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CHAPTER 1

Introduction to mycoheterotrophy

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BACKGROUND

Mycorrhizal fungi are allies of more than 90% of the half a million land plant species on Earth (Wang & Qiu 2006; Corlett 2016), establishing one of the most widespread and ecologically important mutualisms. The mycorrhizal symbiosis consists of associations between plant roots and specialized soil fungi, where plants transfer photosynthetically fixed carbon to their fungal partners, which in turn facilitate the uptake of limiting soil nutrients, mainly phosphorus and nitrogen (Smith & Read 2008). All major lineages of land plants, with the exception of mosses (Pressel *et al.* 2010), form associations with fungi belonging to the phyla Mucoromycota, Basidiomycota or Ascomycota (van der Heijden *et al.* 2015; Spatafora *et al.* 2016). The classification of mycorrhizal types depends on the morphological features and the identity of the partners within the interaction (van der Heijden *et al.* 2015). There are four main types: arbuscular mycorrhiza (AM), ectomycorrhiza (EM), ericoid mycorrhiza (ErM) and orchid mycorrhiza (OrM). Figure 1 provides a summary of the phylogenetic distribution of these mycorrhizal types and the number of plant and fungal taxa involved in the mycorrhizal symbiosis.

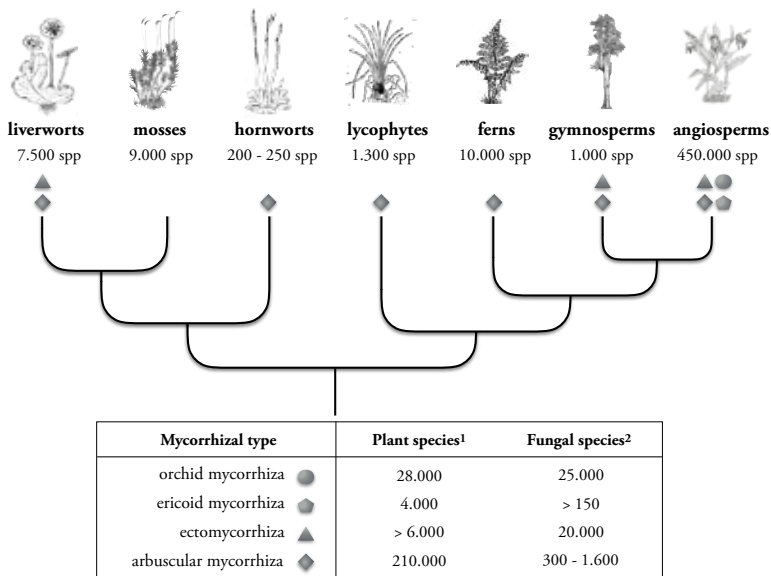


Figure 1 | Representation of the best supported hypothesis of the relationships between the major lineages of land plants (Puttick *et al.* 2018). Symbols represent the different mycorrhizal types. References for the estimated number of species per lineage are the following: angiosperms (Pimm & Joppa 2015), gymnosperms (Christenhusz *et al.* 2011), ferns (Ranker & Sundue 2015), lycophytes and mosses (Magill 2010), hornworts (Villarreal *et al.* 2010), and liverworts (Von Konrat *et al.* 2014). Total number of plant¹ (after Brundrett 2017) and fungal² species (after van der Heijden *et al.* 2015) estimated as taking part in the mycorrhizal symbiosis are depicted in the table.

The AM fungi colonize the roots of about 71% plant species including most families of vascular plants (Brundrett & Tedersoo 2018). These fungi penetrate the root cells and form arbuscules from inter-cellular hyphae or from neighbouring cells. The AM fungi belong to the phylum Mucoromycota, in which most mycorrhizal taxa are included in the sub-phylum Glomeromycotina (former phylum Glomeromycota; Spatafora *et al.* 2016). The EM fungi usually form a Hartig net with a differential hyphal mantle that surrounds the roots cells, without intracellular colonization. The EM type occurs in 2% of plant species, within few families of gymnosperms and angiosperms. The fungi involved belong to distantly-related lineages in the phyla Ascomycota and Basidiomycota. The ErM fungi colonize the plants by penetrating the root cells forming hyphal coils with each individual cell being colonized from the root surface. The ErM type is restricted to members of the Ericaceae family and the fungal partners are mostly Ascomycota. Finally, the OrM fungi form hyphal pelotons within the root cells and colonization occurs from root surface mycelia or from neighbouring cells. This mycorrhizal type occurs exclusively within members of the Orchidaceae, and involves mostly Basidiomycota fungi (Brundrett & Tedersoo 2018).

The mycorrhizal fungi have the potential to link plants from different species creating extensive underground networks. These underground mycorrhizal networks are complex to understand, yet play crucial roles in the ecosystem. The mycorrhizal symbiosis has great impact at the ecosystem level since plants allocate between 10 to 30% of their fixed carbon to their fungal partners, and receive from them up to 90% of their nutritional demands (Drigo *et al.* 2010; van der Heijden *et al.* 2015). Furthermore, AM networks are estimated to retain up to 20% carbon that plants fixate through photosynthesis (Parniske 2008), while EM networks are estimated to retain 30-70% (Leake *et al.* 2004; Clemmensen *et al.* 2013). Besides, it has been shown that within the EM symbiosis plants transfer carbon to each other from mother trees to tree seedlings (Simard & Perry 1997), but also between trees of different species, representing up to 4% of the forest net primary productivity. Thus, mycorrhizal networks are, most likely, responsible for tree to tree carbon transfer (Klein *et al.* 2016).

Evolution and stability of the mycorrhizal symbiosis

The majority of land plants associate with AM fungi and this symbiosis is believed to have been instrumental in the colonization of land by plants (Read *et al.* 2000; Bonfante & Selosse 2010). The AM symbiosis has evolved since the colonisation of land by plants,

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representing a great example of evolutionary stability (Selosse & Le Tacon 1998), which has been extensively studied during the past decades. Fossil evidence shows the presence of arbuscule-like structures resembling the current morphological features formed by arbuscular mycorrhizal fungi for at least 407 Mya (Field *et al.* 2015; Strullu-Derrien *et al.* 2018). Moreover, molecular evolutionary analysis revealed the presence of conserved genes required for the establishment of mycorrhizal symbiosis in the common ancestor of land plants (Wang *et al.* 2010). With the colonization of land by plants, environmental conditions drastically changed due to the substantial reduction of carbon dioxide and increase of oxygen towards the atmosphere composition of the present day (Selosse *et al.* 2015). Thus, the dawn of mycorrhizal symbiosis represents a key event that allowed the evolution of life on Earth as we know today.

The evolutionary persistence of the mycorrhizal mutualism demonstrates the stability of cooperation between species, despite the selfish interest of individuals (Kiers & van der Heijden 2006). The maintenance of a stable interaction is expected by the reciprocal reward between partners (Kiers *et al.* 2011; Fellbaum *et al.* 2014). Mycorrhizal fungi, particularly AM fungi, are strictly dependent on the carbon provided by the plant for their growth. Kiers *et al.* (2011) has demonstrated that the exchange of nutrients for carbon is reciprocally regulated and the most beneficial partner is rewarded with the most resources in return. This mutualistic association is thus maintained due to the reciprocal exchange of goods between partners where both benefit, suggesting that a biological market dynamics have been critical for the stability of the interaction over the course of evolution.

According to the biological market theory (Noë & Hammerstein 1995) applied to the mycorrhizal symbiosis, the trading partners – plants and mycorrhizal fungi – have the ability to regulate resource supply by discriminating between different mutualistic partners and, thus, allocating resources preferentially towards more beneficial partners (Kiers & van der Heijden 2006; Bever *et al.* 2009; Kiers *et al.* 2011). Besides the preferential allocation of resources rewarding most beneficial partners, this mechanism implies the application of sanctions to the least cooperative ones (Kiers & Denison 2008). This theory leads to the expectation of low specificity among partners, as is observed globally in the AM symbiosis, supporting partner choice as an evident component in maintaining the stability of the mycorrhizal mutualism (Kiers & van der Heijden 2006; Kiers & Denison 2008; Kiers *et al.* 2011; Walder & van der Heijden 2015). However, the processes of recognition and discrimination of the best partners from both plants and fungi still remain unclear.

Cheaters

Mycorrhizal symbiosis contains relationships that likely span a mutualism-parasitism continuum of plant-fungus interactions. The mutual reciprocal interactions between plants and fungi during the mycorrhizal symbiosis can be regarded as the midpoint, while the exploitation from each of the partners represent the endpoints of the mutualism-parasitism continuum (Bronstein 1994; Egger & Hibbett 2004). Additionally, mutualisms are predicted to persist when the costs to obtain resources are lower than the benefits gained from it (Foster & Wenseleers 2006). The costs to obtain resources within the mycorrhizal symbiosis show temporal fluctuation, such as seasonal or circadian cycles, and vary according to environmental conditions that determine resource availability (García & Mendoza 2008; Hernandez & Allen 2013; Klabi *et al.* 2014). Hence, resource acquisition can become costly, and cheating may arise. Antagonistic interactions are, thus, predicted to occur on both sides of the interaction.

Plant growth reduction in response to AM fungi inoculation has been recorded in several experiments (see, for example, Johnson *et al.* 1997; Graham & Abbott 2000). Despite the negative impact in the host plants, high colonisation of AM fungi in the roots was detected in some cases (Veiga *et al.* 2011, amongst others), suggesting a parasitic interaction of the fungi towards the host plants. However, due to the interlink between partner performance and environmental influence, which has high dependency on plant and fungi identity (Grman 2012; Walder & van der Heijden 2015), it is challenging to find substantial evidence of exclusive fungal parasitism in natural mycorrhizal systems. Despite the context-dependent outcome of mycorrhizal interactions, several mechanisms have been put forth to explain growth depression scenarios in plants influenced by AM fungi (Jin *et al.* 2017). Overall, the effectiveness of AM fungal performance between the mutualistic and the parasitic outcome has been attributed to light availability (Reinhard *et al.* 1993), which in turn is dependent on host identity (Stonor *et al.* 2014), and in combination with the relative availability of nutrients in the soil (Johnson 2010). Possible natural scenarios of plant growth depression related to AM performance include the negative effect of AM fungi in the growth of seedlings established near non-conspecific adult plants (Burke 2012); the low performance of AM partners which can be masked by the presence of more effective partners within the total fungal community (Hart *et al.* 2013); and stress conditions where AM fungi increase their carbon demands due to preferentially allocation of resources to vesicles (Johnson 1993). In addition, high fertility environments combined with a luxurious supply of

phosphorus have been hypothesized to benefit the rise of parasitic associations within the AM symbiosis. In this situation, fungal growth is only limited by carbon leading to an increase of carbon demand to a point that may depress plant growth (Thrall *et al.* 2007; Johnson 2010).

Besides the parasitism of AM fungi towards host plants, plants can also exploit their mycorrhizal partners – a phenomenon in which the plants are described as mycoheterotrophic plants (Leake 1994). Although it is difficult to measure the reduction of fitness of fungi, mycoheterotrophic plants cannot perform photosynthesis and, subsequently, there is no exchange of carbon for nutrients; thus, these plants are considered cheaters of the mycorrhizal symbiosis (Bidartondo 2005b). It remains unclear whether mycoheterotrophic plants provide benefits other than carbon to their mycorrhizal partners, such as vitamins or protection, engaging still in a mutualistic interaction, but no evidence for this has been presented so far (Selosse & Rousset 2011). Conversely, evidence points to a biotrophic parasitic nutritional mode of mycoheterotrophic plants due to the apparent digestion of the AM fungi colonising their roots (Imhof 1999). Yet, the mechanism underpinning the persistence of mycoheterotrophy through which plants subvert the carbon flux from their fungal partners to themselves still remains unknown.

Mycoheterotrophy

Leake (1994) coined the term mycoheterotrophy to define plants that rely on their fungal partners for the uptake of carbon (Bidartondo *et al.* 2002). Stable isotope natural abundance analyses have revealed that mycoheterotrophic plants are enriched in the heavy isotopes of carbon ^{13}C and nitrogen ^{15}N compared to the surrounding autotrophic species, pointing out for a carbon source coming from the mycorrhizal network, with numerous examples of partial mycoheterotrophy where plants combine both autotrophic and mycoheterotrophic strategies (for example, Bidartondo *et al.* 2004; Hynson *et al.* 2009; Gebauer *et al.* 2016; Schiebold *et al.* 2018). Both AM (Merckx *et al.* 2010) and EM (Hynson *et al.* 2009) fungi can participate in mycoheterotrophic interactions.

Some plants exhibit mycoheterotrophic mode only at initial stages of their life, called initial mycoheterotrophy (Merckx 2013). This is the case for the gametophytes of some ferns and lycopophytes which rely on mycorrhizal fungi for carbon (Leake 1994; Pressel *et al.* 2016). A possible explanation for the fungi to engage in mycoheterotrophic

relationships at this stage has been suggested as a ‘take now, pay later’ strategy in which the fungi invest carbon in supporting the mycoheterotrophic gametophytes and subterranean sporophytes (Cameron *et al.* 2008), leading to the fungi to be repaid once these plants have established as autotrophs. This has been shown for the fern *Ophioglossum vulgatum* (Field *et al.* 2015). Such strategy has also been extensively suggested for orchids, which, by having dust seeds with no endosperm, rely on carbon from mycorrhizal fungi in their early life stages. Later in their development, most orchids develop leaves gaining the ability to perform photosynthesis. However, for many orchid species it is not clear whether adult plants become totally autotrophic, since isotopic analysis of several lineages have recently suggested a partial mycoheterotrophic mode for a wider number of orchids than previously expected (Gebauer *et al.* 2016). In this



Figure 2 | Examples of mycoheterotrophic plants: (a) *Voyria spruceana*, Colombia; (b) *Sciaphila* sp., Malaysia; (c) *Apteria aphylla*, French Guiana; (d) *Gastrodia cunninghamii*, New Zealand; (e) *Afrothismia hydra*, Cameroon; (f) *Burmannia hexaptera*, Cameroon; (g) *Epirixanthes pallida*, Malaysia; (h) *Voyria* sp. nov., French Guiana; (i) *Thismia clavarioides*, Australia; (j) *Corsia* cf. *brassii*, Papua New Guinea; (k) *Monotropia hypopitys*, Netherlands; (l) *Petrosavia stellaris*, Malaysia; (m) *Pterospora andromedea*, USA. Photo credits: a, c, h, k by Sofia Gomes; b, d, e, f, g, i, l, m by Vincent Merckx; j by Stephanie Lyon.

case, orchids are able to shift the dependence on their mycorrhizal partners depending on their nutritional demands and light availability in the environment, and can vary throughout their life cycle.

Fully mycoheterotrophic plants (see Figure 2) remain on the extreme end of the spectrum of the mycorrhizal mutualism-parasitism continuum throughout their development. These plants have completely lost their ability to photosynthesize and depend entirely on their fungi to meet their carbon demands, which ultimately rely on the surrounding autotrophic plants as carbon sources (epiparasitic mycoheterotrophs), or in rare cases obtain carbon from saprotrophic activity (Hynson *et al.* 2013). The focus of this thesis is on the epiparasitic mycoheterotrophs.

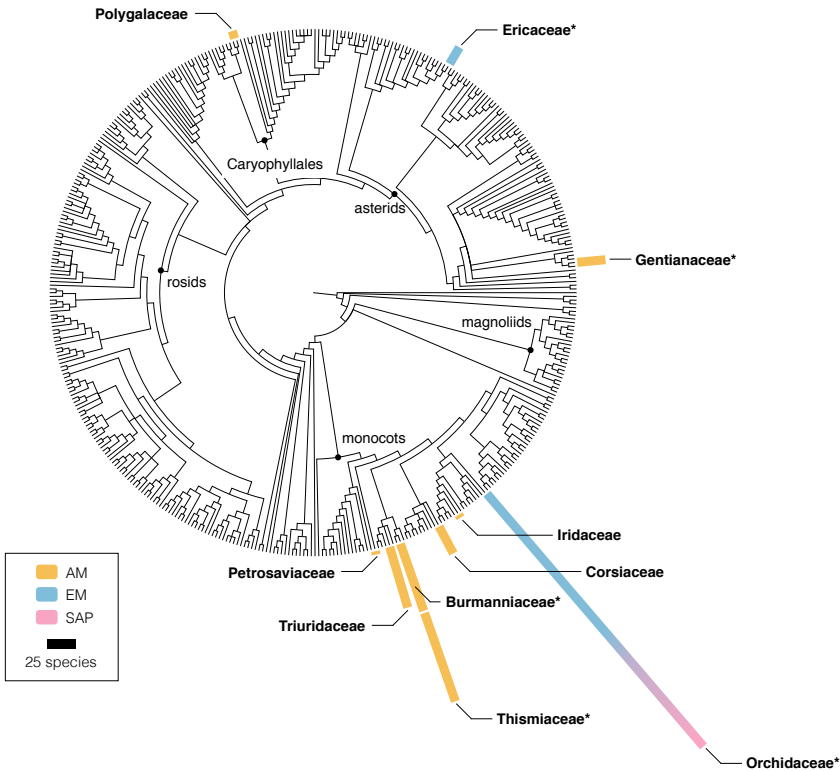


Figure 3 | Phylogenetic relationships of families within the flowering plants based on Gastauer & Meira Neto 2017, including the APG IV relationships of orders (Chase *et al.* 2016). Number of species of fully mycoheterotrophic plants described to date are represented by the coloured bars according to their mycorrhizal associations (yellow, with AM fungi; blue, with EM fungi; pink, with saprotrophic fungi). Asterisks indicates families in which there are multiple independent evolutionary origins of full mycoheterotrophy.

Diversity of mycoheterotrophic plant species

Considering the half a million land plant species, the emergence and establishment of full mycoheterotrophy is a rare event in evolution (Corlett 2016b; Merckx 2013). Yet, it evolved multiple times independently harbouring a wide variety of taxa, and it is present in almost every major group of land plants with a major presence within the flowering plants (Fig. 3). The conspicuous habit of mycoheterotrophic plants does not pass unnoticed, however their reduced size and unpredictable flowering intervals hinder their discovery even in frequently visited places, thus new species to science are discovered every year mainly in tropical rainforests. Within the flowering plants, the first complete overviews of mycoheterotrophic species estimated ca. 400 species (Furman & Trappe 1971; Leake 1994). This number has increased to ca. 515 species (Merckx 2013), and currently there are 579 mycoheterotrophic species described (Merckx 2013; Tsukaya *et al.* 2016; WCSP 2016). Of these, 530 are in 7 families of monocots, and 49 in 3 families of eudicots.

Fungal diversity

The ecology and evolution of mycoheterotrophic plants is tightly linked to their associated fungi. The fungi involved in mycoheterotrophic interactions were first investigated with molecular methods to belong to EM fungi in temperate regions (Cullings *et al.* 1996; Taylor & Bruns 1997; McKendrick *et al.* 2000; Bidartondo & Bruns 2001; Bidartondo & Bruns 2002). A decade later, Bidartondo *et al.* (2002), investigated for the first time using molecular methods the nature of fungal associations of non-ectomycorrhizal mycoheterotrophs and confirmed the involvement of AM fungi in these cheating interactions, as suggested by morphological observations (see, for example, Janse 1897; Imhof 1999).

Probably because the mycorrhizal symbiosis has evolved in the three main phyla within the fungal kingdom – Mucoromycota, Ascomycota and Basidiomycota – cheating the mycorrhizal symbiosis occurred in all these lineages. Actually, among the mycorrhizal lineages, only within the sub-phylum Mucoromycotina, two orders within the sub-phylum Glomeromycotina, and one class of Ascomycota have not been yet found being cheated by mycoheterotrophic plants (Hynson & Bruns 2010). Due to the wide range of mycoheterotrophic interactions fungi engage in, it does not appear that specific fungal lineages are particularly more prone to cheating than others (Leake

& Cameron 2010), suggesting that mycoheterotrophic plants do not necessarily target particular clades of 'naïve' fungi (Merckx 2013).

All mycoheterotrophic plant lineages evolved from mutualistic mycorrhizal ancestors. In most cases, these ancestors were likely to have mutualistic interactions with mycorrhizal fungi of the same type than the extant mycoheterotrophic descendants (Merckx *et al.* 2013). However, in some plant clades, the evolutionary transition from autotrophy to mycoheterotrophy has been described to follow evident shifts in the identity of the associated mycorrhizal fungi (Motomura *et al.* 2010; Ogura-Tsujita *et al.* 2012; Yagame *et al.* 2016). Within Orchidaceae, some mycoheterotrophic species recruited non-mycorrhizal fungal lineages including wood-rotting and parasitic fungi (Brundrett 2002; Ogura-Tsujita *et al.* 2009). Mycoheterotrophic orchids exclusively dependent on saprotrophic fungi are quite widespread (Martos *et al.* 2009), including tropical rainforests, and also temperate forests (Hynson *et al.* 2013). So far, only orchids have been observed to be able to associate with fungi other than mycorrhizal ones, however there obviously remain mycoheterotrophic species to be studied, especially in the tropics.

Plant-fungus specificity

In general, host-parasite relationships tend to present a high degree of specificity (Page 2003). As a parasitic interaction, mycoheterotrophy is generally expected to exhibit greater host specificity with decreasing nestedness of their mycorrhizal networks compared to the mutualistic autotrophic plants (Bidartondo & Bruns 2005; van der Heijden *et al.* 2015; Pölme *et al.* 2018). Although this frequent high specificity is not fully understood, there are exceptions that show lack of specificity (Hynson & Bruns 2009; Roy *et al.* 2009). Also, even in partial mycoheterotrophy, high fungal specificity and fidelity are frequently observed throughout the entire life cycle of plants, probably due to selection for net overall fitness benefits for both partners over their lifetimes (Field, Leake, *et al.* 2015). Such an interaction for the fungi makes them vulnerable to plants not rewarding them back at any time of their life, favouring cheating to arise. In the case of fully mycoheterotrophs, where plants never pay back their fungi during their entire lifetime, plants may benefit from fine-tuning their physiological responses to match interactions with specific hosts, thus fostering broad host shifts unlikely (Hynson & Bruns 2010). Other mechanism such as partner filtering may occur instead, restricting these plants to cheat particular fungal lineages (Egger & Hibbett 2004).

There is evidence that mycoheterotrophic plants often present a high degree of host-fungus specificity at least within the EM symbiosis (Taylor *et al.* 2002; Bidartondo 2005a). The first indication of extreme fungal specificity has been shown in Monotropoideae where fidelity is displayed at species level among closely related species within the broad geographic distribution of the plant taxa, suggesting a complex geographical mosaic of specificity (Bidartondo & Bruns 2001). In many mycoheterotrophic species of Orchidaceae, sequencing data shows high specificity patterns (Ogura-Tsujita & Yukawa 2008; Ogura-Tsujita *et al.* 2009), and even some species e.g. *Corallorhiza*, *Gastrodia* and *Galeola* may only associate with a single fungal genus (Brundrett 2002; Taylor *et al.* 2004; Barrett *et al.* 2010). Mycoheterotrophic orchids exclusively dependent on saprotrophic fungi are still highly specific in their fungal partners (Ogura-Tsujita & Yukawa 2008; Ogura-Tsujita *et al.* 2009),

Testing specificity in AM mycoheterotrophic interactions remains poorly studied compared to EM mycoheterotrophs. On one hand, germination of seeds together with the AM partners represents a serious challenge since the system has not been cultured axenically successfully yet, hampering the functional characterization of both partners in the interaction. For EM mycoheterotrophs, culture in-vitro is also not possible, but transplantation experiments are feasible (Bidartondo & Bruns 2005). On the other hand, species delimitation in AM fungi is still a major challenge, impeding an objective way to evaluate the extent of diversity of their mycorrhizal interactions. The mycoheterotrophic interactions with AM fungi have been described to vary from very specialized to relatively general targeting a wide variety of taxa within the Glomeromycotina (Merckx *et al.* 2012). Moreover, AM fungi from different families within Glomeromycotina have been occasionally sequenced in the roots of some mycoheterotrophs, suggesting an ample diversity in their associations. Yet, due to the non-consistent recovery of more diverse communities in the majority of individuals, the hypothesis is that they may represent facultative associations (Merckx *et al.* 2012; Renny *et al.* 2017; Merckx *et al.* 2017). Specificity in mycorrhizal associations within AM mycoheterotrophs have been mostly investigated from a phylogenetic perspective. *Petrosavia* species show higher mycorrhizal specificity comparing to their green plant relatives suggesting a selection and specialization towards their AM partners (Yamato *et al.* 2014). Similarly, certain *Burmanningia* species have been found to associate with narrow and unique phylogenetic ranges of AM fungi (Yamato *et al.* 2011; Ogura-Tsujita *et al.* 2013), as well as in *Voyria* (Bidartondo *et al.* 2002) and the temperate *Thismia* clade, where species were found to target very narrow fungal lineages (Merckx *et al.* 2017).

Additionally, the African genera such as *Kupea* and *Afrothismia* were also found to be quite specific in their fungal interactions (Franke *et al.* 2006; Merckx & Bidartondo 2008).

Distribution of mycoheterotrophic plants

Fully mycoheterotrophic plant species are almost always found in forests, usually with a closed canopy that produces deep shade. As a consequence, their non-photosynthetic mode of life is often regarded as an adaptation to the low-light conditions of forest understories (Bidartondo *et al.* 2004; Bidartondo 2005b). Nevertheless, mycoheterotrophy is not exclusive to forest habitats. In the neotropics, several arbuscular mycorrhizal mycoheterotrophic species are reported to grow in wet grasslands (Maas *et al.* 1986). Specimens of *Arachnitis* have been found on the treeless East Falkland Island, ‘growing in sand amongst rocks on an eroded sandstone ridge’ (Cribb *et al.* 1995). In Africa, mycoheterotrophic orchids occur in woodland and wooded grassland (Cheek & Williams 1999). And, in the northern hemisphere, mycoheterotrophic Ericaceae species are often found in open vegetation, such as dune slacks (Wallace 1985; Leake 1994). Besides their general preference for forest habitats, mycoheterotrophic plants seem to prefer microhabitats with high soil moisture and humidity, acidic soils, and a thick layer of decaying leaf litter (Wallace 1985; Maas *et al.* 1986; Merckx 2013). Due to the latter, they have been traditionally described as ‘saprophytes’ (Leake 2005).

The majority of fully mycoheterotrophic species are found in tropical rainforests, but they have a global distribution, occurring on all continents except Antarctica. The species in the families Burmanniaceae, Thismiaceae, Corsiaceae, Triuridaceae, Petrosaviaceae, Iridaceae, Polygalaceae, and Gentianaceae, are – a few exceptions notwithstanding – all restricted to tropical and subtropical forests, where they grow on arbuscular mycorrhizal fungi (Leake 1994; Merckx 2013). Mycoheterotrophic Orchidaceae species also occur in tropical and subtropical forests where they are associated either with ectomycorrhizal (Roy *et al.* 2009) or saprotrophic fungi (Martos *et al.* 2009). In terms of species diversity, tropical Asia harbors most fully mycoheterotrophic species, closely followed by tropical South America. In comparison, tropical Africa is relatively poor in fully mycoheterotrophic species. While some tropical mycoheterotrophic species have very restricted occurrences, many species reach relatively widespread distributions, spanning many countries and in some cases almost an entire continent (Maas *et al.* 1986; Cheek & Williams 1999). Each species, however, is confined to a single continent. On the other hand, many genera and families (e.g. Burmanniaceae, Triuridaceae, Thismiaceae)

occupy multiple continents. Outside the tropics, fully mycoheterotrophic plants species of Orchidaceae and Ericaceae occur throughout the temperate and boreal forests of the northern hemisphere, where they associate mainly with ectomycorrhizal fungi. In contrast, the temperate forests of the southern hemisphere lack mycoheterotrophic Ericaceae species, and within these forests mycoheterotrophic species of Orchidaceae grow on saprotrophic fungi. The liverwort *Aneura mirabilis*, the only non-angiosperm full mycoheterotroph, is restricted to temperate forests in Europe, where it obtains carbon from surrounding trees through shared ectomycorrhizal fungi (Bidartondo *et al.* 2003).

Despite the relatively widespread distributions of some species, most mycoheterotrophic plants have highly patchy distributions and are locally rare (Wallace 1985; Leake 1994; Merckx 2013). The fact that multiple mycoheterotrophic species are often found growing at the same site (see, for example, Maas *et al.* 1986; Cheek & Williams 1999) but in association with different fungi, possibly indicates that distribution of mycoheterotrophic species is restricted by adaptations to similar microhabitats (Merckx 2013). These microhabitats can be characterized by certain abiotic factors such as soil type, humidity, and water availability, or biotic factors, such as the presence of certain mycorrhizal fungi, pollinators, or seed dispersers (Swarts & Dixon 2009).

Challenges in mycoheterotrophy research

As expected for cheaters in general, mycoheterotrophic plants are commonly considered to have specialized fungal interactions. In fact, evidence indeed piles towards a general increase of specificity in fungal interactions by mycoheterotrophs, but also with clear exceptions (Hynson & Bruns 2009; Roy *et al.* 2009). At the same time, the fungal diversity harbored by these plants is known for a few species only, and for the large majority of species, information on the identity of the fungal partners and range of associations is still lacking (see, for example, Bidartondo *et al.* 2002; Yamato *et al.* 2011; Renny *et al.* 2017). The high observed specificity can be potentially biased by the sampling of only few specimens, or populations, due to the rarity of these plants, and thus limited to the fungal pool available locally. Broader inventories of fungal diversity in areas where mycoheterotrophic plants occur are needed to address questions regarding for example partner choice.

Furthermore, the placement of these cheater plants in the mycorrhizal network that links autotrophic plants is still obscure. Evidence for the presence of this mycorrhizal

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network supporting mycoheterotrophic plants is now being unraveled (Bidartondo *et al.* 2002; Yamato *et al.* 2011). Due to the reliance of mycoheterotrophic plants on their fungal partners, this type of information is of importance to understand how biotic interactions shape the distribution of mycoheterotrophic plants, including their typical co-occurrence patterns where distantly related species are often seen in the vicinity of each other (Merckx 2013). This may be due to sharing similar fungi, or by integrating the mycorrhizal networks in different modules. Thus, having a broader overview on the fungal interactions of these plants, including the available pool of fungal species found locally, can give us valuable insights about the influence of the biotic interactions for the mechanisms promoting the occurrence and coexistence of these plants.

Compared to their biotic interactions, our knowledge on the abiotic preferences of mycoheterotrophic plants is even more fragmented. Their rare and patchy distribution, together with their ephemeral flowering periods hinder the effective design of studies to target this subject. There is a general idea that mycoheterotrophic plants are adapted to low light conditions inside deep dark, humid forests, but no empirical data on this topic has been presented yet (Leake 1994; Bidartondo *et al.* 2004; Bidartondo 2005b; Merckx 2013). Moreover, little is known about the environmental conditions that influence the outcome of symbiotic mycorrhizal interactions. Even less is known about the conditions where plants are able to subvert the mutualistic interaction with their associated fungi.

Moreover, knowing the range of ecological settings under which cheating is prone to occur is essential to predict whether certain adaptations promoting mutualistic or antagonistic relationships are likely to evolve in a given interaction. Deepening our knowledge on the biotic and abiotic factors that drive the occurrence of mycoheterotrophic plants will allow us to obtain novel insights on the ecological preferences of these plants, and also allow us to predict the environmental conditions where the mutualistic mycorrhizal interaction has more chances to be cheated.

THESIS OUTLINE

The aim of my thesis is to shed light on the diversity, ecology and distribution of mycoheterotrophic interactions. For that, I combine perspectives from different levels of ecological complexity in mycoheterotrophic plants' systems which can give us valuable insights into the occurrence of cheating in mycorrhizal interactions (Figure

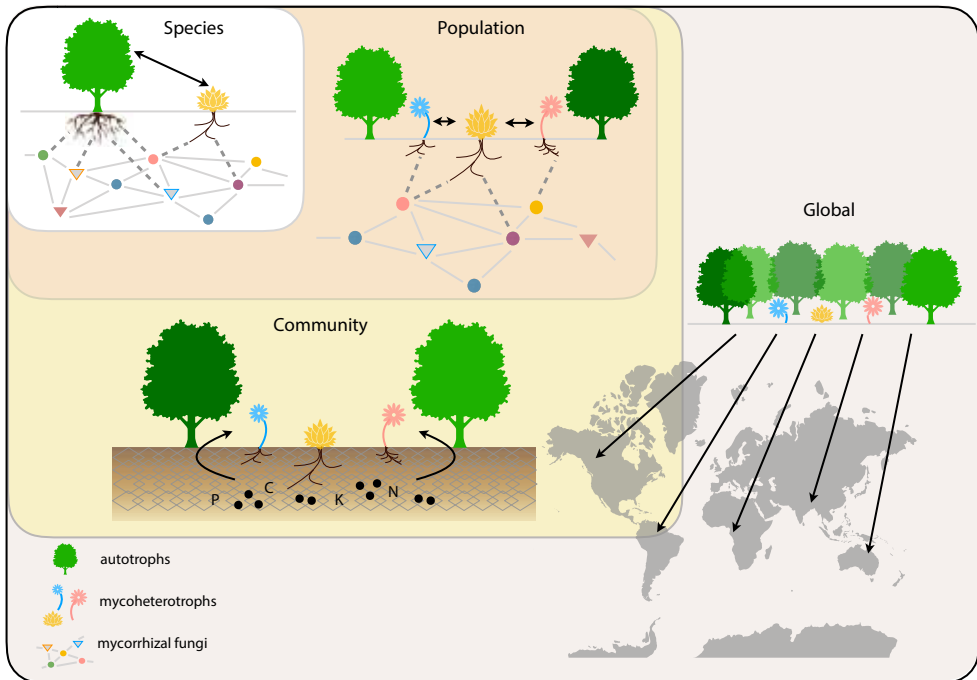


Figure 4 | Illustration of the different levels of ecological organization based on the chapters of this thesis to study the diversity, ecology and distribution of mycoheterotrophy. From single organism and its biotic interactions (chapter 2), to populations where species of mycoheterotrophic plants coexist with each other (chapter 3), to communities of mycoheterotrophic plants integrated in the environment (chapter 4), to finally niches at a global scale preferred by these plants (chapter 5).

4). Plant species together with their obligatory interactions with mycorrhizal fungi comprise the smallest organisational level (chapter 2). Followed by the interactions among distantly-related mycoheterotrophic plants searching for general mechanisms explaining fungal interaction patterns at the population level (chapter 3). Next, I investigate the edaphic properties where communities of mycoheterotrophic plants occur (chapter 4). Finally, I am interested in the general environmental drivers for the distribution of mycoheterotrophy at global scale (chapter 5).

Chapter 2

Mycoheterotrophic plants have an obligatory dependence on their mycorrhizal fungal partners. Previous studies have suggested a general predisposition for increased specialization in the associated AM fungi in the course of evolution from autotrophic

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to parasitic interactions (Yamato *et al.* 2014). These studies have compared the fungal range of interactions of mycoheterotrophic taxa with their closely-related autotrophic relatives. However, most of these studies disregard the local ecological conditions where the plants occur, which can directly influence the range of available mycorrhizal partners, since the habitats of closely-related autotrophs are often divergent. In the chapter: ‘Arbuscular mycorrhizal interactions of mycoheterotrophic *Thesium* are more specialized than in autotrophic plants’ (Gomes *et al.* 2017a), I target a highly specialized clade of mycoheterotrophic plants, confined to Australia and New Zealand, and compare the arbuscular mycorrhizal interactions of these plants with the surrounding autotrophic plants, testing the hypothesis of phylogenetic niche conservatism in their fungal interactions.

Chapter 3

Curious enough is the patchy distribution displayed by mycoheterotrophs, but the co-occurrence of distantly-related taxa within these patches is even more remarkable (Leake 1994; Merckx 2013). Possible explanations for this phenomenon range from the existence of suitable environmental conditions compatible with the heterogeneity of soil resources availability (which will be dealt with in chapter 4) to a constrained distribution due to the local availability of selected fungi that can be cheated. Yet, if the presence of the AM partners is the main driving force for the co-occurrence of mycoheterotrophic plants, then we would expect that some cheater plants would outcompete others. Alternatively, these plants may rely on mechanisms to avoid competition and allow for a stable coexistence. This chapter entitled ‘Fungal-host diversity among mycoheterotrophic plants increases proportionally to their fungal-host overlap’ (Gomes *et al.* 2017b) explores the mycorrhizal host range of 20 mycoheterotrophs collected in French Guiana, and proposes a comprehensive framework of their co-occurrence.

Chapter 4

Abiotic factors, specifically edaphic characteristics, can be very heterogeneous even at a small scale. Due to the patchy distribution that mycoheterotrophic plants often exhibit, it is likely that particular soil parameters play a role in their occurrence. With this chapter: ‘Environmental drivers of cheating arbuscular mycorrhizas in tropical rainforests’ (submitted), I intend to unveil the soil preferences that allow the occurrence of mycoheterotrophic plants at local scale. For that, I focus on tropical mycoheterotrophic

plants occurring in two bioregions in Colombia, particularly including the Amazon and the Chocó rainforests, in the northwest part of South America separated by the Andes mountains. These two regions have peculiar biogeographical histories and harbour quite unique flora and fauna. In this chapter, I compare soil nutrients and chemistry of plots where mycoheterotrophs are found with plots where they are absent. By integrating both sites, I expect to find a more general overview on the local drivers of mycoheterotrophic plants' occurrence.

Chapter 5

Going from local to global scale, the environmental drivers that drive the distribution of mycoheterotrophic plants are expected to vary. Thus, in the chapter entitled 'Global distribution of mycoheterotrophic plants' (submitted), I examine large-scale patterns of distribution of these plants considering the environmental preferences of the 15 independent lineages where mycoheterotrophy evolved within the flowering plants (Figure 3).

Chapter 6

In this final chapter, I integrate the knowledge about mycoheterotrophy obtained within my PhD study. Final considerations are made and future steps that in my view would greatly contribute to increase the understanding of this fascinating system are proposed.