varepsilon^{13}C = 5.5 \pm 0.8‰, n = 16$) is, however, significantly lower than in EM-MH orchids ($U = 40.5$, $p < 0.001$), EM-MH Ericaceae ($U = 393.5$, $p = 0.002$), and SAP-MH orchids ($U = 4$, $p < 0.001$). Similar trends were found by Courty et al. (2011) for *V. aphylla* and *V. tenella* (Gentianaceae) as well as *Aptera aphylla* and *Gymnosiphon sphaerocarpus* (Burmanniaceae) from a Caribbean island, La Guadeloupe. In addition, the latter study recovered spores from soil to investigate the isotope signature of AM fungal propagules, which proved similar to that of leaves of canopy trees. Thus, while the relative ^{13}C enrichment of EM-MH plants compared to surrounding autotrophic plants is primarily related to the ^{13}C enrichment of their fungal associates, the enrichment of AM-MH plants compared to surrounding understory autotrophic plants is likely unrelated to differences in AM fungal C acquisition and rather due to differences in photosynthetic rates between canopy trees and understory plants. Because of low photosynthetic rates and more humid conditions supporting longer and more numerous stomatal openings, understory plants are more depleted in ^{13}C than canopy trees (see Gebauer and Schulze 1991). Noteworthy is the finding of no significant ^{15}N enrichment in

the AM-MH plants by both Merckx et al. (2010) (mean $\varepsilon^{15}N = -0.4 \pm 3.3‰$, $n = 16$; see Fig. 8.1) and Courty et al. (2011) who, additionally, reported that the ^{15}N signature was similar in AM fungi. Courty et al. (2011) also showed that, compared to their accompanying reference plants, the investigated AM-MH plants have similar total N concentrations as compared to green plants and fungi, whereas higher total N concentrations are common among EM-MH plants (Gebauer and Meyer 2003; Julou et al. 2005; Liebel et al. 2010; Stöckel et al. 2011; see also Sect. 8.6.1). Lack of differentiation in ^{15}N natural abundance and total N concentrations between AM-MH plants and reference plants suggests utilization by all of these plants of similar N sources, presumably inorganic N compounds obtained through their AM fungal partners. Taken together, these observations, which deserve replicates from other sites and taxa, suggest that the matter exchanged between fungus and host plant differs in EM-MH vs. AM-MH plants, a situation not fully unexpected owing to the ancient divergence between AM Glomeromycota and EM-forming taxa of Asco- and Basidiomycota.

8.4 Partial Mycoheterotrophy

8.4.1 Evolution of Partial and Full Mycoheterotrophy

PMH plant lineages add to a long list of taxa where heterotrophic abilities evolved from autotrophic ancestors. In the broadest sense, such a strategy is a kind of mixotrophy. The word mixotrophy has been used as a synonym for partial mycoheterotrophy, but is a more encompassing term than partial mycoheterotrophy, because mixotrophic strategies and mechanisms are very diverse. For example, many independent phyla of planktonic algae are mixotrophic, either by uptake of dissolved organic matter (Kamjunke and Tittel 2009) or by phagotrophy on unicellular preys (Jones 2000). Uptake of C or phagotrophy is a plesiomorphic condition in these algae, since ancestors of plastid-bearing taxa are considered to have been heterotrophs (phagotrophy likely being the process through which plastids were indeed acquired). In contrast, mixotrophy in land plants is secondarily evolved, i.e., represents subversion from full autotrophy, and its ecological relevance is yet to be estimated in terrestrial ecosystems. Here we use the phrase "partial mycoheterotrophy" whenever mixotrophy is achieved in a green plant by partial use of C from a mycorrhizal fungus.

In land plants, mixotrophic strategies encompasses partial mycoheterotrophy, but also the use of C from prey (Adamec 1997) and of the host sap in hemiparasitic plants (Schulze et al. 1991; Press and Graves 1995; Těšitel et al. 2010). The latter are green plants that obtain some nourishment, especially mineral nutrients and sometimes C, by parasitizing other plants: C is obtained from connection to the xylem sap, or even to the phloem sap. For example, mistletoes (Loranthaceae) derive up to 63% of their C from their host (Schulze et al. 1991; Bannister and Strong 2001); *Olax phyllanthi* (Olacaceae) derives 19–30% (Tennakoon and Pate 1996); *Rhinanthus alectorolophus* (Orobanchaceae) derives up to 50% (Těšitel et al. 2011). In the latter case, as is true for some PMH species (see Sect. 8.4.3), shading enhanced the contribution of host-derived C; moreover, achlorophyllous variants of *Striga hermonthica* (Orobanchaceae) can survive (Press and Graves 1995).

When considering mycoheterotrophy in a phylogenetic framework, some fully MH species are nested within PMH lineages, e.g., in the tribe Neottieae (Fig. 8.2a; Abadie et al. 2006; Selosse and Roy 2009) and in the genus *Cymbidium* (Motomura et al. 2010); and the same may have happened in the genus *Platanthera* (Yagame et al. 2012). Pyroloids (= tribe Pyroleae) are closely related to the MH Monotropoideae (Monotropeae and Pterosporeae; Kron et al. 2002), suggesting a similar scenario (Tedersoo et al. 2007); however, the subtribe relationships in Monotropoideae deserve new analyses and a basal position of Pyroleae remains uncertain. Interestingly, in pyroloids, there are two well-supported clades (Freudenstein 1999; Kron et al. 2002), *Pyrola* + *Orthilia* on the one hand and *Moneses* + *Chimaphila* on the other; the second clade tends to encompass less frequent reports of

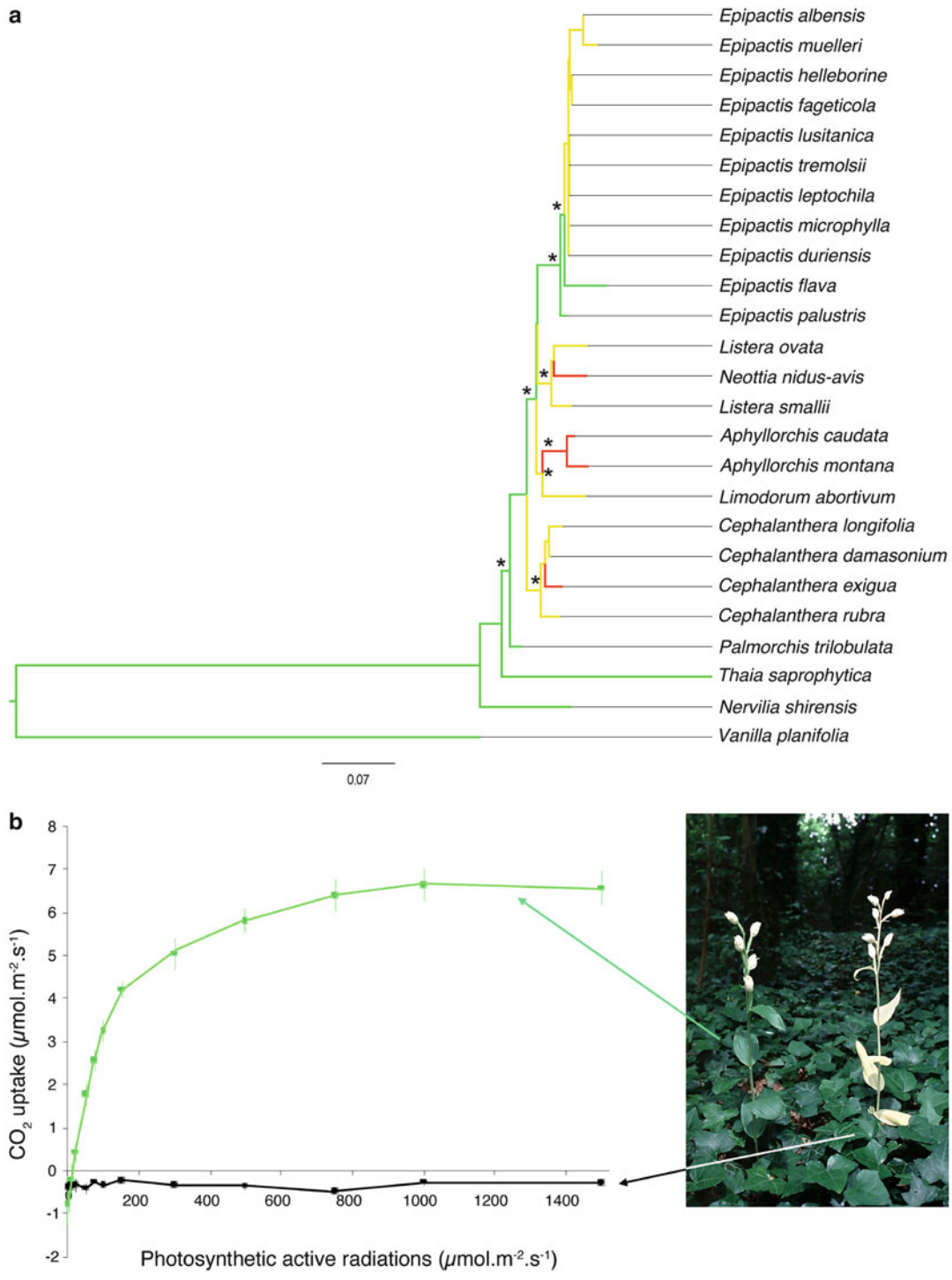

Fig. 8.2 (**a**) A phylogeny of Neottieae (based on ITS + rbcL phylogenies, inferred by Maximum Likelihood and 1,000 bootstrap repetitions, using a GTR model, from Roy et al. 2009a) with reconstruction inferred ancestral trophic status, assuming the most parsimonious scenario for its evolution. *Green*, autotrophic nutrition; *yellow*, partially mycoheterotrophic (PMH) nutrition; *red*, mycoheterotrophic nutrition. *Stars* indicate node supported by >80% bootstrap support and (**a**) indicates green species for which albino, i.e., fully achlorophyllous variants are reported. (**b**) CO_2 exchanges at various light levels in *Cephalanthera damasonium*, a green species where rare fully non-chlorophyllous albinos exist. Each *curve* is the mean of five individuals (M. Roy and M.-A. Selosse, unpublished data; see Julou et al. 2005 for site, material and methods). On the *right* a typical green *C. damasonium* individual together with a non-chlorophyllous variant

Table 8.1 A convergent scenario for the evolution of heterotrophy in plants through mixotrophic steps that exploit a living source for mineral nutrients

Nutrition	Evolution to parasitism in plants:	Evolution to mycoheterotrophy	Positively selected for
#1 Autotrophy	Free-living, autotrophic non-mycorrhizal plant.		
#2 Autotrophy	Hemiparasitic xylem tapping plants of its host mainly for mineral nutrition.	Mineral nutrition by mycorrhizal fungi shared with surrounding plants that also contribute to fungal nutrition.	improved and less costly mineral nutrition.
#3 Mixotrophy (low to high heterotrophy)[a]	Hemiparasitic xylem tapping plant tapping xylem accessing both minerals and carbon from its host.	Mixotrophic plant deriving mineral nutrients and some carbon from shared mycorrhizal fungi, but still photosynthetic.	improved carbon nutrition, improved tolerance to low light.
#4 Mixotrophy (high heterotrophy)[a]	Mixotrophic hemiparasitic plant with reduced photosynthetic abilities with improved growth and reproduction when associated with it's host plant.	Partially mycoheterotrophic plant with reduced photosynthetic abilities.	improved carbon nutrition in low light.
#5 Full heterotrophy	Heterotrophic, nongreen plant, obtaining all its mineral nutrient and carbon supply by tapping the xylem and phloem of its host plant.	Mycoheterotrophic, nongreen plant obtaining all its mineral and carbon supply from fungi.	Complete carbon nutrition, even in absence of light.

[a]Possibility of survival for albino (=achlorophyllous) variants

partial MH than the first, which harbors the fully MH *P. aphylla* (Zimmer et al. 2007; Hynson et al. 2009b) and PMH *P. japonica* (Matsuda et al. 2012).

The evolution to full mycoheterotrophy through partial mycoheterotrophy is thus reminiscent of the evolution of holoparasitic plants (= full heterotrophs) from hemiparasitic ancestors, as reported in the Orobanchaceae (Bennett and Mathews 2006) and Convolvulaceae (McNeal et al. 2007). A common scenario for evolution of plant heterotrophy can be suggested (Table 8.1; Cameron and Leake 2007; Selosse and Roy 2009), where biological interactions which only formerly selected for mineral nutrition allow (1) some indirect mixotrophy, and then (2) select for emergences of full heterotrophy, directly targeting C. Basically, this is an exaptation from mineral to C nutrition by "baiting the feeding hand." This scenario awaits confirmation from other taxa such as AM-MH plants that associate with one of the oldest extant mycorrhizal lineages of fungi on earth, the Glomeromycota (Selosse and Roy 2009).

In terms of selective mechanisms, evolutionary transition to mycoheterotrophy can be fuelled by the fact that some PMH plants, at least in some environments, grow in young forests whose initially open canopies allows photosynthesis but tend to close due to ongoing succession. Thus, they undergo increasing shading that selects for more light-independent C supply (Selosse and Roy 2009); indeed, this may explain the convergent evolution to full MH observed within the Neottieae (Fig. 8.2a; Roy et al. 2009a). In this tribe, full MH is never shown to be evolutionarily reversible, maybe due to loss of photosynthetic genes both in the plastid and nucleus. However, there are possible reversions from partial mycoheterotrophy at adulthood to autotrophy at adulthood. For example, the *Epipactis palustris-gigantea* clade (Fig. 8.2a) is autotrophic at adulthood, as shown by survival upon transplantation

(Sadovsky 1965), ^{13}C abundances indicative of autotrophy (Fig. 8.3), and association with rhizoctonia fungi that are common associates of initially MH orchids (Bidartondo et al. 2004; Zimmer et al. 2007; Illyés et al. 2009). Similarly, some green *Listera* species, despite close taxonomic relatedness to *Neottia nidus-avis* and Asian MH species (Roy et al. 2009a), are likely autotrophic as adults based on their ^{13}C abundances (Fig. 8.3) and associations with rhizoctonias (Bidartondo et al. 2004). Although Neottieae phylogenies remain poorly resolved, these clades are unlikely to be basal (Pridgeon et al. 2008; Roy et al. 2009a), making reversion from partial mycoheterotrophy to autotrophy at adulthood the most parsimonious scenario, while multiple shifts to partial mycoherotrophy remain a possible, but less parsimonious, alternative. Reversion to autotrophy at adulthood is not unexpected, since photosynthetic abilities remain in partial mycoheterotrophs.

Despite their successful survival, albinos observed in some orchid species show reduced fitness (Salmia 1986, 1989b; Roy et al. 2012), reduced demography as compared to green individuals (Abadie et al. 2006; Tranchida-Lombardo et al. 2010), and more or less impaired vegetative traits (Salmia 1989b; Julou et al. 2005). Their lower basal metabolic rates (Julou et al. 2005; see CO_2 evolution in the dark on Fig. 8.2b) suggest C limitation. They often display higher rates of mycorrhizal colonization (Salmia 1989a; Selosse et al. 2004; Abadie et al. 2006), a fact that could compensate for absence of photosynthesis, but it is unknown if more extensive colonization is linked to greater carbon gains. In a study of two *C. damasonium* populations with albino and green individuals (Roy et al. 2012), albinos comparatively displayed (1) more frequent shoot drying at time of fruiting, possibly due to stomatal dysfunctions, (2) higher frequency of dormancy, and (3) fewer seeds, with lower germination capacity. This results in a 500–1,000× fitness reduction as compared to green individuals. Among other factors, two observed features were proposed to cause a C limitation and fitness reduction in these albinos: they displayed higher pathogen and herbivore load, and a sharp reduction of mycorrhizal colonization at time of fruiting, which would likely be compensated by photosynthesis in green individuals, but may be critical for the survival of albinos (Roy et al. 2012). Albinos likely represent unique snapshots of failed transitions from partial mycoheterotrophy to full mycoheterotrophy, and the analysis by Roy et al. (2012) suggests that successful transitions at least require degeneration of leaves and stomata, optimization of the temporal pattern of fungal colonization and shoot sprouting, and new defences against pathogens and herbivores. In Neottieae, albinos suggest that the transition from partial to full mycoheterotrophy cannot be sudden, and that additional traits are required to become successfully MH. Moreover, their absence in PMH Ericaceae suggests that the transition to full mycoheterotrophy can occur in lineages devoid of albinos, so that they are not a necessary step toward full mycoheterotrophy. Albinos are ecological equivalents to mutants in genetics, i.e., their dysfunctions may suggest what makes mycoheterotrophy successful. Although their determinism remains unknown, they offer fascinating models for comparing the physiology of mixo- and autotrophs within very similar genetic backgrounds. The options for physiological investigations on the transition from autotrophy to full mycoheterotrophy become even wider when including MH species possessing variegated leaves. In addition to the frequent green and rare albino forms of some orchid species, individuals with a continuous range between these extremes have episodically been found (Renner 1938; Salmia 1989b; Stöckel et al. 2011).

8.4.2 Initial Mycoheterotrophy-Autotrophy

In contrast with full mycoheterotrophs, many plants are MH during and after seed/spore germination, but eventually develop into autotrophic individuals. While the duration of mycoheterotrophy in such species is relatively short, it remains an obligate and critical part of their life cycle. Most orchids (aside from a few hundred species

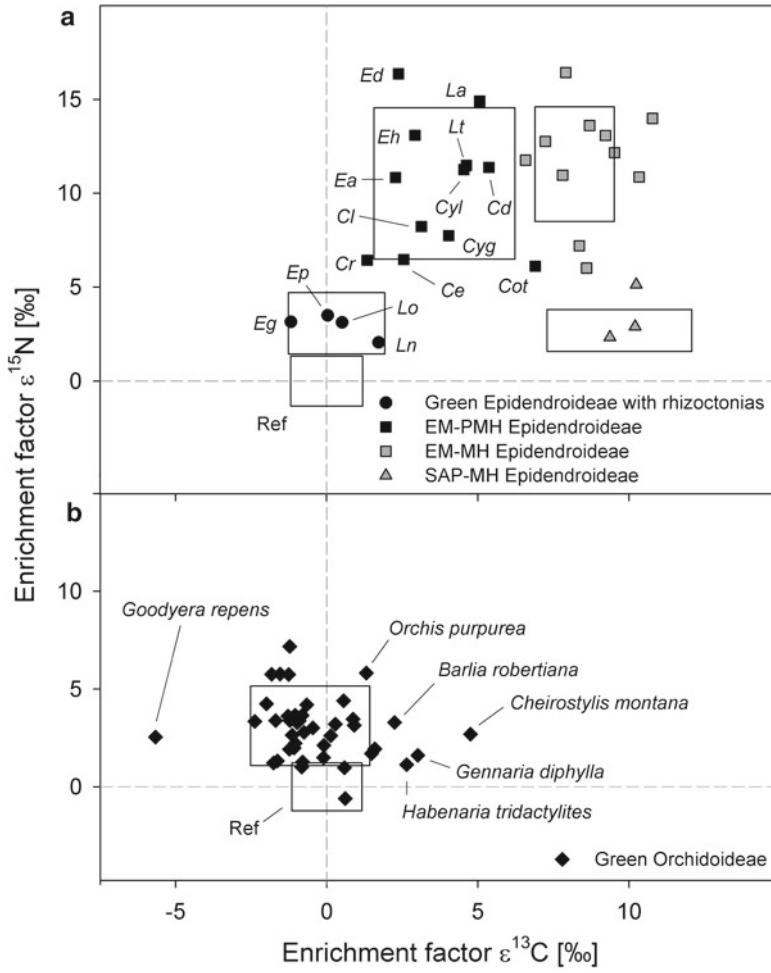

Fig. 8.3 Mean enrichment factors (ε, see Box 8.3) for ^{13}C and ^{15}N of (**a**) 16 green orchid species (*black symbols*) and 14 fully mycoheterotrophic (MH) orchid species (*grey symbols*, for further details see Fig. 8.1) belonging to the subfamily Epidendroideae and associated either with fungi from the polyphyletic rhizoctonia group or, fungi simultaneously forming ectomycorrhizas with surrounding trees (EM-PMH and EM-MH) or with saprotrophic wood-decomposer fungi (SAP-MH) and of autotrophic reference plants (Ref, $n = 765$) collected together with each of the respective orchids and (**b**) of 44 green orchid species belonging to the subfamily Orchidoideae and of autotrophic reference plants (Ref, $n = 863$) collected together with each of the respective orchids. The *boxes* represent one SD of the mean ε values for the different groups of green and MH orchids and for the autotrophic reference plants. Abbreviations of the green orchid species belonging to the Epidendroideae and numbers of replicates (n) as following: *Cd* = *Cephalanthera damasonium* ($n = 21$); *Ce* = *C. erecta* ($n = 3$); *Cyl* = *C. longifolia* ($n = 19$); *Cr* = *C. rubra* ($n = 7$); *Cot* = *Corallorhiza trifida* ($n = 9$); *Cyg* = *Cymbidium goeringii* ($n = 7$); *Cl* = *C. lancifolium* ($n = 6$); *Ea* = *Epipactis atrorubens* ($n = 11$); *Ed* = *E. distans* ($n = 4$); *Eg* = *E. gigantea* ($n = 5$); *Eh* = *E. helleborine* ($n = 18$); *Ep* = *E. palustris* ($n = 4$); *La* = *Limodorum abortivum* ($n = 10$); *Lt* = *L. trabutianum* ($n = 5$); *Ln* = *Liparis nervosa* ($n = 3$); *Lo* = *Listera ovata* ($n = 25$). The data set of the green orchids belonging to the Orchidoideae includes the following species and replicates (n): *Aceras anthropophorum* ($n = 10$), *Anacamptis laxiflora* ($n = 5$), *Barlia metlesicsiana* ($n = 5$), *B. robertiana* ($n = 5$), *Cheirostylis montana* ($n = 2$), *Dactylorhiza majalis* ($n = 4$), *D. sambucina* ($n = 11$), *Gennaria diphylla* ($n = 10$), *Goodyera oblongifolia* ($n = 18$), *G. repens* ($n = 5$), *G. schlechtendaliana* ($n = 1$), *Gymnadenia conopsea* ($n = 8$), *Habenaria tridactylites* ($n = 5$), *Ludisia discolour* ($n = 5$), *Neotinea maculata* ($n = 5$), *Ophrys fuciflora* ($n = 9$), *O. insectifera* ($n = 12$), *O. apifera* ($n = 5$), *O. incubacea* ($n = 5$), *O. sicula* ($n = 5$), *O. sphegodes* ($n = 5$), *Orchis brancifortii* ($n = 5$), *O. canariensis* ($n = 5$), *O. ichnusae* ($n = 5$), *O. laxiflora* ($n = 5$), *O. longicornu* ($n = 5$), *O. mascula* ($n = 18$), *O. morio* ($n = 5$), *O. papilionacea* ($n = 5$), *O. pauciflora* ($n = 5$), *O. provincialis* ($n = 5$), *O. purpurea* ($n = 10$), *O. tridentata* ($n = 5$), *O. ustulata* ($n = 5$), *Platanthera bifolia* ($n = 7$), *P. chlorantha* ($n = 4$), *P. leucostachys* ($n = 13$), *Serapias cordigera* ($n = 5$), *S. lingua* ($n = 5$), *S. nurrica* ($n = 5$), *S. parviflora* ($n = 10$), *S. vomeracea* ($n = 10$), *Spiranthes spiralis* ($n = 5$), and *Zeuxine agyokuana* ($n = 1$). Data compiled from Gebauer and Meyer (2003), Bidartondo et al. (2004), Julou et al. (2005), Abadie et al. (2006), Tedersoo et al. (2007), Zimmer et al. (2007, 2008), Hynson et al. (2009a), Roy et al. (2009a), Liebel et al. (2010), Motomura et al. (2010) and Girlanda et al. (2011)

that remain fully or PMH as adults) appear to be initially mycoheterotrophic-autotrophic (IMH), although direct evidence is available for only a small number of species. With over 20,000 species in the Orchidaceae, the number of IMH species is likely far greater than all fully and PMH species combined. Initial mycoheterotrophy-autotrophy is not limited to the orchid family; however, additional taxa within the Ericaceae (Pyroleae), Lycopodiaceae, and several fern families appear to be IMH, as well (see Chap. 2).

MH seedlings and sporelings of IMH plants are typically subterranean and non-photosynthetic; however, despite their cryptic nature, such germlings belonging to the Orchidaceae (Salisbury 1804), Pyroleae (Irmisch 1855), Ophioglossaceae (Hofmeister 1857), and Lycopodiaceae (Mettenius 1856) had already been observed by the nineteenth century. Like fully MH plants, they were often erroneously described as "saprophytic" (Leake 2005). The understanding of IMH orchid seedlings advanced in the late nineteenth to early twentieth centuries, with discoveries by Noël Bernard (Bernard 1899) that fungal symbionts are necessary for the heterotrophic growth of orchid seedlings, Beau (1920) that growth of symbiotic orchid seedlings occurs only when the fungi have access to a C source, and Bernard (1908) and Knudson (1922) that orchid seedlings exhibit asymbiotic growth on media enriched with simple sugars. More recently, studies involving labeled and naturally abundant isotopes have indicated that adult plants of photosynthetic orchid spp., as has generally been assumed, are usually autotrophic (Gebauer and Meyer 2003; Cameron et al. 2006, 2008; Hynson et al. 2009a; Liebel et al. 2010; Girlanda et al. 2011). Autotrophy at adulthood is indicated by ^{13}C natural abundance for the majority of green species from the subfamily Orchidoideae investigated thus far (Fig. 8.3b) and for species from the subfamily Epidendroideae that are solely associated with fungi of the polyphyletic rhizoctonia group (Fig. 8.3a). While isotopic evidence for initial mycoheterotrophy-autotrophy is not yet available for most putatively IMH taxa, this lifestyle can be inferred by characteristic dust seed morphology (in angiosperms), subterranean and non-photosynthetic germling development, consistent association of germlings with fungi, absence of hemiparasitic interactions, and photosynthetic, readily cultivated adults.

8.4.2.1 Initial Mycoheterotrophy-Autotrophy in the Orchidaceae

Like fully MH angiosperms, initially mycoheterotrophic-autotrophic orchids (IMHOs) have highly reduced "dust seeds" which contain an embryo but no endosperm (see Chap. 5). Germination occurs when the embryo enlarges enough to rupture the testa, with the seedling at this stage known as a protocorm. Seeds of IMHOs may be stimulated to germinate by host or non-host fungi, or may germinate in the absence of fungi all together (Downie 1959; Hadley 1970; Warcup 1973; Rasmussen 1995); however, further protocorm growth is usually dependent on mycoheterotrophy. Protocorms of most terrestrial IMHOs are subterranean, non-photosynthetic, and unambiguously MH. While protocorms of many epiphytic and some terrestrial species are superficial and green, they usually cannot progress beyond germination without a fungal host (or exogenous supply of sugar). A small number of terrestrial species (e.g., in the genera *Disa* (Section *Disa*, *Bletilla*, and *Sobralia*) have been observed to germinate and develop to the leafy stage without host fungi (Burgeff 1959). However, it has been suggested that seedling development in some of these taxa occurs more rapidly with fungal symbionts than without.

Shoot production in symbiotically cultured seedlings frequently occurs within several months of germination (Warcup 1973; Muir 1989; reviewed in Rasmussen 1995), though the first leaf may not emerge until the second growing season in some temperate spp. (e.g., Zettler et al. 2001; Sharma et al. 2003); production of the first root usually occurs concurrently or shortly thereafter. Early field reports suggesting IMHO seedlings remain underground and MH for several years (e.g., Curtis 1943) were based on conjectural interpretation of seedling "growth segments"; as MH periods of similar duration have not been observed in symbiotic cultural studies, it would appear that such claims are exaggerated. Whether seedlings

become fully autotrophic upon emergence of the first leaf or remain PMH for a period of time thereafter is not known. However, asymbiotic seedlings propagated following Knudson's (1922) protocol are commonly removed from sugar-enriched media when they have well-developed shoots and roots, apparently transitioning readily from partial/full heterotrophy to full autotrophy.

With very few exceptions (e.g., McCormick et al. 2004), host fungi of IMHO seedlings belong to *Ceratobasidium sensu lato* (incl. *Thanatephorus*), *Tulasnella*, and the Sebacinales (Dearnaley et al. 2012). Before Warcup and Talbot (1967) identified the perfect states of host fungi in culture, the identity of such fungi was hidden behind the veil of the morphologically defined, asexual genus *Rhizoctonia*, now known to be polyphyletic. Identification of host fungi has at times been further confounded by the propensity of IMHO seeds to germinate—and even for protocorms to undergo limited MH development—with non-host fungi, and of adult plants to allow peloton-formation of such fungi in their roots. These fungi may include rhizoctonia strains capable of hosting seedlings of other orchid species (Harvais and Hadley 1967), as well as Basidio- and Ascomycota not known to host seedlings of any orchid species (Currah et al. 1997; Vujanovic et al. 2000). Consequently, true host fungi of IMHOs are most appropriately identified as those supporting seedling development to the first leaf stage (e.g., Warcup 1973; Zettler and Hofer 1998).

Host specificity of IMHO seedlings is variable; while some IMHO species are highly host-specific (e.g., Wright et al. 2010; Phillips et al. 2011), compatibility with multiple strains of *Ceratobasidium* and/or *Tulasnella* is not uncommon (Hadley 1970). However, compatibility with *Tulasnella* and Sebacinales has only been observed in *Microtis* spp. (Warcup 1981; Milligan and Williams 1988; Bonnardeaux et al. 2007). *Ceratobasidium* and *Tulasnella*, by far the most common hosts of IMHOs, are thought to be predominantly SAP and/or pathogenic (Rasmussen 2002), though some lineages can be EM (Bidartondo et al. 2003; Yagame et al. 2012), and the ecology of these genera deserves further study. Sebacinales are known as hosts of IMHO seedlings within the Caladeniinae and occasionally the Prasophyllinae and Acianthinae (three closely related and predominantly Australian subtribes within the Diuridae). Sebacinales have been implicated in a wide variety of mycorrhizal and non-mycorrhizal (endophytic) interactions with plants (Selosse et al. 2009; Weiss et al. 2011), though notably, almost all such hosts of IMHOs belong to Sebacinales clade B, a group that is endophytic in many plants (Weiss et al. 2004; Selosse et al. 2009).

In symbiotic culture, growth of IMHO seedlings commonly occurs when C is available to host fungi in the form of starch or cellulose, and there is field evidence that organic matter may enhance germination in the presence of host fungi (McCormick et al. 2012). The extent to which C from living plants—acquired by orchid host fungi as pathogens or endophytes—may contribute to IMHO seedling development in nature is unknown. Unlike *Ceratobasidium* and *Tulasnella* spp., Sebacinales are often difficult to culture, suggesting relatively poor SAP capability. Nevertheless, they may consume dead plant tissues (Zuccaro et al. 2011) and are capable of supporting IMHO seedling development in vitro via utilization of starch (Warcup 1981; Ramsay et al. 1986; Bonnardeaux et al. 2007; Wright et al. 2009). Seedlings of *Microtis* spp., which are sometimes compatible with EM sebacinoid hosts (Warcup 1988), are the only known examples of EM-hosted IMHOs. However, given the recent discovery of EM *Ceratobasidium* and *Tulasnella* strains hosting fully and PMH orchids (Mursidawati 2004; Bougoure et al. 2009, 2010; Yagame et al. 2008b, 2012) and a fully MH liverwort (Bidartondo et al. 2003), respectively, it is possible that IMHO seedlings are hosted by EM fungi in additional genera.

8.4.2.2 Isotopic Evidence for Initial Mycoheterotrophy-Autotrophy in Orchidaceae

Cameron et al. (2006, 2008) demonstrated reciprocal transport of labeled C between adult, photosynthetic plants of *Goodyera repens* and their *Ceratobasidium* symbiont, with more than five

times as much C transferred from plant to fungus as in the opposite direction. These findings suggest the direction of net C flow may reverse as seedlings transition from mycoheterotrophy to autotrophy. Although Cameron et al. did not confirm that the fungal symbiont associated with adult plants is capable of hosting MH development of conspecific seedlings, this has frequently been found in other studies of *Goodyera* spp. (Rasmussen 1995; McCormick et al. 2004). It should be noted, however, that the placement within the subfamily Orchidoideae of taxa capable of reciprocal C transfer is not sufficient to support that the trait is ancestral to the orchid family; isotopic data from taxa in the Apostasioideae, Vanilloideae, and Cypripedioideae are needed in order to determine the evolutionary polarity of this trait. Further, the repeated acquisition of EM hosts by partially and fully MH orchids, as stated above, suggests an innate ability to exploit C of fungi with which they had no apparent preexisting mutualism.

Natural abundance ^{13}C and ^{15}N data indicate that adult IMHOs may, as expected, exhibit ^{13}C and ^{15}N abundances equivalent to autotrophic reference plants (Fig. 8.3). More often, however, they are significantly depleted in ^{13}C and/or enriched in ^{15}N (Fig. 8.3). Hynson et al. (2009a) suggest that because adult individuals of *Goodyera* spp. exhibit such ^{13}C depletion (Fig. 8.3b), it may result from the net C transfer from plant to fungus, as documented in *G. repens* by Cameron et al. (2006, 2008). Given that ^{13}C depletion has been found in other spp. within the Orchideae (Liebel et al. 2010), it is possible that reciprocal C transfer occurs in additional taxa.

Why C transfer from orchids to fungi might result in ^{13}C depletion relative to autotrophic reference plants (which also participate in such transfer) is not known. It is possible that physiological differences in C transfer pathways between orchids and SAP *Ceratobasidium*/*Tulasnella* fungi and non-orchid reference plants and EM fungi result in different patterns of ^{13}C natural abundance in these groups of plants. The mechanism by which adults of some IMHOs are enriched in ^{15}N, typically to a level intermediate between autotrophic and MH reference plants (Fig. 8.3), is also unknown. Liebel et al. (2010) suggest such species may continue to obtain N via a pathway analogous to that in fully MH plants, resulting in ^{15}N enrichment, but that concurrent ^{13}C enrichment of plant tissue may be counteracted by simultaneous transfer of ^{13}C from plant to fungus. This suggestion is supported by the finding of significantly increased total N concentrations in many IMHOs in comparison to accompanying reference plants (Gebauer and Meyer 2003; Abadie et al. 2006; Liebel et al. 2010; see also Sect. 8.6.1).

While it has been assumed that cultivable, *Ceratobasidium*/*Tulasnella*-hosted orchid species are invariably IMH, Liebel et al. (2010) and Girlanda et al. (2011) found levels of natural ^{13}C enrichment consistent with partial mycoheterotrophy in adult plants of several such species (Fig. 8.3b). While these taxa represent a minority of the orchids investigated thus far, the discovery of their trophic status suggests the total number of IMHO species may be significantly smaller than previously expected. Further, the known cultivability of some of the study taxa suggests that partial mycoheterotrophy in the adults is facultative rather than obligate, in which case initial mycoheterotrophy-autotrophy and initial mycoheterotrophy-partial mycoheterotrophy may not represent mutually exclusive categories. Additionally, the association of these orchids with *Ceratobasidium* and *Tulasnella* suggest that partial mycoheterotrophy in adult orchids may not always be accompanied by a switch to EM hosts, though the ability to form mycorrhizal networks among the strains identified by Liebel et al. (2010) and Girlanda et al. (2011) is not yet known.

Among putatively IMHOs, natural abundance stable isotope analyses have largely been limited to species that are (1) hosted by *Ceratobasidium* and/or *Tulasnella*, (2) members of the Orchidoideae and Epidendroideae subfamilies, and (3) terrestrial. Given the utility of these analyses in confirming the trophic status of photosynthetic adults, they deserve to be more widely applied to species that are hosted by Sebacinales members of the Apostasioideae, Vanilloideae, and Cypripedioideae subfamilies; and/or epiphytic species. However, a potentially confounding

factor for examining partial mycoheterotrophy among epiphytic orchids is that many of these species are either obligate or facultative CAM (crassulacean acid metabolism) plants (Neales and Hew 1975; Motomura et al. 2008; Silvera et al. 2010). Plants that rely on the CAM photosynthetic pathway are enriched in ^{13}C compared to those that utilize C3 photosynthesis. Thus, based on their C stable isotope profiles some epiphytic CAM orchids may, like PMH orchids, be enriched in ^{13}C even though they do not rely on fungi to meet their C demands. While analyses of IMHO seedlings have not yet been published, it would seem that these seedlings are likely to exhibit enrichment in ^{13}C and ^{15}N similar to fully MH plants. Enrichment in ^{13}C and ^{15}N compared to surrounding autotrophs has been observed in some adult orchids associated with *Ceratobasidium* and *Tulasnella* (Liebel et al. 2010; Girlanda et al. 2011), the most common hosts of IMHO seedlings.

8.4.2.3 Initial Mycoheterotrophy-Autotrophy in Pyroleae

The Pyroleae have dust seeds that, like most terrestrial orchids, germinate underground and develop into MH seedlings. While such subterranean, non-photosynthetic seedlings, made up of root-like organs, have been observed in culture (Lihnell 1942) and in the field (Irmisch 1855; Velenovsky 1892), the duration of initial mycoheterotrophy remains unknown. The few data available indicate that individual seedlings of two species of Pyroleae, *Pyrola chlorantha* and *Orthilia secunda,* are hosted by fungi in Sebacinales clade B (*sensu* Weiss et al. 2004), and a suite of EM fungi (N.A. Hynson unpublished; Smith and Read 2008). When investigating the fungal hosts to seedlings of *P. asarifolia* in Japan, Hashimoto et al. (2012) found a higher degree of host specificity for fungi only in Sebacinales clade B. However, seedlings of *Pyrola chlorantha* and *Orthilia secunda* have also been found to associate with ectomycorrhizal Sebacinales fungi from clade A (N.A. Hynson unpublished). It is surprising that single seedlings associate with single fungal hosts, and that some appear to be rather specific to non-EM fungi given that adult Pyroleae commonly associate with a diversity of EM fungi (Tedersoo et al. 2007; Zimmer et al. 2007; Vincenot et al. 2008; Hynson and Bruns 2009; Toftegaard et al. 2010).

With the exception of the fully MH *Pyrola aphylla* (Zimmer et al. 2007; Hynson et al. 2009b), many adult Pyroleae are leafy and primarily dependent on photosynthesis. Partial mycoheterotrophy in adult plants is frequently inconsistent between conspecific populations, with some individuals significantly more enriched in ^{13}C than autotrophic reference plants and others not (see Sect. 8.4.3; Tedersoo et al. 2007; Zimmer et al. 2007; Hynson et al. 2009b). Nevertheless, the populations that appear to be primarily dependent on photosynthetic C gains upon reaching adulthood, together with the survival of adult plants in cultivation (e.g., Hunt and Hope-Simpson 1990), suggests that some adult Pyroleae may be facultatively, if not consistently, autotrophic. Clearly, this group of plants deserves further investigation into the trophic status of adult individuals.

8.4.2.4 Initial Mycoheterotrophy-Autotrophy in Other Taxa

A number of taxa in the Lycopodiaceae, Psilotaceae, Ophioglossaceae, Schizaeaceae, and Gleicheniaceae have unambiguously MH gametophytes and preemergent sporophytes (Boullard 1979). With few exceptions, adult sporophytes are consistently photosynthetic, and many can be cultivated. It appears that many of these taxa may be IMH, although the trophic status of adult sporophytes under field conditions has yet to be investigated (see Sect. 8.4.3).

8.4.3 Initial Mycoheterotrophy-Partial Mycoheterotrophy

Contrasting with the previous scenario where adult plants are fully autotrophic, several species that turn green at adulthood after MH seedling development were discovered to remain PMH, i.e., maintain a C flow from the fungus to the plant over their whole lifespan. Although orchids were instrumental in the emergence of the concept, the

Table 8.2 Mean proportional C and N gains from fungi via mycoheterotrophy (Cd [%] and Ndf [%] ± 1 SD) by green orchids from the subfamily Epidendroideae and green pyroloids (Ericaceae) based on the data of all so far published literature (see Figs. 8.3 and 8.5) and on the linear two-source isotopic mixing model (Box 8.4) with fully EM-MH orchids or Ericaceae species used as the upper end point of the model representing 100% C and N gains via mycoheterotrophy, respectively

Species	Association with EM fungi	N_{df} (%)	C_{df} (%)	*n*
Orchidaceae				
Cephalanthera damasonium	+	*98 ± 21*	65 ± 24	21
C. erecta	?	56 ± 17	30 ± 6	3
C. longifolia	+	71 ± 21	38 ± 22	19
C. rubra	+	56 ± 8	16 ± 14	7
Corallorhiza trifida	+	53 ± 10	84 ± 12	9
Cymbidium goerginii	+	67 ± 33	49 ± 32	7
C. lancifolium	+	*97 ± 9*	55 ± 26	6
Epipactis atrorubens	+	*94 ± 30*	28 ± 19	11
E. distans	+	142 ± 32	*29 ± 23*	4
E. helleborine	+	*113 ± 38*	36 ± 33	18
Limodorum abortivum	+	129 ± 27	62 ± 6	10
L. trabutianum	+	*99 ± 30*	56 ± 13	5
Epipactis gigantea	–	27 ± 6	–14 ± 8	5
E. palustris	–	30 ± 3	*0 ± 7*	4
Liparis nervosa	–	18 ± 10	*21 ± 21*	3
Listera ovata	–	27 ± 16	*6 ± 20*	25
Ericaceae				
Chimaphila umbellata	+	61 ± 19	*0 ± 20*	36
Orthilia secunda	+	45 ± 16	33 ± 20	17
Pyrola chlorantha	+	67 ± 26	20 ± 18	17
P. minor	+	33 ± 13	*–4 ± 12*	9
P. picta	+	73 ± 13	*6 ± 23*	54
P. rotundifolia	+	68 ± 5	55 ± 7	6

Note: N_{df} data in *italics* are not significantly distinguished from 100%. C_{df} data in *italics* are not significantly distinguished from 0%

phenomenon is now suspected, and partly demonstrated, to be more widespread (Tedersoo et al. 2007; Zimmer et al. 2007; Cameron and Bolin 2010; reviewed in Selosse and Roy 2009).

8.4.3.1 Discovery of Partial Mycoheterotrophy in Adult Orchids

The suspicion of partial mycoheterotrophy came from two lines of observation in species of the Neottieae orchid tribe: unique C and N isotope natural abundances compared to autotrophic reference plants, and existence of achlorophyllous, albino individuals in otherwise green species. Gebauer and Meyer (2003) discovered unexpected isotope abundances in some forest orchids, with ^{13}C and ^{15}N abundances intermediate between those of autotrophic reference plants and full mycoheterotrophs from the same site. This was confirmed for additional European, North American and Asian species by several studies (Fig. 8.3a; Bidartondo et al. 2004; Julou et al. 2005; Abadie et al. 2006; Tedersoo et al. 2007; Zimmer et al. 2007; Liebel et al. 2010; Motomura et al. 2010). Stable isotope analyses from putative PMH plants have also been used to calculate these plants degree of heterotrophy (Table 8.2, Box 8.4).

Independent observations that some fully achlorophyllous individuals (= albinos), with colors ranging from white to pinkish due to anthocyanins (Fig. 8.4a, b), indicated partial

Box 8.4 The Linear Two-Source Mixing Model Approach

Stable isotope natural abundance data not only contain qualitative information about the C and N origin from different sources in the various types of MH plants, but are also a tool to estimate the proportions of C and N gained from different sources by PMH plants. Stable isotope natural abundance data and mixing model approaches have already frequently been used in a broad range of ecological field investigations to partition the origin of different nutrient sources utilized by various kinds of organisms (see Fry 2006). In our specific case we have to distinguish between two kinds of sources for C or N. For C the sources are quite obvious. PMH plants can gain C from photosynthesis and organic C compounds from their fungal hosts. For N the nature of the sources is less clear, because N uptake through the roots of terrestrial plants is usually mediated by mycorrhizal fungi irrespective of whether these plants are autotrophs or full or partial mycoheterotrophs. Nonetheless, it is obvious that there must be a distinction between N compounds gained by autotrophic and MH plants through their fungal associates. Otherwise, autotrophic and fully MH plants would not be distinguished by their N isotope signatures. Though the nature of N compounds utilized by autotrophic and MH plants is not fully understood, we can use their different N isotope signatures to estimate the contribution of either of these N fractions to cover the N demand of PMH plants. The fact that we have to consider only two kinds of sources provides the opportunity to use the most simple type of mixing model approaches, namely the linear two-source mixing model. This model requires as endpoints information about the isotopic composition of the two respective sources potentially utilized by a PMH plant and assumes that these sources are mixing in a linear manner in the tissue of the target plant. The mixing model approach in our case furthermore assumes (1) that the isotopic composition of the C source from photosynthesis and the isotopic composition of the N gained by autotrophic plants as one of the two endpoints are *represented* by the isotopic composition of fully autotrophic reference plants living in close spatial proximity and under identical micro climate conditions as the PMH target plant (=0% C or N gain of MH origin) and (2) that the isotopic composition of C and N gained from MH nutrition through the fungal source as the other endpoint is *represented* by fully MH orchids or Ericaceae, respectively (=100% C or N gain of mycoheterotrophic origin). According to this model the C and N isotope signature of PMH plants should range between the two endpoints and the proportional C and N gain from the source utilized by mycoheterotrophs can be calculated according to

$$\%x_{df} = \frac{(\delta x_{PMH} - -\delta x_{Ref})}{\varepsilon_{MH}} 100$$

with $\%x_{df}$ as percentage of C or N in the tissue of a PMH plant derived through MH nutrition from fungi, δx_{PMH} as the individual $\delta^{13}C$ or $\delta^{15}N$ value of a PMH plant, δx_{Ref} as the mean $\delta^{13}C$ or $\delta^{15}N$ value of autotrophic reference plants from a certain study site, and ε_{MH} as the mean enrichment factor of fully MH plants relative to obligate autotrophic reference plants from the same site (Gebauer and Meyer 2003).

mycoheterotrophy in some orchids. The surviving of such individuals in species that are normally green, especially from the Neottieae tribe, hinted at partial heterotrophy in these species. Albinos occur especially frequently in the genera *Epipactis* (Beau 1920; Renner 1938; Salmia 1986, 1989a, b; Selosse et al. 2004) and *Cephalanthera* (Renner 1938; Julou et al. 2005; Abadie et al. 2006; Stöckel et al. 2011). In some populations, the phenotype remains stable for green individuals and nearby albinos over many years (Renner 1938; Tranchida-Lombardo et al.

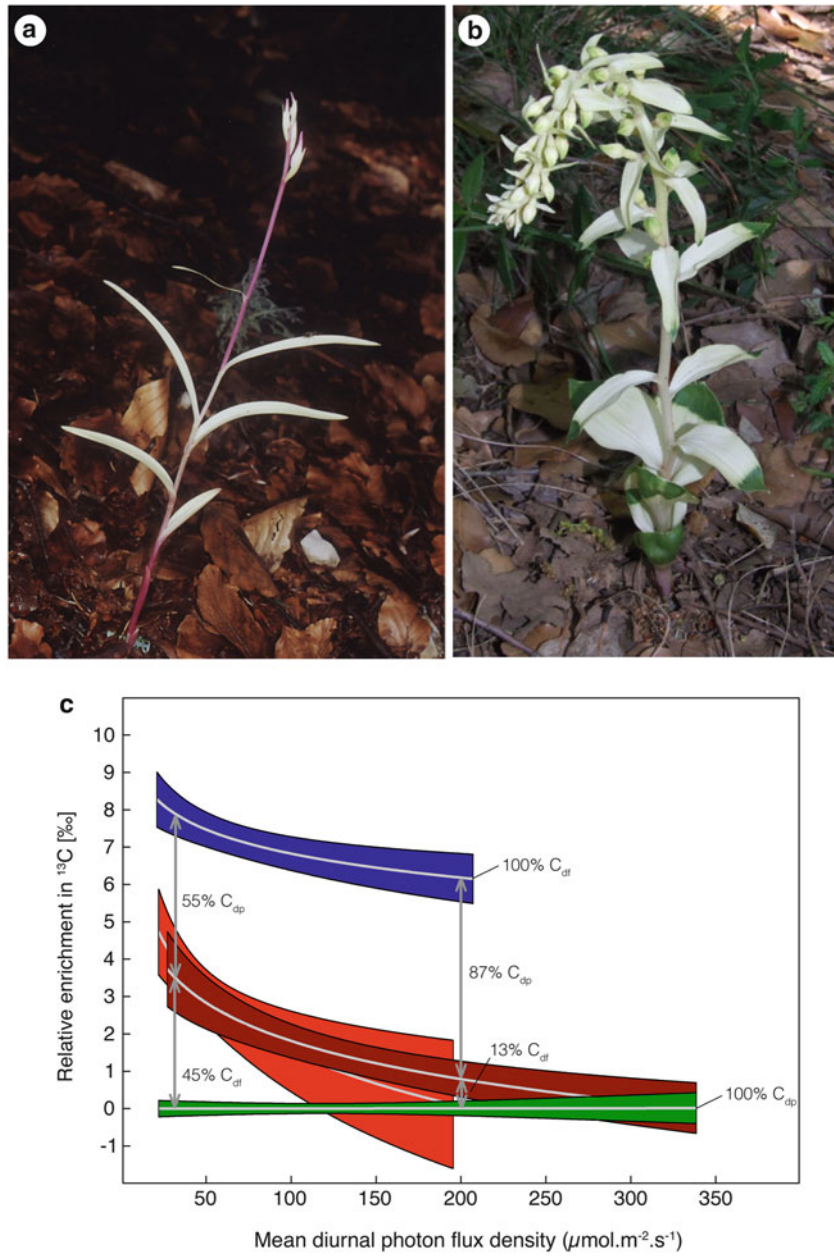

Fig. 8.4 (**a**) Albino *Cephalanthera rubra* (courtesy of J.-P. Amardeihl); see also Fig. 8.2b for albino *C. damasonium*. (**b**) Variegated individual of *Epipactis helleborine* (photo M.-A. Selosse). (**c**) Correlation between relative enrichments in ^{13}C (ε, see Box 8.3) and mean light availability for two PMH and one fully mycoheterotrophic (MH) orchid species and for autotrophic reference plants. Regression lines (±95% confidence intervals) represent the range of isotope signatures of autotrophic plants including the initially mycoheterotrophic (IMH) orchid *Cypripedium calceolus* (*green*), of the two PMH orchids *Cephalanthera damasonium* (*light red*) and *C. rubra* (*dark red*) and of the MH orchid *Neottia nidus-avis* (*blue*). *Arrows* indicate the variable proportions of C derived from fungi (C_{df}) or photosynthesis (C_{dp}), respectively (reproduced with permission from Preiss et al. 2010)

2010), up to 14 years for albinos (Abadie et al. 2006), while in others, variegated individuals or reversion to green shoots can occur (Salmia 1986; Stöckel et al. 2011; Fig. 8.4a, b). Although they tend to perform less well than green individuals (as stated above, see Sect. 8.4.1), some albinos form flowers and fruits (Salmia 1986, 1989a, b; Julou et al. 2005; Tranchida-Lombardo et al. 2010). Albinos were suggested to depend on their mycorrhizal fungi for C nutrition (Selosse et al. 2004): Beau (1920), having observed albino *Epipactis* and *Cephalanthera* spp. nearly one century ago, wrote that "the exact complementation of the photosynthetic function by the symbiosis permits us to understand how green orchids can exceptionally grow and flower in more or less etiolating conditions" (see also Renner 1938). Indeed, most of these species tend to inhabit shaded forest sites. Albinos' mycoheterotrophy is now further corroborated by the demonstration of their low chlorophyll content and lack of CO_2 absorption in the light (Fig. 8.4b; Julou et al. 2005); congruently, they display ^{13}C enrichment similar to that of fully MH plants (Fig. 8.1; Julou et al. 2005; Abadie et al. 2006). This supported the likelihood of partial mycoheterotrophy in green conspecifics (Selosse et al. 2004; Julou et al. 2005). Accordingly, survival of albinos is also reported from green parasitic plants such as *Striga hermonthica* (Press et al. 1991) that use other plants' sap to support part of their C needs (see Sect. 8.4.1 and Table 8.1).

Moreover, some green orchids were found to have high ^{13}C abundance, which correlates with the potential for mycoheterotrophy via their EM fungal partners (Bidartondo et al. 2004; see also Dearnaley et al. 2012, for review). These orchid species also have a trend to low or no colonization by rhizoctonias. However, many orchids from more or less open environments associate with rhizoctonias mainly but occasionally display EM fungi, e.g. *Cypripedium* (Shefferson et al. 2005, 2007), *Gymnadenia* (Stark et al. 2009), or *Orchis* (Liebel et al. 2010; Lievens et al. 2010; Girlanda et al. 2011). These associates were likely hidden in studies based on fungal cultivation, because EM fungi do not grow easily in culture, and may even be discarded as "molecular scraps" in some molecular studies (Selosse et al. 2010), but, as suggested by Girlanda et al. (2011), they could allow a C flow to the plant, at least in some light environments.

PMH Neottieae display various levels of specificity to non-rhizoctonia fungi that are known to form EM associations with forest trees: most *Epipactis* species show a preference for Pezizomycetes related to truffles, sometimes with additional fungi (Bidartondo et al. 2004; Selosse et al. 2004; Ouanphanivanh et al. 2008; Ogura-Tsujita and Yukawa 2008; Shefferson et al. 2008; Liebel et al. 2010); *Cephalanthera* species display a large fungal spectrum including Cortinariaceae, Hymenogastraceae and Thelephoraceae (Bidartondo et al. 2004; Julou et al. 2005; Abadie et al. 2006; Matsuda et al. 2008; Yamato and Iwase 2008) whereas *Russula* are specific associates of *Limodorum* species (Girlanda et al. 2006; Liebel et al. 2010). This is much reminiscent of the EM fungal associates in many fully MH orchids (see Sect. 8.3.1). A similar feature occurs in Japanese *Cymbidium* species (Motomura et al. 2010; Ogura-Tsujita et al. 2012). The MH *C. macrorhizon* and *C. aberrans* exclusively associate with EM taxa (Russulaceae, Thelephoraceae, and Sebacinaceae), the green *C. lancifolium* and *C. goeringii*, which have ^{13}C abundances indicative of partial mycoheterotrophy (Fig. 8.3a), associate simultaneously with EM taxa and Tulasnellaceae. However, there is evidence that Ceratobasidiaceae species alone can support the ex situ growth of *C. goeringii*, although the heterotrophy level of these individuals and ecology of these fungi remain unknown Wu et al. 2010). The pattern is further complicated by the fact that some orchids associated with potentially EM taxa have not demonstrated partial mycoheterotrophy (Waterman et al. 2011), and that a few rhizoctonia lineages within the Sebacinales (Selosse et al. 2004), the Tulasnellaceae (Bidartondo et al. 2003) or the Ceratobasidiaceae (Bougoure et al. 2009) have EM abilities. Indeed, EM *Ceratobasidium* associate with the PMH *Plantanthera minor* and autotrophic trees such as *Pinus densiflora* (Yagame et al. 2008b, 2012). Surrounding trees

are thus likely to be the ultimate C source of all known PMH orchids, and the availability of EM fungi could be a limitation for the geographic distribution of partial mycoheterotrophy (Liebel et al. 2010). The reason why non-EM rhizoctonia, which support MH seedling development in green orchids, only support minor MH gains in adult orchids (Liebel et al. 2010; Girlanda et al. 2011) remains unclear: one interpretation is that they may not be capable of transferring a sufficient amount of C to support adult orchid individualss (Taylor and Bruns 1997; Martos et al. 2009), but there is no direct evidence for this.

Experiments carried out by Sadovsky (1965), at a time European protection laws did not forbid destructive manipulation of native orchids, and in repeated attempts to transplant various orchids, he listed some species that did not survive the process. The list interestingly mixes full mycoheterotrophs (such as *Neottia nidus-avis*) and PMH species (*Corallorhiza trifida*, *Cephalanthera*, *Limodorum*, and *Epipactis* spp.), suggesting that in these cases, disconnecting the fungus from its resources (=nearby mycorrhizal tree roots) entailed plant death.

Within the Epidendroideae (Fig. 8.3a), partial mycoheterotrophy is not limited to the Neottieae. It also occurs in the genus *Cymbidium* (*tribe* Cymbidieae) as stated above, and in the interesting case of *Corallorhiza trifida* (tribe Calypsoeae). Despite being leafless and having only chlorophyllous stems and seed capsules *Corallorhiza trifida*, in contrast to all other species of its genus (Freudenstein and Doyle 1994), is not fully MH upon reaching adulthood (Zimmer et al. 2008; see also Sect. 8.5.1). PMH species with apparently low heterotrophy levels were found among Mediterranean and Macaronesian orchids from the tribe Orchideae (such as *Barlia robertiana*, *Gennaria diphylla*, *Habenaria tridactylites*, and *Orchis purpurea*; Fig. 8.3b; Liebel et al. 2010; Girlanda et al. 2011). Interestingly, *G. diphylla* is associated with EM fungi (Liebel et al. 2010), and it is possible that some Tulasnellaceae and Ceratobasidiaceae found in association with other orchid taxa (Liebel et al. 2010; Girlanda et al. 2011) are EM as well.

The situation is more complicated in Orchidoideae (Fig. 8.3b), where some green species show ^{13}C depletion compared to surrounding autotrophs (Hynson et al. 2009a). This is especially pronounced for *Goodyera repens* (Fig. 8.3b), which is demonstrated to be autotrophic and to transfer C to its fungal associate (Cameron et al. 2008). Finding a relevant autotrophic baseline in terms of ^{13}C abundance is thus difficult for Orchidoideae phylogenetically related to *Goodyera* spp.: using the surrounding green plants that are enriched in ^{13}C relative to autotrophic *Goodyera* spp. may underestimate the heterotrophy level of adult PMH plants in some Orchidoideae. Nevertheless, even using such a baseline, partial mycoheterotrophy was successfully demonstrated in the Japanese *Platanthera minor* (Yagame et al. 2012), and in *Cheirostylis montana* from Thailand (Roy et al. 2009a; Fig. 8.3b), suggesting the need for more investigations to find PMH Orchidoideae within North America and Europe. Thus, many, if not all, orchid taxa appear predisposed to partial mycoheterotrophy (as well as to full mycoheterotrophy), perhaps because of their required MH germination.

We currently have no data on the impact of adult PMH plants on their associated fungi (exact load of the C loss, existence of any reward such as nutrients and/or protection). If one assumes, as suggested by C flow, that the plant is parasitic on the fungus, then some conflicts may occur and fungi may undergo selection to avoid this parasitism. In initial mycoheterotrophy-autotrophy, the interaction maybe positively selected on the fungal side, since investing C in a seedling may later allow it to recover C from the adult roots (see below) and thus compensate the C flow to germinating seedlings. Thus, one may speculate that initial mycoheterotrophy-autotrophy systems are more evolutionarily stable, which is corroborated by this strategy being more ancient in orchids than initial mycoheterotrophy-partial mycoheterotrophy. However, one might also speculate that initial mycoheterotrophy-autotrophy is more evolutionarily stable because the plant—by being MH only at the seedling stage—limits the duration and

magnitude of the parasitism, thereby reducing the selective pressure on host fungi to evolve resistance.

8.4.3.2 Partial Mycoheterotrophy in Adult Pyroloids

Until recently, orchids have been the primary models for investigations of partial mycoheterotrophy, but this nutritional mode is more widespread, and some Ericaceae were essential in demonstrating that this strategy had evolved convergently in other plant lineages. Pyroloids, for instance, are small shade-tolerant perennial shrubs, often with impressive underground rhizome networks, and grow in temperate, alpine and boreal forests. They are candidates for partial mycoheterotrophy for three reasons: (1) they have a close, although not fully resolved, phylogenetic relationship with the MH Monotropeae and Pterosporeae (all members of the Monotropoideae are MH; Kron et al. 2002; Tedersoo et al. 2007; Matsuda et al. 2012). As stated in Sect. 8.3.1, one species of Pyroleae, *Pyrola aphylla*, is even fully MH (Fig. 8.1; Zimmer et al. 2007; Hynson et al. 2009b), although its recognition as a separate species, or simply a leafless variant of the green *P. picta*, remains unclear (Camp 1940; Haber 1987; Freudenstein 1999). (2) As in orchids, seeds are very small, with few C reserves (Eriksson and Kainulainen 2011), and pyroloids undergo an MH subterranean germination (Christoph 1921; Lihnell 1942) where various fungi have been shown to provide C nutrition (Smith and Read 2008). (3) As in many other basal Ericaceae (Selosse et al. 2007), pyroloids form EM-like arbutoid associations: the fungus often forms a sheath around the root and a Hartig net, but in addition, hyphal coils penetrate root cells (Khan 1972; Robertson and Robertson 1985; Vincenot et al. 2008; Hynson and Bruns 2009). The fungal taxa involved are also capable of forming typical EM associations with surrounding tree roots (Robertson and Robertson 1985; Tedersoo et al. 2007; Zimmer et al. 2007; Vincenot et al. 2008; Hynson and Bruns 2009; Toftegaard et al. 2010; Matsuda et al. 2012), thus offering a link to autotrophic trees.

Investigations on stable isotope abundances of leafy Pyroleae species have provided variable results. An investigation in two boreal Estonian forests revealed that some pyroloids had higher ^{13}C and ^{15}N abundances and leaf N concentrations than surrounding autotrophs, including other plants from Ericaceae (Fig. 8.5; Tedersoo et al. 2007). Based on a linear mixing model using *Hypopitys monotropa* as the fully MH end-member (Box 8.4), 10–68% of C was of fungal origin in *Orthilia secunda*, *Pyrola chlorantha*, *P. rotundifolia*, and *Chimaphila umbellata*. However, the latter species did not differ from autotrophs in one of the investigated sites, and the levels of conspecific heterotrophy did not correlate among sites, indicating a complex regulation of this parameter among species. In a second set of investigations in more southern sites from Germany and California, (Fig. 8.5; Zimmer et al. 2007; Hynson et al. 2009b), ^{15}N abundance was intermediate between autotrophic and MH plants for *O. secunda*, *C. umbellata*, *P. chlorantha*, *P. minor,* and *P. picta*; however, based on ^{13}C abundance, only *O. secunda* showed significant C gain via mycoheterotrophy at a single low irradiance site. Given the broad range of irradiances in investigated sites, light availability is unlikely to be the only driver of the heterotrophy level in the study by Zimmer et al. (2007). Conversely, Matsuda et al. (2012) demonstrated that the Japanese *P. japonica* derived 50% of it's C from fungi, and that individuals growing in most shaded forest microsites and in darker months (due to canopy closure) tended to display higher ^{15}N and ^{13}C abundances, and higher leaf N concentrations. This was linked to a more specific association to *Russula* spp. in these conditions, a feature speculated to allow a better supply of fungal C. In a recent study by Hynson et al. (2012), the first truly experimental study on PMH in pyroloids, light and access to mycorrhizal networks, was manipulated in the field for two species of Pyroleae, *P. picta* and *C. umbellata*. The C stable isotope values from these species' leaf soluble sugars were then used to track changes in their relative dependency on MH C gains over the course of a growing season. The major findings of this study were that (1) *P. picta* and

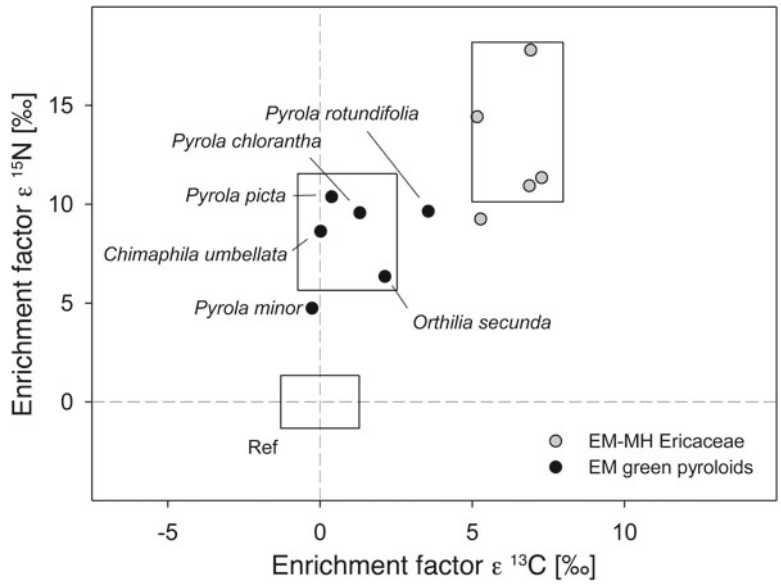

Fig. 8.5 Mean enrichment factors (ε, see Box 8.3) for ^{13}C and ^{15}N of six green species belonging to the tribe Pyroleae within the subfamily Monotropoideae of the Ericaceae (*black symbols*) and five fully mycoheterotrophic (MH) species belonging to three different tribes within the subfamily Monotropoideae of the Ericaceae (*grey symbols*, for further details see Fig. 8.1) and of autotrophic reference plants (Ref, $n = 392$) collected together with each of the respective target species. The *boxes* represent one SD of the mean ε values for the different groups of green and fully MH Monotropoideae and for the autotrophic reference plants. Number of replicates for the six target species as following: *Chimaphila umbellata* ($n = 36$), *Orthilia secunda* ($n = 38$), *Pyrola chlorantha* ($n = 17$), *P. minor* ($n = 9$), *P. picta* ($n = 54$), *P. rotundifolia* ($n = 6$). Data compiled from Tedersoo et al. (2007), Zimmer et al. (2007), Hynson et al. (2009b) and Liebel et al. (2009)

C. umbellata respond differently to a decrease in light availability: due to an increase in ^{13}C abundance over the course of the experiment *P. picta* showed signs of partial mycoheterotrophy, whereas *C. umbellata* did not. (2) Assaying the leaf soluble sugars from the two target species rather than bulk tissue revealed subtle changes along the autotrophy-mycoheterotrophy continuum in these species that would have otherwise gone undetected. And (3), the relative dependency of *P. picta* compared to *C. umbellata* on MH C gains does not appear to be solely driven by light availability.

In the cases where light levels do not drive the heterotrophy level, it was suggested that the C gains were not solely based on C needs, but could arise as a "side-product" of the N and P nutrition (Selosse and Roy 2009; see stage #3 in Table 8.1). Some fungal C could move along with organic forms of N, which is the case in mixotrophic algae where C gains arise as a consequence of mineral nutrients from prey (see Sect. 8.4.1). Indeed, a specific strategy to obtain fungal organic N in pyroloids may also explain their unusual ^{15}N abundance (Fig. 8.5), as well as their elimination after anthropogenic N deposition (Allen et al. 2007). A study of two species (*O. secunda* and *P. asarifolia*) in Canada confirmed high ^{15}N abundance and leaf N concentrations (Kranabetter and MacKenzie 2010), but the responses of these parameters to an edaphic gradient of N availability strongly suggested difference in N metabolism, not only as compared to other plants, but also between these two species. Much remains to be studied on N and C acquisition and metabolism in pyroloids. Interestingly, no evidence for lysis or degradation of intracellular hyphae is reported in living root cells of pyroloids (Tedersoo et al. 2007; Vincenot et al. 2008), adding to the idea, against previous postulates, that hyphal lysis may not be necessary for C transfer.

Furthermore, a ^{14}C labeling experiment was claimed to show transfer from autotrophs to pyroloids, in dual pot cultures of *Larix kaempferi* seedlings and *Pyrola incarnata* from Japan (Kunishi et al. 2004; Hashimoto et al. 2005), but this experiment and its controls remain unpublished. Carbon gains from fungi may explain other ecophysiological features of pyroloids, such as the few C reserves in winter (the fungal C may contribute to development of new organs in spring for *P. incarnata*; Isogai et al. 2003), the low capacity for adjusting vegetative growth and leaf area after shading (in *P. rotundifolia* at least; Hunt and Hope-Simpson 1990), or the sensitivity to forest disturbances that affect mycorrhizal networks (logging, burning; Zimmer et al. 2007). However, at least some green pyroloids survive, at least temporarily, outplanting to glasshouse conditions (e.g., Hunt and Hope-Simpson 1990). In contrast with PMH orchids, the use of fungal C in pyroloids may be more facultative, allowing disconnections from mycorrhizal network in some conditions; If one assumes that populations display variable level of dependency on mycorrhizal networks, this may also resolve the discrepancies between studies reporting variable levels of heterotrophy (Tedersoo et al. 2007; Zimmer et al. 2007; Hynson et al. 2009b).

8.4.3.3 Partial Mycoheterotrophy in Plants Associated with Arbuscular Mycorrhizal Fungi

The two lineages investigated above, orchids and Pyroleae, present two unusual features among MH lineages: (1) they associate with Asco- or Basidiomycota (EM or SAP) and (2) are primarily from temperate ecosystems. Yet, the majority of fully MH species are from tropical regions, and associate with the obligatorily biotrophic AM fungi (Leake 1994, 2004). Is there some evidence of partial mycoheterotrophy in these or other families mycorrhizal with Glomeromycota? Indeed, competition for light is high in wet tropical forests, and may select for partial mycoheterotrophy. In these conditions, some families contain both green and MH species, such as Burmanniaceae (Merckx et al. 2006, 2010), Gentianaceae (Struwe et al. 2002; Cameron and Bolin 2010), Polygalaceae (Imhof 2008), Iridaceae (Reeves et al. 2001), Pandanales (Rudall and Bateman 2006), and Petrosaviaceae (Cameron et al. 2003). Also, some green species have reduced leaves that suggest that photosynthesis is not their sole C source (Cameron and Bolin 2010).

There are unique challenges to assessing partial mycoheterotrophy in AM plants. For instance, it is difficult to sample the tiny, submillimetric spores of individual AM fungi (that do not form conspicuous fruitbodies) for stable isotope analyses, although this has sometimes been done (Allen and Allen 1990; Nakano et al. 1999; Courty et al. 2011). Also, ^{15}N abundances and N concentrations are similar in AM-MH plants and nearby green plants (Merckx et al. 2010; Courty et al. 2011; see Sect. 8.3.3): this suggests similar N nutrition and forbids any specific detection of mixotrophy on these parameters only—at least in Burmanniaceae and Gentianaceae. Finally, Courty et al. (2011) found few or no difference between canopy leaves, Glomeromycota spores, and AM-MH plants, so that specific ^{13}C enrichment is not generally expected for AM-partial mycoheterotrophy. Only in dense forests, light-starved understory plants may display some differences with AM-partial mycoheterotrophy (see Sect. 8.3.3). In more open conditions (field or canopy-open forest), similar ^{13}C enrichment in potential AM-PMH and surrounding autotrophic plants can be expected. Moreover, in some non-forest ecosystems, the occurrence of C4 plants as partners of AM fungi may further complicate the pattern, as these have lower ^{13}C depletion than the C3 plants.

Evidence for partial mycoheterotrophy among AM-MH plants, although expected (e.g., Selosse and Roy 2009), thus remains unclear. Investigating two Gentianaceae species with reduced leaves from a Virginia hardwood forest, Cameron and Bolin (2010) found no ^{13}C enrichment, but higher leaf N concentrations in *Bartonia virginica* and higher ^{15}N abundance in *Obolaria virginica* as compared to nearby autotrophs. Given the statements above, it is difficult to conclude whether this reflects some heterotrophy or some physiological particularity of N nutrition. Investigating the potential candidate for AM-partial mycoheterotrophy

Burmannia capitata from French Guiana forest gaps, Merckx et al. (2010) showed no enrichment in ^{13}C as compared to nearby herbaceous plants. Moreover, this species and three other from the same genus were successfully grown from seeds in isolated pots under controlled conditions (i.e., no mycorrhizal network). Thus, partial mycoheterotrophy is lacking, or at least facultative in these cases.

Green relatives of fully AM-MH plants could provide promising targets for future mechanistic studies on mycoheterotrophy. If partial mycoheterotrophy is found among AM plants, these plants target the most widespread mycorrhizal symbiosis, and could prove ideal for further studies of the ecophysiology of MH plants. Field and laboratory studies of the tripartite symbiosis between autotrophic host plant, mycorrhizal fungus and mycoheterotroph that have been difficult with EM mycoheterotrophs (but see Yagame et al. 2012) may receive new attention among AM mycoheterotrophs. Although many AM fungi cannot be grown axenically in culture, the putative autotrophic C source for AM mycoheterotrophs are relatively smaller than the trees that host EM fungi, and are possibly annuals which can be grown in combination with their AM mutualists. Therefore, these systems lend themselves to both in situ and laboratory experiments that follow the fate of C as it is passed from the autotrophic host, onto its associated fungus and finally the mycoheterotroph. Owing to these factors and, the plasticity of specificity among AM mycoheterotrophs, the physiological pathways and biochemical signaling between mycoheterotrophs and fungi may finally gain traction in the field of MH research.

8.5 Challenges in the Study of Partial Mycoheterotrophy

8.5.1 Gas Exchange Measurements and Photosynthesis in Partially Mycoheterotrophic Plants

Investigations of photosynthesis and in situ gas exchange support partial heterotrophy for some green orchids. CO_2 exchanges in *C. damasonium* revealed that, as expected from their phenotype, albinos were fully heterotrophic and did not respond to light, while green individuals exhibited a normal photosynthetic response to light (Fig. 8.4b; Julou et al. 2005). After in situ ^{13}C labeling, *C. damasonium* showed reduced CO_2 assimilation compared to surrounding green plants, while *C. trifida* showed nearly no assimilation, close to the level of the fully MH control (Cameron et al. 2009). This was in agreement with chlorophyll content and fluorescence values (reflecting proper assembly of pigments into photosystems): these parameters suggested subnormal and very reduced potential for photosynthesis in *C. damasonium* and *C. trifida* respectively (Fig. 8.6; Cameron et al. 2009). In *C. trifida*, the chlorophyll content is only 1% of that of *C. damasonium* (Zimmer et al. 2008). However, Montfort and Küsters (1940), measuring CO_2 exchange in inflorescences and maturing infrutescences, found that CO_2 assimilation was 2.2 times higher than respiration—whether differences in technology or plant tissue origin explains this remains unclear. Intrinsic photosynthetic limitations also exist in *Limodorum abortivum*, where photosynthetic abilities do not compensate respiration even in full light (Girlanda et al. 2006). In some *C. damasonium* populations, there is evidence that low light conditions force the plant to survive near its compensation point, where C losses through respiration are equal to C gains from photosynthesis (Julou et al. 2005). Based on variegated albinos, *C. damasonium* individuals with a mosaic of chlorophyllous and achlorophyllous tissues, Stöckel et al. (2011) found a positive correlation between leaf chlorophyll concentrations and degree of mycoheterotrophy, as shown by ^{13}C abundance. However, the recent finding that some meadow Mediterranean orchids, living in places devoid of canopy, are PMH (Girlanda et al. 2011) demonstrates that light limitation is not the sole driver of this nutritional strategy. Thus, both intrinsic and environmental factors determine partial mycoheterotrophy, depending on the species and site. Partial mycoheterotrophy may also allow buffering against herbivory or shading damage: in a defoliation and shading experiment involving the autotrophic

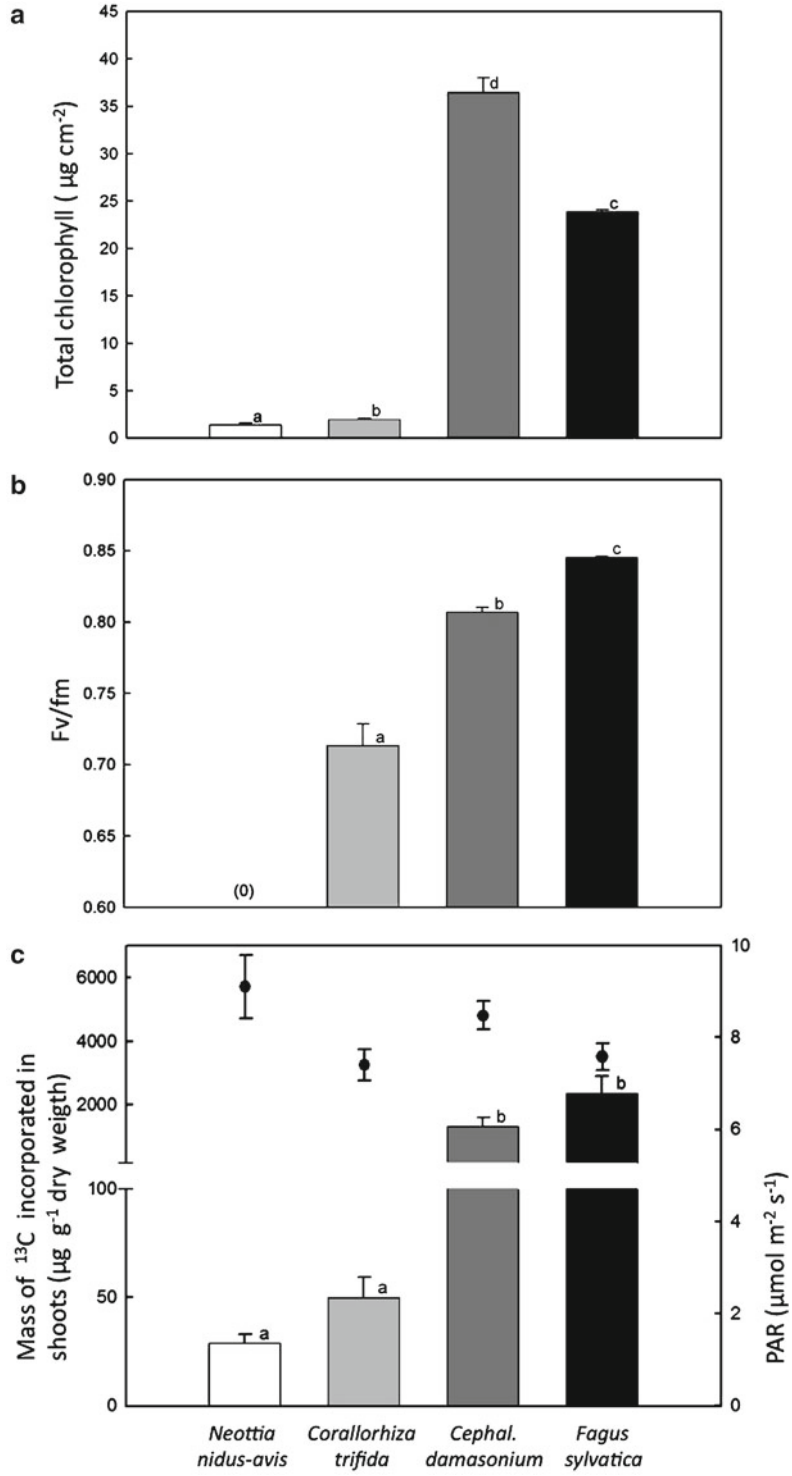

Fig. 8.6 A comparison of photosynthetic abilities between *Neottia nidus-avis* (mycoheterotroph) and *Corallorhiza trifida* (leafless partial mycoheterotroph) and the leaves of *Cephalanthera damasonium* (partial mycoheterotroph) and *Fagus sylvatica* (autotroph), reproduced with permission from Cameron et al. (2009). (**a**) Total chlorophyll content as a function of surface area of the organ (stems for *Corallorhiza trifida*, which is leafless, and leaves in all other cases). (**b**) Maximum quantum yield (Fv/Fm). (**c**) Total amount of ^{13}C present in plant shoots after a 4-h exposure to a $^{13}CO_2$ atmosphere; mean photosynthetically active radiation (PAR) is given above each bar (values on the *right*). In each panel, bars with differing letters are significantly different

Cypripedium calceolus and the PMH *C. longifolia*, Shefferson et al. (2006) demonstrated that both treatments led to significant declines in vegetative and reproductive vigor of *C. calceolus*, but had more limited impacts in *C. longifolia.*

Measurements of photosynthetic activity and stable isotope natural abundance are both powerful tools for investigating partial mycoheterotrophy. However, the information gained from these techniques differs. Gas exchange measurements provide snapshot information about photosynthetic activity, while stable isotope natural abundance data integrate the sources of C gain over the entire life history of a plant or plant organ (Liebel et al. 2010). This difference may explain the diverging results for *C. trifida*, where isotopic abundances suggest partial mycoheterotrophy (Zimmer et al. 2008) whereas CO_2 fixation was detected (Montfort and Küsters 1940) or not (Cameron et al. 2009). However, one advantage of stable isotope analyses is that they can be used in a quantitative manner way to compare levels of heterotrophy: the percent of C derived from the fungi can be estimated from a linear mixing model (Box 8.4, Table 8.2, but see 8.5.2 for considerations when applying linear mixing models to the study of PMH). Not unexpectedly, this reveals a continuum from autotrophy to full mycoheterotrophy (as also suggested in Fig. 8.3). Moreover, a given species shows a variable heterotrophy level from one site to another: for the well-studied *C. damasonium*, this level ranges for green individuals from 33% in an open, sunny pine forest to 85% in a dark beech forest (Gebauer and Meyer 2003; Bidartondo et al. 2004; Gebauer 2005).

8.5.2 Considerations for the Application of Linear Isotope Mixing Models to Mycoheterotrophic Food Webs

With the revelation of partial mycoheterotrophy, two primary challenges for researchers have been (1) determining the degree of dependence on fungal C gains in these plants and (2) elucidating the factors that select for partial mycoheterotrophy in nature. Attempts to quantify the percent fungal C and N gains in PMH plants have been made through the application of an isotope mixing model (Box 8.4; Gebauer and Meyer 2003; Bidartondo et al. 2004; Tedersoo et al. 2007; Zimmer et al. 2007). The two end-members of this linear mixing model are the ^{13}C and ^{15}N isotope abundances of fully MH plants that theoretically receive all of their C and mineral nutrients from fungi, and the ^{13}C and ^{15}N isotope abundances of autotrophic plants, which are theoretically not receiving any C from their associated mycorrhizal fungi, but meeting basically all of their N demands via their mycorrhizal fungi (Gebauer and Meyer 2003). However, while this model generates estimates of the potential dependency on fungal derived C, it also involves numerous assumptions about the isotopic behavior of MH plants, many of which are not yet fully understood. For instance, it is unknown if the relationship between the ^{13}C isotope signatures of partial mycoheterotrophs and autotrophs across environmental gradients is linear. Most PMH orchids and some pyroloids have been found living in low-light environments (<20 μmol photons m^{-2} s^{-1}; Gebauer and Meyer 2003; Bidartondo et al. 2004; Julou et al. 2005; Abadie et al. 2006; Zimmer et al. 2007; Liebel et al. 2010). Under low light conditions, fully autotrophic plants in the understory would theoretically become more depleted in ^{13}C. This depletion is owed to a combination of biochemical and leaf-level processes such as a decrease in photosynthetic rate, along with an increase in C_i/C_a (the ratio of the CO_2 concentration inside the leaf to the atmosphere outside the leaf) which would lead to greater discrimination against $^{13}CO_2$ by the primary caboxylating enzyme for C3 photosynthesis, Rubisco (Farquhar et al. 1989). In contrast, PMH often have significant increases in δ13C in low light conditions (Zimmer et al. 2007; Liebel et al. 2010; Preiss et al. 2010; Hynson et al. 2012; Fig. 8.4b). Thus the assumption that the $\delta^{13}C$ of target species and the autotrophic end-members of the mixing model are linearly related may be invalid, especially if they are from different light environments or have differing rates of photosynthesis (Fig. 8.2b). In fact, PMH species are likely have

lower photosynthesis rates than autotrophic plants, resulting in better equilibration of $^{13}CO_2$ concentration between environmental air and stomatal chamber, therefore entailing increased ^{13}C discrimination (= lower ^{13}C abundance). They may also use respired CO_2 in relatively larger amounts as compared to plants with higher CO_2 need; and this respiratory CO_2 is more depleted in ^{13}C than atmospheric CO_2. Thus the photosynthetic contributions to PMH's δ13C may be more depleted in ^{13}C than surrounding autotrophic plants. The linear relationship between leaf chlorophyll concentration (= photosynthetic capacity) and $\delta^{13}C$ observed in the variegated leaves of *Cephalanthera damasonium* (Stöckel et al. 2011) suggests that these mechanisms may have limited effects. However, it should be kept in mind that CO_2 equilibrium effects and respiratory effects may increase ^{13}C depletion in leaves, thus potentially reducing the apparent heterotrophy level of some PMH plants.

Furthermore, the factors that lead to interspecific differences in the $\delta^{13}C$ and $\delta^{15}N$ of fully MH plants are unclear (Fig. 8.1; Kranabetter and MacKenzie 2010). Therefore, the species of fully MH plants used as end-members in the mixing model can greatly affect the estimates of percent C or N gains via mycoheterotrophy. For example, in the case of the MH *Pyrola aphylla* (Ericaceae), if the percent C and N gains via fungi are calculated (Hynson et al. 2009b) using the mixing model approach with an MH end-member based on the mean relative ^{13}C enrichment of seven fully MH plants associated with EM fungi (Table 8.2, Preiss and Gebauer 2008) the estimated percent C derived from fungi would be 96 ± 12%. This indicates that *P. aphylla* essentially gains all of its C from a source that is similar to other fully MH plants associated with EM fungi. In contrast, if the same mixing model is used to calculate percent N gain via mycoheterotrophy, *P. aphylla* would gain over 100% (149 ± 18%) of its N from the source(s) utilized by the MH end-members. Thus, the MH species used as end-members in this scenario do not fully represent the extent of variability in ^{15}N signatures of mycoheterotrophs. However, from the meta-analysis of MH plant enrichment factors (Fig. 8.1), we have found significant differences in C and N stable isotope enrichment between EM-MH Orchidaceae and Ericaceae. In order to improve the resolution of the mixing model approach, we recommend that for future quantifications of mycoheterotrophy among orchidaceous and ericaceous species the MH end-member of the mixing model should be chosen based on the test species' respective plant family.

Other potential issues with using a mixing model approach to quantify the degree of mycoheterotrophy are the types of compounds that are being analyzed for their stable isotope abundances. To date, all mixing model calculations of partial mycoheterotrophy except a single study by Hynson et al. (2012), have been based on structural (bulk) leaf or stem tissue isotope signatures. Because these values represent a time-integrated measurement of C and other nutrient gains over the lifespan of a leaf, they may mask short-term fluctuations of mycoheterotrophy with organ or plant developmental stage, or with season. This factor may be particularly important for long-lived evergreen species such as Pyroleae species or orchids that have long belowground dormancy periods. For example, in the latter case, when the dormancy period ends and aboveground shoots are formed, the C for building these tissues will be derived from a heterotrophic source—either fungal or the mobilization of stored C such as starch. Both these scenarios would lead to ^{13}C enrichment in newly formed orchid leaves, potentially leading to erroneous conclusions regarding the MH status of the plants at time of observation (although the conclusion is exact regarding the C origin). The only study to date to have targeted compounds other than bulk C and N is by Hynson et al. (2012) where the leaf soluble sugars of two Pyroleae species were used to track changes in relative levels of mycoheterotrophy throughout a single growing season. Though the identities of the transfer compounds from fungi to MH plants remain unknown, sugars are reasonable candidates. Sugars are the major transport materials in biological systems, including the mycorrhizal symbiosis, due to their high energy and chemical stability (Smith and Read 2008). The results of

the work by Hynson et al. (2012) revealed that indeed sugars were a sensitive assay for shifts of C assimilation over short time periods, whereas bulk C gave an average value for the global origin of the C in the investigated plants' biomass.

8.5.3 Cryptic Mycoheterotrophy

One additional gap remaining in the current literature are labeling experiments directly demonstrating the C transfer from plant to (1) fungus and (2) PMH orchid: thus, the fact that nearby trees are the ultimate C source remains to be formally demonstrated. There are two indirect additional sources of evidence for the use of mycorrhizal fungi as trophic source: ^{15}N abundance and N concentrations in plant tissues (N content, best expressed as mmol N gdw^{-1}, also expressed as C/N ratios in some papers) are high in PMH orchids as compared to surrounding autotrophs (Gebauer and Meyer 2003; Abadie et al. 2006; Tedersoo et al. 2007; Selosse and Roy 2009; Liebel et al. 2010). However, these values are lower than in fully MH plants, supporting a mixotrophic strategy (see also Sect. 8.6.1). Stöckel et al. (2011) demonstrated a linear relationship between MH C gain and leaf N concentration in *Cephalanthera damasonium* populations with albinos displaying various level of variegation with green tissues. The same studies also demonstrate that some orchids considered as autotrophic based on their ^{13}C abundance exhibit higher (although less extreme) ^{15}N abundance (Fig. 8.3) and N concentrations than other autotrophs, again raising the possibility of a cryptic partial mycoheterotrophy.

8.6 Future Directions and Unanswered Questions

8.6.1 Nitrogen

Soil N availability is for many plants in natural terrestrial habitats a growth-limiting factor (e.g., Marschner 1995). Therefore, plants repeatedly developed strategies to gain access to N sources alternative to soil-born mineral N. For example, legumes through their symbiosis with N_2-fixing bacteria use atmospheric N and carnivorous plants use their preys as alternative N sources. The N availability to plants is mirrored by the N concentration in their tissues (Gebauer et al. 1988). Legumes have N concentrations in their tissues that are up to twice as high as in non-legumes living in the same environment. A major part of N, specifically in photosynthetic plant tissues, is invested in the enzyme Rubisco (Feller et al. 2008), which is directly involved in photosynthetic C fixation. Thus, the capacity for leaf photosynthesis and plant productivity increases with increasing leaf total N concentration (Schulze et al. 2005).

As already mentioned above (see Sects. 8.3.3 and 8.5.3) many MH and PMH plants, specifically those associated with EM fungi, but also orchids associated with rhizoctonias, have surprisingly high total N concentrations in their tissues (Gebauer and Meyer 2003; Abadie et al. 2006). Liebel et al. (2010) investigated orchids and autotrophic reference plant species in the Mediterranean and Macaronesian regions and found that representatives of the tribe Neottieae (subfamily Epidendroideae) mostly associated with EM fungi were PMH or even MH had total N concentrations of 2.9 ± 0.6(SD) mmol N gdw^{-1} ($n = 42$). Conversely, species belonging to the subfamily Orchidoideae that were mostly associated with rhizoctonias did not show signs of mycoheterotrophy based on their bulk stable isotope signatures, but had tissue total N concentrations of 1.7 ± 0.4(SD) mmol N gdw^{-1} ($n = 150$). Both groups of orchids' total N concentrations are significantly distinguished from each other and significantly above those found for non-orchid reference plants from the same environments: 1.4 ± 0.5(SD) mmol N gdw^{-1} ($n = 513$). The finding of increased tissue N concentrations in orchids associated with rhizoctonias supports the idea of a cryptic C gain from the fungus through organic N compounds (see Sect. 8.5.3). Moreover, the high total N concentrations in PMH and MH orchids raise the question as to the function of these high N concentrations in their

tissues. Use of increased photosynthetic activity as in "normal" autotrophic plants seems to be unlikely, because PMH plants mostly live under conditions under which light availability limits their photosynthetic activity (Preiss et al. 2010) and fully MH plants have given up photosynthetic activity altogether. Furthermore, in variegated individuals of *Cephalanthera damasonium*, Stöckel et al. (2011) documented a linear increase in leaf total N concentration with decreasing chlorophyll concentration and thus, decreasing photosynthetic capacity. At the moment we only can speculate about the reasons why MH and PMH plants accumulate unusually high N concentrations in their tissues. One possible reason may be related to the fact that fungal tissues and specifically those of EM fungi also have total N concentrations far above those known for the majority of autotrophic plants (Gebauer and Dietrich 1993; Gebauer and Taylor 1999) and MH plants may have an N metabolism more similar to their fungal hosts than other plants. Future studies that focus specifically on the N assimilation strategies among MH plants are greatly needed. These studies may in turn elucidate modes of C acquisition among MH plants because the two nutrients are inherently linked in their organic forms.

8.6.2 Liverworts, Lycopods, and Ferns

Gametophytes of most species in the liverwort family Aneuraceae are photosynthetic, readily grown in asymbiotic culture, and lack a morphologically unambiguous MH stage. However, in the field, most species maintain specialized relationships with tulasnelloid fungi, exhibiting patterns of fungal peloton formation and digestion that appear cytologically identical to those in the confamilial MH liverwort *Aneura mirabilis* and in IMH orchid seedlings (Ligrone et al. 1993). As in other liverworts, the sporophytes of the Aneuraceae are minute, ephemeral, dependent on the gametophytes, and uncolonized by mycorrhizal fungi. While it appears likely that gametophytes of many species in the Aneuraceae exhibit facultative partial mycoheterotrophy, their trophic status has yet to be investigated via isotope analyses.

Most members of the Lycopodiaceae, all members of the Ophioglossaceae and Psilotaceae, some members of the Schizaeaceae, and one member of the Gleicheniaceae have subterranean, non-photosynthetic, and unambiguously MH gametophytes and preemergent sporophytes. Both gametophytes and sporophytes in the Lycopodiaceae (Winther and Friedman 2008), Ophioglossaeae (Winther and Friedman 2007), and Psilotaceae (Winther and Friedman 2009) have specialized associations with Glomeromycota. Adult sporophytes of all species are typically photosynthetic, although albino and/or non-emergent forms have been observed in *Lycopodium clavatum* (Bruce and Beitel 1979), *Psilotum nudum* (Whittier 1988), and *Botrychium mormo* (Johnson-Groh 1998; Johnson-Groh and Lee 2002). In *B. paradoxum*, sporophyte leaves, while green, are reduced to small, bladeless, sporangia-bearing stipes (Wagner and Wagner 1981). Adult sporophytes of Lycopodiaceae, Ophioglossaceae, and Psilotaceae are commonly cultivated in the absence of AM host plants, although some *Botrychium* spp. are noted to be uncultivable (Donald Farrar, pers. comm.). In contrast, adult sporophytes of the Gleicheniaceae are difficult to cultivate, and those of the Schizaeaceae are rarely, if ever, cultivated.

Basic life history data, such as the duration of initial mycoheterotrophy, are unknown for most fern and lycopod taxa. The identities of autotrophic plants supporting MH development of ferns and lycopods via AM fungal networks are also largely unknown. Adult sporophytes are apparently autotrophic or PMH, depending on the taxa, but this has yet to be investigated via isotope analyses. Whether surficial, green gametophytes possessed by some taxa in the Lycopodiaceae, Schizaeaceae, and Gleicheniaceae are autotrophic or PMH is also uncertain. While gametophytes belonging to some species in the Lycopodiaceae (Whittier and Renzaglia 2005) and Gleicheniaceae (Stokey 1950) have been cultured asymbiotically

on sugar-free media (indicating they are at least facultatively autotrophic), relatively few such taxa have been studied and the trophic status of gametophytes under field conditions remains unknown. While this represents an interesting avenue for further research, collecting isotope data from minute—and in some cases, filamentous—gametophytes may prove difficult.

8.6.3 Fungi

Because the study of PMH and MH plants reveals constraints that we currently are unable to explain, future studies should also focus on the physiology of soil mycelia in regards to carbon and nutrient cycling. For example, apparently only in wet climates can SAP fungi support fully MH orchids. Although, as mentioned above (see Sect. 8.3.2), this may be linked to longer periods of C assimilation (growing seasons) and higher efficiency of fungal exoenzymes, which allow the access to larger C amounts, we must acknowledge these ideas are speculative, and face the reality that current knowledge of fungal ecophysiology may limit our understanding of these processes. Similarly, although SAP, parasitic and endophytic rhizoctonias support MH seedling development in most orchid species and some pyroloids, they are never used by fully MH orchids. Here again, we currently can speculate that the maximal C provided by rhizoctonias is not sufficient (Taylor and Bruns 1997; Hashimoto et al. 2012), but we lack direct evidence for this. In the future, simple designs that are tractable in the lab, or data from the growing number of sequenced fungal genomes, that reveal metabolic abilities (Martin and Selosse 2008) may help understanding these observations through the knowledge gained on the physiology of fungal partners.

8.6.4 An Ecological View

Up to now, as exemplified in this chapter, our approach to the study of partial and full mycoherotrophy is centered on the side of plants receiving C, as well as on the description of the fungal associates. We now need to know more about the overlooked components of the association. First, what is the impact on the fungus? Although widespread opinion holds mycoheterotrophy as a parasitism on the fungus, and EM-MH/AM-MH nutrition as "epiparasitism," on the autotrophic host plant the final evidence for this would be to show that the fungal or autotrophic host plant fitness, i.e., growth and/or sporulation, is impaired. Again here, tractable ex situ designs, which may be the most plausible on plants using SAP fungi, may help to resolve the costs to fungi associating with MH plants. It may be discovered that there is a variable level of impact of mycoheterotrophy on fungal fitness, or even some compensation by MH plants to their fungi: This compentation could possibly in the form of nutrients, growth factors (as early suggested by Björkman 1960) or shelter provided to the fungi in the roots of MH plants.

Furthermore, future studies should address the impact of PMH and MH plants on surrounding green plant(s) that provide C to these plants, in every case where a mycorrhizal fungus is providing C. From these ideas comes the question of the ecological relevance of MH nutrition. Parasitic plants are well known to impact ecosystem productivity (Westwood et al. 2010) and hemiparasitic plants, by influencing dominance of plants, have important impact on diversity of plant communities (Watson 2009). It is fascinating to observe how common some MH plants are in some tropical and temperate forests while completely absent from forests that appear similar in both their above and belowground biotic and abiotic conditions. Future studies, will hopefully reveal the conditions that select for the presence of MH plants in nature, and discern the impacts that they have on ecosystem dynamics.

References

Abadie J-C, Püttsepp Ü, Gebauer G, Faccio A, Bonfante P, Selosse M-A (2006) *Cephalanthera longifolia* (Neottieae, Orchidaceae) is mixotrophic: a comparative study between green and non-photosynthetic individuals. Can J Bot 84:1462–1477

Adamec L (1997) Mineral nutrition of carnivorous plants: a review. Bot Rev 63:273–299

Allen EB, Allen MF (1990) The mediation of competition by mycorrhizae in successional and patchy environments.

In: Grace JR, Tilman D (eds) Perspectives on plant competition. Academic, New York, pp 367–389
Allen EB, Temple PJ, Bytnerowicz A, Arbaugh MJ, Sirulnik AG, Rao LE (2007) Patterns of understory diversity in mixed coniferous forests of southern California impacted by air pollution. ScientificWorldJournal 7, http://www.thescientificworld.com
Baldrian P (2009) Ectomycorrhizal fungi and their enzymes in soils: is there enough evidence for their role as facultative soil saprotrophs? Oecologia 161:657–660
Bannister P, Strong GL (2001) Carbon and nitrogen isotope ratios, nitrogen content and heterotrophy in New Zealand mistletoes. Oecologia 126:10–20
Beau C (1920) Sur le role trophique des endophytes d'orchid6es. Comp Rend Acad Sci 171:675–677
Bennett JR, Mathews S (2006) Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. Am J Bot 93:1039–1051
Bernard N (1899) Sur la germination du *Neottia nidus-avis*. C R Hebd Seances Acad Sci 128:1253–1255
Bernard N (1908) La culture des orchidées dans ses rapports avec la symbiose. Société Royale d'Agriculture et de Botanique de Gand, Gand
Bidartondo MI, Bruns TD (2005) On the origins of extreme mycorrhizal specificity in the Monotropoideae (Ericaceae): performance trade-offs during seed germination and seedling development. Mol Ecol 14:1549–1560
Bidartondo MI, Read DJ (2008) Fungal specificity bottlenecks during orchid germination and development. Mol Ecol 17:3707–3716
Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Dominguez L, Sérsic A, Leake JR, Read DJ (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature 419:389–392
Bidartondo MI, Bruns TD, Weiß M, Sérgio C, Read DJ (2003) Specialized cheating of the ectomycorrhizal symbiosis by a epiparasitic liverwort. Proc Biol Sci 270:835–842
Bidartondo MI, Burghardt B, Gebauer G, Bruns TD, Read DJ (2004) Changing partners in the dark: Isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc Biol Sci 271:1799–1806
Björkman E (1960) *Monotropa hypopitys* L.—an epiparasite on tree roots. Physiol Plant 13:308–327
Bonnardeaux Y, Brundrett M, Batty A, Dixon K, Koch J, Sivasithamparam K (2007) Diversity of mycorrhizal fungi of terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions. Mycol Res 111:51–61
Booth MG (2004) Mycorrhizal networks mediate overstorey-understorey competition in a temperate forest. Ecol Lett 7:538–546
Bougoure J, Ludwig M, Brundrett M, Grierson P (2009) Identity and specificity of the fungi forming mycorrhizas with the rare mycoheterotrophic orchid *Rhizanthella gardneri*. Mycol Res 113:1097–1106
Bougoure JJ, Brundrett MC, Grierson PF (2010) Carbon and nitrogen supply to the underground orchid, *Rhizanthella gardneri*. New Phytol 186:947–956
Boullard B (1979) Considérations sur la symbiose fongique chez les Ptéridophytes. Syllogeus 19:1–58
Bruce JG, Beitel JM (1979) A community of *Lycopodium* gametophytes in Michigan. Am Fern J 69:33–41
Bruns TD, Read DJ (2000) In vitro germination of non-photosynthetic, myco-heterotrophic plants stimulated by fungi isolated from the adult plants. New Phytol 148:335–342
Burgeff H (1932) Saprophytismus und Symbiose. Studien an tropischen Orchideen. Gustav Fischer, Jena, Germany
Burgeff H (1936) Samenkeimung der Orchideen. Gustav Fischer, Jena, Germany
Burgeff H (1959) Mycorrhiza of orchids. In: Withner CL (ed) The orchids. Ronald Press, New York, pp 361–395
Cameron DD, Bolin JF (2010) Isotopic evidence of partial mycoheterotrophy in the Gentianaceae: *Bartonia virginica* and *Obolaria virginica* as case studies. Am J Bot 97:1272–1277
Cameron DD, Leake JR (2007) A different kind of parasitic plant: a brief history of mycoheterotrophy and epi-parasitism. Haustorium 50:4–6
Cameron KM, Chase MW, Rudall PJ (2003) Recircumscription of the monocotyledonous family Petrosaviaceae to include *Japanolirion*. Brittonia 55:214–225
Cameron DD, Leake JR, Read DJ (2006) Mutualistic mycorrhiza in orchids: evidence from the plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. New Phytol 171:405–416
Cameron DD, Johnson I, Read DJ, Leake JR (2008) Giving and receiving: measuring the carbon cost of mycorrhizas in the green orchid, *Goodyera repens*. New Phytol 180:176–184
Cameron DD, Preiss K, Gebauer G, Read DJ (2009) The chlorophyll-containing orchid *Corallorhiza trifida* derives little carbon through photosynthesis. New Phytol 183:358–364
Camp WH (1940) Aphyllous forms in pyrola. Bull Torrey Bot Club 67:453–465
Campbell EO (1962) The mycorrhiza of *Gastrodia cunninghamii* Hook. F. Trans R Soc New Zealand 1:289–296
Campbell EO (1964) The fungal association in a colony of *Gastrodia sesamoides*. R Br Trans R Soc New Zealand 2:237–246
Cha JY, Igarashi T (1995) *Armillaria* species associated with *Gastrodia elata* in Japan. Eur J Forest Pathol 25:319–326
Cha JY, Igarashi T (1996) *Armillaria jezoensis*, a new symbiont of *Galeola septentrionalis* (Orchidaceae) in Hokkaido. Mycoscience 37:21–24
Chou H (1974) In propagation of *Gastrodia elata* Bl. Acta Bot Sin 16:288–290
Christoph H (1921) Untersuchungen über mykotrophen Verhältnisse der "Ericales" und die Keimung von Pirolaceen. Beihefte Botan Centralblatt 38:115–157

Courty P-E, Walder F, Boller T, Ineichen K, Wiemken A, Selosse M-A (2011) C and N metabolism in mycorrhizal networks and mycoheterotrophic plants of tropical forests: a stable isotope analysis. Plant Physiol 156:952–961

Currah RS, Zelmer CD, Hambleton S, Richardson KA (1997) Fungi from orchid mycorrhizas. In: Arditti J, Pridgeon AM (eds) Orchid biology: reviews and perspectives, VII. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 117–170

Curtis JT (1943) Germination and seedling development in five species of *Cypripedium*. Am J Bot 30:199–206

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–559

Dearnaley JDW (2006) The fungal endophytes of *Erythrorchis cassythoides*—is this orchid saprophytic or parasitic? Aust Mycol 25:51–57

Dearnaley JDW, Bougoure JJ (2010) Isotopic and molecular evidence for saprotrophic Marasmiaceae mycobionts in rhizomes of *Gastrodia sesamoides*. Fungal Ecol 3:288–294

Dearnaley JDW, Martos F, Selosse M-A (2012) Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects. In: Hock B (ed) Fungal associations, The mycota IX, 2nd edn. Springer-Verlag, Berlin

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Douhan GW, Vincenot L, Selosse M-A (2011) Population genetics of ectomycorrhizal fungi: from current knowledge to emerging directions. Fungal Biol 115:569–597

Downie DG (1959) *Rhisoctonia solani* and orchid seed. Trans Bot Soc Edinb 37:279–285

Eriksson O, Kainulainen K (2011) The evolutionary ecology of dust seeds. Perspect Plant Ecol Evol Syst 13:73–87

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Feller U, Anders I, Mae T (2008) Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. J Exp Bot 59:1615–1624

Finlay RD, Read DJ (1986) The structure and function of the vegetative mycelium of ectomycorrhizal plants: 1. Translocation of C-14-labeled carbon between plants interconnected by a common mycelium. New Phytol 103:143–156

Fitter AH, Graves JD, Watkins NK, Robinson D, Scrimgeour C (1998) Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. Funct Ecol 12:406–412

Francis R, Read DJ (1984) Direct transfer of carbon between plants connected by vesicular arbuscular mycorrhizal mycelium. Nature 307:53–56

Frank AB (1885) Neue Mittheilungen über die Mycorrhiza der Bäume und der *Monotropa hypopitys*. Ber Dtsch Bot Ges 3:27–33

Freudenstein JV (1999) Relationships and character transformation in Pyroloideae (Ericaceae) based on ITS sequences, morphology, and development. Syst Bot 24:398–408

Freudenstein JV, Doyle JJ (1994) Character transformation and relationships in *Corallorhiza* (Orchidaceae: Epidendroideae). I. Plastid DNA. Am J Bot 81:1449–1457

Fry B (2006) Stable isotope ecology. Springer, New York, 308pp

Gebauer G (2005) Partnertausch im dunklen Wald—Stabile Isotope geben neue Einblicke in das Ernährungsverhalten von Orchideen. In: Bayer (ed) Auf Spurensuche in der Natur: Stabile Isotope in der ökologischen Forschung. Rundgespräche der Kommission für Ökologie Bd. 30. Akademie der Wissenschaften. Verlag Dr. Friedrich Pfeil, München, Germany, pp 55–67

Gebauer G, Dietrich P (1993) Nitrogen isotope ratios in different compartments of a mixed stand of spruce, larch and beech trees and of understorey vegetation including fungi. Isotopenpraxis 29:35–44

Gebauer G, Meyer M (2003) ^{15}N and ^{13}C natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. New Phytol 160:209–223

Gebauer G, Schulze E-D (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. Oecologia 87:198–207

Gebauer G, Taylor AFS (1999) ^{15}N natural abundance in fruit bodies of different functional groups of fungi in relation to substrate utilization. New Phytol 142:93–101

Gebauer G, Rehder H, Wollenweber B (1988) Nitrate, nitrate reduction and organic nitrogen in plants from different ecological and taxonomic groups of Central Europe. Oecologia 75:371–385

Girlanda M, Selosse M-A, Cafasso D, Brilli F, Delfine S, Fabbian R, Ghignone S, Pinelli P, Segreto R, Loreto F, Cozzolino S, Perotto S (2006) Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. Mol Ecol 15:491–504

Girlanda M, Segreto R, Cafasso D, Liebel HT, Rodda M, Ercole E, Cozzolino S, Gebauer G, Perotto S (2011) Photosynthetic Mediterranean meadow orchids feature partial mycoheterotrophy and specific mycorrhizal associations. Am J Bot 98:1148–1163

Gleixner G, Danier H-J, Werner RA, Schmidt H-L (1993) Correlations between the ^{13}C content of primary and secondary plant products in different cell compartments and that in decomposing fungi. Plant Physiol 102:1287–1290

Haber E (1987) Variability distribution and systematics of pyrola-picta sensu-lato Ericaceae in Western North America. Syst Bot 12:324–335

Hadley G (1970) Non-specificity of symbiotic infection in orchid mycorrhiza. New Phytol 69:1015–1023
Hamada M (1939) Studien über die Mykorrhiza von *Galeola septentrionalis* Reichb. F. — Ein neuer Fall der Mycorrhizabildung durch intraradicale Rhizomorpha. Jpn J Bot 10:151–211
Hamada M, Nakamura S (1963) Wurzelsymbiose von *Galeola altissima* Reichb. F., einer chlorophyllfreien Orchidee, mit dem holzzerstörenden Pilz *Hymenochaete crocicreas* Berk et Br. Science Reports of the Tohoku University, Fourth Series (Biology), vol 29, pp 227–238
Harvais G, Hadley G (1967) The relation between host and endophyte in orchid mycorrhiza. New Phytol 66:205–215
Hashimoto Y, Kunishi A, Hasegawa S (2005) Interspecific C transfers from *Larix kaempferi* Carr. to *Pyrola incarnata* Fischer by way of mycorrhizal fungi. Inoculum (Supplement to Mycologia) 56:23–24
Hashimoto Y, Fukukawa S, Kunishi A, Suga H, Richard F, Sauve M, Selosse M-A (2012) Mycoheterotrophic germination of *Pyrola asarifolia* dust seeds reveals convergences with germination in orchids. New Phytol 195(3):620–630
Hofmeister W (1857) Beiträge zur Entwicklungsgeschichte der Gefässkryptogamen. II. Abh Math Phys Cl Königl Sächs Ges Wiss 3:601–682, Taf. 1–13
Högberg P (1997) Tansley review no. 95. ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203
Högberg P, Plamboeck AH, Taylor AFS, Fransson PMA (1999) Natural ^{13}C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. Proc Natl Acad Sci U S A 96:8534–8539
Hunt R, Hope-Simpson JF (1990) Growth of *Pyrola rotundifolia* ssp. *maritima* in relation to shade. New Phytol 114:129–137
Hynson NA, Bruns TD (2009) Evidence of a myco-heterotroph in the plant family Ericaceae that lacks mycorrhizal specificity. Proc Biol Sci 276:4053–4059
Hynson NA, Bruns TD (2010) Fungal hosts for mycoheterotrophic plants: a nonexclusive, but highly selective club. New Phytol 185:598–601
Hynson NA, Preiss K, Gebauer G (2009a) Is it better to give than receive? A stable isotope perspective to orchid-fungal carbon transport in the green orchid species *Goodyera repens* and *Goodyera oblongifolia*. New Phytol 182:8–11
Hynson NA, Preiss K, Gebauer G, Bruns TD (2009b) Isotopic evidence of full and partial myco-heterotrophy in the plant tribe Pyroleae (Ericaceae). New Phytol 182:719–726
Hynson NA, Mambelli S, Amend AS, Dawson TE (2012) Measuring carbon gains from fungal networks in understory plants from the tribe Pyroleae (Ericaceae): a field manipulation and stable isotope approach. Oecologia 169:307–317. doi:10.1007/s00442-011-2198-3
Illyés Z, Halász K, Rudnóy S, Ouanphanivanh N, Garay T, Bratek Z (2009) Changes in the diversity of the mycorrhizal fungi of orchids as a function of the water supply of the habitat. J Appl Bot Food Qual 83:28–36
Imhof S (2008) Specialized mycorrhizal colonization pattern in achlorophyllous *Epirixanthes* spp. (Polygalaceae). Plant Biol 9:786–792
Irmisch T (1855) Bemerkungen über einige Pflanzen der deutschen Flora. Flora 13:625–638
Isogai N, Yamamura Y, Mariko S, Nakano T (2003) Seasonal pattern of photosynthetic production in a subalpine evergreen herb, *Pyrola incarnata*. J Plant Res 116:199–206
Johnson-Groh CL (1998) Population demographics, underground ecology and phenology of *Botrychium mormo*. In: Berlin N, Miller P, Borovansky J, Seal US, Byers O (eds) Population and habitat viability assessment (PHVA) for the goblin fern (*Botrychium mormo*), Final Report. Conservation Biology Specialist Group, Apple Valley, Minnesota, pp 103–108
Johnson-Groh CL, Lee JM (2002) Phenology and demography of two species of *Botrychium* (Ophioglossaceae). Am J Bot 89:1624–1633
Jones RI (2000) Mixotrophy in planktonic protists: an overview. Freshwater Biol 45:219–226
Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse M-A (2005) Mixotrophy in orchids: insights from a comparative study of green individuals and non-photosynthetic mutants of *Cephalanthera damasonium*. New Phytol 166:639–653
Kamienski F (1881) Die Vegetationsorgane der *Monotropa hypopitys* L. Bot Zeit 29:458–461
Kamjunke N, Tittel J (2009) Mixotrophic algae constrain the loss of organic carbon by exudation. J Phycol 45:807–811
Khan AG (1972) Mycorrhizae in the Pakistan Ericales Pak. J Bot 4:183–194
Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Felbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882
Kikuchi G, Higuchi M, Yoshimura H, Morota T, Suzuki A (2008a) In vitro symbiosis between *Gastrodia elata* Blume (Orchidaceae) and *Armillaria* Kummer (Tricholomataceae) species isolated from the orchid tuber. J Jpn Bot 83:77–87
Kikuchi G, Higuchi M, Morota T, Nagasawa E, Suzuki A (2008b) Fungal symbiont and cultivation test of *Gastrodia elata* Blume (Orchidaceae). J Jpn Bot 83:88–95
Knudson L (1922) Nonsymbiotic germination of orchid seeds. Bot Gaz 73:1–25
Kohzu A, Yoshioka T, Ando T, Takahashi M, Koba K, Wada E (1999) Natural ^{13}C and ^{15}N abundance of field-collected fungi and their ecological implications. New Phytol 144:323–330
Kranabetter JM, MacKenzie WH (2010) Contrasts among mycorrhizal plant guilds in foliar nitrogen concentration and $d^{15}N$ along productivity gradients of a boreal forest. Ecosystems 13:108–117

Kron KA, Judd WS, Stevens PF, Crayn DM, Anderberg AA, Gadek PA, Quinn CJ, Luteyn JL (2002) Phylogenic classification of Ericaceae: molecular and morphological evidence. Bot Rev 68:335–423

Kunishi A, Hasegawa S, Hashimoto Y (2004) Effects of mycorrhiza on *Pyrola incarnata* growing in dark forest floor. In: Proceedings of the 51st annual meeting of the Ecological Society of Japan (JES51)

Kusano S (1911) *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. J Coll Agric Imp Univ Tokyo 4:1–65

Leake JR (1994) Tansley review No. 69. The biology of myco-heterotrophic ('saprophytic') plants. New Phytol 127:171–216

Leake JR (2004) Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. Curr Opin Plant Biol 7:422–428

Leake JR (2005) Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the "saprophytic" plant myth. Mycologist 19:113–122

Lerat S, Gauci R, Catford JG, Vierheilig H, Piché Y, Lapointe L (2002) ^{14}C transfer between the spring ephemeral *Erythronium americanum* and sugar maple saplings *via* arbuscular mycorrhizal fungi in natural stands. Oecologia 132:181–187

Levin I, Kromer B (1997) Twenty years of atmospheric $^{14}CO_2$ observations at Schauinsland Station, Germany. Radiocarbon 39:205–218

Liebel HT, Gebauer G (2011) Stable isotope signatures confirm carbon and nitrogen gain through ectomycorrhizas in the ghost orchid *Epipogium aphyllum* Swartz. Plant Biol 13:270–275

Liebel HT, Preiss K, Gebauer G (2009) Parsiell mycoheterotrofi i norske vintergrønnarter – relevans for vernetiltak av truede vintergrønnarter (partial myco-heterotrophy in Norwegian wintergreen species—relevance for endangered species protection). Blyttia J Norw Bot Soc 67:138–143

Liebel HT, Bidartondo MI, Preiss K, Segreto R, Stöckel M, Rodda M, Gebauer G (2010) C and N isotope signatures reveal constraints to nutritional modes in orchids of the Mediterranean and Macaronesia. Am J Bot 97:903–912

Lievens B, van Kerckhove S, Justé A, Cammue BPA, Honnay O, Jacquemyn H (2010) From extensive clone libraries to comprehensive DNA arrays for the efficient and simultaneous detection and identification of orchid mycorrhizal fungi. J Microbiol Methods 80:76–85

Ligrone R, Pocok K, Duckett JG (1993) A comparative ultrastructural study of endophytic basidiomycetes in the parasitic achlorophyllous hepatic *Cryptothallus mirabilis* and the closely allied photosynthetic species *Aneura pinguis* (Metzgeriales). Can J Bot 71:666–679

Lihnell D (1942) Keimungsversuche mit Pyrola-Samen. Symb Bot Upsal 6:1–37

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London, UK

Martin F, Selosse M-A (2008) The *Laccaria* genome: a symbiont blueprint decoded (Tansley review—introduction to a special issue). New Phytol 180:296–310

Martos F, Dulormne M, Pailler T, Bonfante P, Faccio A, Fournel J, Dubios M-P, Selosse M-A (2009) Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. New Phytol 184:668–681

Matsuda Y, Amiya A, Ito S-I (2008) Colonization patterns of mycorrhizal fungi associated with two rare orchids, *Cephalanthera falcata* and *C. erecta.*. Ecol Res 24:1023–1031

Matsuda Y, Shimizu S, Mori M, Ito S-I, Selosse M-A (2012) Seasonal and spatial changes of mycorrhizal associations and heterotrophy levels in mixotrophic *Pyrola japonica* growing under different light environments. In revision for Am. J, Bot

Matsushita N, Fukuda K, Nagasawa E, Terashita T, Suzuki K (1996) *Armillaria* species in Japan identified by isozyme patterns with special reference to the biological species of the Northern hemisphere. J For Res 1:155–160

Mayor JR, Schuur EAG, Henkel TW (2009) Elucidating the nutritional dynamics of fungi using stable isotopes. Ecol Lett 12:171–183

McCormick MK, Whigham DF, O'Neill J (2004) Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol 163:425–438

McCormick MK, Taylor DL, Juhaszova K, Burnett RK, Whigham DF, O'Neill JP (2012) Limitations on orchid recruitment: not a simple picture. Mol Ecol 21:1511–1523

McGuire KL (2007) Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. Ecology 88:567–574

McKendrick SL, Leake JR, Read DJ (2000) Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. New Phytol 145:539–548

McKendrick SL, Leake JR, Taylor DL, Read DJ (2002) Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. New Phytol 154:233–247

McNeal JR, Arumugunathan K, Kueh JV, Boore JL, dePamphilis CW (2007) Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus *Cuscuta* (Convolvulaceae). BMC Biol 5:55

Merckx V, Schols P, Van De Kamer HM, Maas HM, Huysmans S, Smets E (2006) Phylogeny and evolution of Burmaniaceae (Dioscoreales) based on nuclear and mitochondrial data. Am J Bot 93:1684–1698

Merckx V, Stöckel M, Fleischmann A, Bruns TD, Gebauer G (2010) ^{15}N and ^{13}C natural abundance of two mycoheterotrophic and a putative partially mycoheterotrophic species associated with arbuscular mycorrhizal fungi. New Phytol 188:590–596

Mettenius G (1856) Filices Horti Botanici Lipsiensis. L. Voss, Leipzig

Milligan MJ, Williams PG (1988) The mycorrhizal relationship of multinucleate rhizoctonias from

non-orchids with *Microtis* (Orchidaceae). New Phytol 108:205–209
Montfort C, Küsters E (1940) Saprophytismus und Photosynthese. I. Biochemische und physiologische Studien an Humus-Orchideen. Bot Arch 40: 571–633
Motomura H, Yukawa T, Ueno O, Kagawa A (2008) The occurrence of Crassulacean acid metabolism in *Cymbidium* (Orchidaceae) and its ecological and evolutionary implications. J Plant Res 121:163–177
Motomura H, Selosse M-A, Martos F, Kagawa A, Yukawa T (2010) Mycoheterotrophy evolved from mixotrophic ancestors: evidence in *Cymbidium* (Orchidaceae). Ann Bot 106:573–581
Muir HJ (1989) Germination and mycorrhizal fungus compatibility in European orchids. In: Pritchard HW (ed) Modern methods in orchid conservation: the role of physiology, ecology and management. Cambridge University Press, England, pp 39–56
Mursidawati S (2004) Mycorrhizal association, propagation and conservation of the myco-heterotrophic orchid *Rhizanthella gardneri*. MSc thesis, The University of Western Australia
Nakano A, Takahashi K, Kimura M (1999) The carbon origin of arbuscular mycorrhizal fungi estimated from δ13C values of individual spores. Mycorrhiza 9:41–47
Nara K (2006) Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytol 169:169–178
Neales TF, Hew CS (1975) Two types of carbon fixation in tropical orchids. Planta 123:303–306
Ogura-Tsujita Y, Yukawa T (2008) High mycorrhizal specificity in a widespread mycoheterotrophic plant, *Eulophia zollingeri* (Orchidaceae). Am J Bot 95:93–97
Ogura-Tsujita Y, Gebauer G, Hashimoto T, Umata H, Yukawa T (2009) Evidence for novel and specialized mycorrhizal parasitism: the orchid *Gastrodia confusa* gains carbon from saprotrophic *Mycena*. Proc Biol Sci 276:761–767
Ogura-Tsujita Y, Yokoyama J, Miyoshi K, Yukawa T (2012) Shifts in mycorrhizal fungi during the evolution of autotrophy to mycoheterotrophy in *Cymbidium* (Orchidaceae). Am J Bot 99(7):1158–1176
Ota Y, Intini M, Hattori T (2000) Genetic characterization of heterothallic and non-heterothallic *Armillaria mellea* sensu stricto. Mycol Res 104:1046–1054
Ouanphanivanh N, Merényi Z, Orczán ÁK, Bratek Z, Szigeti Z, Illyés Z (2008) Could orchids indicate truffle habitats? Mycorrhizal association between orchids and truffles. Acta Biol Szeg 52:229–232
Parrent JL, James TY, Vasaitis R, Taylor AFS (2009) Friend or foe? Evolutionary history of glycoside hydrolase family 32 genes encoding for sucrolytic activity in fungi and its implications for plant-fungal symbioses. BMC Evol Biol 9:148
Pfeffer PE, Douds DD, Bucking H, Schwartz DP, Shachar-Hill Y (2004) The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. New Phytol 163:617–627
Phillips RD, Barrett MD, Dixon KW, Hopper SD (2011) Do mycorrhizal symbioses cause rarity in orchids? J Ecol 99:858–869
Preiss K, Gebauer G (2008) A methodological approach to improve estimates of nutrient gains by partially myco-heterotrophic plants. Isotopes Environ Health Stud 44:393–401
Preiss K, Adam IKU, Gebauer G (2010) Irradiance governs exploitation of fungi: fine-tuning of carbon gain by two partially myco-heterotrophic orchids. Proc Biol Sci 277:1333–1336
Press MC, Graves JD (1995) Parasitic plants. Chapmann & Hall, London
Press MC, Smith JD, Stewart GR (1991) Carbon acquisition and assimilation relations in parasitic plants. Funct Ecol 5:278–283
Pridgeon A, Cribb PJ, Chase MM (2008) Genera orchidacearum: vol 4: Epidendroidae. Oxford University Press, New York
Ramsay RR, Dixon KW, Sivasithamparam K (1986) Patterns of infection and endophytes associated with Western Australian orchids. Lindleyana 1:203–214
Rasmussen HN (1995) Terrestrial orchids from seed to mycotrophic plant. Cambridge University Press, Cambridge, UK
Rasmussen HN (2002) Recent developments in the study of orchid mycorrhiza. Plant Soil 244:149–163
Rayner MC (1927) Mycorrhiza: an account of non-pathogenic infection by fungi in vascular plants and bryophyes. New phytologist reprint no. 15. Wheldon & Wesley, London, UK
Reeves P, Chase MW, Goldblatt P, Rudall P, Fay MF, Cox AV, Lejeune B, Souza-Chies T (2001) Molecular systematics of Iridaceae: evidence from four plastid DNA regions. Am J Bot 88:2074–2087
Renner O (1938) Über blasse, saprophytische *Cephalanthera alba* und *Epipactis latifolia*. Flora 132:225–233
Robertson DC, Robertson JA (1985) Ultrastructural aspects of *Pyrola* mycorrhizae. Can J Bot 63:1089–1098
Robinson D, Fitter A (1999) The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. J Exp Bot 50:9–13
Roy M, Watthana S, Stier A, Richard F, Vessabutr S, Selosse M-A (2009a) Two mycoheterotrophic orchids from Thailand tropical dipterocarpacean forests associate with a broad diversity of ectomycorrhizal fungi. BMC Biol 7:51
Roy M, Yagame T, Yamato M, Iwase K, Heinz C, Faccio A, Bonfante P, Selosse M-A (2009b) Ectomycorrhizal *Incybe* species associate with the mycoheterotrophic orchid *Epipogium aphyllum* but not its asexual propagules. Ann Bot 104:595–610
Roy M, Gonneau C, Rocheteau A, Berveiller D, Thomas J-C, Damesin C, Selosse M-A (in press) Why do mixotrophic plants stay green? A comparison between

green and achlorophyllous orchid individuals *in situ*. Ecol Monographs

Rudall PJ, Bateman R (2006) Morphological phylogenetic analysis of Pandanales: testing contrasting hypotheses of floral evolution. Syst Bot 31:223–238

Sadovsky O (1965) Orchideen im eigenen Garten. Bayerischer Landwirtschaftsverlag, Munich, Germany

Salisbury RA (1804) On the germination of the seeds of Orchidaceae. Trans Linn Soc Lond 7:29–32

Salmia A (1986) Chlorophyll-free form of *Epipactis helleborine* (Orchidaceae) in SE Finland. Ann Bot Fenn 23:49–57

Salmia A (1989a) Features of endomycorrhizal infection of chlorophyll-free and green forms of *Epipactis helleborine* (Orchidaceae). Ann Bot Fenn 26:15–26

Salmia A (1989b) General morphology and anatomy of chlorophyll-free and green forms of *Epipactis helleborine* (Orchidaceae). Ann Bot Fenn 26:95–105

Schimel DS (1993) Theory and application of tracers. Academic, San Diego, USA

Schulze E-D, Lange OL, Ziegler H, Gebauer G (1991) Carbon and nitrogen isotope ratios of mistletoes growing on nitrogen fixing and non-nitrogen fixing hosts and on CAM plants in the Namib desert confirm partial heterotrophy. Oecologia 88:457–462

Schulze E-D, Beck E, Müller-Hohenstein K (2005) Plant ecology. Springer, Berlin

Sekizaki H, Kuninaga S, Yamamoto M, Asazu SN, Sawa S, Kojoma M, Yokosawa R, Yoshida N (2008) Identification of *Armillaria nabsnona* in *Gastrodia* tubers. Biol Pharm Bull 31:1410–1414

Selosse M-A, Rousset F (2011) The plant-fungal marketplace. Science 333:828–829

Selosse M-A, Roy M (2009) Green plants eating fungi: facts and questions about mixotrophy. Trends Plant Sci 14:64–70

Selosse M-A, Weiss M, Jany JL, Tillier A (2002) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring treeectomycorrhizae. Mol Ecol 11:1831–1844

Selosse M-A, Faccio A, Scappaticci G, Bonfante P (2004) Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. Microb Ecol 47:416–426

Selosse M-A, Richard F, He X, Simard SW (2006) Mycorrhizal networks: des liaisons dangereuses? Trends Ecol Evol 22:621–628

Selosse M-A, Setaro S, Glatard F, Richard F, Urcelay C, Weiß M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. New Phytol 174:864–878

Selosse M-A, Dubois M-P, Alvarez N (2009) Do Sebacinales commonly associate with plant roots as endophytes? Mycol Res 113:1062–1069

Selosse M-A, Martos F, Perry BA, Padamsee M, Roy M, Pailler T (2010) Saprotrophic fungal symbionts in tropical achlorophyllous orchids: finding treasures among the 'molecular scraps'? Plant Signal Behav 5:1–5

Selosse M-A, Boullard B, Richardson D (2011) Noel Bernard (1874–1911): orchids to symbiosis in a dozen years, one century ago. Symbiosis 54:61–68

Sharma J, Zettler LW, Van Sambeek JW, Ellersieck MR, Starbuck CJ (2003) Symbiotic seed germination and mycorrhizae of federally threatened *Platanthera praeclara* (Orchidaceae). Am Midl Nat 149:104–120

Shefferson RP, Weiß M, Kull T, Taylor DL (2005) High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. Mol Ecol 14:613–626

Shefferson RP, Kull T, Tali K (2006) Demographic response to shading and defoliation in two woodland orchids. Folia Geobot 41:95–106

Shefferson RP, Taylor DL, Weiß M, Garnica S, McCormick MK, Adams S, Gray HM, McFarland JW, Kull T, Tali K, Yukawa T, Kawahara T, Miyoshi K, Lee Y-I (2007) The evolutionary history of mycorrhizal specificity among lady's slipper orchids. Evolution 61:1380–1390

Shefferson RP, Kull T, Tali K (2008) Mycorrhizal interactions of orchids colonizing Estonian mine tailings hills. Am J Bot 95:156–164

Silvera K, Santiago LS, Cushman JC, Winter K (2010) The incidence of crassulacean acid metabolism in Orchidaceae derived from carbon isotope ratios: a checklist of the flora of Panama and Costa Rica. Bot J Linn Soc 163:194–222

Simard SW, Durall DM (2004) Mycorrhizal networks: a review of their extent, function, and importance. Can J Bot 82:1140–1165

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388:579–582

Smith SE (1966) Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. New Phytol 65:488–499

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London, UK

Stark JM (2000) Nutrient transformations. In: Sala OE, Jackson RB, Mooney HA, Howarth RW (eds) Methods in ecosystem science. Springer, New York, pp 215–234

Stark C, Babik W, Durka W (2009) Fungi from the roots of the common terrestrial orchid *Gymnadenia conopsea*. Mycol Res 113:952–959

Stöckel M, Meyer C, Gebauer G (2011) The degree of mycoheterotrophic carbon gain in green, variegated and vegetative albino individuals of *Cephalanthera damasonium* is related to leaf chlorophyll concentrations. New Phytol 189:790–796

Stokey AG (1950) The gametophyte of the Gleicheniaceae. Bull Torrey Bot Club 77:323–339

Struwe L, Kadereit J, Klackenberg J, Nilsson S, Thiv M, von Hagen KB, Albert VA (2002) Systematics, character evolution, and biogeography of Gentianaceae, including a new tribal and subtribal classification. In: Struwe L, Albert VA (eds) Gentianaceae—systematics and natural history. Cambridge University Press, Cambridge, pp 21–309

Sung JM, Jung BS, Yang KJ, Lee HK, Harrington TC (1995) Production of *Gastrodia elata* tuber using *Armillaria* spp. Korean J Mycol 23:61–70

Taylor DL, Bruns TD (1997) Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proc Natl Acad Sci U S A 94:4510–4515

Taylor AFS, Fransson PM, Högberg P, Högberg MN, Plamboeck AH (2003) Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. New Phytol 159:757–774

Taylor AFS, Gebauer G, Read DJ (2004) Uptake of nitrogen and carbon from double-labelled (^{15}N and ^{13}C) glycine by mycorrhizal pine seedlings. New Phytol 164:383–388

Tedersoo L, Pellet P, Kõljalg U, Selosse M-A (2007) Parallel evolutionary paths to mycohetereotrophy in understorey Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyroleae. Oecologia 151:206–217

Tennakoon KU, Pate JS (1996) Heterotrophic gain of carbon from hosts by the xylem-tapping root hemiparasite *Olax phyllanthi* (Olacaceae). Oecologia 105:369–376

Terashima K, Kawashima Y, Cha JY, Miura K (1998) Identification of *Armillaria* species from Hokkaido by analysis of the intergenic spacer (IGS) region of ribosomal DNA using PCR-RFLP. Mycoscience 39:179–183

Terashita T, Chuman S (1987) Fungi inhabiting wild orchids in Japan. IV. *Armillariella tabescens*, a new symbiont of *Galeola septentrionalis*. Trans Mycol Soc Jpn 28:145–154

Terashita T, Chuman S (1989) Armillarias, isolated from the wild orchid, *Galeola septentrionalis*. In: Proceedings of the 7th IUFRO international conference on root and butt rots of forest trees, pp 364–370

Těšitel J, Plavcová L, Cameron DD (2010) Interactions between hemiparasitic plants and their hosts. The importance of organic carbon transfer. Plant Signal Behav 5:1072–1076

Těšitel J, Lepš J, Vráblová M, Cameron DD (2011) The role of heterotrophic carbon acquisition by the hemiparasitic plant *Rhinanthus alectorolophus* in seedling establishment in natural communities: a physiological perspective. New Phytol 192:188–199

Tesitelova T, Těšitel J, Jersáková J, Říhová G, Selosse M-A (2012) Symbiotic germination capability of four *Epipactis* species (Orchidaceae) is broader than expected from adult ecology. Am J Bot 99(6):1020–1032

Teste FP, Simard SW, Durall DM, Guy RD, Jones MD, Schoonmaker AL (2009) Access to mycorrhizal networks and roots of trees: importance for seedling survival and resource transfer. Ecology 90:2808–2822

Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R, Benabdellah K, Colard A, Croll D, Da Silva C, Gomez SK, Koul R, Ferrol N, Fiorilli V, Formey D, Franken P, Helber N, Hijri M, Lanfranco L, Lindquist E, Liu Y, Malbreil M, Morin E, Poulain J, Shapiro H, van Tuinen D, Waschke A, Azcón-Aguilar C, Bécard G, Bonfante P, Harrison MJ, Küster H, Lammers P, Paszkowski U, Requena N, Rensing SA, Roux C, Sanders IR, Shachar-Hill Y, Tuskan G, Young JP, Gianinazzi-Pearson V, Martin F (2012) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol 193:755–769

Toftegaard T, Iason GR, Alexander IJ, Rosendahl S, Taylor AFS (2010) The threatened plant intermediate wintergreen (*Pyrola media*) associates with a wide range of biotrophic fungi in native Scottish pine woods. Biodivers Conserv 19:3963–3971

Tranchida-Lombardo V, Roy M, Bugot E, Santoro G, Püttsepp U, Selosse M-A, Cozzolino S (2010) Spatial repartition and genetic relationship of green and albino individuals in mixed populations of *Cephalanthera* orchids. Plant Biol 12:659–667

Trudell SA, Rygiewicz PT, Edmonds RL (2003) Nitrogen and carbon stable isotope abundances support the myco-heterotrophic nature and host-specificity of certain achlorophyllous plants. New Phytol 160: 391–401

Trumbore SE (2000) Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecol Appl 10:399–411

Umata H (1995) Seed germination of *Galeola altissima*, an achlorophyllous orchid, with aphyllophorales fungi. Mycoscience 36:369–372

Umata H (1997) Formation of endomycorrhizas by an achlorophyllous orchid, *Erythrorchis ochobiensis*, and *Auricularia polytricha*. Mycoscience 38:335–339

Umata H (1998a) In vitro symbiotic association of an achlorophyllous orchid, *Erythrorchis ochobiensis*, with orchid and non-orchid fungi. Memoirs of the Faculty of Agriculture, Kagoshima University, vol 34, pp 97–107

Umata H (1998b) A new biological function of shiitake mushroom, *Lentinula edodes*, in a myco-heterotrophic orchid, *Erythrorchis ochobiensis*. Mycoscience 39: 85–88

Umata H (1999) Germination and growth of *Erythrorchis ochobiensis* (Orchidaceae) accelerated by monokaryons and dikaryons of *Lenzites betulinus* and *Trametes hirsuta*. Mycoscience 40:367–371

Umata H, Kaneko M, Miyagi T, Nakahira Y (2007) The application and utilization of fungi for the propagation of the endangered achlorophyllous plant, *Erythrorchis ochobiensis* (Hayata) Garay (Orchidaceae) in natural situations. Research Bulletin of the Kagoshima University Forests 35:31–48

Velenovsky J (1892) Über die Biologie und Morphologie der Gattung *Moneses*. Rozpravy Královské české společnosti nauk, řada, matematicko-přírodovědná 11:147–159

Vincenot L, Tedersoo L, Richard F, Horcine H, Kõljalg U, Selosse M-A (2008) Fungal associates of *Pyrola rotundifolia*, a mixotrophic Ericaceae, from two Estonian boreal forests. Mycorrhiza 19:15–25

Vujanovic V, St-Arnaut M, Barab D, Thibeault G (2000) Viability testing of orchid seed and the promotion of colouration and germination. Ann Bot 86:79–86

Wagner WH Jr, Wagner FS (1981) New species of moonworts, *Botrychium* subg. *Botrychium* (Ophioglossaceae), from North America. Am Fern J 71:20–30

Warcup JH (1973) Symbiotic germination of some Australian terrestrial orchids. New Phytol 72:387–392

Warcup JH (1981) The mycorrhizal relationships of Australian orchids. New Phytol 87:371–381

Warcup JH (1988) Mycorrhizal associations of isolates of *Sebacina vermifera*. New Phytol 110:227–231

Warcup JH, Talbot PHB (1967) Perfect states of rhizoctonias associated with orchids. New Phytol 66:631–641

Waterman RJ, Bidartondo MI, Stofberg J, Combs JK, Gebauer G, Savolainen V, Barraclough TG, Pauw A (2011) The effects of above- and belowground mutualism in orchid speciation and coexistence. Am Nat 177:E54–E68

Watson DM (2009) Parasitic plants as facilitators: more dryad than dracula? J Ecol 97:1151–1159

Weiss M, Selosse M-A, Rexer K, Urban A, Oberwinkler F (2004) Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol Res 108:1003–1010

Weiss M, Sýkorová Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D (2011) Sebacinales everywhere: previously overlooked ubiquitous fungal endophytes. PLoS One 6:e16793

Westwood JH, Yoder JI, Timko MP, dePamphilis CW (2010) The evolution of parasitism in plants. Trends Plant Sci 15:227–235

Whittier P (1988) Dark-grown psilotum. Am Fern J 78:109–116

Whittier DP, Renzaglia KS (2005) The young gametophyte of *Lycopodiella lateralis* and the role of the intermediate shaft in development of *Lycopodiella* gametophytes. Am Fern J 95:153–159

Winther JL, Friedman WE (2007) Arbuscular mycorrhizal symbionts in *Botrychium* (Ophioglossaceae). Am J Bot 94:1248–1255

Winther JL, Friedman WE (2008) Arbuscular mycorrhizal associations in Lycopodiaceae. New Phytol 177:790–801

Winther JL, Friedman WE (2009) Phylogenetic affinity of arbuscular mycorrhizal symbionts in *Psilotum nudum*. J Plant Res 122:485–496

Wright M, Cross R, Dixon K, Huynh T, Lawrie A, Nesbitt L, Pritchard A, Swarts N, Thomson R (2009) Propagation and reintroduction of *Caladenia*. Aust J Bot 57:373–387

Wright MM, Cross R, Cousens RD, May TW, McLean CB (2010) Taxonomic and functional characterisation of fungi from the *Sebacina vermifera* complex from common and rare orchids in the genus *Caladenia*. Mycorrhiza 20:375–390

Wu BY, Nara K, Hogetsu T (2001) Can C-14-labeled photosynthetic products move between *Pinus densiflora* seedlings linked by ectomycorrhizal mycelia? New Phytol 149:137–146

Wu J, Ma H, Lü M, Han S, Zhu Y, Jin H, Liang J, Liu L, Xu J (2010) *Rhizoctonia* fungi enhance the growth of the endangered orchid *Cymbidium goeringii*. Botany 88:20–29

Xu J, Guo S (2000) Retrospect on the research of the cultivation of *Gastrodia elata* Bl, a rare traditional Chinese medicine. Chin Med J 113:686–692

Yagame T, Yamato M, Mii M, Suzuki A, Iwase K (2007) Developmental processes of achlorophyllous orchid, *Epipogium roseum*: from seed germination to flowering under symbiotic cultivation with mycorrhizal fungus. J Plant Res 120:229–236

Yagame T, Fukiharu T, Yamato M, Suzuki A, Iwase K (2008a) Identification of a mycorrhizal fungus in *Epipogium roseum* (Orchidaceae) from morphological characteristics of basidiomata. Mycoscience 49: 147–151

Yagame T, Yamato M, Suzuki A, Iwase K (2008b) Ceratobasidiaceae mycorrhizal fungi isolated from nonphotosynthetic orchid *Chamaegastrodia sikokiana*. Mycorrhiza 18:97–101

Yagame T, Orihara T, Selosse M-A, Yamato M, Iwase K (2012) Mixotrophy of *Platanthera minor*, an orchid associated with ectomycorrhiza-forming Ceratobasidiaceae fungi. New Phytol 193:178–187

Yamato M, Iwase K (2008) Introduction of asymbiotically propagated seedlings of *Cephalanthera falcata* (Orchidaceae) into natural habitat and investigation of colonized mycorrhizal fungi. Ecol Res 23:329–337

Yamato M, Yagame T, Suzuki A, Iwase K (2005) Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). Mycoscience 46:73–77

Zettler LW, Hofer CJ (1998) Propagation of the little club-spur orchid (*Platanthera clavellata*) by symbiotic seed germination and its ecological implications. Environ Exp Bot 39:189–195

Zettler LW, Stewart SL, Bowles ML, Jacobs KA (2001) Mycorrhizal fungi and cold-assisted symbiotic germination of federally threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nuttall) Lindley. Am Midl Nat 145:168–175

Zimmer K, Hynson NA, Gebauer G, Allen EB, Allen MF, Read DJ (2007) Wide geographical and ecological distribution of nitrogen and carbon gains from fungi in pyroloids and monotropoids (Ericaceae) and in orchids. New Phytol 175:166–175

Zimmer K, Meyer C, Gebauer G (2008) The ectomycorrhizal specialist orchid *Corallorhiza trifida* is a partial myco-heterotroph. New Phytol 178:395–400

Zuccaro A, Lahrmann U, Güldener U, Langen G, Pfiffi S, Biedenkopf D, Wong P, Samans B, Grimm C, Basiewicz M, Murat C, Martin F, Kogel K-H (2011) Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. PLoS Pathog 7:e1002290