Cheating belowground interactions : diversity, ecology and distribution of mycoheterotrophy

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CHAPTER 5

Global distribution patterns of mycoheterotrophy

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ABSTRACT

Mycoheterotrophy is a mode of life where plants cheat the mycorrhizal symbiosis, receiving carbon via their mycorrhizal partners. Despite being widespread, mycoheterotrophic plants are often locally rare, hampering the understanding of their global environmental drivers. Here, we explore the global environmental preferences of mycoheterotrophy, and investigate the environmental drivers of differential habitat preferences of mycoheterotrophic plants associated with arbuscular (AM) and ectomycorrhizal (EM) fungi.

We compiled the currently largest global dataset of mycoheterotrophic plant species occurrences and examined which environmental factors, including soil type, climate, vegetation type and distribution patterns of host mycorrhizal plants relate to occurrence patterns of mycoheterotrophic plant species associated with AM and EM fungi. Mycoheterotrophic plant species avoid cold and highly seasonal climates and show a strong preference for forests. AM-associated mycoheterotrophs are predominantly found in broadleaved tropical evergreen forests whereas EM-associated mycoheterotrophs occur in temperate regions and mostly in broadleaved deciduous and evergreen needleleaved forests. The abundance of AM and EM host plants was a worse predictor for mycoheterotrophs occurrences than forest type. Temperature and precipitation variables - but not edaphic factors - were the best predictors explaining the distribution patterns of AM and EM mycoheterotrophs after accounting for the effects of forest type. For individual lineages, major differences in environmental preferences (often related to edaphic factors) occurred which were significantly associated with plant evolutionary relationships, indicating that these cheater plants have limited adaptive capabilities.

The strong global geographic segregation of AM and EM mycoheterotrophs does not reflect the abundance of their green hosts, but seems to be driven by differential climate and habitat preferences. Our results highlight the non-trivial nature of mycorrhizal interactions, and indicate that identity of the partners is not enough to understand the underlying mechanisms that promote plant-mycorrhizal interactions.

Introduction

Mycoheterotrophy represents the breakdown of one of the most widespread and ecologically important mutualisms on Earth – the mycorrhizal symbiosis, where green plants exchange photosynthesized carbohydrates for mineral nutrients obtained by mycorrhizal fungi in the soil (Smith & Read 2008). In this trophic strategy, 'cheater' plants obtain carbon from their mycorrhizal partners. Mycoheterotrophic plants can use mycoheterotrophy in combination with autotrophy, or rely exclusively on their mycorrhizal fungi to obtain carbon, becoming fully mycoheterotrophic, and losing the ability to perform photosynthesis (Leake 1994; Gebauer *et al.* 2016). Mycoheterotrophy has evolved multiple times independently in flowering plants (Merckx 2013), and occurs within the two most common mycorrhizal types: the arbuscular mycorrhizal (AM) and the ectomycorrhizal (EM) fungi (Leake 1994; Smith & Read 2008).

Fully mycoheterotrophic plants occur on every continent except Antarctica (Bidartondo & Bruns, 2002; Merckx 2013) and comprise around 500 species (Leake 1994; Merckx *et al.* 2009). AM mycoheterotrophic plants occur mostly in tropical rainforests but occasionally grow in subtropical and even temperate regions, while EM mycoheterotrophs occur mostly in temperate zones but occasionally reach lower latitudes in mountain ranges (Merckx 2013). Thus, this suggests a tropical vs temperate distribution of mycoheterotrophic plants according to their mycorrhizal type, which indicates that climate plays a major role in their distribution. Nonetheless, regardless of occurring in tropical or temperate areas, all mycoheterotrophic plants seem to share a preference for humid forests with dense overstory in deep shade, with a thick layer of leaf litter on the forest floor where the occurrence of herbaceous plants is restricted (Leake 1994). Despite being widespread, mycoheterotrophic plants are often locally rare. However, when such plant is found in the field, there is a high probability to find other distantly related mycoheterotrophic species in the vicinity (Leake 1994; Merckx 2013). This suggests that mycoheterotrophic plants share environmental preferences both within and across tropical and temperate areas that still remain unexplored.

Because mycoheterotrophic plants obtain their carbon through a belowground fungal network, and ultimately from surrounding green plants (Bidartondo *et al.* 2002; Yamato *et al.* 2016; Gomes *et al.* 2017a), the distribution of mycoheterotrophy might be limited by the abundance of green host plants that act as a carbon source for their mycorrhizal fungi. Furthermore, besides the ecological drivers, the evolutionary history of mycoheterotrophic plants may also play an important role in their global distribution

patterns since species tend to be restricted to biogeographical realms (Jonker, 1938).

Here, we explore global environmental preferences of mycoheterotrophy. Specifically, we test whether the differential distribution of mycoheterotrophic plants associated with AM and EM fungi can be better explained by soil and climate, by distinct types of vegetation, or by the distribution of their host plants that share the same mycorrhizal type, i.e. AM vs EM dominant vegetation. Moreover, we explore potential drivers for the distribution of mycoheterotrophic plant lineages within each mycorrhizal type to investigate the habitat ranges that these lineages occupy. Understanding global preferences of full mycoheterotrophy will give us new insights in the environmental conditions where mycorrhizal cheating is likely to occur and therewith will enlarge our understanding of the ecology of mycorrhizas.

Materials and Methods

Mycoheterotrophic plant species data

To study the global distribution of mycoheterotrophic plants we compiled a dataset with worldwide observations of a large majority of fully mycoheterotrophic flowering plant species known to date (Merckx 2013). We combined the records from the Global Biodiversity Information Facility (GBIF, http://www.gbif.org) for the whole globe, the Botanical Information and Ecology Network (BIEN, http://bien.nceas.ucsb.edu/bien/) for America, the African Plant Database (http://www.ville-ge.ch) for Africa, and personal datasets (SI, Table S1). We also consulted the database of ex-Soviet Union territory (Akhmetzhanova *et al.* 2012) but there were no records of mycoheterotrophic plants available for that region. Our dataset included 21 species in Gentianaceae, 11 in Ericaceae, 5 in Polygalaceae, 15 in Liliales, 2 in Petrosaviaceae, 2 in Iridaceae, 79 in Dioscoreales, and 40 in Orchidaceae. After removing potentially incorrect occurrences, the compiled dataset contained 22,853 records. We assigned the mycorrhizal types AM and EM to the mycoheterotrophic plants in our dataset based on literature descriptions compiled in Merckx 2013, and excluded those records of species associated with saprotrophic fungi or unverified mycorrhizal type. We created a 1 km^2 worldwide grid and recorded presences and absences of these plants in each grid cell considering their mycorrhizal types. In total, 1,935 (8.5%) individuals were associated with AM fungi and 20,918 (91.5%) with EM fungi. When plants associated with AM and EM fungi were

present in a single grid cell, they were assigned to both AM and EM individual datasets in the subsequent analyses.

Global drivers of mycoheterotrophic plants distribution

We generated histograms of the distribution of mycoheterotrophic plants overlain with global patterns of climatic and edaphic conditions to highlight the environmental preferences of mycoheterotrophs in its entirety (SI, Figure S1). Mycoheterotrophic plants were shown to occur at a global scale with a clear dichotomy of tropics vs temperate regions in their distribution according to mycorrhizal type (see results, Figure 1). Therefore, we focused our analysis on the drivers underpinning the differential distribution of AM and EM-associated mycoheterotrophic plants. Given the obvious differences in temperature and precipitation regimes characteristic for tropics and temperate zones we did not examine the global environmental drivers promoting the differential distribution of AM and EM mycoheterotrophs. Instead, we examined if AM and EM mycoheterotrophs had distinct preferences for a specific type of vegetation. This would reflect the common description of mycoheterotrophic plants as understory plants in closed canopy forests (Leake 1994; Merckx 2013). Alternatively, mycoheterotrophic occurrences may be associated with preference for habitats dominated by hosts of the same mycorrhizal type. This would reflect the reliance of these plants on the belowground mycorrhizal network for carbon uptake (Trudell *et al.* 2003). To investigate these alternative hypotheses, we considered the land class categories from the CCI Land Cover maps (ESA 2015) to infer vegetation type. For the association with the host green plant featuring the same mycorrhizal type, we used the global maps of abundance of green plants associated with AM and EM fungi, respectively (Soudzilovskaia *et al.* 2018). The Land Cover maps were obtained with a spatial resolution of 300 m, which we rescaled to the 1 km^2 grid used in this study. Maps on the abundance of mycorrhizal host types were obtained at a resolution of 10 min. To reduce noise in our dataset caused by potential imprecision of coordinates, and by overlaying the vegetation and plant abundance maps, we excluded all records that were found in areas with no vegetation since mycoheterotrophs need to be associated with mycorrhizal fungi that are subsequently associated to surrounding green plants.

Climatic and edaphic factors are known to be important predictors of plant species and mycorrhizal fungi assemblages at large scales (Tedersoo *et al.* 2014; Davison *et al.* 2015). Hence, we tested the relative importance of these potential drivers for the

distribution of mycoheterotrophic plants after accounting for the effects of vegetation type or abundance of hosts. The climatic data, obtained from the WorldClim database at 1 km^2 resolution (Hijmans *et al.* 2005), describe temperature and precipitation annual trends, seasonality and extreme or limiting environmental factors worldwide. For the soil data, since these plants have generally shallow root systems (Leake 1994; Merckx 2013), we considered only edaphic variables in the top-soil layer from the Harmonized World Soil Database (Batjes *et al.* 2009), which is a set of spatial databases of derived soil properties at a global scale. Furthermore, it is often assumed that these plants are sensitive to desiccation (Leake 1994; Merckx 2013), and therefore we also considered the actual evaporation, the evaporation stress factor, the root zone soil moisture and the surface soil moisture (GLEAM maps; Martens et al. 2017) as potential drivers.

Environmental preferences of individual mycoheterotrophic lineages

Mycoheterotrophic plant species and genera are often restricted to particular biogeographical regions (Jonker, 1938; Merckx 2013; Mennes *et al.* 2015a), suggesting that evolutionary relationships may shape the distribution patterns of these plants. Therefore, we tested whether the evolutionary history of mycoheterotrophic species limits their occurrence to particular ecozones. For this purpose, we considered the 15 independent shifts towards mycoheterotrophy represented by our data (SI Table S2). Based on recent phylogenetic insights we considered four independent shifts in Gentianaceae represented by the genera *Voyria, Voyriella, Exacum* and *Exochaenium* (Merckx et al. 2013), two shifts in Ericaceae including Monotropoideae and Pyrola (Freudenstein *et al.* 2016), and a single shift in Polygalaceae: *Epirixanthes* (Mennes et al. 2015a), Liliales: Corsiaceae (Mennes et al. 2015b), Petrosaviaceae: *Petrosavia* (Cameron *et al.* 2003), Triuridaceae (Mennes *et al.* 2013) and Iridaceae: *Geosiris* (Goldblatt *et al.* 2008). In Dioscoreales we recognized three shifts: *Afrothismia*, Thismiaceae s.s., and Burmanniaceae (Merckx *et al.* 2017). The latter group also contains chlorophyllous species, but recent evidence indicates that these are partially mycoheterotrophic (Bolin *et al.* 2015), suggesting the presence of a strong predisposition for mycoheterotrophy in the most recent common ancestor of the family. Similarly, since all Orchidaceae are initially mycoheterotrophic and many are potentially partially mycoheterotrophic (Gebauer *et al.* 2016), we considered all species in this family to be part of a single lineage. To better understand the observed dichotomy in distribution of AM and EM mycoheterotrophic plant species, we explored environmental preferences separately for each type.

Statistical analyses

To test whether mycoheterotrophic plants had a stronger preference for particular forest types or for association with host green plants with the same mycorrhizal type, we examined four alternative univariate generalized linear models testing the occurrence of AM and EM mycoheterotrophs, respectively vs. (1) eight forest, and (2) the host green plant abundance. We selected the most parsimonious models based on the highest adjusted R^2 and the Bayesian Information Criteria (BIC) (Aho *et al.* 2014). Once the variance explained by the selected predictor in the most parsimonious model was accounted for, we used hierarchical partitioning on the residuals of this model to understand if mycoheterotrophic plants had further preferences for particular environmental conditions. All predictors were standardized to avoid scaling variance issues due to different measurement scales. The selection of the predictors to be included in the models was performed in two steps. First, we excluded variables with $R^2 \leq 0.05$ in univariate linear regressions to avoid spurious correlations. Then, we assessed collinearity among variables by calculating the variance inflation factors (VIF) in a stepwise manner, discarding the variable with the highest VIF at each step, until all the variables maintained in the final model had VIF < 4 (Zuur *et al.* 2010) and Pearson correlation < |0.7| (Dormann *et al.* 2013). To evaluate the importance of each predictor, we calculated the omega squared (ω^2) as an unbiased effect size estimate on the amount of variance explained by each of the individual predictors in the models (Olejnik & Algina, 2000).

We used a one-way permutational multivariate analysis of variance (perMANOVA with 99 permutations), in which Euclidean distance between the lineages was employed as dependent variable and ecozone (to reflect biogeographical preferences) as independent. The analysis was run for the AM and EM datasets separately. Subsequently, multiple testing using BH corrections (Benjamini & Hochberg, 1995) suitable for large datasets was performed as posthoc test. To visualize the environmental preferences of the mycoheterotrophic lineages, we applied principal component analysis (PCA) on the standardized environmental variables for each dataset separately, labelling the occurrence of each lineage in PCA-space.

All analyses were carried out in R 3.4.1 (R Core Team, 2016) with the LME4, LSR, RVAIDEMEMOIRE, and VEGAN packages.

RESULTS

Global distribution patterns of mycoheterotrophy

The global distribution of mycoheterotrophic occurrences showed a clear dichotomy with the AM plants mainly occurring in the tropics and EM plants in temperate areas (Figure 1). The Palearctic is the most well represented region in our study comprising 71.1% of the total number of records, followed by the Neartic with 14.7%, Neotropic with 7.3%, Australasia with 4.3%, Afrotropics with 1.4% and finally Indomalaya with 1.2% of the total records.

When comparing the distribution of mycoheterotrophic plants with patterns in global climate and soil variables, we observed that in general mycoheterotrophy has no strong preference for particular conditions except for avoiding very cold and seasonal climates (Figure 2; see the other variables in SI Figure S1). The majority of mycoheterotrophs occur in forests (Figure 3), with clear preferences for particular forest types: AM mycoheterotrophs occur mostly in broadleaved evergreen forest (Figure 4a), while EM mycoheterotrophs occur mostly in other forest types, preferring needleleaved evergreen forests, broadleaved deciduous forests, forests with mixed leaf habits, and forests with shrub cover (Figure 4b). AM and EM mycoheterotrophic plants showed clear preferences for climatic conditions coinciding with their tropical and temperate distribution, respectively, but not for particular soil conditions (SI, Figure S1). The selection of the most parsimonious models resulted in the evergreen forests (BIC: 6936; Adj R^2: 0.49 for AM; BIC: 6784; Adj R^2: 0.48 for EM) as being the best predictor among all forest types.

The global abundance of mycoheterotrophic plants seems to follow the global trend of AM hosts abundance (Figure 4c), better than the abundance of EM hosts (Figure 4d). However, models based on forest type were consistently significantly better than models including the host plants associated with AM type (BIC: 7428.2; Adj R^2: 0.45 for AM; BIC: 7026.2; Adj R^2: 0.46 for EM).

The hierarchical partitioning analyses on the residuals of the best models - which had evergreen forest as single predictor - showed that evergreen forests were the main predictors of the distribution of mycoheterotrophic plants, with climate and soil variables hardly showing any explanatory power, since only one climatic variable showed medium importance (ω^2 >0.06; Cohen 1988) for either of the mycorrhizal

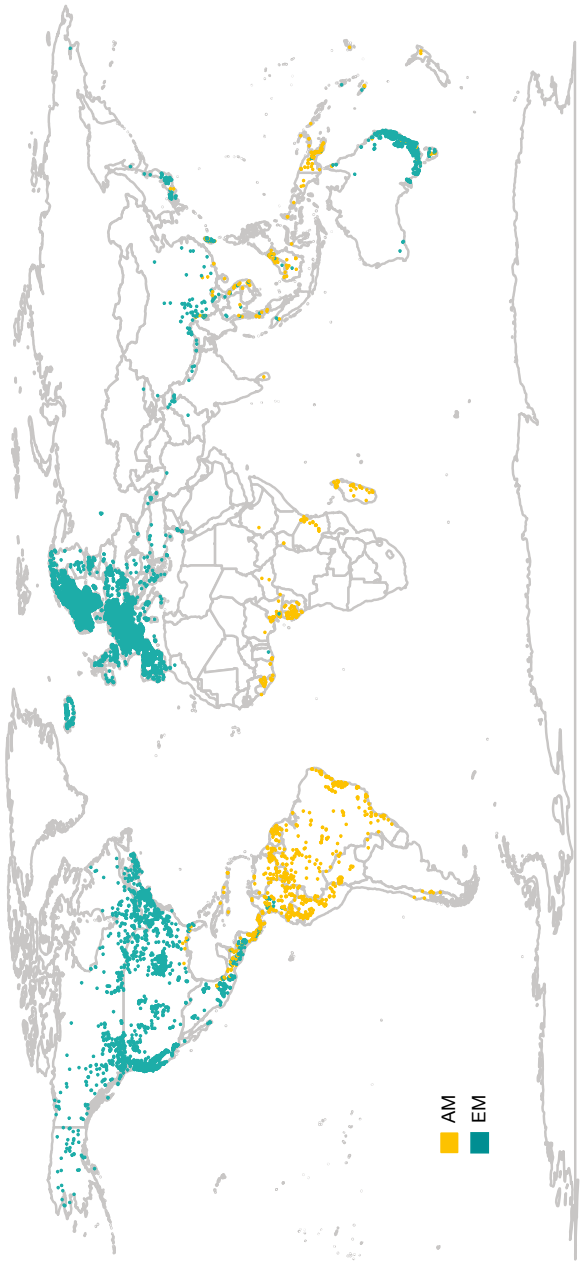


Figure 1 | Global distribution of mycoheterotrophic plants associated with arbuscular (AM) and ectomycorrhizal (EM) fungi. Records were obtained from public databases (see methods).

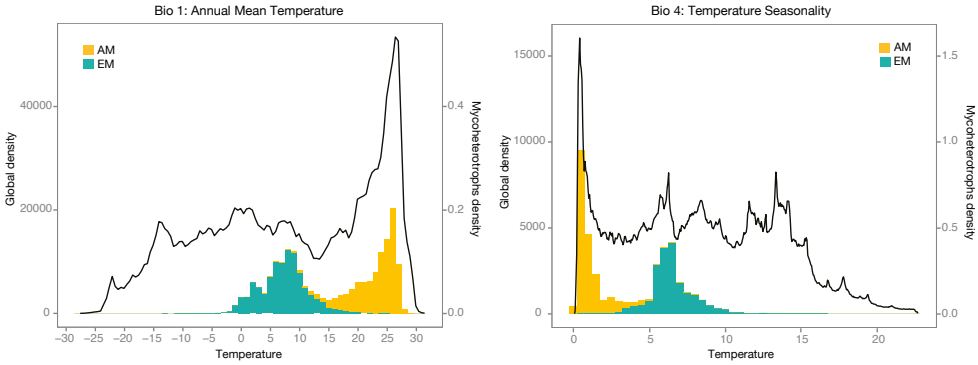
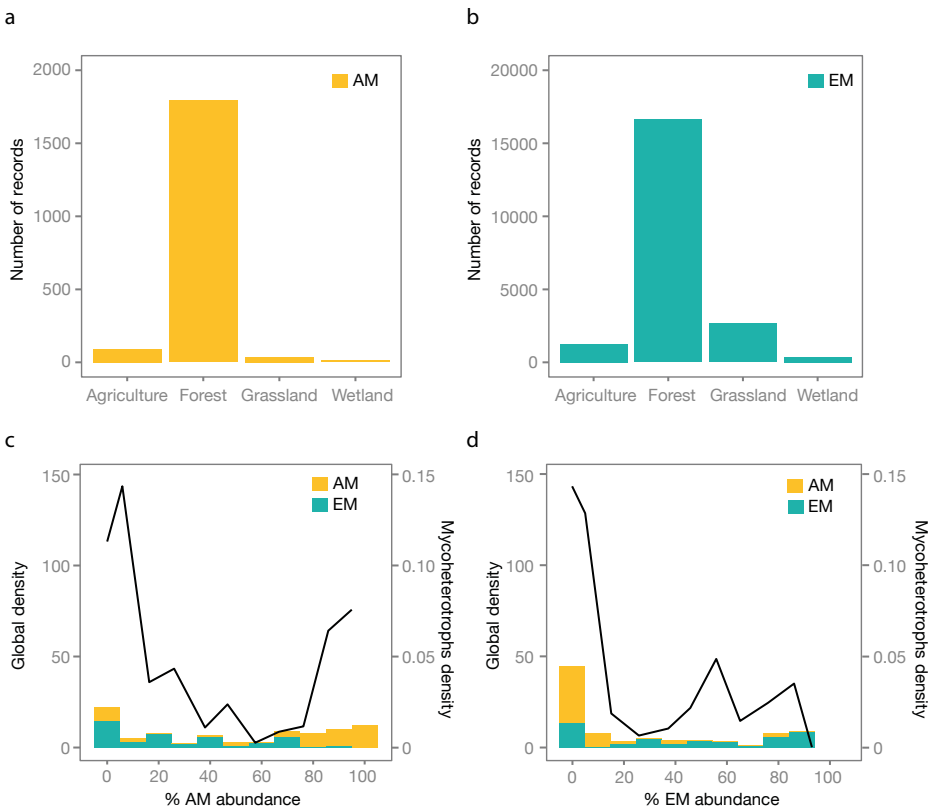


Figure 2 | Climatic preferences of mycoheterotrophic plants (histograms) and global trend of plants (solid line) for the WorldClim dataset (Hijmans et al., 2005) variables annual mean temperature (bio 1) and temperature seasonality (bio 4).



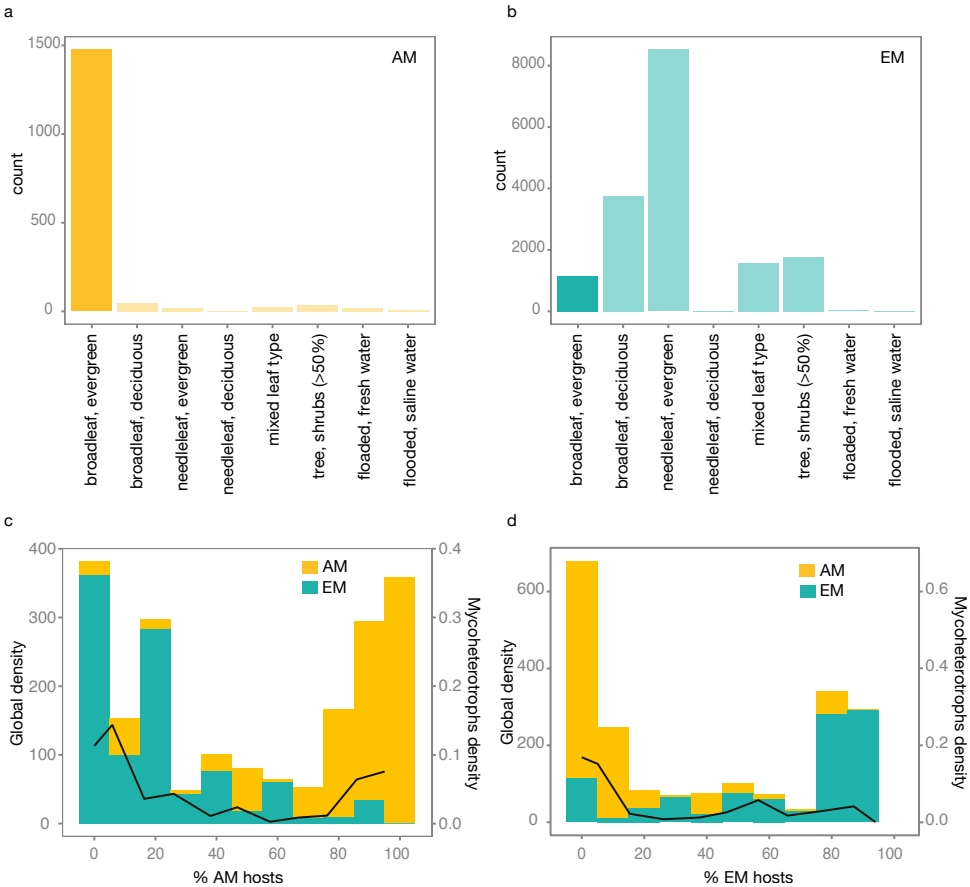


Figure 4 | Mycoheterotrophic plants habitat preference within forest types based on the categories of the CCI Land Cover maps for AM (a) and EM (b) mycoheterotrophs. Full bars highlight the forest type that best predictor of the dichotomic distribution pattern among mycorrhizal types of these plants. Global abundance of green plants (hosts) associated with AM (c) and EM fungi (d), and respective abundance of records of mycoheterotrophic plants per mycorrhizal type in our dataset.

Figure 3 (previous page) | Land cover preference of mycoheterotrophic plants. The land cover categories were obtained using the CCI Land Cover maps for AM (a) and EM (b) mycoheterotrophs.

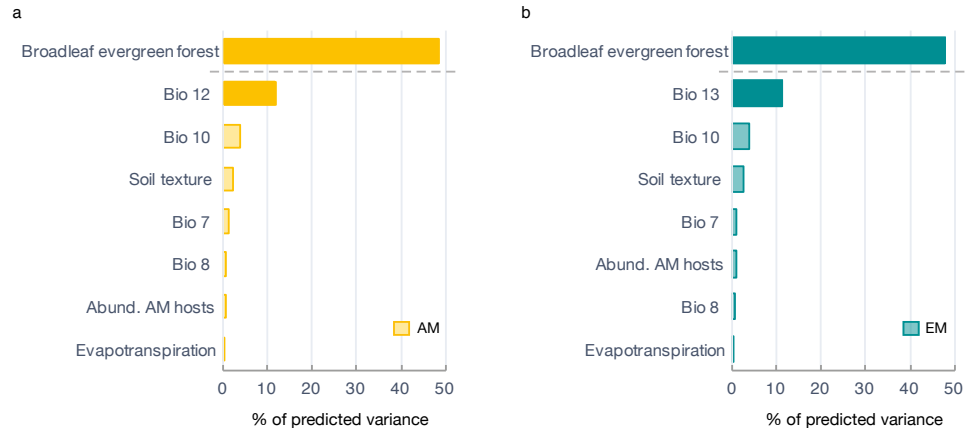


Figure 5 | Ranking of selected predictors explaining the distribution of mycoheterotrophic plants associated with AM and EM fungi as a result of the GLM (above dashed lines) and the hierarchical partitioning analyses (below dashed lines). Predictors are ranked according to the % of predicted variance reflecting their importance in the models. Important predictors are represented as full bars.

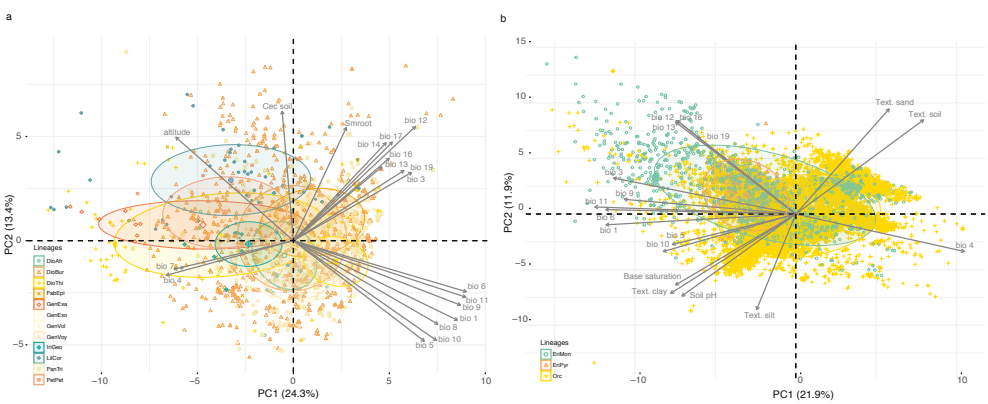


Figure 6 | Principal Components Analysis of the environmental space occupied by mycoheterotrophic plants associated with AM (a) and EM (b) fungi. Only the predictors significantly associated with the first and second principal components are shown. Length of the arrows represent the relative importance of predictors. Clouds of points are the records in the dataset. Ellipses delimitate the environmental space occupied by each lineage.

types, namely annual precipitation (model coefficient: 0.12; $\omega^2 = 0.12$) and the mean precipitation of the wettest month (model coefficient: -0.16; $\omega^2 = 0.11$) for the AM and EM mycoheterotrophs, respectively. All the other predictors in the models had $\omega^2 < 0.06$ (Figure 5).

Environmental preferences of lineages

The one-way perMANOVA indicated that mycoheterotrophic lineages showed preferences for specific ecozones, both for AM (*df* = 11, *Pseudo-F* = 9.0, $R^2 = 0.05$, *P* = 0.01) and for EM fungi (*df* = 2, *Pseudo-F* = 315.8, $R^2 = 0.03$, *P* = 0.01). Pairwise permutation comparisons of lineage indicated differences between all AM lineages (*P* = 0.013 – 0.044), except for *Exochaenium* with *Afrothismia, Burmanniaceae, Exacum, Voyria, Voyriella* (*P* = 0.054 – 0.760), and *Petrosavia* with *Exacum* (*P* = 0.213). This exception can be an artifact due to the low number of records for Exochaenium in our dataset. For the EM dataset, pairwise permutation comparisons of lineage indicated significant differences among all the three EM lineages (*P* = 0.010).

The Principal Component Analyses indicated that mycoheterotrophic lineages associated with AM and EM fungi have different habitat preferences within tropical and temperate areas, respectively. For the AM mycoheterotrophs, the first two components of the PCA explained 37.7% of the total variance, and contain mainly variables related to temperature and precipitation. The cation exchange capacity and the root zone soil moisture also showed a significant contribution (Figure 6a). The clustering per lineage within these environmental variables is significant (see perMANOVA above) but not strong (see diffuse separation in the PCA, Figure 6a). Furthermore, we checked PC3 and PC4 for their potential to explain variance not accounted for by PC1 and PC2, but their additional explanatory power was low compared to PC1 and PC2. For the EM mycoheterotrophs, the first two components of the PCA explained 33.8% of the total variance. We observed an influence of mostly temperature variables, and then precipitation ones. For mycoheterotrophs within this mycorrhizal type, edaphic variables such as soil texture, density, base saturation and pH also provide a suitable range of habitats (Figure 6b). Compared to AM, we observed that EM mycoheterotrophs showed a significant (see perMANOVA above) and stronger (see the clearer clustering between lineages in the PCA, Figure 6b) association per lineage within climatic space.

Discussion

This study is the first global study to assess the biogeography of mycoheterotrophs, taking into account both ecological and evolutionary aspects. According to our study, the trophic strategy of non-photosynthetic plants to obtain carbon from mycorrhizal networks occurs in forests worldwide, following Leake's (1994) hypothesis, without specific environmental preferences except for avoiding very cold and seasonal climates. Apart from occurring in broadleaved evergreen forests (AM mycoheterotrophs) or avoiding them (EM mycoheterotrophs), hardly any environmental predictor contributed to the segregated distribution of mycoheterotrophic plants according to their mycorrhizal type within tropical vs temperate forests. Climatic predictors such as annual precipitation and precipitation during the wettest month were the only variables that explained some variance in AM and EM mycoheterotrophic plant occurrence which had remained unexplained by forest type in the hierarchical models for AM- and EM-associated mycoheterotrophs, respectively. However, these variables had low explanatory power. Thus, humidity was revealed to be the only marginally important factor explaining the occurrence of mycoheterotrophy within evergreen (AM mycoheterotrophs) and other forests (EM mycoheterotrophs). The nearly exclusive occurrence in forests may be the result of a competitive advantage over other plants that grow in low-light conditions. Alternatively, forests in general may offer specific micro-habitats, such as favorable humidity levels, supporting the patchy distribution of these plants (Leake 1994), which are difficult to disentangle in a global scale analysis.

The evident dichotomic distribution of mycoheterotrophic plants according to mycorrhizal type between tropical and temperate forests (Figure 1) suggests a major importance of climate conditions in explaining this pattern, even though it coincides with a minimal importance of these same factors for explaining the distribution of AM and EM mycoheterotrophic plants within their preferred forest types. Hence, climatic conditions do not restrict the wide range of niches that the mycoheterotrophic life strategy occupies. Beyond climatic characteristics, our results show that AM mycoheterotrophs withstand a wide range of root zone soil moisture, being not so restricted to the most humid areas as previously predicted (Leake 1994). Also, these plants have been described to mainly occur in humus rich soils, which was not apparent from our analyses, perhaps due to the patchy character of soils at small scales that is not reflected in a global analysis.

The reliance of mycoheterotrophy on specific fungal partners for carbon uptake suggests that mycoheterotrophic plants could occur everywhere where the suitable fungal partner is present. AM fungi are abundant inside and outside the tropics and constitute important components of temperate forests (Phillips *et al.* 2013). At the same time, EM fungi are widespread in the tropics besides their predominant abundance in temperate areas (Roy *et al.* 2016). However, in our study, mycoheterotrophs with AM mycoheterotrophs occur predominantly in AM-dominated forests in the tropics, while EM mycoheterotrophs avoid AM-dominated forests in temperate forests (see Figure 4). Therefore, the observed dichotomy in the distribution of AM and EM mycoheterotrophic plants does not reflect the global distribution pattern of AM and EM fungi, indicating that the distribution of particular mycorrhizal types does not constrain the global distribution of mycoheterotrophic plants. Previous studies, focusing on a finer taxonomic scale, suggested that the abundance of mycoheterotrophic plant species is related to the abundance of their specific fungal partners (Hazard *et al.* 2012; Yamato *et al.* 2016). This indicates that the mere presence of a suitable fungal partner is not sufficient to promote a mycoheterotrophic relationship of a plant with its mycorrhizal partners, even though the abundance of host green plants supports the required mycorrhizal type. The habitat preferences associated to particular forest types likely restrict the distribution of mycoheterotrophic plants to a subset of environmental conditions of their fungal partners. Other factors that may constrain the occurrence of mycoheterotrophic plants is that they are likely to grow and reproduce only if their associated fungus is able to provide enough carbon from co-associated plants (Taylor & Bruns 1997). This may be influenced by the dynamics within fungal networks, including their size, and the age, identity, and fitness of its associated green plants (Merckx 2013; Fellbaum *et al.* 2014). In addition, competition between fungal species may influence their ability to obtain photosynthesized carbohydrates as well (Bever *et al.* 2009; Kiers *et al.* 2011), and only permit the presence of cheaters under particular conditions.

Despite the ubiquitous occurrence of mycoheterotrophic plants in forests, individual lineages show clear preferences for particular environmental conditions, resulting in a significant clustering of mycoheterotrophic lineages in environmental space. EM mycoheterotrophic lineages were clustered based on preferences for particular soil texture, soil base saturation and soil pH, while AM mycoheterotrophic lineages were separated based on soil cation exchange capacity. This suggests that edaphic factors are more relevant for the local distribution of individual mycoheterotrophic species than previously expected, and should be studied more in detail.

The clustering corresponds to a pattern of phylogenetic niche conservatism (Wiens *et al.* 2010), indicating spatial aggregation of related species. The strength of this spatial aggregation should depend on dispersal ability of species (Cavender-Bares *et al.* 2009), suggesting that mycoheterotrophic plants have limited dispersal capabilities. Despite their small seed size conferring a potential advantage promoting seed dispersal (Eriksson & Kainulainen, 2011), their habitat preference for dense close-canopy forests reduces their potential to disperse over large distances via wind (Wapstra *et al.* 2005). Many lineages, particularly those endemic to a single continent, are estimated to have evolved long after the breakup of Gondwana, further reducing their chances for effective intercontinental dispersal (Merckx 2013). Thus, low dispersal capability together with the divergence history of these plants can be a viable explanation for the observed restricted distribution of certain clades to specific ecozones (Jonker 1938; Mennes et al. 2015). This suggests an intricate connection between environmental factors and evolutionary history to explain the distribution patterns of mycoheterotrophic lineages.

The temperate versus tropical distribution pattern of AM and EM mycoheterotrophs also seems to have an evolutionary component. Interestingly, in the common ancestor of the Ericaceae clade, which includes the Monotropoideae and *Pyrola* lineages, there was a shift in mycorrhizal associations from AM fungi to specialized ericoid mycorrhizas formed by Ascomycota and Basidiomycota, a feature that may have been instrumental in their niche expansion (Lallemand *et al.* 2016). Similarly, for Orchidaceae, the specialized association with orchid mycorrhiza involving members of Ascomycota and Basidiomycota was the result of a shift from the ancestral AM associations in the common ancestor of the family (Yukawa *et al.* 2009). Moreover, the orchid's ability to recruit free-living saprotrophic fungi into novel mycorrhizae may also have led to niche expansions and radiations, also into temperate habitats (Givnish *et al.* 2016). Yet remarkably, nearly all mycoheterotrophic lineages within both Ericaceae and Orchidaceae have shifted from specialized ericoid and orchid mycorrhiza respectively towards an association with EM fungi. The only exceptions are a few tropical fully mycoheterotrophic orchids in Southeast Asia (Waterman *et al.* 2013). Thus, ericoid and orchid mycorrhizas fail to support full mycoheterotrophy, despite their participation in partial mycoheterotrophic interactions (Gebauer *et al.* 2016). Furthermore, mycoheterotrophy in temperate regions evolved in lineages with pre-adaptations to form mycorrhizas with Ascomycota and Basidiomycota fungi, and nearly exclusively occurs through shifts towards EM fungi, but not AM fungi. One explanation might be that in temperate forests only EM networks provide sufficient conditions of carbon availability to sustain mycoheterotrophic plants.

We may also use this evolutionary perspective to understand the wide distribution of mycoheterotrophy across many climatic zones as their association with EM fungi could have provided an advantage to plants to expand their niche from the tropics to colder and more seasonal areas (Wang *et al.* 2017). These colder and more seasonal climatic conditions are described to have been the main limitation for land plants to adapt and migrate out of the tropics during the Tertiary, potentially generating the latitudinal diversity gradient observed nowadays at a global scale (Wiens & Donoghue 2004). This latitudinal diversity gradients also seems present for mycoheterotrophy. From the about 500 species described to date, most of the species occupy tropical areas (Merckx *et al.* 2009). This suggests that mycoheterotrophic plants may have been under similar climatic pressures as host green plants in the colonization of temperate regions.

In conclusion, our study demonstrates that the global distribution of full mycoheterotrophy is mainly determined by forest occurrence and type, while the occurrence of mycoheterotrophic plants is further limited by their evolutionary history and mycorrhizal type of their associations. Thus, cheating belowground networks is only possible under particular conditions, and the vulnerability of the mycorrhizal symbiosis to be cheated by plants differs among climatic regimes in the globe. AM networks are more prone to be cheated in the tropics, and EM networks in temperate areas, despite the distribution of both mycorrhizal types across these regions. This suggests that the mutualistic stability of mycorrhizal networks is context dependent, and thus we should not expect to find a single underlying mechanism to understand the dynamics of plant-mycorrhizal interactions.

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Supporting Information

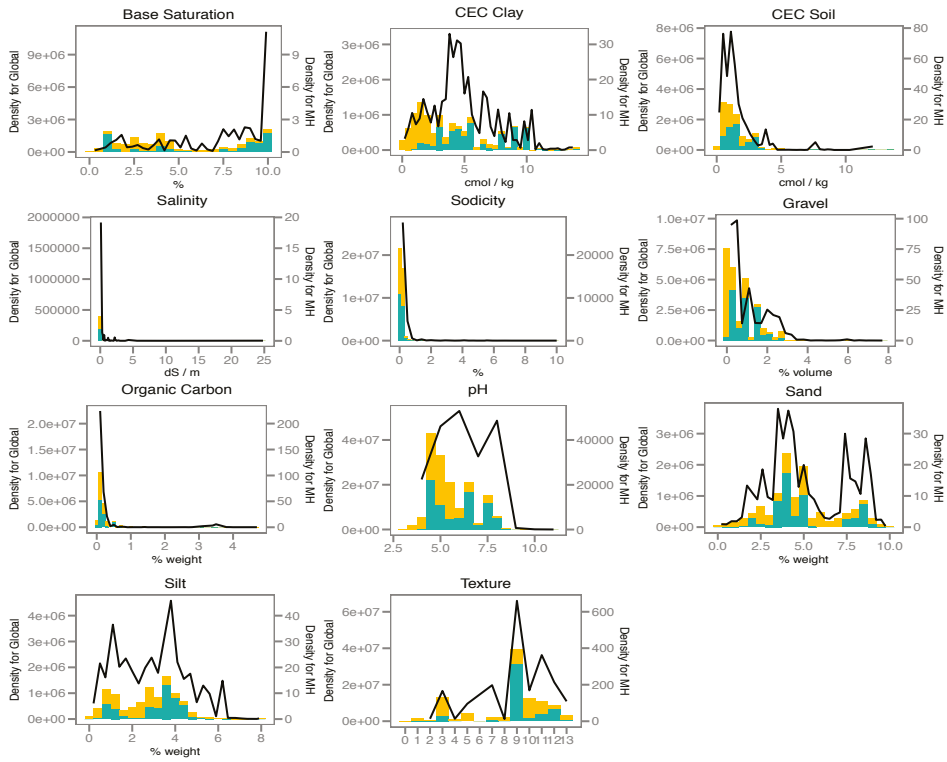

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Figure S1 | Representation of the environmental space occupied by the mycoheterotrophic plant species (histograms) and global trend of plants (solid lines), for the 19 BioClim (World Clim database) variables and the 12 top-soil variables (Harmonized World Soil Database). The colors of the histograms represent the mycorrhizal type association. The x-axis represents the range of values for each environmental variable and two y-axis represent the density of records with those conditions for the global trend of plants (left) and mycoheterotrophic plants (right).

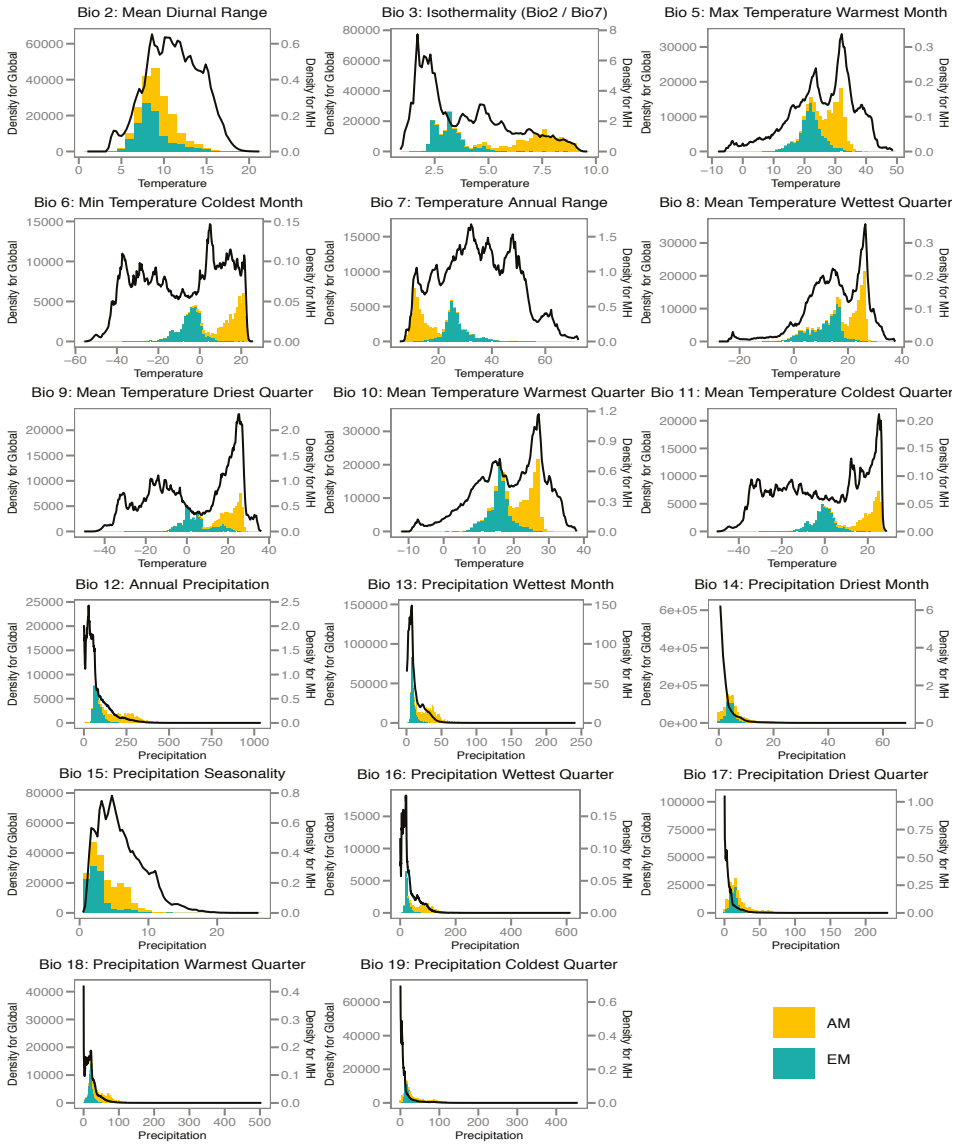
Bio 2: Mean Diurnal Range
Bio 3: Isothermality (Bio2 / Bio7)
Bio 5: Max Temperature Warmest Month
Bio 6: Min Temperature Coldest Month
Bio 7: Temperature Annual Range
Bio 8: Mean Temperature Wettest Quarter
Bio 9: Mean Temperature Driest Quarter
Bio 10: Mean Temperature Warmest Quarter
Bio 11: Mean Temperature Coldest Quarter
Bio 12: Annual Precipitation
Bio 13: Precipitation Wettest Month
Bio 14: Precipitation Driest Month
Bio 15: Precipitation Seasonality
Bio 16: Precipitation Wettest Quarter
Bio 17: Precipitation Driest Quarter
Bio 18: Precipitation Warmest Quarter
Bio 19: Precipitation Coldest Quarter
Density for Global
Density for MH
Temperature
Precipitation
AM
EM

Table S1 | MH dataset final. Dataset with the records of mycoheterotrophic plants.

fungi	Species	**family**	**lineage**
AMF	*Afrothismia amietii*	Burmanniaceae	DioAfr
AMF	*Afrothismia baerae*	Burmanniaceae	DioAfr
AMF	*Afrothismia fungiformis*	Burmanniaceae	DioAfr
AMF	*Afrothismia gesnerioides*	Burmanniaceae	DioAfr
AMF	*Afrothismia hydra*	Burmanniaceae	DioAfr
AMF	*Afrothismia insignis*	Burmanniaceae	DioAfr
AMF	*Afrothismia korupensis*	Burmanniaceae	DioAfr
AMF	*Afrothismia mhoroana*	Burmanniaceae	DioAfr
AMF	*Afrothismia mvileyi*	Burmanniaceae	DioAfr
AMF	*Afrothismia pusilla*	Burmanniaceae	DioAfr
AMF	*Afrothismia saingei*	Burmanniaceae	DioAfr
AMF	*Afrothismia sp*	Burmanniaceae	DioAfr
AMF	*Afrothismia winkleri*	Burmanniaceae	DioAfr
AMF	*Apteria aphylla*	Burmanniaceae	DioBur
AMF	*Burmannia championii*	Burmanniaceae	DioBur
AMF	*Burmannia cryptopetala*	Burmanniaceae	DioBur
AMF	*Burmannia geelvinkiana*	Burmanniaceae	DioBur
AMF	*Burmannia indica*	Burmanniaceae	DioBur
AMF	*Burmannia itoana*	Burmanniaceae	DioBur
AMF	*Burmannia kalbreyeri*	Burmanniaceae	DioBur
AMF	*Burmannia micropetala*	Burmanniaceae	DioBur
AMF	*Burmannia nepalensis*	Burmanniaceae	DioBur
AMF	*Burmannia sphagnoides*	Burmanniaceae	DioBur
AMF	*Burmannia tenella*	Burmanniaceae	DioBur
AMF	*Burmannia wallichii*	Burmanniaceae	DioBur
AMF	*Campylosiphon congestus*	Burmanniaceae	DioBur
AMF	*Campylosiphon purpurascens*	Burmanniaceae	DioBur
AMF	*Cymbocarpa refracta*	Burmanniaceae	DioBur
AMF	*Cymbocarpa saccata*	Burmanniaceae	DioBur
AMF	*Cymbocarpa sp*	Burmanniaceae	DioBur
AMF	*Dictyostega orobanchoides*	Burmanniaceae	DioBur
AMF	*Gymnosiphon sp*	Burmanniaceae	DioBur
AMF	*Gymnosiphon aphyllus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon bekensis*	Burmanniaceae	DioBur
AMF	*Gymnosiphon brachycephalus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon breviflorus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon capitatus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon constrictus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon cymosus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon dangyanus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon divaricatus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon fimbriatus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon guianensis*	Burmanniaceae	DioBur
AMF	*Gymnosiphon longistylus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon minutus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon panamensis*	Burmanniaceae	DioBur
AMF	*Gymnosiphon recurvatus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon samoritoureanus*	Burmanniaceae	DioBur

Table S1 | MH dataset final. Dataset with the records of mycoheterotrophic plants (continued).

fungi	Species	**family**	**lineage**
AMF	*Gymnosiphon sp*	Burmanniaceae	DioBur
AMF	*Gymnosiphon suaveolens*	Burmanniaceae	DioBur
AMF	*Gymnosiphon tenellus*	Burmanniaceae	DioBur
AMF	*Gymnosiphon usambaricus*	Burmanniaceae	DioBur
AMF	*Hexapterella gentianoides*	Burmanniaceae	DioBur
AMF	*Hexapterella steyermarkii*	Burmanniaceae	DioBur
AMF	*Oxygyne sp*	Burmanniaceae	DioThi
AMF	*Thismia abei*	Burmanniaceae	DioThi
AMF	*Thismia alba*	Burmanniaceae	DioThi
AMF	*Thismia annamensis*	Burmanniaceae	DioThi
AMF	*Thismia arachnites*	Burmanniaceae	DioThi
AMF	*Thismia betung-kerihuensis*	Burmanniaceae	DioThi
AMF	*Thismia brunneomitra*	Burmanniaceae	DioThi
AMF	*Thismia clavarioides*	Burmanniaceae	DioThi
AMF	*Thismia clavigera*	Burmanniaceae	DioThi
AMF	*Thismia glaziovii*	Burmanniaceae	DioThi
AMF	*Thismia gongshanensis*	Burmanniaceae	DioThi
AMF	*Thismia haxagona*	Burmanniaceae	DioThi
AMF	*Thismia hillii*	Burmanniaceae	DioThi
AMF	*Thismia hongkongensis*	Burmanniaceae	DioThi
AMF	*Thismia huangii*	Burmanniaceae	DioThi
AMF	*Thismia hyalina*	Burmanniaceae	DioThi
AMF	*Thismia lauriana*	Burmanniaceae	DioThi
AMF	*Thismia megalongensis*	Burmanniaceae	DioThi
AMF	*Thismia mucronata*	Burmanniaceae	DioThi
AMF	*Thismia mullerensis*	Burmanniaceae	DioThi
AMF	*Thismia nigricans*	Burmanniaceae	DioThi
AMF	*Thismia okahensis*	Burmanniaceae	DioThi
AMF	*Thismia panamensis*	Burmanniaceae	DioThi
AMF	*Thismia puberula*	Burmanniaceae	DioThi
AMF	*Thismia rodwayi*	Burmanniaceae	DioThi
AMF	*Thismia saulensis*	Burmanniaceae	DioThi
AMF	*Thismia singeri*	Burmanniaceae	DioThi
AMF	*Thismia sp*	Burmanniaceae	DioThi
AMF	*Thismia taiwanensis*	Burmanniaceae	DioThi
AMF	*Thismia tentaculata*	Burmanniaceae	DioThi
AMF	*Tiputinia foetida*	Burmanniaceae	DioThi
ECM	*Hemitomes congestum*	Ericaceae	EriMon
ECM	*Monotropa hypopitys*	Ericaceae	EriMon
ECM	*Monotropa sp*	Ericaceae	EriMon
ECM	*Monotropa uniflora*	Ericaceae	EriMon
ECM	*Monotropastrum humile*	Ericaceae	EriMon
ECM	*Monotropastrum sciaphilum*	Ericaceae	EriMon
ECM	*Monotropastrum sp*	Ericaceae	EriMon
ECM	*Monotropsis odorata*	Ericaceae	EriMon
ECM	*Pityopus californica*	Ericaceae	EriMon
ECM	*Pleuricospora fimbriolata*	Ericaceae	EriMon
ECM	*Sarcodes sanguinea*	Ericaceae	EriMon

Table S1 | MH dataset final. Dataset with the records of mycoheterotrophic plants (continued).

fungi	Species	**family**	**lineage**
ECM	*Pyrola picta*	Ericaceae	EriPyr
AMF	*Epirixanthes cylindrica*	Polygalaceae	FabEpi
AMF	*Epirixanthes elongata*	Polygalaceae	FabEpi
AMF	*Epirixanthes kinabaluensis*	Polygalaceae	FabEpi
AMF	*Epirixanthes pallida*	Polygalaceae	FabEpi
AMF	*Epirixanthes papuana*	Polygalaceae	FabEpi
AMF	*Epirixanthes sp*	Polygalaceae	FabEpi
AMF	*Exacum paucisquamum*	Gentianaceae	GenExa
AMF	*Exacum tenue*	Gentianaceae	GenExa
AMF	*Exochaenium oliganthum*	Gentianaceae	GenExo
AMF	*Voyriella parviflora*	Gentianaceae	GenVol
AMF	*Voyria acuminata*	Gentianaceae	GenVoy
AMF	*Voyria aphylla*	Gentianaceae	GenVoy
AMF	*Voyria aurantiaca*	Gentianaceae	GenVoy
AMF	*Voyria caerulea*	Gentianaceae	GenVoy
AMF	*Voyria chionea*	Gentianaceae	GenVoy
AMF	*Voyria clavata*	Gentianaceae	GenVoy
AMF	*Voyria corymbosa*	Gentianaceae	GenVoy
AMF	*Voyria flavescens*	Gentianaceae	GenVoy
AMF	*Voyria obconica*	Gentianaceae	GenVoy
AMF	*Voyria parasitica*	Gentianaceae	GenVoy
AMF	*Voyria pittieri*	Gentianaceae	GenVoy
AMF	*Voyria primuloides*	Gentianaceae	GenVoy
AMF	*Voyria rosea*	Gentianaceae	GenVoy
AMF	*Voyria sp*	Gentianaceae	GenVoy
AMF	*Voyria spruceana*	Gentianaceae	GenVoy
AMF	*Voyria tenella*	Gentianaceae	GenVoy
AMF	*Voyria tenuiflora*	Gentianaceae	GenVoy
AMF	*Voyria truncata*	Gentianaceae	GenVoy
AMF	*Geosiris aphylla*	Iridaceae	IriGeo
AMF	*Geosiris sp*	Iridaceae	IriGeo
AMF	*Arachnitis uniflora*	Corsiaceae	LilCor
AMF	*Corsia arfakensis*	Corsiaceae	LilCor
AMF	*Corsia boridiensis*	Corsiaceae	LilCor
AMF	*Corsia brassii*	Corsiaceae	LilCor
AMF	*Corsia clypeata*	Corsiaceae	LilCor
AMF	*Corsia cornuta*	Corsiaceae	LilCor
AMF	*Corsia huonensis*	Corsiaceae	LilCor
AMF	*Corsia lamellata*	Corsiaceae	LilCor
AMF	*Corsia merimantaensis*	Corsiaceae	LilCor
AMF	*Corsia ornata*	Corsiaceae	LilCor
AMF	*Corsia papuana*	Corsiaceae	LilCor
AMF	*Corsia purpurata*	Corsiaceae	LilCor
AMF	*Corsia sp*	Corsiaceae	LilCor
AMF	*Corsia torricellensis*	Corsiaceae	LilCor
AMF	*Corsia unguiculata*	Corsiaceae	LilCor
AMF	*Corsia wubungu*	Corsiaceae	LilCor
ECM	*Aphyllorchis alpina*	Orchidaceae	Orc

Table S1 | MH dataset final. Dataset with the records of mycoheterotrophic plants (continued).

fungi	Species	**family**	**lineage**
ECM	*Aphyllorchis anomala*	Orchidaceae	Orc
ECM	*Aphyllorchis caudata*	Orchidaceae	Orc
ECM	*Aphyllorchis elata*	Orchidaceae	Orc
ECM	*Aphyllorchis montana*	Orchidaceae	Orc
ECM	*Aphyllorchis pallida*	Orchidaceae	Orc
ECM	*Aphyllorchis queenslandica*	Orchidaceae	Orc
ECM	*Aphyllorchis simplex*	Orchidaceae	Orc
ECM	*Aphyllorchis sp*	Orchidaceae	Orc
ECM	*Cephalanthera austiniae*	Orchidaceae	Orc
ECM	*Chamaegastrodia inverta*	Orchidaceae	Orc
ECM	*Chamaegastrodia sp*	Orchidaceae	Orc
ECM	*Corallorhiza bulbosa*	Orchidaceae	Orc
ECM	*Corallorhiza macrantha*	Orchidaceae	Orc
ECM	*Corallorhiza maculata*	Orchidaceae	Orc
ECM	*Corallorhiza mertensiana*	Orchidaceae	Orc
ECM	*Corallorhiza odontorhiza*	Orchidaceae	Orc
ECM	*Corallorhiza sp*	Orchidaceae	Orc
ECM	*Corallorhiza striata*	Orchidaceae	Orc
ECM	*Corallorhiza trifida*	Orchidaceae	Orc
ECM	*Corallorhiza wisteriana*	Orchidaceae	Orc
ECM	*Dipodium hamiltonianum*	Orchidaceae	Orc
ECM	*Dipodium roseum*	Orchidaceae	Orc
ECM	*Dipodium variegatum*	Orchidaceae	Orc
ECM	*Epipogium aphyllum*	Orchidaceae	Orc
ECM	*Epipogium roseum*	Orchidaceae	Orc
ECM	*Hexalectris arizonica*	Orchidaceae	Orc
ECM	*Hexalectris brevicaulis*	Orchidaceae	Orc
ECM	*Hexalectris grandiflora*	Orchidaceae	Orc
ECM	*Hexalectris nitida*	Orchidaceae	Orc
ECM	*Hexalectris parviflora*	Orchidaceae	Orc
ECM	*Hexalectris sp*	Orchidaceae	Orc
ECM	*Hexalectris spicata*	Orchidaceae	Orc
ECM	*Hexalectris warnockii*	Orchidaceae	Orc
ECM	*Limodorum abortivum*	Orchidaceae	Orc
ECM	*Limodorum sp*	Orchidaceae	Orc
ECM	*Limodorum trabutianum*	Orchidaceae	Orc
ECM	*Neottia acuminata*	Orchidaceae	Orc
ECM	*Neottia camtschatea*	Orchidaceae	Orc
ECM	*Neottia listeroides*	Orchidaceae	Orc
ECM	*Neottia nidus-avis*	Orchidaceae	Orc
ECM	*Rhizanthella gardneri*	Orchidaceae	Orc
ECM	*Rhizanthella omissa*	Orchidaceae	Orc
ECM	*Rhizanthella slateri*	Orchidaceae	Orc
AMF	*Kihansia jengiensis*	Triuridaceae	PanTri
AMF	*Kihansia lovettii*	Triuridaceae	PanTri
AMF	*Kupea jonii*	Triuridaceae	PanTri
AMF	*Kupea martinetugei*	Triuridaceae	PanTri
AMF	*Lacandonia brasiliana*	Triuridaceae	PanTri

Table S1 | MH dataset final. Dataset with the records of mycoheterotrophic plants (continued).

fungi	Species	**family**	**lineage**
AMF	*Lacandonia schismatica*	Triuridaceae	PanTri
AMF	*Peltophyllum luteum*	Triuridaceae	PanTri
AMF	*Sciaphila africana*	Triuridaceae	PanTri
AMF	*Sciaphila albescens*	Triuridaceae	PanTri
AMF	*Sciaphila arfakiana*	Triuridaceae	PanTri
AMF	*Sciaphila corallophyton*	Triuridaceae	PanTri
AMF	*Sciaphila corymbosa*	Triuridaceae	PanTri
AMF	*Sciaphila densiflora*	Triuridaceae	PanTri
AMF	*Sciaphila janthina*	Triuridaceae	PanTri
AMF	*Sciaphila japonica*	Triuridaceae	PanTri
AMF	*Sciaphila ledermannii*	Triuridaceae	PanTri
AMF	*Sciaphila oligantha*	Triuridaceae	PanTri
AMF	*Sciaphila picta*	Triuridaceae	PanTri
AMF	*Sciaphila purpurea*	Triuridaceae	PanTri
AMF	*Sciaphila quadribullifera*	Triuridaceae	PanTri
AMF	*Sciaphila rubra*	Triuridaceae	PanTri
AMF	*Sciaphila schwackeana*	Triuridaceae	PanTri
AMF	*Sciaphila secundiflora*	Triuridaceae	PanTri
AMF	*Sciaphila sp*	Triuridaceae	PanTri
AMF	*Sciaphila tenella*	Triuridaceae	PanTri
AMF	*Sciaphila thaidanica*	Triuridaceae	PanTri
AMF	*Sciaphila winkleri*	Triuridaceae	PanTri
AMF	*Seychellaria africana*	Triuridaceae	PanTri
AMF	*Seychellaria madagascariensis*	Triuridaceae	PanTri
AMF	*Seychellaria sp*	Triuridaceae	PanTri
AMF	*Soridium spruceanum*	Triuridaceae	PanTri
AMF	*Triuridopsis intermedia*	Triuridaceae	PanTri
AMF	*Triuris hexophthalma*	Triuridaceae	PanTri
AMF	*Triuris hyalina*	Triuridaceae	PanTri
AMF	*Triuris sp*	Triuridaceae	PanTri
AMF	*Petrosavia sinii*	Petrosaviaceae	PetPet
AMF	*Petrosavia sp*	Petrosaviaceae	PetPet
AMF	*Petrosavia stellaris*	Petrosaviaceae	PetPet

Table S2 | Summary of lineages of mycoheterotrophic plants included in this study and their occurrence per ecozone.

Fungi	Lineage	Ecozones (*)	# genera
AM	Dioscoreales, Afrothismia	Afrotropics (1)	1
AM	Dioscoreales, Burmanniaceae	Afrotropics (2), Australasia (1), Indomalaya (2), Neartic (7)	7
AM	Dioscoreales, Thismiaceae	Australasia (1), Indomalaya (2), Neartic (2), Paleotropic (1)	3
EM	Ericales, Monotropaceae	Indomalaya (1), Neartic (6), Paleotropic (2)	7
EM	Ericales, Pyrolaceae	Neartic (1)	1
AM	Fabales, Epirixanthes	Australasia (1), Indomalaya (1)	1
AM	Gentianales, Exacum	Indomalaya (1), Paleotropic (1)	1
AM	Gentianales, Exochaenium	Afrotropics (1)	1
AM	Gentianales, Voyria	Afrotropics (1), Neartic (1)	1
AM	Gentianales, Voyriella	Neartic (1)	1
AM	Iridaceae, Geosiris	Afrotropics (1)	1
AM	Liliales, Corsiaceae	Australasia (1), Neartic (1)	2
EM	Orchidaceae	Afrotropics (1), Australasia (3), Indomalaya (3), Neartic (3), Paleotropic (5)	10
AM	Pandanales, Triuridaceae	Afrotropics (3), Australasia (1), Indomalaya (1), Neartic (6), Paleotropic (1)	9
AM	Petrosaviales, Petrosavia	Indomalaya (1)	1