FULL PAPER

Plant cheaters preferentially target arbuscular mycorrhizal fungi that are highly connected to mutualistic plants

Sofia IF Gomes¹, Miguel A Fortuna², Jordi Bascompte², Vincent SFT Merckx^{3,4}

¹ Institute of Environmental Sciences, Leiden University, Leiden, the Netherlands

² Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland.

⁴ Naturalis Biodiversity Center, Leiden, the Netherlands

³ Department of Evolutionary and Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands

Abstract

- To address how arbuscular mycorrhizal networks sustain cheaters – mycoheterotrophic plants that obtain both carbon and soil nutrients from fungi – here we investigate how mutualistic and antagonistic mycorrhizal networks are interlinked.
- We sampled root tips of mutualistic and cheater plants in two tropical forest plots and assembled the combined network between fungi linked to mutualistic and cheater plants (i.e., tripartite network) using DNA sequencing. We compared the interactions of the fungi in the mutualistic and antagonistic networks and searched for motifs (simple subgraphs that are overrepresented in complex networks) representing simultaneous links of particular mutualist and cheater plants to pairs of fungi to investigate fungal association preferences.
- Within the tripartite network, cheater plants are highly connected and interact with the majority of available fungal partners (74%), and we find that fungi that share interactions with the same mutualistic plants also share interactions with sets of cheaters. The motif analysis demonstrates that cheaters preferentially interact with sets of fungi that are linked to particular plant species.
- Our findings indicate that cheaters preferentially interact with fungi that are well-connected to particular mutualistic plants. We hypothesize that these non-random interactions may result from trait-based selection and that this strategy maximizes carbon availability for cheaters.

Introduction

Since the early history of life, interspecific mutualisms have been paramount in the functioning of ecosystems (Thompson, 2005; Bascompte & Jordano, 2013). Mutualisms can form complex networks of interdependence between dozens or even hundreds of species. A prime example of this ‘web of life’ is the 450-million-year-old mutualism between the majority of land plants and arbuscular mycorrhizal (AM) fungi (Smith & Read, 2008). In this interaction plants supply root-associated Glomeromycotina fungi with carbohydrates, essential for fungal survival and growth. In return, the fungi provide their host plants with mineral nutrients and water from the soil (Smith & Read, 2008). One of the key characteristics of the arbuscular mycorrhizal interaction is its low specificity. A mycorrhizal plant typically associates simultaneously with multiple fungi and a mycorrhizal fungus often associates simultaneously with multiple plants. This creates complex underground networks in which plants of different species are linked by shared arbuscular mycorrhizal fungi (Toju *et al.*, 2015; Chen *et al.*, 2017).

Experiments demonstrate that the arbuscular mycorrhizal mutualism is evolutionarily stable because control is bidirectional, and partners offering the best rate of exchange are rewarded (Bever *et al.*, 2009; Kiers *et al.*, 2011). These results suggest that arbuscular mycorrhizal networks consist of ‘fair trade’ interactions, which are akin to trade in a market economy (Kiers *et al.*, 2011; Wyatt *et al.*, 2014). However, the universal importance of these market dynamics in arbuscular mycorrhizal interactions is challenged by the occurrence of cheaters (Selosse & Rousset, 2011). Mycoheterotrophic plants receive not only soil nutrients but also carbon from arbuscular mycorrhizal fungi (Leake, 1994; Merckx, 2013). Hence, mycoheterotrophic plants exploit the arbuscular mycorrhizal symbiosis for nutrients and carbon without being sanctioned by the fungal partner (Walder & Van Der Heijden, 2015). Therefore, it has been suggested that mycoheterotrophic plants may display a truly biotrophic parasitic mode, digesting the fungus colonizing their roots (Imhof *et al.*, 2013). The mechanism underpinning carbon transfer from arbuscular mycorrhizal fungi to mycoheterotrophic plants remains unclear, but it is unlikely that resource exchange in these mycoheterotrophic associations relies on reciprocal dynamics.

Phylogenetic studies demonstrate that these cheaters evolved from mutualistic ancestors (Merckx *et al.*, 2013). Within obligate mutualisms, the critical barrier to mutualism breakdown and to the evolutionary stability of the resulting cheater species is thought to be a requirement for three-species coexistence: a cheater species relies on a mutualistic partner which simultaneously interacts with a mutualistic species (Pellmyr & Leebens-Mack, 1999). In species-rich mutualisms such as the arbuscular mycorrhizal symbiosis, where multi-species coexistence is the rule, a high potential for the occurrence of these tripartite linkages (cheater–fungus–mutualistic plant) is expected (Merckx & Bidartondo, 2008). Indeed, while evolution of cheating in specialized obligate mutualisms is relatively rare (Sachs & Simms, 2006), cheating in the arbuscular mycorrhizal symbiosis evolved in more than a dozen of plant clades, including over 250 species which together cover nearly all tropical and subtropical forests (Gomes *et al.*, 2019a).

Previous work shows that cheaters target a subset of the mycorrhizal fungi available in the local community (e.g Bidartondo *et al.* (2002); Gomes *et al.* (2017a); Sheldrake *et al.* (2017)). Their associated fungal communities can vary in specificity (Merckx *et al.*, 2012), and in communities of co-occurring cheaters their fungal diversity appears to increase proportionally to their fungal overlap (Gomes *et al.*, 2017b). However, which arbuscular mycorrhizal fungi cheaters select from the available community is not well understood. From an evolutionary perspective there are two mechanisms that may be involved: (1) cheaters, because of their increasingly parasitic interaction with fungi, have been ‘denied’ access to some members of the fungal community except for fungal lineages that fail to detect or exclude the cheaters, and (2) cheaters have selected from the potential fungal community the best target to meet their nutrient demands (Bruns *et al.*, 2002; Egger & Hibbett, 2004; Bidartondo, 2005). We address the potential importance of the latter mechanism and hypothesise that mycoheterotrophic plants preferentially associate with ‘keystone’ (Mills & Doak, 1993) fungi that are connected to many different mutualistic plants, since these fungi are potentially more resilient to perturbation (Bascompte & Jordano, 2007) and may be the most reliable source of carbon (Waterman *et al.*, 2013). In addition, since fungal traits play an important role in the arbuscular mycorrhizal symbiosis – phylogenetically related AM fungi (assumed to have similar functional traits), preferentially interact with similar plant species (Chagnon *et al.*, 2015) – we hypothesize that if partner selection in tripartite networks is trait-

driven we will be able to detect an influence of the phylogenetic relationships of the fungi. Here we test these hypotheses on a combined tripartite mycorrhizal network of co-occurring mycoheterotrophic and surrounding mutualistic plants linked by shared arbuscular mycorrhizal fungi compiled by high-throughput DNA sequencing.

Materials and methods

Sampling

Since mycoheterotrophic plants are relatively rare and often have patchy distributions (Gomes *et al.*, 2019b), we sampled two proximate 4 x 4 m plots (plot 1 and plot 2) in a coastal rainforest in French Guiana (5°28'25"N 53°34'51"W) on 28 July 2014, with overlapping mycoheterotrophs as replicates. In both plots, root tips of mycoheterotrophic plants (cheaters) and surrounding autotrophic plants (mutualists) were sampled, cleaned with water, and stored on CTAB buffer at -20°C until further processing. Plot 1 contained the cheater species *Dictyostegia orobanchoides* (3 specimens), *Gymnosiphon breviflorus* (2 specimens), *Voyria aphylla* (3 specimens), *Voyriella parviflora* (7 specimens), and *Soridium spruceanum* (6 specimens). Plot 2 contained *D. orobanchoides* (2 specimens), *G. breviflorus* (5 specimen), *V. parviflora* (15 specimens), and *S. spruceanum* (2 specimens). Around each cheater, 10 root tips of mutualistic plants were collected. In addition, we sampled 5 additional mutualistic plant root tips randomly from each quadrant of each plot aiming to represent the belowground mutualistic community. Root tips of mutualistic plant species were identified by sequencing the genetic markers *matK* or *trnL* as described in (Gomes *et al.*, 2017a). Fungi associated with the roots of mutualist and cheater plants were sequenced as described in Gomes *et al.* (2017b) in two separate runs, following the same bioinformatics methods to process the raw reads from the two runs combined until clustering into 97% Operational Taxonomic Units (OTUs). The OTUs that were represented by less than five reads in each sample were excluded to avoid spurious OTUs (Lindahl *et al.*, 2013). The taxonomical assignment of OTUs was done by querying against the UNITE database (Kõljalg *et al.*, 2013). Because the mycoheterotrophic plants of this study are associated with fungi that belong to the sub-phylum Glomeromycotina (Merckx *et al.*, 2012), we only retained fungal OTUs from this sub-phylum in the subsequent analysis. In this study, we sequenced the fungal

communities of mutualistic and cheater plants, which are partially and totally representative of the entire root systems, respectively, and may vary in the degree of specificity towards their fungal interactions (Yamato *et al.*, 2014; Mennes *et al.*, 2015; Gomes *et al.*, 2017a,b). Thus, because rarifying the OTU matrix has been shown to greatly increase the false positive rate of OTUs per sample (McMurdie & Holmes, 2014), we decided not to rarify the data, and only used incidence data, since our main interest was to determine which interactions can be established and not how abundant they are. Hereafter we refer to OTUs as ‘fungi’. The simultaneous presence of fungi in the roots of both cheater and mutualistic plants does not necessarily reflect that these fungi are functionally active but is indicative of a potential link between plants.

Tripartite network compilation

We built three tripartite networks: (1) the tripartite network of plot 1 with 21 mutualists, 5 cheaters, and 108 fungi; (2) the tripartite network of plot 2 with 16 mutualists, 4 cheaters, and 99 fungi; and (3) the combined tripartite network of plot 1 and plot 2 with 33 plants (28 mutualists and 5 cheaters) and 121 fungi, of which 89 (74%) are shared between mutualists and cheaters. The tripartite network and the corresponding plant-plant interaction strength network including mutualists and cheater plants were visualized with the *igraph* R package (Csardi & Nepusz, 2006). Plot 1 and plot 2 overlapped in 9 mutualists (32%), 4 cheaters (80%), and 86 fungi (71%). In each network, a plant was linked to a fungus if that fungus was found in the roots of that plant. To examine the congruence between the mycorrhizal interactions of plot 1 and plot 2, we calculated the normalized interaction degree of plants and fungi of both tripartite networks using the *bipartite* R package (Dormann *et al.*, 2018). We measured the correlation between the normalized interaction degree of plants and fungi which occur in both plots using the Pearson correlation coefficient.

Phylogenetic signal

For the combined tripartite network, we infer the phylogenetic relationships between the fungi following the strategy of Gomes *et al.* (2017b). Phylogenetic relationships of the plants were derived from the Angiosperm Phylogeny Website (Stevens, 2012). To test the influence

of the evolutionary relationships of the interacting partners in both mutualists and cheater mycorrhizal networks, the phylogenetic signal on each bipartite network was evaluated using an estimated generalized least squares (EGLS) analysis that fits the phylogenetic variance–covariance matrix to the plant-fungi association matrix (Ives & Godfray, 2006). Using this EGLS method, we calculated the independent phylogenetic signals of the plant (d_{plant}) and fungi (d_{fungi}) phylogenies on the binary association matrix and assessed the overall strength of the signal of both phylogenies combined using mean-squared error (MSE) values.

Comparing mutualistic and antagonistic networks

To understand how fungi are preferentially linked to the cheater and mutualistic plants, first we compared the fungal strength association of the individual mutualistic and antagonistic bipartite interactions of the combined tripartite network. The fungal strength association of a pair of fungi represents similarity in their interactions through shared plants. This measure is a proxy for the ecological similarity between fungi. We selected the fungal OTUs shared between both interaction types in the subsequent analysis, and calculated dissimilarity matrices between the fungi linked to mutualistic and cheater plants separately, using two different measures. The first measure was the Jaccard index, and the second was the Overlap measure (Saavedra *et al.*, 2013), which corresponds to the number of shared plants relative to the maximum number of plants that can be shared. We computed the Mantel test correlation between the distance matrices for the mutualistic and antagonistic interactions using the two different measures. Because a single fungal species is often not represented exclusively by an individual OTU, and multiple OTUs can in fact represent a single species (Flynn *et al.*, 2015), we accounted for the phylogenetic relatedness among the fungi: we computed the partial Mantel test correlation between the distance matrices calculated using the Jaccard and Overlap measures, controlling for the phylogenetic relatedness of the shared fungi. The phylogenetic distances among the shared fungi were calculated by pruning the fungal phylogenetic tree to the fungi shared by the mutualistic and antagonistic interaction networks and summing up the total branch lengths using the *ape* R package (Paradis & Schliep, 2019).

Then, we searched for simple motifs in the combined tripartite network (reduced to only include the fungal OTUs shared between both interaction types). Motifs are subgraphs of interconnections that appear significantly more often than expected by chance in complex networks (Milo *et al.*, 2002; Bascompte & Melián, 2005; Stouffer *et al.*, 2007). We aimed to test whether the plants in the mutualistic and antagonistic networks had simultaneous preferential links to specific fungi, or each interaction type was disconnected in terms of their fungal association preferences. Therefore, we selected motifs that translate the simultaneous link of a mutualist and antagonist plants to pairs of fungi (Fig. 1a). We generated null models to evaluate the fidelity of plants in associating with specific fungi (consistency in partner choice). Both datasets have imbalanced number of plant species (5 cheaters and 28 mutualistic plants), and because the nature of the plant-fungus interactions is unknown for each plant type, null models were generated separately for cheater and mutualistic plants. We generated randomly resampled null matrices using the *shuffle.web* method in *bipartite* (Dormann *et al.*, 2009), where each fungal OTU and plant species keeps exactly the same number of interactions as in the observed network, resampling 1000 times. Mutualistic and antagonistic random matrices were combined each time before counting the number of motifs. We compared the empirical motif result to the null model's 95% confidence interval. An empiric result above the null confidence interval indicates that pairs of fungi are shared between particular cheater and mutualistic plants more often than expected by chance, reflecting a simultaneous preferential association to particular fungi between species in the antagonist and mutualist network. An empirical result below the null confidence interval indicates that pairs of fungi are shared between particular cheater and mutualistic plants less often than expected by chance, reflecting separate dynamics and disconnection between antagonist and mutualist networks.

Results

Plant – fungus interactions

In total, we successfully sequenced 145 root tips samples of 28 mutualistic and 5 antagonistic plant species (see Table S1 for detailed species list). From all the root tips analyzed, the Ion Torrent sequencing runs produced 10,453,711 quality filtered reads, of which 368,005 were

classified as Glomeromycotina and represented by 121 OTUs (Fig. 1b). Mutualist plants were associated with 110 fungi (51% of the reads) and cheaters were associated with 101 fungi (49% of reads). The plant-plant interaction strength network is shown in Fig. 1c. Plants belong to 21 different families (20 angiosperms, 1 fern), arbuscular mycorrhizal fungi are part of Gigasporaceae (4 fungi), Acaulosporaceae (14 fungi), and Glomeraceae (103 fungi). We find a strong significant linear correlation between the interaction degree of overlapping fungi of plots 1 and 2 ($R^2 = 0.81$, $p < 0.001$; Fig 2), but not for the overlapping plants ($R^2 = 0.01$, $p = 0.318$; Fig 2). Due to the similarities in fungal interaction strength, the subsequent analyses were performed on the combined tripartite network.

Phylogenetic signal

We measured a low significant phylogenetic signal of the mutualistic plant phylogeny ($d_{\text{plant}} = 0.14$; 95% CI: 0.11 – 0.19) and a strong significant signal of the fungal phylogeny ($d_{\text{fungi}} = 0.34$; 95% CI: 0.29 – 0.40; Fig 3) on the mutualistic interactions of the combined tripartite network. The overall strength of the phylogenetic signal for the linear model fitted to the actual data ($\text{MSE}_d = 0.18$) was closer to that found under the assumption of no phylogenetic covariances ($\text{MSE}_{\text{star}} = 0.18$) than for the assumption of maximum phylogenetic signal ($\text{MSE}_b = 0.28$). We measured a low but significant phylogenetic signal of the cheater phylogeny on the antagonistic network ($d_{\text{plant}} = 1.05 \times 10^{-9}$; 95% CI: 3.85×10^{-14} – 5.96×10^{-4}) and a strong significant signal of the fungal phylogeny on the interactions ($d_{\text{fungi}} = 0.59$; 95% CI: 0.32– 0.85). The overall strength of the phylogenetic signal for the linear model fitted to the actual data ($\text{MSE}_d = 0.25$) was closer to that found under the assumption of no phylogenetic covariances ($\text{MSE}_{\text{star}} = 0.25$) than for the assumption of maximum phylogenetic signal ($\text{MSE}_b = 0.67$).

Comparison between mutualistic and antagonistic plant networks

The mutualists and cheaters shared 89 fungal OTUs. We found a similar fungal association strength between both interaction types (Mantel tests: Jaccard $r = 0.31$, $p = 0.001$; Overlap $r = 0.28$, $p = 0.001$), and also when accounting for the phylogenetic relatedness among the shared fungi (partial Mantel tests: Jaccard $r = 0.29$, $p = 0.001$; Overlap $r = 0.24$, $p = 0.001$). The tripartite network had 23,261 motifs ($z\text{-score} = 2.675$, $p = 0.005$), suggesting that particular

pairs of fungi are being preferentially targeted within the antagonist and mutualistic networks simultaneously.

Discussion

The tripartite networks from the two sampled plots share the majority of their fungi and interestingly, these overlapping fungi have a similar interaction degree in both plots (Fig. 2a). The ability of fungi to interact with many plant species simultaneously therefore seems a taxon-specific characteristic. In contrast, the shared mutualistic plant species differ in fungal richness in each plot (Fig. 2b), indicating that for mutualistic plants the level of specificity may be context dependent, and preferential association with particular arbuscular mycorrhizal fungi may be independent from their identity or position in the interaction network. Alternatively, our sampling method may have failed to identify all fungi linked to each plant.

In our study, the cheater plant community is highly connected to arbuscular mycorrhizal fungi, interacting with 74% of the available fungi. This is congruent with previous studies which found communities of co-occurring cheaters of the same families from tropical America to associate with a diverse array of fungi (Gomes *et al.*, 2017b; Sheldrake *et al.*, 2017). Within the combined network, the four most generalist fungi of the mutualistic interaction network, representing 13% of all mutualistic interactions, were also among the highest-ranking generalists in the antagonistic network (Fig. 2). In fact, these Glomeraceae fungi were found to associate with all cheater species (Fig. 3). Glomeraceae are usually not only the most dominant clade in natural arbuscular mycorrhizal communities, often accounting for c. 70% of all species (Montesinos-Navarro *et al.*, 2012), but they also have consistently been found to include the most generalist arbuscular mycorrhizal fungi in other network studies (e.g. Montesinos-Navarro *et al.*, 2012; Chagnon *et al.*, 2015; Chen *et al.*, 2017). The ability to interact with many mutualistic plant species can be a potential reason for why cheaters generally target Glomeraceae fungi (Merckx *et al.*, 2012; Renny *et al.*, 2017). Ecological theory predicts that generalist species tend to have large distribution ranges (Brown, 1984) and, consequently, are less vulnerable to (local) extinction than specialized species (Schleuning *et al.*, 2016). Therefore, associations to generalist fungi may be advantageous for the evolutionary persistence of cheaters. In addition, in the context of mycorrhizal fungi, which

can be linked to different plant species simultaneously, generalist fungi are likely to be more reliable carbon sources for cheaters. Associations with multiple plant partners may increase fungal resilience to disturbance, and mediate temporal fluctuations in carbon flow and interaction dynamics (Bennett *et al.*, 2013) and thus guarantee continuous carbon supply to the cheaters, without pronounced negative effects on particular fungal hosts. An alternative and perhaps not mutually exclusive explanation for why cheaters preferentially target well-connected fungi may be that these fungi are less effective in detecting and excluding non-mutualistic plant partners (van der Heijden & Walder, 2016).

Within Glomeraceae, the most generalist fungi are part of multiple distantly related clades, belonging to different species in at least two distinct genera (*Rhizophagus* and *Glomus*; Fig. 3), potentially representing distinctive functional traits (Chagnon *et al.*, 2015). The tendency of plants to simultaneously associate with distantly-related fungi may reflect functional trait complementarity among co-occurring species (Maherali & Klironomos, 2007). Overall, we measured a significant influence of the fungal phylogenetic relationships on both the mutualistic and antagonistic interactions, showing that closely related fungi interact with similar mutualistic and cheater plants respectively. We detected a lower but significant phylogenetic signal of the mutualistic plants on the mutualistic network, while the phylogenetic signal of the cheater plants of the antagonistic interactions was near zero. The latter likely results from the fact that all cheater plants mainly target similar Glomeraceae fungi. These results reinforce the hypothesis that partner selection in the arbuscular mycorrhizal symbiosis is driven by fungal traits (Chagnon *et al.*, 2015), also for antagonistic interactions.

The positive and statistically significant correlation between the ecological distances of the fungi in the antagonistic and mutualistic networks strongly suggests that fungi that are targeted by the same cheaters tend to interact with a similar set of mutualistic plants species. This result is not the consequence of the phylogenetic signal of the fungi on the interactions reported above as shown by the partial mantel test. Similarly, the motif analysis demonstrates that pairs of fungi that share an interaction with a cheater species have a high chance to also share a link with a particular mutualistic plant. These results indicate that antagonistic and mutualistic mycorrhizal networks are not linked randomly but are symmetrically influenced

by mutualistic and cheater plant identity. Whether this is the consequence of spatial patterns in the distribution of mutualists, cheaters, and fungi remains to be determined. Alternatively, the detected pattern may reflect species-specific association preferences. The plant interaction strength network indicates that each cheater species is linked to several mutualistic plants through shared fungi, and each cheater species tends to be closely connected to a different mutualistic plant (Fig. 1c). Earlier work suggested that antagonistic mycorrhizal interactions can be better explained by understanding cheater–cheater interactions generated by sharing fungal associations (Gomes *et al.*, 2017b), and indicate that antagonistic interactions may respond to an ecological mechanism driven by maximizing co-occurrence and avoiding competitive exclusion among cheaters. The results obtained here expand this view and suggest that not only cheater-cheater interactions drive patterns of co-occurrence of cheater plants, but also the indirect interactions between mutualistic and cheater plants.

Conclusions

This is the first study assessing how plant cheaters are embedded in mutualistic mycorrhizal networks. We find that arbuscular mycorrhizal cheater plants simultaneously interact with a wide range of fungal partners, but generally prefer those that are well-connected to mutualistic plants. The phylogenetic relationships between the fungi, likely a proxy for fungal traits, have a significant influence on these non-random tripartite interactions. We also detect that the antagonistic and mutualistic and mutualistic networks are not linked randomly. Instead, sets of cheaters plants target fungi that are simultaneously linked to similar mutualistic plants. Therefore, we conclude that the persistence of cheaters in arbuscular mycorrhizal networks is dependent on well-connected ‘keystone’ mycorrhizal fungi. The finding that well-connect nodes in arbuscular mycorrhizal networks are more prone to cheating remains to be tested in other diffuse mutualisms.

Acknowledgements

This research was supported by a Veni grant from the Netherlands Organisation of Scientific Research (NWO) to V.S.F.T.M. (863.11.018) and field work was funded by the Royal Academy

of Arts and Sciences of the Netherlands (KNAW) Ecology fund, grant J1606/Eco/G437 to SIFG.
JB is supported by the Swiss National Science Foundation (Grant 31003A-169671 3).

References

- Bascompte J, Jordano P. 2007.** Plant-Animal Mutualistic Networks: The Architecture of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **38**: 567+593.
- Bascompte J, Jordano P. 2013.** *Mutualistic networks*. Princeton: Princeton University Press.
- Bascompte J, Melián CJ. 2005.** Simple trophic modules for complex food webs. *Ecology* **86**: 2868–2873.
- Bennett AE, Daniell TJ, Öpik M, Davison J, Moora M, Zobel M, Selosse MA, Evans D. 2013.** Arbuscular mycorrhizal fungal networks vary throughout the growing season and between successional stages. *PLoS ONE* **8**.
- Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009.** Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12**: 13–21.
- Bidartondo MI. 2005.** The evolutionary ecology of myco-heterotrophy. *New Phytologist* **167**: 335–352.
- Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Domínguez L, Sérsic A, Leake JR, Read DJ. 2002.** Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* **419**: 389–392.
- Brown JH. 1984.** On the Relationship between Abundance and Distribution of Species. *The American Naturalist* **124**: 255–279.
- Bruns TD, Bidartondo MI, Taylor DL. 2002.** Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integrative and comparative biology* **42**: 352–359.
- Chagnon PL, Bradley RL, Klironomos JN. 2015.** Trait-based partner selection drives mycorrhizal network assembly. *Oikos* **124**.
- Chen L, Zheng Y, Gao C, Mi XC, Ma KP, Wubet T, Guo LD. 2017.** Phylogenetic relatedness explains highly interconnected and nested symbiotic networks of woody plants and arbuscular mycorrhizal fungi in a Chinese subtropical forest. *Molecular Ecology* **26**: 2563–2575.
- Csardi G, Nepusz T. 2006.** The igraph software package for complex network research. *InterJournal Complex Systems*: 1695. <http://igraph.org>.

- Dormann C, Fruend J, Gruber B. 2009.** Indices, graphs and null models: analysing bipartite ecological networks. *The Open Ecology Journal* **2**: 7–24.
- Dormann CF, Gruber B, Fruend J. 2018.** Introducing the bipartite Package: Analysing Ecological Networks. *R news* **8**: 8–11.
- Egger KN, Hibbett DS. 2004.** The evolutionary implications of exploitation in mycorrhizas. *Canadian Journal of Botany* **82**: 1110–1121.
- Flynn JM, Brown EA, Chain FJJ, Macisaac HJ, Cristescu ME. 2015.** Toward accurate molecular identification of species in complex environmental samples: Testing the performance of sequence filtering and clustering methods. *Ecology and Evolution*.
- Gomes SIF, Aguirre-Gutiérrez J, Bidartondo MI, Merckx VSFT. 2017a.** Arbuscular mycorrhizal interactions of mycoheterotrophic *Thismia* are more specialized than in autotrophic plants. *New Phytologist* **213**: 1418–1427.
- Gomes SIF, van Bodegom PM, Merckx VSFT, Soudzilovskaia NA. 2019a.** Global distribution patterns of mycoheterotrophy. *Global Ecology and Biogeography*: 1–13.
- Gomes SIF, van Bodegom PM, Merckx VSFT, Soudzilovskaia NA. 2019b.** Environmental drivers for cheaters of arbuscular mycorrhizal symbiosis in tropical rainforests. *New Phytologist* **223**: 1575–1583.
- Gomes SIF, Merckx VSFT, Saavedra S. 2017b.** Fungal-host diversity among mycoheterotrophic plants increases proportionally to their fungal-host overlap. *Ecology and Evolution* **7**: 3623–3630.
- van der Heijden MGA, Walder F. 2016.** Reply to ‘Misconceptions on the application of biological market theory to the mycorrhizal symbiosis’. *Nature Plants* **2**: 16062.
- Imhof S, Massicotte HB, Melville LH, Peterson RL. 2013.** Subterranean morphology and mycorrhizal structures. In: *Mycoheterotrophy: The Biology of Plants Living on Fungi*. 157–214.
- Ives AR, Godfray HC. 2006.** Phylogenetic analysis of trophic associations. *The American naturalist* **168**: E1–14.
- Kiers ET, Duhamel M, Beesetty Y, Mensah J a, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk G a, Hart MM, Bago A, et al. 2011.** Reciprocal Rewards Stabilize in the Mycorrhizal Symbiosis. *Science* **333**: 880–882.
- Köljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, et al. 2013.** Towards a unified paradigm for sequence-

based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Leake JR. 1994. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist* **127**: 171–216.

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjølner R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, et al. 2013. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytologist* **199**: 288–299.

Maherali H, Klironomos JN. 2007. Influence of Phylogeny on Fungal Community Assembly and Ecosystem Functioning. *Science* **316**: 1746–1748.

McMurdie PJ, Holmes S. 2014. Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Computational Biology*.

Mennes CB, Moerland MS, Rath M, Smets EF, Merckx VSFT. 2015. Evolution of mycoheterotrophy in Polygalaceae: the case of *Epirixanthes*. *American Journal of Botany* **102**: 598–608.

Merckx VSFT. 2013. *Mycoheterotrophy: The biology of plants living on fungi*. New York, NY, USA: Springer.

Merckx V, Bidartondo MI. 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. *Proceedings of the Royal Society B* **275**: 1029–1035.

Merckx VSFT, Janssens SB, Hynson NA, Specht CD, Bruns TD, Smets EF. 2012. Mycoheterotrophic interactions are not limited to a narrow phylogenetic range of arbuscular mycorrhizal fungi. *Molecular Ecology* **21**: 1524–1532.

Merckx VSFT, Mennes CB, Peay KG, Geml J. 2013. Evolution and diversification. In: *Mycoheterotrophy: The Biology of Plants Living on Fungi*.

Mills LS, Doak DF. 1993. The Keystone-Species Concept in Ecology and Conservation. *BioScience* **43**: 219–224.

Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. 2002. Network motifs: Simple building blocks of complex networks. *Science* **298**: 824–827.

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M. 2012. The network structure of plant-arbuscular mycorrhizal fungi. *New Phytologist* **194**: 536–547.

Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics (Oxford, England)* **35**: 526–528.

Pellmyr O, Leebens-Mack J. 1999. Forty million years of mutualism: Evidence for eocene origin of the yucca-yucca moth association. *Proceedings of the National Academy of*

450 *Sciences of the United States of America* **96**: 9178–9183.

451 **Renny M, Acosta MC, Cofré N, Domínguez LS, Bidartondo MI, Sérsic AN. 2017.** Genetic
 452 diversity patterns of arbuscular mycorrhizal fungi associated with the mycoheterotroph
 453 *Arachnitis uniflora* Phil. (Corsiaceae). *Annals of Botany* **119**: 1279–1294.

454 **Saavedra S, Rohr RP, Dakos V, Bascompte J. 2013.** Estimating the tolerance of species to
 455 the effects of global environmental change. *Nature Communications* **4**.

456 **Sachs JL, Simms EL. 2006.** Pathways to mutualism breakdown. *Trends in Ecology and*
 457 *Evolution* **21**: 585–592.

458 **Schleuning M, Fründ J, Schweiger O, Welk E, Albrecht J, Albrecht M, Beil M, Benadi G,**
 459 **Blüthgen N, Bruehlheide H, et al. 2016.** Ecological networks are more sensitive to plant than
 460 to animal extinction under climate change. *Nature Communications* **7**: 13965.

461 **Selosse MA, Rousset F. 2011.** The plant-fungal marketplace. *Science* **333**: 828–829.

462 **Sheldrake M, Rosenstock NP, Revillini D, Olsson PA, Wright SJ, Turner BL. 2017.** A
 463 phosphorus threshold for mycoheterotrophic plants in tropical forests. *Proceedings of the*
 464 *Royal Society B: Biological Sciences* **284**: 20162093.

465 **Smith SE, Read DJ. 2008.** *Mycorrhizal Symbiosis*. Cambridge: Academic Press, San Diego, CA.

466 **Stevens PF. 2012.** Angiosperm Phylogeny Website. *Version 12, July 2012*.

467 **Stouffer DB, Camacho J, Jiang W, Amaral LAN. 2007.** Evidence for the existence of a robust
 468 pattern of prey selection in food webs. *Proceedings of the Royal Society B: Biological*
 469 *Sciences* **274**: 1931–1940.

470 **Thompson JN. 2005.** *The Geographic Mosaic of Coevolution*. University of Chicago Press,
 471 Chicago.

472 **Toju H, Guimaraes PR, Olesen JM, Thompson JN. 2015.** Below-ground plant-fungus network
 473 topology is not congruent with above-ground plant-animal network topology. *Science*
 474 *Advances* **1**: e1500291–e1500291.

475 **Walder F, Van Der Heijden MGA. 2015.** Regulation of resource exchange in the arbuscular
 476 mycorrhizal symbiosis. *Nature Plants* **1**.

477 **Waterman RJ, Klooster MR, Hentrich H, Bidartondo MI. 2013.** Species interactions of
 478 mycoheterotrophic plants: Specialization and its potential consequences. In:
 479 *Mycoheterotrophy: The Biology of Plants Living on Fungi*. 267–296.

480 **Wyatt GAK, Toby Kiers E, Gardner A, West SA. 2014.** A biological market analysis of the
 481 plant-mycorrhizal symbiosis. *Evolution* **68**: 2603–2618.

482 **Yamato M, Ogura-Tsujita Y, Takahashi H, Yukawa T. 2014.** Significant difference in
 483 mycorrhizal specificity between an autotrophic and its sister mycoheterotrophic plant
 484 species of Petrosaviaceae. *Journal of Plant Research* **127**: 685–693.

485

486

Figures

Figure 1. Tripartite arbuscular mycorrhizal interactions. (a) Motif depicts pairs of fungi simultaneously linked to a cheater and a mutualistic plant. This motif appears significantly more often than expected by chance in the empirical network; (b) Visualization of the combined tripartite network between cheaters (yellow), arbuscular mycorrhizal fungi (grey), and mutualistic plants (green), where lines represent a connection between a plant and a fungus; (c) The plant-plant interaction strength network, in which lines represent a link through a shared arbuscular mycorrhizal fungus. The thickness of the lines represents the interaction strength between the plants (the thicker, the more fungi are shared). Yellow lines link cheater to mutualistic plants; light grey lines link mutualistic to mutualistic plants; and dark grey lines link cheater to cheater plants. The closer plants are together in the graph, the higher their interactions strength.

Figure 2. Comparison of interaction degree of taxa shared between plot 1 and plot 2. (a) Shared arbuscular mycorrhizal fungi (n = 86) show a strong significant linear correlation in their interaction degree of plot 1 and plot 2; (b) Shared plants do not have similar interaction degrees in the plots. Grey dots represent the 9 shared mutualistic plants and yellow dots represent the 4 shared cheater plants.

Figure 3: Plant-fungus interactions of mutualistic (green rectangles) and cheater (yellow rectangles) plants. Phylogenetic relationships between the fungi are shown on top. The intensity of the green dots on the tips of the fungal phylogeny represent the interaction degree of each fungus in the total network. Plant species are listed on the left, hierarchy clustered dendrogram based on the Bray-Curtis distance of their fungal communities is shown on the right.