

New Phytologist Supporting Information

Article title: Mycoheterotrophic plants preferentially target arbuscular mycorrhizal fungi that are highly connected to autotrophic plants

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Fig S2 | Venn diagrams representing variation explained by plant species identity, plant type and subplot.

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Table S1 | Plant identity of autotrophic and mycoheterotrophic plants from this study.

Methods S1 | Effect of plant identity, plant type and subplot on the structure of fungal communities at the plant individual level:

We explored the effects of plant identity, plant type (antagonist vs mutualist) and subplot origin on the fungal community composition obtained from the roots, at the individual plant level. For that, we rarefied each individual sample to 100 reads, and calculated the variance explained by these three factors using the function *varpart* using the *vegan* (Oksanen *et al.*, 2015) R package. We used two distance matrices by calculating the Bray-Curtis dissimilarities on the Hellinger transformed rarefied counts, and the Unifrac distance (Lozupone & Knight, 2005) to account for fungal relatedness. The variation partitioning analysis revealed that plant species identity captured most of the variation in the data, including the distinction between plant types (antagonist and mutualist), with a minor proportion of the variance explained by subplot (see Supporting Information, [Figure S2](#)), which can be expected due to the high heterogeneity of microbial communities in the soil at the local scale (Jacquemyn *et al.*, 2014). Then, to test for significance of association, we built a distance-based redundancy analysis (db-RDA) model for each matrix with these three factors, and performed model selection with the *ordi2step* function. The best model based showed that fungal communities were significantly structured by plant species identity, both considering their shared interactions only ($F = 1.848$, $P = 0.001$), or simultaneously accounting for the phylogenetic relationships between the shared fungi ($F = 3.015$, $P = 0.001$), explaining 44.8% or 57.7% of the variance in the data, respectively. The effect of plant type and plot were not significant in the db-RDA model.

Methods S2 | Plant root identification:

Plant roots were identified to genus or family level based on *matK* or *trnL* sequencing using BLAST. The genus of the BLAST hit with the highest percent identity to the query sequence was used as the genus identification, unless this genus is not known to occur in the region. Query sequences with a similarity of 99% or higher were considered to belong to the same plant species. A time tree was constructed based on genus identification using TimeTree.org. For groups sequences that could not be identified to genus level with certainty, the genus of the best BLAST hit was used.

Fig S1 | Accumulation curves considering the cumulative number of reads (top panels) and the cumulative number of samples (bottom panel) for autotrophic (left panels) and mycoheterotrophic (right panels) plants.

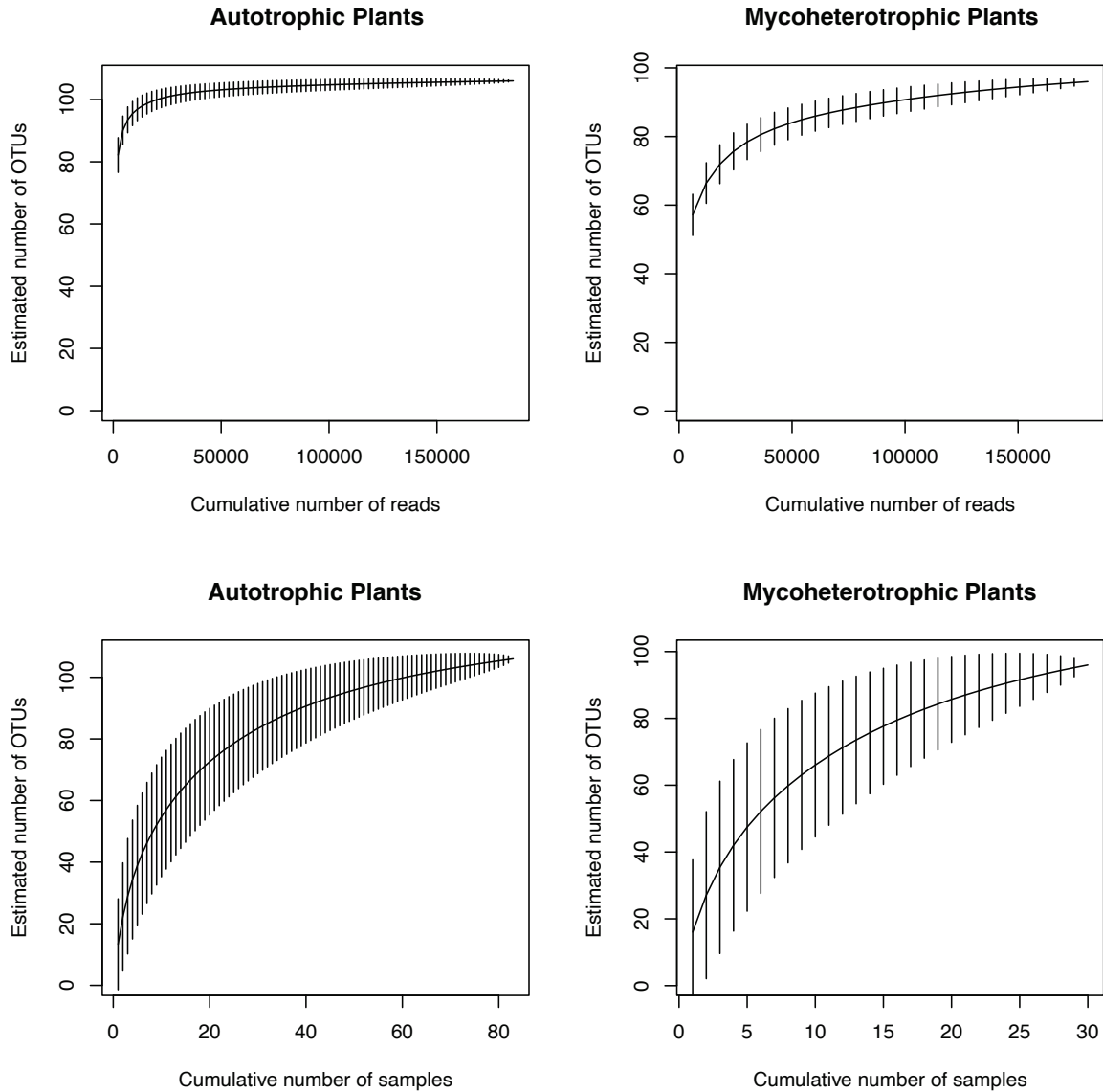


Fig S2 | Venn diagrams representing variation explained by plant species identity, plant type and subplot using variation partitioning.

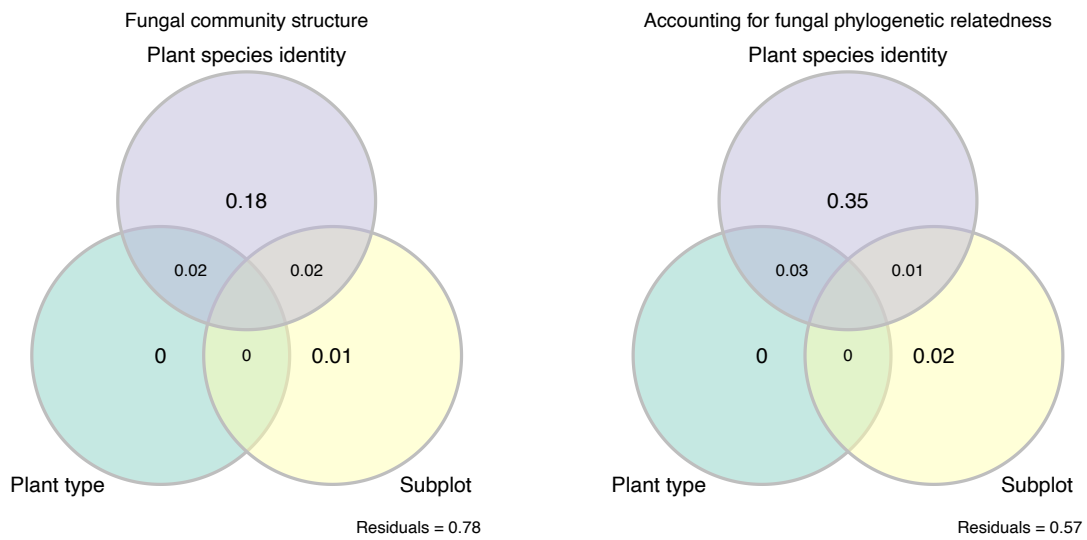


Fig S3 | Phylogenetic signal analysis repeated on multiple rarefaction depths. Mean observed phylogenetic signal of fungi in the antagonistic (top) and mutualistic (bottom) networks for the 100 rarefaction matrices tested per rarefaction depth (left), and corresponding mean p-values (right). Bars represent standard deviation.

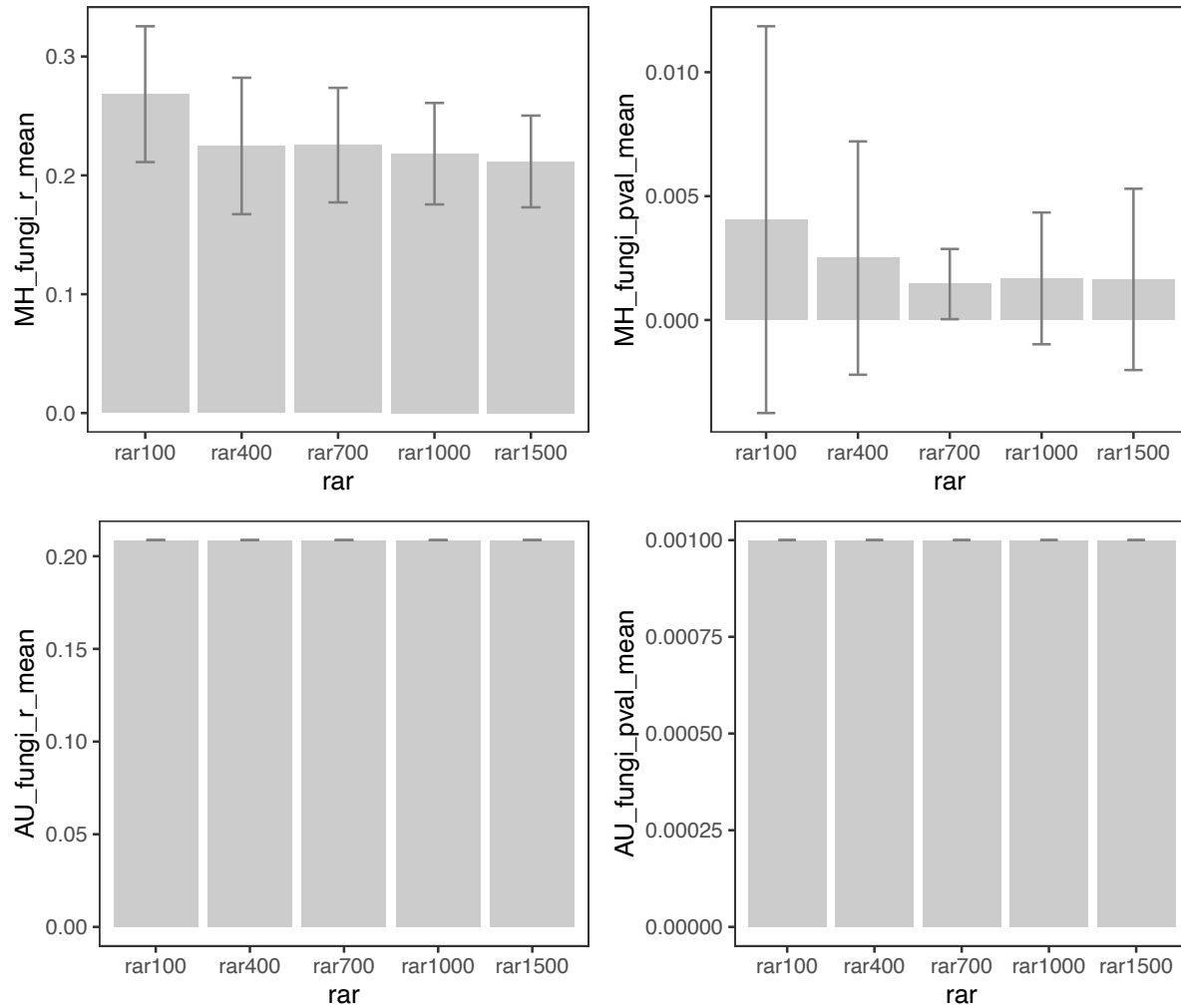


Fig S4 | Motif analysis repeated on multiple rarefaction depths. Mean observed motifs for the 100 rarefaction matrices tested per rarefaction depth (left), and corresponding mean z-scores (right). Dashed lines represent the critical z-score values for 95% ($y = 1.96$) and 99% ($y = 2.58$) confidence levels. Bars represent standard deviation.

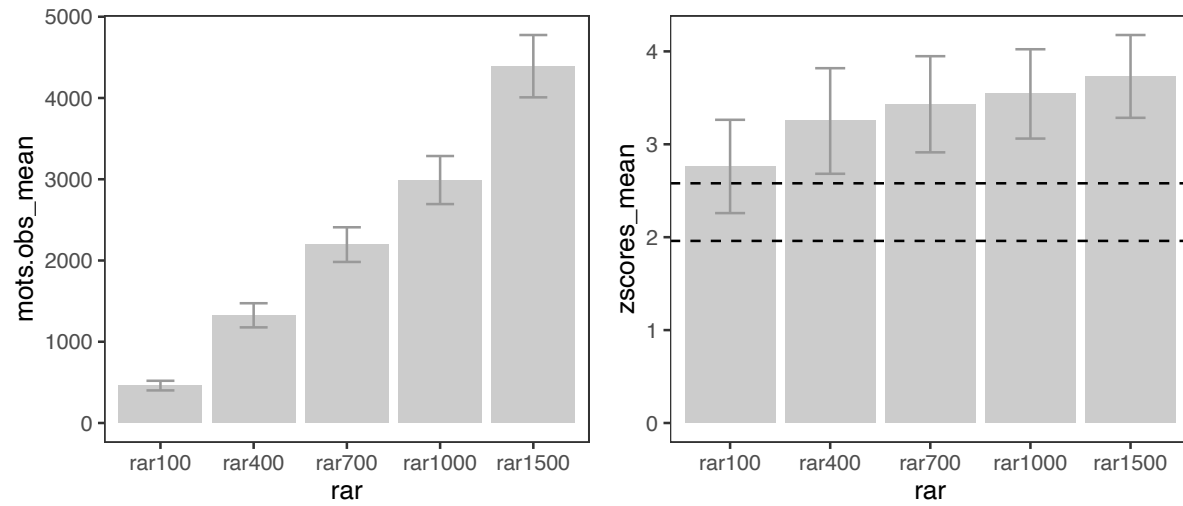


Table S1 | Plant identity of autotrophic (mutualistic) and mycoheterotrophic (antagonistic) plants from this study. In total, we collected 220 autotrophic and 60 mycoheterotrophic root tips samples. For the autotrophic plants, 123 samples could be identified by DNA sequencing; these are listed here. 99 samples among 28 autotrophic, and 45 samples among the five mycoheterotrophic plant species had Glomeromycotina reads. After removing samples with less than 100 reads, and only considering an OTU present in a sample when represented by at least 5 reads, we obtained a total of 77 samples among 21 autotrophic species, and 27 samples among the five mycoheterotrophic species. Rows in grey are excluded taxa due to the absence of Glomeromycotina reads; rows in orange are taxa excluded from subsequent analyses because overall at species level, they had < 500 reads (total number of reads in autotrophic plants in the table excludes the taxa in orange).

Mutualistic Plants	Life form	sequenced samples	samples with AM fungi	excluding samples (< 100 reads)	AM fungi reads
<i>Acacia</i>	tree	1	1	1	6350
Araceae	herb	3	3	3	10456
Araliaceae	shrub	3	2	2	7122
Arecaceae	palm	1	0	0	0
Aspidosperma	tree	23	20	13	13667
Bromeliaceae	herb	1	0	0	0
<i>Clusia</i>	tree	4	4	4	43344
<i>Cupania</i>	tree	2	2	0	126
<i>Dicorynia</i>	tree	4	2	2	2302
Dilleniaceae	tree	1	1	1	2819
<i>Eperua</i>	tree	12	12	11	11483
Fabaceae2	tree*	2	2	2	5771
Fabaceae1	tree**	4	3	3	4441
<i>Hydrochorea</i>	shrub	1	0	0	0
<i>Ilex</i>	tree	1	1	1	844
<i>Inga</i>	tree	3	1	1	19099
<i>Iryanthera</i>	tree	1	0	0	0
Malpighiaceae	vine	12	11	9	20624
Metteniusaceae	tree	7	5	5	4616
<i>Mimosa</i>	shrub	1	1	1	3486
<i>Morus</i>	tree	3	3	3	2821
Myristica	tree	1	1	0	0
<i>Odontadenia</i>	liana	1	1	0	0
<i>Paloue</i>	tree	8	6	6	11635
<i>Peltophorum</i>	tree	1	1	0	401
<i>Protium</i>	tree	5	4	4	6104
Sapindaceae	tree	4	1	1	1285
<i>Sapotaceae</i>	shrub	3	2	1	246
Schizaeaceae	fern	1	1	0	0
<i>Schradera</i>	liana	2	2	2	1882
<i>Tapirira</i>	tree	6	5	1	5124
<i>Urticaceae</i>	tree	1	1	0	293
Total		123	99	77	185275
Antagonistic Plants					
<i>Saridium spruceanum</i>	herb	9	8	4	15290
<i>Dictyostega orobanchoides</i>	herb	7	5	3	5132
<i>Voyria aphylla</i>	herb	3	3	3	8559
<i>Voyriella parviflora</i>	herb	34	22	10	3357
<i>Gymnosiphon breviflorus</i>	herb	7	7	7	148522
Total		60	45	27	180860

Identification of life form of these plants is based on the life form of their best Blast hit:

*best Blast hit (95.9%) is *Martiodendron mediterraneum* (does not occur in the region)

** best Blast hit (99.6%) is *Acaciella chamalensis* / *Enterolobium cyclocarpum* (does not occur in the region).

References

Jacquemyn H, Brys R, Merckx VSFT, Waud M, Lievens B, Wiegand T. 2014. Coexisting orchid species have distinct mycorrhizal communities and display strong spatial segregation. *New Phytologist* **202**: 616-627.

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