CHAPTER 3

Fungal-host diversity among mycoheterotrophic plants increases proportionally to their fungal-host overlap

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Abstract

The vast majority of plants obtain an important proportion of vital resources from soil through mycorrhizal fungi. Generally, this happens in exchange of photosynthetically fixed carbon, but occasionally the interaction is mycoheterotrophic, and plants obtain carbon from mycorrhizal fungi. This process results in an antagonistic interaction between mycoheterotrophic plants and their fungal hosts. Importantly, the fungal-host diversity available for plants is restricted as mycoheterotrophic interactions often involve narrow lineages of fungal hosts. Unfortunately, little is known whether fungal-host diversity may be additionally modulated by plant-plant interactions through shared hosts. Yet, this may have important implications for plant competition and coexistence.

Here we use DNA sequencing data to investigate the interaction patterns between mycoheterotrophic plants and arbuscular mycorrhizal fungi. We find no phylogenetic signal on the number of fungal hosts nor on the fungal hosts shared among mycoheterotrophic plants. However, we observe a potential trend towards increased phylogenetic diversity of fungal hosts among mycoheterotrophic plants with increasing overlap in their fungal hosts. While these patterns remain for groups of plants regardless of location, we do find higher levels of overlap and diversity among plants from the same location.

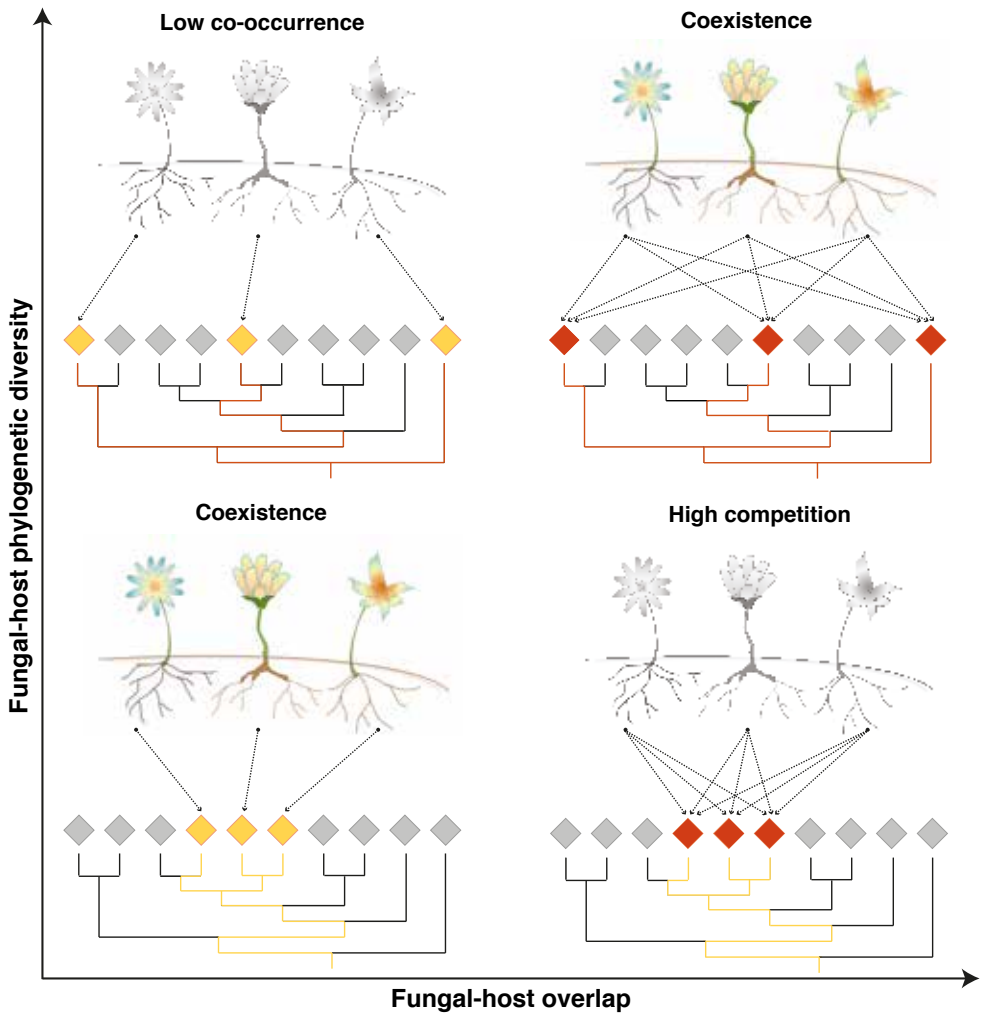
These findings suggest that species coexistence cannot be fully understood without attention to the two sides of ecological interactions.

Introduction

Mycorrhizal fungi play a crucial role for plant survival (Smith & Read, 2008). In mycorrhizal interactions, mycorrhizal fungi facilitate the uptake of essential resources for plant metabolism, such as water and soil minerals (Raven *et al.* 1999). Generally, in exchange, plants transfer photosynthethically fixed carbon to their mycorrhizal partners (Smith & Read, 2008). Occasionally, however, plants do not give back carbon, but instead obtain it from the mycorrhizal fungi as replacement for photosynthesis (Leake, 1994; Merckx *et al.* 2009). This results in an antagonistic interaction between plants and their fungal hosts. Specifically, these interactions are called mycoheterotrophic (MH) interactions and can occur in a single developmental stage (e.g. in orchids, and some ferns and lycopods) or during the entire life cycle of a plant (fully mycoheterotrophic plants) (Winther & Friedman, 2008; Merckx & Freudenstein, 2010). MH interactions represent a non-mutualistic mode of life that occurs in nearly all major lineages of land plants, involving more than 20,000 plant species (Merckx, 2013). In general, the fungal-host diversity available for these plants is restricted as MH interactions often involve more narrow lineages of mycorrhizal fungi than non-MH interactions (Bidartondo *et al.* 2002). Unfortunately, little is known whether fungal-host diversity may be additionally modulated by plant-plant interactions through shared hosts. Yet, this may have important implications for plant competition and coexistence (Bever *et al.* 2010).

Recent studies have shown that the diversity of mycorrhizal fungi is strongly associated with plant community composition (Davison *et al.* 2011; Peay *et al.* 2013; Martínez-García *et al.* 2015) and habitat conditions (Hazard *et al.* 2013). For instance, in the case of MH interactions, a given group of plant species can be exploiting either closely or distantly related fungal hosts (see Figure 1). Additionally, this same group of plants can have either a weak or a strong fungal-host overlap (see Figure 1). The combination of these two factors depends on plant niche, and have been shown to be determinant for plant coexistence (Levins, 1968; Levine & HilleRisLambers, 2009; Rohr *et al.* 2016). According to niche theory (MacArthur & Levins, 1967; Loreau, 2010), species coexistence is a function of their their niche width and niche overlap (Chesson, 2000). Competitive exclusion among species is high when their potential niche overlap is large and their combined niche width is small. Similarly, the chances of co-occurrence among species in the same niche space is low when their potential niche overlap is small and their combined niche width is large. Species coexistence (co-occurrence and no exclusion) then is expected to happen when niche overlap and

niche width are symmetric (Chesson, 2000; Tilman, 2011) (see Figure 1 - diagonal). Niche delimitation is never straightforward due to our often lack of a priori knowledge about the resources and functional traits defining the niche dimensions of a species (Kraft *et al.* 2015). Defining the niche of fungal hosts of mycoheterotrophic plants is as challenging as for other groups of organisms, but one potential hypothesis is that the higher the fungal-host diversity of mycoheterotrophic plants, the broader their niche. Thus, species coexistence may be favored under symmetric patterns of fungal-host overlap and diversity.



To work on the above hypothesis, we use a system where the mycorrhizal interaction involves mycoheterotrophic plants. In addition, these plants are associated with arbuscular mycorrhizal fungi (phylum Glomeromycota), which are associated with more than 80 % of land plants. Therefore, this association represents one of the most ancient and abundant mycorrhizal interaction among plants on a global scale (Smith & Read, 2008; Strullu-Derrien *et al.* 2014). Here, we investigate MH interactions by analyzing the observed patterns of associations between MH plants and their fungal hosts in a niche framework. In particular, we study how the phylogenetic diversity of arbuscular mycorrhizal hosts varies among individual MH plants, and how this diversity is modulated and shared among groups of MH plants.

Material and Methods

Sampling sites and mycoheterotrophic species

The geographic range of MH plants associated with arbuscular mycorrhizal fungi is mostly restricted to tropical rainforests worldwide (Leake, 1994). Neotropical forests harbor the largest species diversity compared to the paleotropical forests. In the neotropics, the two biomes with the highest diversity of MH species are the Amazon forest and the Atlantic forest (Merckx, 2013). We collected MH plants in these two biomes in French Guiana and Brazil, respectively (see Figure S3). The sampled sites in French Guiana were low land coastal plain forests (Guitet *et al.* 2015), and in Brazil were also low lands in ombrophilous dense coastal forests (Veloso *et al.* 1991). Due to the ephemeral nature of MH plants, it is only possible to collect them during their flowering period. Most MH species flower after the rainy season, from July until November. All collections were made during this period.

Figure 1 (previous page) | Illustration of possible fungal-host patterns among mycoheterotrophic plants. On the vertical and horizontal axes, the figure illustrates, respectively, an increase in fungal-host diversity and fungal-host overlap among MH plants. The bottom right panel represents a scenario for plants with high chances of competitive exclusion given by their large fungal-host overlap and their small fungal-host diversity (using similar functional traits). The top left panel represents a scenario for plants with low chances of co-occurring in the same space given by their small fungal-host overlap and their large fungal-host diversity (using different functional traits), which could be difficult to find in a common place. The diagonal panels then represent the scenarios for plants with a higher chance of coexistence given by their symmetry between fungal-host overlap and fungal-host diversity, which could lead to maximize co-occurrence (exploit available resources) and to minimize competitive exclusion

We visited 15 localities, 10 of which in the Amazon forests and 5 in the Atlantic forests. We considered all the individuals of the same species found within 4 x 4 m to be part of the same population. Populations of MH species were separated from each other with a minimum of 30 m. In each population, we collected at least one individual and a maximum of ten individuals per species. We focused on three of the four MH plant families distributed in the sampled area, namely Burmanniaceae, Gentianaceae and Triuridaceae. We did not target species of Thismiaceae, the fourth family of MH plants in the area, since all neotropical species are extremely rare. In the 15 localities, we identified 54 populations of MH species. In total, we collected root samples of 140 specimens of 20 MH plant species, covering more than a quarter of the described arbuscular mycorrhizal MH species for South America. See Supporting Information for further details about the sampling.

Fungal-host diversity in single mycoheterotrophic plants

To study fungal-host patterns, first we investigated the arbuscular mycorrhizal fungal-host diversity that can be potentially associated with single MH plants. This information was obtained through DNA sequencing of roots of arbuscular mycorrhizal MH plants. For each of the 140 specimens, immediately after collection, root samples were washed with distilled water and stored in 2% CTAB buffer at -20ºC until further processing. Subsequently, DNA was extracted using the NucleoSpin Soil kit (Macherey-Nagel Gmbh and Co., Düren, Germany). Next-generation DNA sequencing of each root sample was used to identify the arbuscular mycorrhizal hosts that can be potentially associated with each MH plant species. We sequenced the ITS2 region using the primers fITS7 (5'-GTGARTCATCGAATCTTTG-3') (Ihrmark *et al.* 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). In total, we found 138 operational taxonomic units (OTUs) identified as Glomeromycota by quering against UNITE database (version 6.0, 10.09.2014) using the BLAST algorithm. Hereafter, we refer to the fungal OTUs as fungal hosts. See Supporting Information for more details about the sequencing. Raw sequences are deposited in the NCBI Short Read Archive under the project number PRJNA339563.

To generate the phylogenetic tree for each family of MH plant species, we reconstructed the phylogenetic relationships between the species for each family by reanalyzing previously published datasets of Burmanniaceae (Merckx *et al.* 2010a), Triuridaceae (Mennes *et al.* 2013), and Gentianaceae (Merckx *et al.* 2013a). For

Triuridaceae we included newly sequenced data for *Soridium spruceanum* (GenBank accession number KX756649). We combined the resulting trees based on divergence ages taken from (Magallón *et al.* 2015). Only the 20 taxa from this study were kept in the phylogeny shown in Supplementary Figure S2.

To generate the host phylogenetic tree we used an alignment with the 138 Glomeromycota fungal OTUs with MAFFT 7.017 (Katoh & Standley, 2013) implemented in GENEIOUS PRO 6.1.4 (Biomatters, Auckland, New Zealand). Reference sequences of the accepted genera in the phylum were added as a backbone to the tree to support and better deduce the phylogenetic position of each OTU (Öpik *et al.* 2010; Krüger *et al.* 2012). We reconstructed a Maximum Likelihood tree using the GTR+I+G substitution model as selected with JMODELTEST 2.3.1 (Darriba *et al.* 2012) under the Akaike Information Criterion. The resulting highest-likelihood tree was transformed into an ultrametric tree using COMPUTE.BRLEN and VCV commands in the R-APE package. The phylogeny of the 138 Glomeromycota OTUs is shown in Supplementary Figure S3. The alignment and tree topology are archived in the database TREEBASE (http://www.treebase.org; submission ID 20259).

To calculate the effect of phylogenetic relatedness on the number of fungal hosts among MH plants (phylogenetic signal), we computed the Mantel test correlation between the phylogenetic distance matrix between plants and the dissimilarity matrix between the number of fungal hosts per plant. The phylogenetic distances were extracted from the plants phylogenetic tree, and the dissimilarity matrix was calculated by $|d_i - d_j|$, where d_i and d_j are the number of fungal hosts associated to plant *i* and *j*, respectively (Saavedra *et al.* 2014). Separately, phylogenetic relatedness on the number of fungal hosts was investigated among MH plants species that belong to the same location.

To calculate the phylogenetic signal on the shared fungal hosts among MH plants, we computed the Mantel test correlation between the phylogenetic distance matrix between plants and two dissimilarity matrices between the shared hosts. The phylogenetic distance matrix is the same as above, whereas the dissimilarity matrices here were calculated using two different measures. The Bray-Curtis measure $1 - (2Cij)/(di + dj)$, where C*ij* is the number of shared hosts between plant *i* and *j*, and d_i and d_j are the number of fungal hosts associated to MH plant *i* and *j*, respectively. Note that the Bray-Curtis measure corresponds to the number of shared fungal hosts relative to the total number of fungal hosts. The second measure we used is the overlap measure

$C_{ij}/\min(d_i,d_j)$, where the parameters are the same as above and $\min(d_i,d_j)$ refers to the smallest of the two values (Saavedra *et al.* 2013). The overlap measure corresponds to the number of shared fungal hosts relative to the maximum number of fungal hosts that can be shared. Correlations were computed using the function mantel in the R-VEGAN package. Mantel statistics were tested for significance by permutation 104 trials). Separately, phylogenetic signal on the shared fungal hosts was investigated among MH plants species that belong to the same location.

For each MH plant, the observed fungal-host diversity was calculated using the phylogenetic diversity (PD) of the observed hosts. Phylogenetic diversity was calculated by summing up the branch lengths in the fungal-host phylogenetic tree among all the fungal hosts associated to the MH plant or group. Because the number of fungal hosts determines the branch length, we normalized the PD by calculating the scaled PD as $PD' = (PD - PD_{MIN})/(PD_{MAX} - PD_{MIN})$, where PD_{MAX} and PD_{MIN} correspond, respectively, to the maximum and minimum PD values that can be generated from all the possible combinations of fungal hosts. These combinations are generated by creating groups of fungal hosts of the same number as in the observed case, but the identity of the hosts is changed using the pool of the 138 possible fungi. The MH plants from our study were only found to associate with these 138 fungi, which represent a subset of the total fungal diversity available in the soil. Note that this scaling does not assume a particular generative process, rather it compares the observed phylogenetic diversity to all the possible outcomes with the same number of fungal hosts.

Fungal-host diversity and overlap among mycoheterotrophic plants

We investigated the diversity and overlap patterns among observed co-occurring MH plants in the field, as well as among the artificially-generated groups. In particular, we observed six communities of MH plants that were found co-occurring in the field. To maximize the possibility of co-occurrence and to avoid small-scale niche segregation of mycorrhizal communities (Jacquemyn *et al.* 2014), plants were considered to co-occur when flowering specimens were found growing less than one meter from each other (see Supporting Table S6 for the composition of these communities). Two of the observed communities in the field had 2 plants, three communities had 3 plants, and one community had 5 plants. Additionally, to generate groups of potentially co-occurring plants, we formed all groups with n plant species using the 20 MH collected species. We generated artificial groups with 2, 3, 4 and 5 MH species (mimicking the size of the observed communities in the field).

In every single observed community and generated group, we calculated the combined phylogenetic diversity (PD) of the fungal hosts that can be associated with a given community/group of MH plants. Similarly, to investigate fungal-host overlap among MH plants, we calculated the overlap of fungal hosts among MH plants in a given community/group. This overlap is again calculated as $\sum_{i<j} C_{ij}/\min(d_i,d_j)$, where C_{ij} represents the number of fungal hosts shared between MH plant *i* and *j* that belong to a given community/group, min(di,dj) refers to the smallest of the two values, and the summation is done over all possible pairs of MH plants (Saavedra *et al.* 2013). Note that this overlap measure corresponds to the average number of shared fungal hosts among all pairs of MH plants in given community/group relative to the maximum number of fungal hosts that can be shared. To compare phylogenetic diversity and overlap across communities/groups, we used the scaled PD and scaled overlap, which are the values of the phylogenetic diversity and overlap measures within the range of possible phylogenetic diversity and overlap values generated by all the groups with the same number of plants.

Finally, to investigate the spatial influence of our sampling in the observed patterns of fungal hosts in MH plants, we compared the scaled PD and scaled overlap between MH plants belonging to the same location and MH plants belonging to different locations. Because in nine of the fifteen localities we visited, we found more than one MH plant species (see Figure S1), we generated two categories for each of the groups with 2, 3, 4 and 5 plant species generated above. Only if all plants in a given group were found in a common location, they were considered in category one. Otherwise, the group was considered in category two. For each group and category, we separately calculated the scaled PD and scaled overlap.

RESULTS

Fungal-host diversity in single mycoheterotrophic plants

We found that the number of fungal hosts in each of the 20 MH plant species varies from 2 to 42 (see Fig. 2A). Particularly, we found no phylogenetic signal on the number of fungal hosts among plants (Mantel test: r = -0.050, P = 0.766, df = 19) nor on the fungal hosts shared among plants (Mantel tests: Bray-Curtis r = -0.035, P = 0.682; overlap r = 0.047, P = 0.245; df = 19). Looking at the MH plants that belong to the

same location (Fig. S1), we found no phylogenetic signal on the number of fungal hosts among plants (Mantel test: $r = 0.17$, $P = 0.375$, $df = 3$ for Laussat; $r = -0.20$, $P = 0.650$, $df = 4$ for Elie; $r = -0.21$, $P = 0.717$, $df = 5$ for Singes; $r = 0.37$, $P = 0.089$, $df = 5$ for Virginie) nor on the fungal hosts shared among plants (Mantel test: Bray-Curtis $r = 0.03$, $P = 0.583$; overlap $r = 0.03$, $P = 0.512$; $df = 3$ for Laussat; Bray-Curtis $r = -0.54$, $P = 0.983$; overlap $r = 0.34$, $P = 0.150$; $df = 4$ for Elie; Bray-Curtis $r = -0.22$, $P = 0.794$; overlap $r = 0.08$, $P = 0.472$; $df = 5$ for Singes; Bray-Curtis $r = -0.09$, $P = 0.608$; overlap $r = 0.25$, $P = 0.161$; $df = 5$ for Virginie). Overall, these findings reveal an important variability in MH interactions that can be driven by mechanisms other than evolutionary relationships.

Additionally, we found that fungal-host diversity in each observed plant ranks among the highest when compared to the potential host diversity that can be expected by chance in a single MH plant with the same number of fungal hosts. The majority of plants (14 out of 20) lies in the upper half of the range of possible phylogenetic diversity values (scaled PD > 0.5; Figure 2). These findings imply that individual plants typically have a high fungal-host diversity by exploiting distantly related fungi, regardless of their number. This raises then the question of how plants are sharing their fungal hosts.

Fungal-host diversity and overlap among mycoheterotrophic plants

Mycorrhizal fungi create extensive underground networks that could make MH plants compete to obtain their belowground vital resources via their MH interactions. This makes necessary the study of how the diversity of MH interactions is modulated and shared within groups of plants.

We find that on average the fungal-host diversity (the combined phylogenetic diversity of the associated fungal hosts within the group) is proportional to fungal-host overlap (the average fraction of shared fungal hosts) in groups of MH plants. This pattern was present in both the observed communities in the field (Figure 3A) and in the generated group of plants (Figure 3B). In particular, there is a systematic positive association between scaled PD and scaled overlap in the observed communities (Pearson correlation: $r = 0.805$, $P = 0.053$, $df = 4$) and in the artificially-generated groups (Pearson correlation: $r = 0.497$, $P = 0.001$, $df = 21680$). This positive relationship does not depend on group size (Pearson correlation: $r = 0.377$, $df = 191$, $P = 0.001$ for 2 species, $r = 0.487$, $df = 1138$, $P = 0.001$ for 3 species, $r = 0.493$, $df = 4843$, $P = 0.001$ for

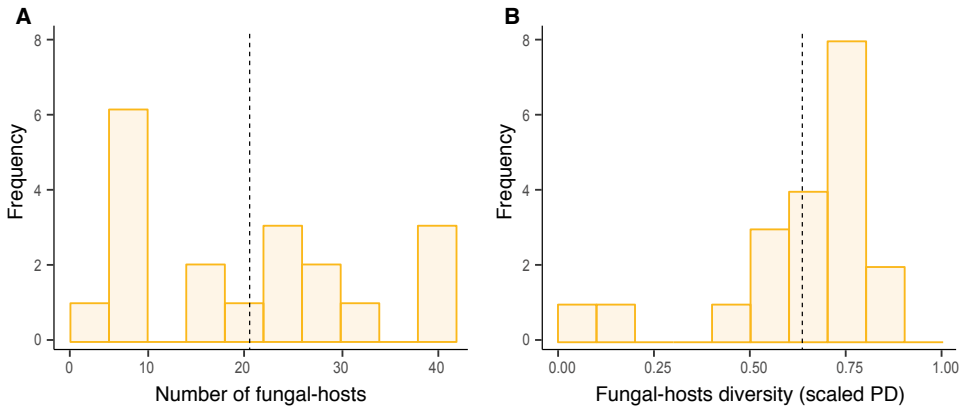


Figure 2 | Fungal- host patterns in single mycoheterotrophic plants. Panel (A) shows the distribution of the total number of fungal hosts associated with each of the 20 observed MH plants. Panel (B) shows the fungal-host diversity (scaled phylogenetic diversity) associated with each of the 20 observed plants. This shows that most of the observed MH plants have a fungal- host diversity that falls in the upper half of the potential range. The dashed lines correspond to the mean values in the distributions.

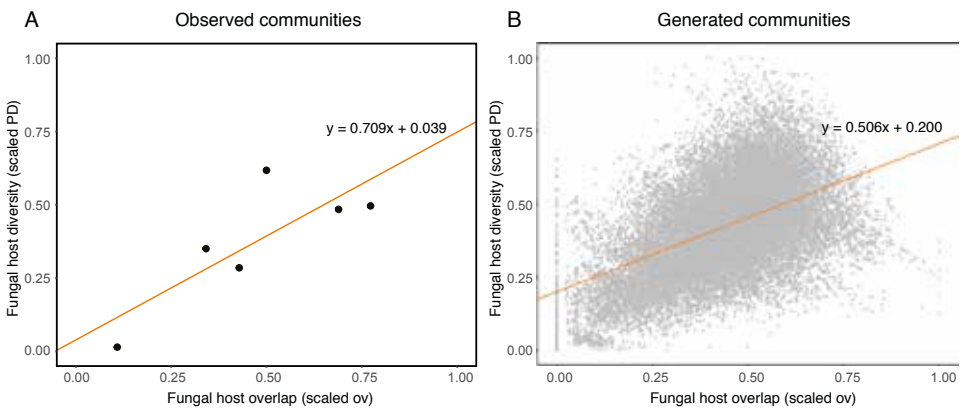


Figure 3 | Fungal-host diversity increases along with fungal-host overlap among mycoheterotrophic plants. The figures show the relationship between fungal-host diversity and fungal-host overlap for both the six observed communities in the field (panel A) and in the artificially generated groups of plants (of the 20 sampled MH species) (panel B). Both panels show the common positive relationship between fungal-host diversity (scaled phylogenetic diversity in y-axis) and fungal-host overlap (scaled overlap in x-axis). Fungal-host diversity and overlap correspond, respectively, to the combined phylogenetic diversity of the hosts associated with the plants in each group normalized by the number of fungal hosts, and the fraction of shared fungal hosts (see Section 2). The solid lines correspond to the linear regression between scaled PD and scaled overlap across all points.

4 species, $r = 0.478$, $df = 15502$, $P = 0.001$ for 5 species).

The results above are also qualitatively the same if scaled PD and scaled overlap values are replaced by their raw values while controlling for the total number of fungal hosts. Because the number of specimens and the OTU richness per MH species are variable among samples and may influence the results (see Supporting Table S1), we computed the partial Pearson correlations between scaled PD and scaled overlap controlling for the number of individuals sampled per species, number of OTUs, and variation in the number of individuals per species within a community (using the Herfindahl index). The obtained correlations remain positive and significant at the 95 % confidence, which confirm that fungal-host diversity within a group of plants increases together with their fungal-host overlap.

Finally, by dividing the categories of MH plants into one in which all plants belong to the same location and another one in which not all plants belong to the same location (see Methods), we found that typically the former group displays higher levels of both scaled PD and scaled overlap across the different group sizes (see Tables 1 and 2). These

Table 1 | Fungal-host diversity is higher in groups of plants that belong to the same location. The table shows the *t*-test results comparing the scaled PD in groups of MH plants (composed by two, three, four, or five species) that belong to the same location and in different locations.

Scaled PD	Mean in same location	Mean in different location	P-value	95 % CI
Two species	0.421	0.297	0.0012	0.05, 0.20
Three species	0.412	0.327	0.0002	0.04, 0.13
Four species	0.479	0.394	0.0009	0.04, 0.39
Five species	0.553	0.440	0.0023	0.05, 0.18

Table 2 | Fungal-host overlap is higher in groups of plants that belong to the same location. The table shows the *t*-test results comparing the scaled PD in groups of MH plants (composed by two, three, four, or five species) that belong to the same location and in different locations.

Scaled PD	Mean in same location	Mean in different location	P-value	95 % CI
Two species	0.358	0.220	$6.6\ e^{-6}$	0.07, 0.21
Three species	0.493	0.362	$3.2\ e^{-8}$	0.09, 0.17
Four species	0.512	0.404	$2.1\ e^{-8}$	0.08, 0.14
Five species	0.577	0.458	$1.3\ e^{-5}$	0.08, 0.15

results suggest that fungal-host diversity increases within a location as a response to a natural increase in fungal-host overlap, which can be expected from a niche framework perspective (MacArthur & Levins, 1967; Levine & HilleRisLambers, 2009; Rohr *et al.* 2016).

Discussion

Previous studies have investigated fungal-host diversity of MH plants in relation to the fungal diversity associated with the surrounding green plants (Cullings *et al.* 1996; Bidartondo *et al.* 2002, 2003; Bougoure *et al.* 2009; Roy *et al.* 2009b; Yamato *et al.* 2011). However, several MH species present vast geographic distributions despite being locally rare. Therefore, these surrounding plants may not be the exclusive factors determining fungal-host diversity in MH plants. Indeed, many studies have reported the occurrence of different species of arbuscular mycoheterotrophs in the field without a clear explanation for this phenomenon (e.g. van der Pijl, 1934; Jonker, 1938; van Royen, 1972; van de Meerendonk, 1984; Maas & Rübsamen, 1986; Cheek & Williams, 1999; Merckx, 2013).

In our study, we have considered potential neighboring effects of MH plants with each other as possible drivers of fungal-host diversity. Because many unmeasured factors can influence MH interactions, we opted to compared the observed patterns against all the possible fungal-host combinations (what we called artificially-generated groups of plants). We have found that individual MH plants have a tendency to exploit more distantly related fungi than expected by chance. This tendency of targeting distantly related fungi has been described in autotrophic plants (Giovannetti *et al.* 2004). Nevertheless, it has been suggested that MH plants have more restricted interactions, since they often show higher specificity towards their fungal-hosts (e.g. Bidartondo *et al.* 2003; Gomes *et al.* 2017a). For example, in *Afrothismia*, five closely related MH plants were found to specialize in five closely related lineages of Glomeromycota fungi (Merckx & Bidartondo, 2008). In contrast, in Monotropoideae, the five MH species in this clade associate with five different distantly related Basidiomycota fungi, but each within the same fungal lineage (Bidartondo & Bruns, 2005). Either way, and despite the processes leading to this extreme level of fungal specificity, it has been suggested that MH plants adapt to the suitable fungal partners that participate in this mycoheterotrophic interaction, and therefore host-jumps to distantly related fungal lineages are unexpected (Bidartondo & Bruns, 2002).

Building on niche theory, our results may reflect a MH plant strategy to increase its fungal-host diversity or niche width, as species with a wider niche may be more likely to obtain different resources and to establish successfully in new habitats (Levins, 1968; Tilman *et al.* 1996; Levine & HilleRisLambers, 2009). Mycoheterotrophic plants require established mycorrhizal networks to persist (Sachs & Simms, 2006; van der Heijden *et al.* 2015). Although each species tend to increase the phylogenetic diversity of their fungal hosts, it is still a limited fraction of the total diversity of arbuscular mycorrhizal fungi that can be part of this interaction (Douglas, 2008; Merckx *et al.* 2009; Gomes *et al.* 2017a), suggesting that these fungi appear to be under selection pressure to be resistant to these cheaters (Douglas, 2008). Therefore, the ability to increase its fungal-host diversity may confer an advantage to increase the opportunities to cheat mycorrhizal networks.

We have found that in communities of co-occurring MH plant species in the field the fungal-host diversity among MH plants appear to increase proportionally to their fungal-host overlap. This same tendency was confirmed among the artificially-generated groups of MH plants showing that the patterns observed are not an artifact of the reduced number of MH communities observed in the field. Moreover, we have found that both fungal-host diversity and overlap are significantly higher among plants that belong to the same geographical location, which could provide an explanation for the lack of phylogenetic signal on the fungal hosts among MH plants. These results indicate that fungus-plant interactions can be better explained by understanding plant-plant interactions generated by sharing resources or fungal hosts. Future studies could explain whether this symmetry between fungal-host diversity and overlap may respond to an ecological mechanism driven by maximizing co-occurrence and avoiding competitive exclusion among MH plants.

A potential bias in our study is the use of ITS2 sequences and future work should consider expanding these sequences (see Supporting Information for more details). Another aspect that deserves particular attention is the influence of abiotic factors that can affect the diversity of fungal hosts for the MH plants. In fact, many other factors can influence diversity, including the surrounding autotrophic plants. Taking everything into account is virtually impossible. However, our findings suggest that species coexistence cannot be fully understood without attention to the two sides of ecological interactions.

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Data accessibility

Data is publicly available as Supporting Information.

Author contribution

SS designed the overall study. VSFTM designed the sampling, DNA study, and provided data. SIFG performed the analysis. SIFG and SS wrote a first draft of the manuscript; all authors contributed to revisions.

Supporting Information

DNA sequencing

Fungal DNA was extracted from root material with KingFisher Flex Magnetic Particle Processors (Thermo Scientific, USA), using the NucleoMag 96 Plant Kit (Machery-Nagel Gmbh and Co., Düren, Germany). The internal transcribed spacer 2 (ITS2) was amplified using the fungal specific primer fITS7 (Ihrmark *et al.* 2012) and the universal primer ITS4 (White *et al.* 1990), which was labeled with 96 different Ion Torrent MID-labels to differentiate individual samples. ITS2 labelled amplicons were sequenced on a Personal Genome Machine with 850 flows (PGM; Life Technologies, Guilford, CT, USA). The next-generation sequencing of the 140 specimens were done in two runs, including other plant species not used for this work. The reads obtained then were processed using USearch v.7 using the UPARSE algorithm (Edgar, 2013). The Ion Torrent runs originated 9 547 370 raw sequences. From these, 156 517 passed our quality control steps (excluding sequences with $Q < 20$, length < 100 bp and global

singletons), originating 37 563 unique sequences. These sequences were clustered at 97% similarity. A chimera check was performed using Uchime Reference Database (3.07.2014 UNITE/INSD; (Edgar *et al.* 2011). Global OTUs singletons and doubletons were excluded, generating a total of 138 Glomeromycota OTUs (represented by 7,227 sequences). The 138 OTUs were identified by BLAST search using the UNITE+INSD database (version 6.0, 10.09.2014) in UPARSE implemented with the current Index Fungorum classification. See Table S2 in Supporting Information for information on the closest match for each OTU. We matched the 138 fungal hosts to the 20 MH plant species. All non-Glomeromycota OTUs were omitted, retaining 138 Glomeromycota OTUs for further analysis. Because the majority of the Glomeromycota hits (see Table S2) matched uncultured Glomeromycota species, we placed the obtained OTUs in a phylogenetic tree (see Figure S3) to better understand their phylogenetic relationships.

To avoid the conflicts that molecular assessments generate in the species delimitation of arbuscular mycorrhizal fungi, due to the current absence of species concept for the fungi in this phylum, we measured the diversity of MH interactions as the phylogenetic diversity among the fungi detected per plant species, instead of considering the number of OTUs.

A potential bias in our study is the use of ITS2 sequences. The marker regions often used for Glomeromycota phylogenetic studies are ribosomal DNA markers, including SSU, ITS and LSU genes, also because rDNA markers are the largest sampled within this group of fungi. Previous studies showed that SSU alone has a limited resolution power (Bruns et al. 1991; Hofstetter et al. 2007), which can introduce a bias towards an under-estimation of AM fungi (Krüger, 2011). The ITS region is known to be a highly variable region, which can also introduce a bias in the opposite direction of the SSU marker, towards an over-estimation of AM fungi. To overcome these problems, Krüger et al. (2009) suggested the amplification of a SSU-ITS-LSU fragment for a phylogenetic analysis with species-level resolution. However, the use of next-generation DNA sequencing techniques only allows amplification of short DNA fragments, which forces us to choose a fragment of one of the three markers. Due to the limited length of Ion Torrent sequencing, the better candidate region chosen was the ITS2. Preliminary data analysis (not shown) based on the SSU region has proven not to discriminate the different fungal lineages associated with these plants. Therefore, the use of ITS2, which is a more variable region, potentially delivers the most phylogenetic informative characters. Because ITS2 is a fast-evolving region we used a backbone alignment

including concatenated reference sequences (Krüger et al. 2012) of partial SSU, whole ITS and partial LSU representative of all the described AM fungi genera (Krüger et al. 2009), adding the two new genera described later on curated in the MaarjAM database (Öpik *et al.* 2010), for a more accurate phylogenetic placement of the generated fungal sequences in this study. To reduce the potential bias due to the under- or over-splitting of fungal taxa in OTUs, we used the phylogenetic distances between the fungal taxa instead of richness of the samples in the downstream analysis.

Figures

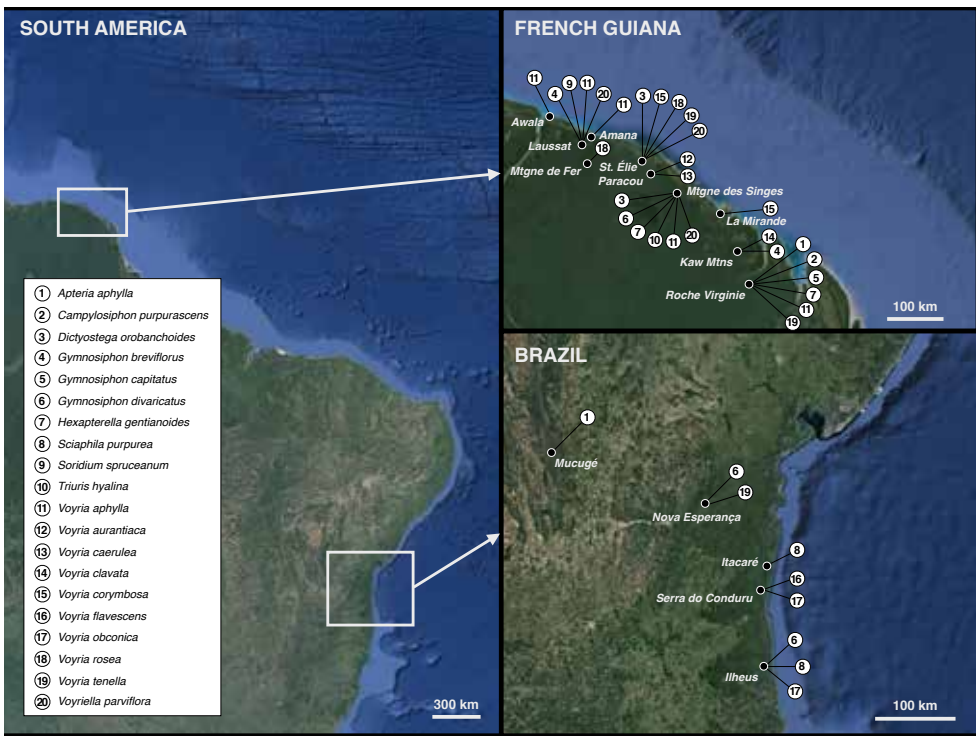

Figure S1 | Map of the 15 sampling locations of our study: 10 in French Guiana and 5 in Brazil. Mycoheterotrophic species (on the left) are represented by numbers in each location that were collected (on the right).

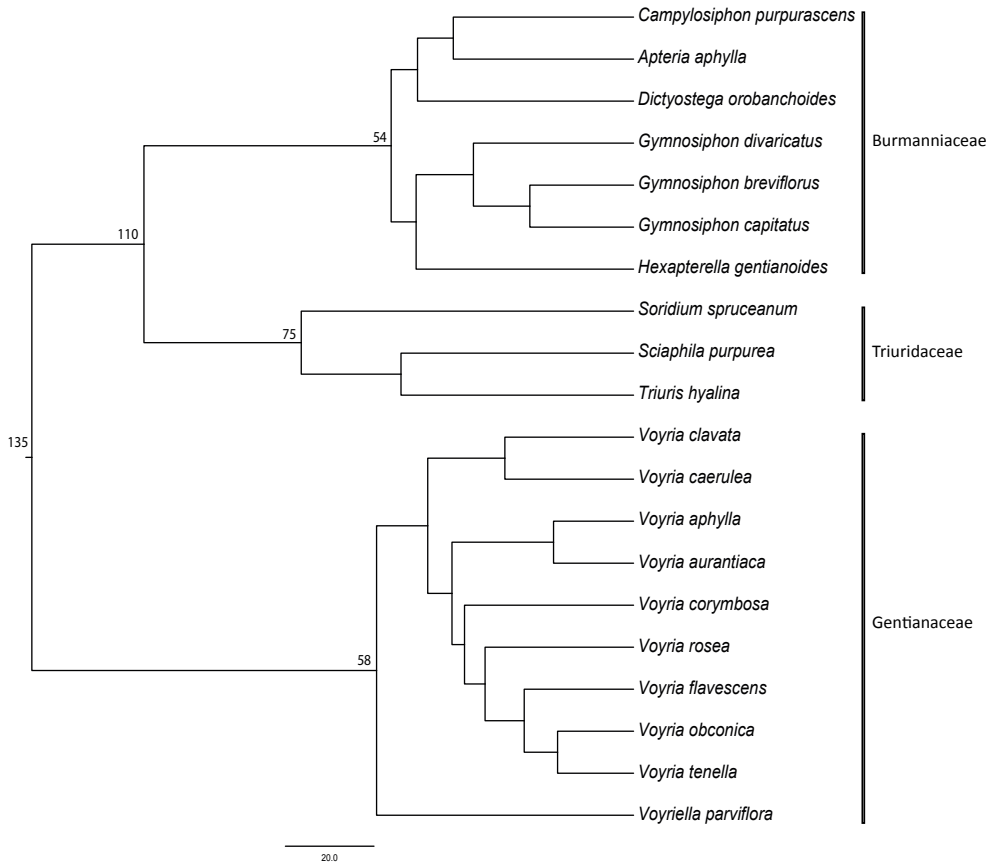

Figure S2 | Phylogeny of MH plants used to infer phylogenetic signal. Branch lengths represent divergence times. Root age and crown node ages of the sampled families are shown (in million years ago).

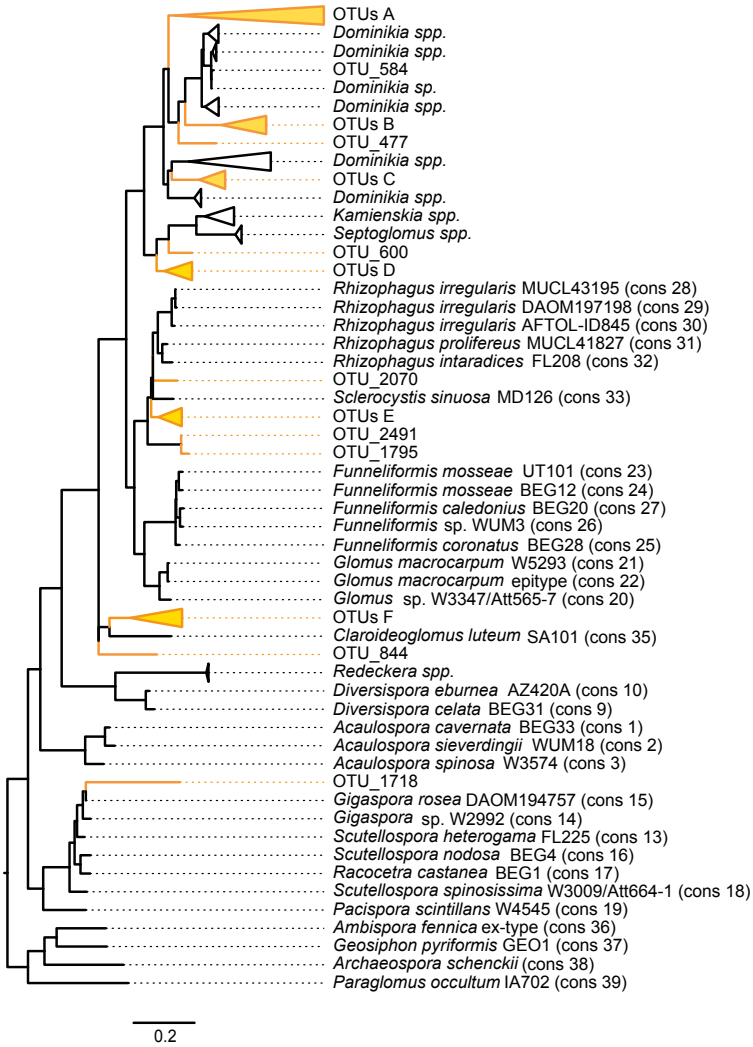

Figure S3 | Phylogeny of the Glomeromycota OTUs found in all the MH plants. Sequences with identification correspond to curated sequences of Glomeromycota (see DNA Sequencing in Supporting Information). Sequences indicated with cons were obtained from the reference dataset of AM fungi built by Krüger et al. (2011). We also included the following genera identified in the MaarjAM database (Öpik et al., 2010): *Dominikia* spp. (HG938301-HG938304, KJ564145-KJ564169, KM05657-KM05665, KR105638-KR105649), *Kamienskia* spp. (KJ564133-KJ564144), *Redeckera* spp. (HG518627-HG518629), *Septoglomus* spp. (HF548853-HF548862). The list of OTU numbers in the collapsed clades is the following OTUs A: 7, 36, 41, 42, 56, 65, 79, 82, 97, 100, 136, 170, 203, 210, 225, 293, 325, 438, 471, 497, 499, 508, 545, 641, 683, 765, 777, 798, 819, 956, 1048, 1109, 1255, 1257, 1353, 1355, 1362, 1594, 1939, 2129, 2182, 2186, 2191, 2239, 2266, 2443, 2509, 2518, 2586, 2660, 2847, 2898, 3037, 3062, 3094, 3239, 3386, 3515, 3581, 3700; OTUs B: 338, 873, 1377, 1625, 2613; OTUs C: 12, 45, 57, 80, 81, 159, 162, 211, 233, 253, 260, 320, 354, 364, 382, 506, 523, 553, 653, 686, 687, 769, 772, 997, 1002, 1034, 1052, 1064, 1079, 1086, 1135, 1357, 1381, 1492, 1512, 1633, 1664, 1788, 2072, 2198, 2304, 2312, 2319, 2402, 2424, 2772, 3171, 4185, 4203; OTUs D: 400, 590; OTUs E: 38, 299, 1349, 1656, 2112, 3260, 3355; OTUs F: 107, 112, 146, 383, 786, 1642, 1677.

Tables

Figure S1 | Identity of MH plant species.

Species	Family	Locality	Date
Apteria aphylla		French Guiana, Savanne Roche Virginie	02-08-2014
		French Guiana, Savanne Roche Virginie	02-08-2014
		Brazil, Mucugé	27-10-2014
Campylosiphon purpurascens		French Guiana, Savanne Roche Virginie	25-08-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
Dictyostega orobanchoides		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
Gymnosiphon breviflorus		French Guiana, Kaw, trail to caves	01-08-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
Gymnosiphon capitatus	Burmanniaceae	French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
		French Guiana, Savanne Roche Virginie	25-08-2014
Gymnosiphon divaricatus		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	02-11-2017
Hexapterella gentianoides		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Savanne Roche Virginie	02-08-2014
		French Guiana, Montagne de Singes	22-07-2014
Sciaphila purpurea		Brazil, Bahia, Ilhaeus	04-11-2014
	Triuridaceae	Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014

Coordinates	Habitat	Code	No OTUs	unique OTUs
4°11'42.6"N, 52°08'58.5"W	Edge of woody vegeation on rock outcrop	GM072_1	4	
4°11'42.6"N, 52°08'58.5"W	Edge of woody vegeation on rock outcrop	GM072_2	2	7
12°59'56"N, 41°20'55"W	In *Sphagnum* on rocks near stream	PM3780_1	4	
4°11'42.6"N, 52°08'58.5"W	Primary forest on swampy soil	Bk1_52	1	
4°11'42.6"N, 52°08'58.5"W	Primary forest on swampy soil	Bk1_53	7	10
4°11'42.6"N, 52°08'58.5"W	Primary forest on swampy soil	Bk1_54	3	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	GM009_1	5	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	GM009_2	1	22
5° 8'7.87"N, 53°2'56.77"W	Primary lowland rainforest	GM012	5	
5° 8'7.87"N, 53°2'56.77"W	Primary lowland rainforest	GM018	12	
4°33'2.13"N, 52°10'28.71"W	Primary rainforest on lateritic rocks	GM057	6	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	GM048	7	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_GB1	13	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_GB4	4	42
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_GB1	5	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_GB2	6	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_GB3	31	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_GB4	12	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	GM004	10	
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest on steep slope	Bk2_16	6	
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest on steep slope	Bk2_31	2	29
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest on steep slope	Bk2_63	7	
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest on steep slope	Bk2_70	17	
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest on steep slope	Bk2_104	9	
15°14."53"S, 39°04'05"W	Primary Atlantic rain forest	PM3931B	4	
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_1	7	
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_2	10	
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_3	1	
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_4	15	42
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_5	13	
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_6	12	
13°34'48"S, 39°41'44"W	Primary Atlantic rain forest	PM3854_7	5	
13°34'05"S, 39°42'25"W	Primary Atlantic rain forest	PM3890	7	
5°28'18"N, 53°34'38"W	Primary Atlantic rain forest	GM005_1	13	
5°28'18"N, 53°34'38"W	Primary Atlantic rain forest	GM005_2	1	23
4°11'42.6"N, 52°08'58.5"W	Primary Atlantic rain forest	GM065	3	
5°03'59"N, 52°41'50"W	Primary Atlantic rain forest	P3_HG1	15	
15°14'53"S, 39°04'05"W	Primary Atlantic rain forest	PM3928_1	1	
15°14'53"S, 39°04'05"W	Primary Atlantic rain forest	PM3928_2	7	26
15°14'53"S, 39°04'05"W	Primary Atlantic rain forest	PM3928_3	15	

Figure S1 | Identity of MH plant species (continued).

Species	Family	Locality	Date
Sciaphila purpurea		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Itacaré	06-11-2014
		Brazil, Bahia, Itacaré	06-11-2014
		Brazil, Bahia, Itacaré	06-11-2014
Soridium spruceanum		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
	Triuridaceae	French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
Triuris hyalina		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
Voyria aphylla		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Reserve Amana, zone B	26-07-2014
		French Guiana, Reserve Amana, zone B	26-07-2014
		French Guiana, Reserve Amana, zone B	26-07-2014
		French Guiana, Reserve Amana, zone B	26-07-2014
		French Guiana, Reserve Amana, zone B	26-07-2014
		French Guiana, Kiwala, Awala Reserve	27-07-2014
	Gentianaceae	French Guiana, Kiwala, Awala Reserve	27-07-2014
		French Guiana, Savanne Roche Virginie	02-08-2014
		French Guiana, Laussat	28-07-2014
Voyria aurantiaca		French Guiana, Paracou	24-07-2014
		French Guiana, Paracou	24-07-2014
		French Guiana, Paracou	24-07-2014
		French Guiana, Paracou	24-07-2014
		French Guiana, Paracou	24-07-2014
		French Guiana, Paracou	24-07-2014
Voyria caerulea		French Guiana, Paracou	24-07-2014

Coordinates	Habitat	Code	No OTUs	unique OTUs
15°14'53"S, 39°04'05"W	Primary Atlantic rain forest	PM3928_4	6	
15°14'53"S, 39°04'05"W	Primary Atlantic rain forest	PM3928_5	1	
15°14'53"S, 39°04'05"W	Primary Atlantic rain forest	PM3928_6	1	
14°13'40"S, 39°00'58"W	Coastal Atlantic forest	PM3994_1	2	
14°13'40"S, 39°00'58"W	Coastal Atlantic forest	PM3994_2	2	
14°13'40"S, 39°00'58"W	Coastal Atlantic forest	PM3994_3	3	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	GM049_1	7	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	GM049_2	12	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_SS1	7	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_SS2	13	30
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_SS3	5	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_SS6	10	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_SS7	8	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	GM006_1	4	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	GM006_2	13	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH1	7	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH2	8	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH3	2	24
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH4	1	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH5	2	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH6	1	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	P3_TH7	1	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	GM008_1	6	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	GM008_2	2	
5°32'38"N, 53°29'49"W	Coastal forest on white sand	GM037_1	6	
5°32'38"N, 53°29'49"W	Coastal forest on white sand	GM037_2	2	
5°32'00"N, 53°33'57"W	Coastal forest on white sand	GM040_1	13	
5°32'00"N, 53°33'57"W	Coastal forest on white sand	GM040_2	5	31
5°32'00"N, 53°33'57"W	Coastal forest on white sand	GM040_3	2	
5°44'45.1"N 53°56'07.0"W	Coastal forest on white sand	GM041	7	
5°44'45.1"N 53°56'07.0"W	Coastal forest on white sand	GM042	5	
4°11'42.6"N, 52°08'58.5"W	In shrubby vegetation on rock outcrop	GM070	6	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_VA1	2	
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM028_1	5	
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM028_2	5	
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM032_1	4	9
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM032_2	1	
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM034_1	6	
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM034_2	5	
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM033_1	3	5

Figure S1 | Identity of MH plant species (continued).

Species	Family	Locality	Date
Voyria caerulea	Gentianaceae	French Guiana, Paracou	24-07-2014
Voyria clavata		French Guiana, Montagne de Singes	22-07-2014
Voyria corymbosa		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Savanne Roche Virginie	25-07-2014
Voyria flavescens		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
Voyria obconica		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Serra do Conduru	05-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
		Brazil, Bahia, Ilhaeus	04-11-2014
Voyria rosea		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Montagne de Fer	27-07-2014
		French Guiana, Montagne de Fer	27-07-2014
Voyria tenella		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Savanne Roche Virginie	02-08-2014
		French Guiana, Savanne Roche Virginie	02-08-2014
		French Guiana, Savanne Roche Virginie	02-08-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014

Coordinates	Habitat	Code	No OTUs	unique OTUs
5°16'48.06"N 52°55'4.56"W	Primary lowland rainforest	GM033_2	3	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	GM053	2	2
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM015	3	8
4°51'58.7"N 52°20'45.6"W	Primary lowland rainforest	GM036	5	
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_1	6	
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_2	4	
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_3	3	
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_4	5	17
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_5	10	
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_6	5	
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3974_7	5	
15°14'53"S, 39°04'05"W	Primary lowland rainforest	PM3931_1	3	
15°14'53"S, 39°04'05"W	Primary lowland rainforest	PM3931_2	1	
15°14'53"S, 39°04'05"W	Primary lowland rainforest	PM3931_3	2	
15°14'53"S, 39°04'05"W	Primary lowland rainforest	PM3931_4	4	
15°14'53"S, 39°04'05"W	Primary lowland rainforest	PM3947b	2	10
14°28'50"S, 39°06'29"W	Primary lowland rainforest	PM3947c	1	
15°14'53"S, 39°04'05"W	Restinga forest on sandt soil	PM3950_1	3	
15°14'53"S, 39°04'05"W	Restinga forest on sandt soil	PM3950_2	1	
15°14'53"S, 39°04'05"W	Restinga forest on sandt soil	PM3950_3	3	
15°14'53"S, 39°04'05"W	Restinga forest on sandt soil	PM3950_4	6	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM011_1	3	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM011_2	1	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM014	1	8
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM016	1	
5°18'0" N, 53°36'0" W	Primary forest on swampy soil	GM043_1	2	
5°18'0" N, 53°36'0" W	Primary forest on swampy soil	GM043_2	6	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM019_1	9	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM019_2	2	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM019_3	5	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM019_4	1	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM019_5	2	
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest	GM063_1	1	
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest	GM063_2	5	41
4°11'42.6"N, 52°08'58.5"W	Primary lowland rainforest	GM063_3	7	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_1	7	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_2	4	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_3	3	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_4	1	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_5	3	

Figure S1 | Identity of MH plant species (continued).

Species	Family	Locality	Date
Voyria tenella		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	01-11-2014
		Brazil, Bahia, Nova Esperança	02-11-2014
		Brazil, Bahia, Nova Esperança	02-11-2014
		Brazil, Bahia, Nova Esperança	02-11-2014
Voyriella parviflora		French Guiana, Montagne de Singes	22-07-2014
		French Guiana, Piste de St. Ellie, Sentier Botanique	23-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
	Gentianaceae	French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014
		French Guiana, Laussat	28-07-2014

Table S1 | Identity of MH plant species. Detailed sample localities with GPS coordinatesand collection dates are presented for each sampled specimen. Specimens coded with thesame collection number followed by underscore and specimen number were collected lessthan 1 m apart from each other; different collection numbers indicate that specimens werecollected isolated. It is represented the number of reads generated by next generationsequencing (after filtering steps), OTUs detected per plant specimen and total unique OTUs per plant species (table above).

Table S2 | BLAST hits for the Glomeromycota OTUs based on the UNITE database. Foreach OTU, the closest match is presented (available at https://tinyurl.com/yajewo7p).

Table S3 | Overview of the number of OTUs and number of sequences generated per sample.Presence of each OTU is shown per sample (available at https://tinyurl.com/y7o5hoz4).

Coordinates	Habitat	Code	No OTUs	unique OTUs
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_6	6	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3865_7	1	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3889_1	10	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3889_2	7	
13°34'05"S, 39°42'25"W	Primary lowland rainforest	PM3889_3	9	
5°03'59"N, 52°41'50"W	Primary lowland rainforest	GM007	4	
5°18'7.87"N 53°2'56.77"W	Primary lowland rainforest	GM023	1	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_VP1	4	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_VP2	1	
5°28'25"N, 53°34'51"W	Primary lowland rainforest	P1_VP4	3	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP1	4	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP2	5	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP3	2	18
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP5	4	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP7	2	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP8	1	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP9	2	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP10	1	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP14	1	
5°28'18"N, 53°34'38"W	Primary lowland rainforest	P2_VP15	1	

Table S4 | Species composition of the co-occurring MH plant communities. Plants were considered to co-occur when flowering specimens were found growing less than 1 meter apart from each other. We observed six communities of co-occurring species.

Communities	Plant species
A	*G. breviflorus, V. parviflora*
B	*A. aphylla, V. aphylla*
C	*C. purpurascens, D. orobanchoides, V. aphylla*
D	*G. breviflorus, H. gentianodes, T. hyalina*
E	*V. clavata, V. corymbosa, V. rosea*
F	*D. orobanchoides, G. breviflorus, S. spruceanum, V. aphylla, V. parviflora*