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1	FULL PAPER
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3	Plant cheaters preferentially target arbuscular mycorrhizal fungi that are highly connected
4	to mutualistic plants
5	
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15 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.

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36 Introduction

37

Since the early history of life, interspecific mutualisms have been paramount in the 38 39 functioning of ecosystems (Thompson, 2005; Bascompte & Jordano, 2013). Mutualisms can 40 form complex networks of interdependence between dozens or even hundreds of species. A 41 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 42 43 interaction plants supply root-associated Glomeromycotina fungi with carbohydrates, 44 essential for fungal survival and growth. In return, the fungi provide their host plants with 45 mineral nutrients and water from the soil (Smith & Read, 2008). One of the key characteristics 46 of the arbuscular mycorrhizal interaction is its low specificity. A mycorrhizal plant typically associates simultaneously with multiple fungi and a mycorrhizal fungus often associates 47 48 simultaneously with multiple plants. This creates complex underground networks in which 49 plants of different species are linked by shared arbuscular mycorrhizal fungi (Toju et al., 2015; 50 Chen et al., 2017).

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52 Experiments demonstrate that the arbuscular mycorrhizal mutualism is evolutionarily stable 53 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 54 networks consist of 'fair trade' interactions, which are akin to trade in a market economy 55 56 (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 57 58 (Selosse & Rousset, 2011). Mycoheterotrophic plants receive not only soil nutrients but also 59 carbon from arbuscular mycorrhizal fungi (Leake, 1994; Merckx, 2013). Hence, 60 mycoheterotrophic plants exploit the arbuscular mycorrhizal symbiosis for nutrients and carbon without being sanctioned by the fungal partner (Walder & Van Der Heijden, 2015). 61 62 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 63 mechanism underpinning carbon transfer from arbuscular mycorrhizal fungi to 64 mycoheterotrophic plants remains unclear, but it is unlikely that resource exchange in these 65 mycoheterotrophic associations relies on reciprocal dynamics. 66

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68 Phylogenetic studies demonstrate that these cheaters evolved from mutualistic ancestors 69 (Merckx et al., 2013). Within obligate mutualisms, the critical barrier to mutualism breakdown 70 and to the evolutionary stability of the resulting cheater species is thought to be a 71 requirement for three-species coexistence: a cheater species relies on a mutualistic partner 72 which simultaneously interacts with a mutualistic species (Pellmyr & Leebens-Mack, 1999). In 73 species-rich mutualisms such as the arbuscular mycorrhizal symbiosis, where multi-species 74 coexistence is the rule, a high potential for the occurrence of these tripartite linkages 75 (cheater-fungus-mutualistic plant) is expected (Merckx & Bidartondo, 2008). Indeed, while 76 evolution of cheating in specialized obligate mutualisms is relatively rare (Sachs & Simms, 77 2006), cheating in the arbuscular mycorrhizal symbiosis evolved in more than a dozen of plant 78 clades, including over 250 species which together cover nearly all tropical and subtropical 79 forests (Gomes et al., 2019a).

80

81 Previous work shows that cheaters target a subset of the mycorrhizal fungi available in the 82 local community (e.g Bidartondo et al. (2002); Gomes et al. (2017a); Sheldrake et al. (2017)). 83 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 84 85 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 86 87 an evolutionary perspective there are two mechanisms that may be involved: (1) cheaters, 88 because of their increasingly parasitic interaction with fungi, have been 'denied' access to 89 some members of the fungal community except for fungal lineages that fail to detect or 90 exclude the cheaters, and (2) cheaters have selected from the potential fungal community 91 the best target to meet their nutrient demands (Bruns et al., 2002; Egger & Hibbett, 2004; 92 Bidartondo, 2005). We address the potential importance of the latter mechanism and 93 hypothesise that mycoheterotrophic plants preferentially associate with 'keystone' (Mills & 94 Doak, 1993) fungi that are connected to many different mutualistic plants, since these fungi 95 are potentially more resilient to perturbance (Bascompte & Jordano, 2007) and may be the 96 most reliable source of carbon (Waterman *et al.*, 2013). In addition, since fungal traits play an 97 important role in the arbuscular mycorrhizal symbiosis – phylogenetically related AM fungi (assumed to have similar functional traits), preferentially interact with similar plant species 98 99 (Chagnon et al., 2015) – we hypothesize that if partner selection in tripartite networks is trait100 driven we will be able to detect an influence of the phylogenetic relationships of the fungi.

101 Here we test these hypotheses on a combined tripartite mycorrhizal network of co-occurring

mycoheterotrophic and surrounding mutualistic plants linked by shared arbuscularmycorrhizal fungi compiled by high-throughput DNA sequencing.

104

105 Materials and methods

- 106
- 107 Sampling
- 108

109 Since mycoheterotrophic plants are relatively rare and often have patchy distributions 110 (Gomes et al., 2019b), we sampled two proximate 4 x 4 m plots (plot 1 and plot 2) in a coastal 111 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 112 113 (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 114 115 cheater species Dictyostega orobanchoides (3 specimens), Gymnosiphon breviflorus (2 specimens) Voyria aphylla (3 specimens), Voyriella parviflora (7 specimens), and Soridium 116 117 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 118 119 cheater, 10 root tips of mutualistic plants where collected. In addition, we sampled 5 120 additional mutualistic plant root tips randomly from each quadrant of each plot aiming to 121 represent the belowground mutualistic community. Root tips of mutualistic plant species 122 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 123 124 described in Gomes et al. (2017b) in two separate runs, following the same bioinformatics 125 methods to process the raw reads from the two runs combined until clustering into 97% 126 Operational Taxonomic Units (OTUs). The OTUs that were represented by less than five reads 127 in each sample were excluded to avoid spurious OTUs (Lindahl et al., 2013). The taxonomical 128 assignment of OTUs was done by querying against the UNITE database (Kõljalg et al., 2013). 129 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 130 131 this sub-phylum in the subsequent analysis. In this study, we sequenced the fungal 132 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 133 their fungal interactions (Yamato et al., 2014; Mennes et al., 2015; Gomes et al., 2017a,b). 134 135 Thus, because rarifying the OTU matrix has been shown to greatly increase the false positive 136 rate of OTUs per sample (McMurdie & Holmes, 2014), we decided not to rarify the data, and 137 only used incidence data, since our main interest was to determine which interactions can be 138 established and not how abundant they are. Hereafter we refer to OTUs as 'fungi'. The 139 simultaneous presence of fungi in the roots of both cheater and mutualistic plants does not 140 necessarily reflect that these fungi are functionally active but is indicative of a potential link 141 between plants.

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143 Tripartite network compilation

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145 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, 146 147 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 148 149 and cheaters. The tripartite network and the corresponding plant-plant interaction strength 150 network including mutualists and cheater plants were visualized with the *igraph* R package 151 (Csardi & Nepusz, 2006). Plot 1 and plot 2 overlapped in 9 mutualists (32%), 4 cheaters (80%), 152 and 86 fungi (71%). In each network, a plant was linked to a fungus if that fungus was found 153 in the roots of that plant. To examine the congruence between the mycorrhizal interactions 154 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 155 156 the correlation between the normalized interaction degree of plants and fungi which occur in 157 both plots using the Pearson correlation coefficient.

158

159 Phylogenetic signal

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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.

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172 Comparing mutualistic and antagonistic networks

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174 To understand how fungi are preferentially linked to the cheater and mutualistic plants, first 175 we compared the fungal strength association of the individual mutualistic and antagonistic 176 bipartite interactions of the combined tripartite network. The fungal strength association of 177 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 178 179 between both interaction types in the subsequent analysis, and calculated dissimilarity 180 matrices between the fungi linked to mutualistic and cheater plants separately, using two 181 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 182 183 to the maximum number of plants that can be shared. We computed the Mantel test 184 correlation between the distance matrices for the mutualistic and antagonistic interactions 185 using the two different measures. Because a single fungal species is often not represented 186 exclusively by an individual OTU, and multiple OTUs can in fact represent a single species 187 (Flynn et al., 2015), we accounted for the phylogenetic relatedness among the fungi: we 188 computed the partial Mantel test correlation between the distance matrices calculated using 189 the Jaccard and Overlap measures, controlling for the phylogenetic relatedness of the shared 190 fungi. The phylogenetic distances among the shared fungi were calculated by pruning the 191 fungal phylogenetic tree to the fungi shared by the mutualistic and antagonistic interaction 192 networks and summing up the total branch lengths using the ape R package (Paradis & 193 Schliep, 2019).

195 Then, we searched for simple motifs in the combined tripartite network (reduced to only 196 include the fungal OTUs shared between both interaction types). Motifs are subgraphs of 197 interconnections that appear significantly more often than expected by chance in complex 198 networks (Milo et al., 2002; Bascompte & Melián, 2005; Stouffer et al., 2007). We aimed to 199 test whether the plants in the mutualistic and antagonistic networks had simultaneous 200 preferential links to specific fungi, or each interaction type was disconnected in terms of their 201 fungal association preferences. Therefore, we selected motifs that translate the simultaneous 202 link of a mutualist and antagonist plants to pairs of fungi (Fig. 1a). We generated null models 203 to evaluate the fidelity of plants in associating with specific fungi (consistency in partner 204 choice). Both datasets have imbalanced number of plant species (5 cheaters and 28 205 mutualistic plants), and because the nature of the plant-fungus interactions is unknown for 206 each plant type, null models were generated separately for cheater and mutualistic plants. 207 We generated randomly resampled null matrices using the *shuffle.web* method in *bipartite* 208 (Dormann et al., 2009), where each fungal OTU and plant species keeps exactly the same 209 number of interactions as in the observed network, resampling 1000 times. Mutualistic and 210 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. 211 212 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, 213 214 reflecting a simultaneous preferential association to particular fungi between species in the 215 antagonist and mutualist network. An empirical result below the null confidence interval 216 indicates that pairs of fungi are shared between particular cheater and mutualistic plants less 217 often than expected by chance, reflecting separate dynamics and disconnection between antagonist and mutualist networks. 218

- 219
- 220 Results

221

- 222 Plant fungus interactions
- 223

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 227 228 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 229 230 to 21 different families (20 angiosperms, 1 fern), arbuscular mycorrhizal fungi are part of 231 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 232 plots 1 and 2 ($R^2 = 0.81$, p < 0.001; Fig 2), but not for the overlapping plants ($R^2 = 0.01$, p =233 234 0.318; Fig 2). Due to the similarities in fungal interaction strength, the subsequent analyses 235 were performed on the combined tripartite network.

236

237 Phylogenetic signal

238

239 We measured a low significant phylogenetic signal of the mutualistic plant phylogeny ($d_{plant} =$ 240 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. 241 242 The overall strength of the phylogenetic signal for the linear model fitted to the actual data $(MSE_d = 0.18)$ was closer to that found under the assumption of no phylogenetic covariances 243 244 (MSE_{star} = 0.18) than for the assumption of maximum phylogenetic signal (MSE_b = 0.28). We measured a low but significant phylogenetic signal of the cheater phylogeny on the 245 antagonistic network ($d_{plant} = 1.05 \times 10^{-9}$; 95% CI: 3.85 x $10^{-14} - 5.96 \times 10^{-4}$) and a strong 246 247 significant signal of the fungal phylogeny on the interactions ($d_{\text{fungi}} = 0.59$; 95% CI: 0.32– 0.85). 248 The overall strength of the phylogenetic signal for the linear model fitted to the actual data 249 $(MSE_d = 0.25)$ was closer to that found under the assumption of no phylogenetic covariances (MSE_{star} = 0.25) than for the assumption of maximum phylogenetic signal (MSE_b = 0.67). 250

251

252 Comparison between mutualistic and antagonistic plant networks

253

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-score* = 2.675, p = 0.005), suggesting that particular pairs of fungi are being preferentially targeted within the antagonist and mutualistic networkssimultaneously.

261

262 Discussion

263

264 The tripartite networks from the two sampled plots share the majority of their fungi and 265 interestingly, these overlapping fungi have a similar interaction degree in both plots (Fig. 2a). 266 The ability of fungi to interact with many plant species simultaneously therefore seems a 267 taxon-specific characteristic. In contrast, the shared mutualistic plant species differ in fungal 268 richness in each plot (Fig. 2b), indicating that for mutualistic plants the level of specificity may 269 be context dependent, and preferential association with particular arbuscular mycorrhizal fungi may be independent from their identity or position in the interaction network. 270 271 Alternatively, our sampling method may have failed to identify all fungi linked to each plant.

272

273 In our study, the cheater plant community is highly connected to arbuscular mycorrhizal fungi, 274 interacting with 74% of the available fungi. This is congruent with previous studies which 275 found communities of co-occurring cheaters of the same families from tropical America to 276 associate with a diverse array of fungi (Gomes et al., 2017b; Sheldrake et al., 2017). Within 277 the combined network, the four most generalist fungi of the mutualistic interaction network, 278 representing 13% of all mutualistic interactions, were also among the highest-ranking 279 generalists in the antagonistic network (Fig. 2). In fact, these Glomeraceae fungi were found 280 to associate with all cheater species (Fig. 3). Glomeraceae are usually not only the most 281 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 282 283 to include the most generalist arbuscular mycorrhizal fungi in other network studies (e.g. 284 Montesinos-Navarro et al., 2012; Chagnon et al., 2015; Chen et al., 2017). The ability to 285 interact with many mutualistic plant species can be a potential reason for why cheaters 286 generally target Glomeraceae fungi (Merckx et al., 2012; Renny et al., 2017). Ecological theory 287 predicts that generalist species tend to have large distribution ranges (Brown, 1984) and, 288 consequently, are less vulnerable to (local) extinction than specialized species (Schleuning et al., 2016). Therefore, associations to generalist fungi may be advantageous for the 289 290 evolutionary persistence of cheaters. In addition, in the context of mycorrhizal fungi, which 291 can be linked to different plant species simultaneously, generalist fungi are likely to be more 292 reliable carbon sources for cheaters. Associations with multiple plant partners may increase fungal resilience to disturbance, and mediate temporal fluctuations in carbon flow and 293 294 interaction dynamics (Bennett et al., 2013) and thus guarantee continuous carbon supply to 295 the cheaters, without pronounced negative effects on particular fungal hosts. An alternative 296 and perhaps not mutually exclusive explanation for why cheaters preferentially target well-297 connected fungi may be that these fungi are less effective in detecting and excluding non-298 mutualistic plant partners (van der Heijden & Walder, 2016).

299

300 Within Glomeraceae, the most generalist fungi are part of multiple distantly related clades, 301 belonging to different species in at least two distinct genera (*Rhizophagus* and *Glomus*; Fig. 302 3), potentially representing distinctive functional traits (Chagnon *et al.*, 2015). The tendency 303 of plants to simultaneously associate with distantly-related fungi may reflect functional trait 304 complementarity among co-occurring species (Maherali & Klironomos, 2007). Overall, we 305 measured a significant influence of the fungal phylogenetic relationships on both the 306 mutualistic and antagonistic interactions, showing that closely related fungi interact with 307 similar mutualistic and cheater plants respectively. We detected a lower but significant 308 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 309 310 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 311 312 mycorrhizal symbiosis is driven by fungal traits (Chagnon *et al.*, 2015), also for antagonistic 313 interactions.

314

315 The positive and statistically significant correlation between the ecological distances of the 316 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. 317 318 This result is not the consequence of the phylogenetic signal of the fungi on the interactions 319 reported above as shown by the partial mantel test. Similarly, the motif analysis demonstrates 320 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 321 322 mutualistic mycorrhizal networks are not linked randomly but are symmetrically influenced

323 by mutualistic and cheater plant identity. Whether this is the consequence of spatial patterns 324 in the distribution of mutualists, cheaters, and fungi remains to be determined. Alternatively, the detected pattern may reflect species-specific association preferences. The plant 325 326 interaction strength network indicates that each cheater species is linked to several 327 mutualistic plants through shared fungi, and each cheater species tends to be closely 328 connected to a different mutualistic plant (Fig. 1c). Earlier work suggested that antagonistic mycorrhizal interactions can be better explained by understanding cheater-cheater 329 330 interactions generated by sharing fungal associations (Gomes et al., 2017b), and indicate that 331 antagonistic interactions may respond to an ecological mechanism driven by maximizing co-332 occurrence and avoiding competitive exclusion among cheaters. The results obtained here 333 expand this view and suggest that not only cheater-cheater interactions drive patterns of co-334 occurrence of cheater plants, but also the indirect interactions between mutualistic and cheater plants. 335

336

337 Conclusions

338 This is the first study assessing how plant cheaters are embedded in mutualistic mycorrhizal 339 networks. We find that arbuscular mycorrhizal cheater plants simultaneously interact with a 340 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 341 342 traits, have a significant influence on these non-random tripartite interactions. We also detect 343 that the antagonistic and mutualistic and mutualistic networks are not linked randomly. 344 Instead, sets of cheaters plants target fungi that are simultaneously linked to similar 345 mutualistic plants. Therefore, we conclude that the persistence of cheaters in arbuscular mycorrhizal networks is dependent on well-connected 'keystone' mycorrhizal fungi. The 346 347 finding that well-connect nodes in arbuscular mycorrhizal networks are more prone to 348 cheating remains to be tested in other diffuse mutualisms.

349

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351

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357 **References**

- 358 Bascompte J, Jordano P. 2007. Plant-Animal Mutualistic Networks: The Architecture of
- Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **38**: 567+593.
- 360 **Bascompte J, Jordano P. 2013**. *Mutualistic networks*. Princeton: Princeton University Press.
- 361 **Bascompte J, Melián CJCJ. 2005**. Simple trophic modules for complex food webs. *Ecology*
- **86**: 2868–2873.
- 363 Bennett AE, Daniell TJ, Öpik M, Davison J, Moora M, Zobel M, Selosse MA, Evans D. 2013.
- 364 Arbuscular mycorrhizal fungal networks vary throughout the growing season and between
- 365 successional stages. *PLoS ONE* **8**.
- 366 Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009. Preferential allocation
- to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology*
- 368 *Letters* **12**: 13–21.
- 369 Bidartondo MI. 2005. The evolutionary ecology of myco-heterotrophy. *New Phytologist*370 167: 335–352.
- 371 Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Domínguez L, Sérsic A, Leake JR,
- 372 Read DJ. 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* 419:
 373 389–392.
- Brown JH. 1984. On the Relationship between Abundance and Distribution of Species. *The American Naturalist* 124: 255–279.
- 376 **Bruns TD, Bidartondo MI, Taylor DL. 2002**. Host specificity in ectomycorrhizal communities:
- 377 what do the exceptions tell us? *Integrative and comparative biology* **42**: 352–359.
- 378 Chagnon PL, Bradley RL, Klironomos JN. 2015. Trait-based partner selection drives
- 379 mycorrhizal network assembly. *Oikos* **124**.
- 380 Chen L, Zheng Y, Gao C, Mi XC, Ma KP, Wubet T, Guo LD. 2017. Phylogenetic relatedness
- 381 explains highly interconnected and nested symbiotic networks of woody plants and
- arbuscular mycorrhizal fungi in a Chinese subtropical forest. *Molecular Ecology* 26: 2563–
- 383 2575.
- 384 **Csardi G, Nepusz T. 2006**. The igraph software package for complex network research.
- 385 InterJournal Complex Systems: 1695. http://igraph.org.

- 386 **Dormann C, Fruend J, Gruber B**. 2009. Indices, graphs and null models: analysing bipartite
- 387 ecological networks. *The Open Ecology Journal* **2**: 7–24.
- 388 Dormann CF, Gruber B, Fruend J. 2018. Introducing the bipartite Package: Analysing
- 389 Ecological Networks. *R news* **8**: 8–11.
- 390 Egger KN, Hibbett DS. 2004. The evolutionary implications of exploitation in mycorrhizas.
- 391 *Canadian Journal of Botany* **82**: 1110–1121.
- 392 Flynn JM, Brown EA, Chain FJJ, Macisaac HJ, Cristescu ME. 2015. Toward accurate
- 393 molecular identification of species in complex environmental samples: Testing the
- 394 performance of sequence filtering and clustering methods. *Ecology and Evolution*.
- 395 Gomes SIF, Aguirre-Gutiérrez J, Bidartondo MI, Merckx VSFT. 2017a. Arbuscular
- 396 mycorrhizal interactions of mycoheterotrophic Thismia are more specialized than in
- autotrophic plants. *New Phytologist* **213**: 1418–1427.
- 398 Gomes SIF, van Bodegom PM, Merckx VSFT, Soudzilovskaia NA. 2019a. Global distribution
- 399 patterns of mycoheterotrophy. *Global Ecology and Biogeography*: 1–13.
- 400 Gomes SIF, van Bodegom PM, Merckx VSFT, Soudzilovskaia NA. 2019b. Environmental
- 401 drivers for cheaters of arbuscular mycorrhizal symbiosis in tropical rainforests. *New*
- 402 *Phytologist* **223**: 1575–1583.
- 403 Gomes SIF, Merckx VSFT, Saavedra S. 2017b. Fungal-host diversity among
- 404 mycoheterotrophic plants increases proportionally to their fungal-host overlap. *Ecology and*
- 405 *Evolution* **7**: 3623–3630.
- 406 van der Heijden MGA, Walder F. 2016. Reply to 'Misconceptions on the application of
- 407 biological market theory to the mycorrhizal symbiosis'. *Nature Plants* **2**: 16062.
- 408 Imhof S, Massicotte HB, Melville LH, Peterson RL. 2013. Subterranean morphology and
- 409 mycorrhizal structures. In: Mycoheterotrophy: The Biology of Plants Living on Fungi. 157–
- 410 214.
- 411 Ives AR, Godfray HC. 2006. Phylogenetic analysis of trophic associations. *The American*
- 412 *naturalist* **168**: E1-14.
- 413 Kiers ET, Duhamel M, Beesetty Y, Mensah J a, Franken O, Verbruggen E, Fellbaum CR,
- 414 Kowalchuk G a, Hart MM, Bago A, et al. 2011. Reciprocal Rewards Stabilize in the
- 415 Mycorrhizal Symbiosis. *Science* **333**: 880–882.
- 416 Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD,
- 417 Bengtsson-Palme J, Callaghan TM, et al. 2013. Towards a unified paradigm for sequence-

- 418 based identification of fungi. *Molecular Ecology* **22**: 5271–5277.
- 419 Leake JR. 1994. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist*
- 420 **127**: 171–216.
- 421 Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen
- 422 **T, Rosendahl S, Stenlid J, et al. 2013**. Fungal community analysis by high-throughput
- 423 sequencing of amplified markers a user's guide. *New Phytologist* **199**: 288–299.
- 424 Maherali H, Klironomos JN. 2007. Influence of Phylogeny on Fungal Community Assembly
- 425 and Ecosystem Functioning. *Science* **316**: 1746–1748.
- 426 McMurdie PJ, Holmes S. 2014. Waste not, want not: why rarefying microbiome data is
- 427 inadmissible. *PLoS Computational Biology*.
- 428 Mennes CB, Moerland MS, Rath M, Smets EF, Merckx VSFT. 2015. Evolution of
- 429 mycoheterotrophy in Polygalaceae: the case of *Epirixanthes*. *American Journal of Botany*
- 430 **102**: 598–608.
- 431 Merckx VSFT. 2013. Mycoheterotrophy: The biology of plants living on fungi. New York, NY,
- 432 USA: Springer.
- 433 Merckx V, Bidartondo MI. 2008. Breakdown and delayed cospeciation in the arbuscular
- 434 mycorrhizal mutualism. *Proceedings of the Royal Society B* **275**: 1029–1035.
- 435 Merckx VSFT, Janssens SB, Hynson NA, Specht CD, Bruns TD, Smets EF. 2012.
- 436 Mycoheterotrophic interactions are not limited to a narrow phylogenetic range of
- 437 arbuscular mycorrhizal fungi. *Molecular Ecology* **21**: 1524–1532.
- 438 Merckx VSFT, Mennes CB, Peay KG, Geml J. 2013. Evolution and diversification. In:
- 439 Mycoheterotrophy: The Biology of Plants Living on Fungi.
- 440 Mills LS, Doak DF. 1993. The Keystone-Species Concept in Ecology and Conservation.
- 441 *BioScience* **43**: 219–224.
- 442 Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. 2002. Network motifs:
- 443 Simple building blocks of complex networks. *Science* **298**: 824–827.
- 444 Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M. 2012. The
- 445 network structure of plant-arbuscular mycorrhizal fungi. *New Phytologist* **194**: 536–547.
- 446 Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and
- 447 evolutionary analyses in R. *Bioinformatics (Oxford, England)* **35**: 526–528.
- 448 Pellmyr O, Leebens-Mack J. 1999. Forty million years of mutualism: Evidence for eocene
- 449 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, Bruelheide H, et al. 2016. Ecological networks are more sensitive to plant than
- 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

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490 Figure 1. Tripartite arbuscular mycorrhizal interactions. (a) Motif depicts pairs of fungi 491 simultaneously linked to a cheater and a mutualistic plant. This motif appears significantly 492 more often than expected by chance in the empirical network; (b) Visualization of the 493 combined tripartite network between cheaters (yellow), arbuscular mycorrhizal fungi (grey), 494 and mutualistic plants (green), where lines represent a connection between a plant and a 495 fungus; (c) The plant-plant interaction strength network, in which lines represent a link 496 through a shared arbuscular mycorrhizal fungus. The thickness of the lines represents the 497 interaction strength between the plants (the thicker, the more fungi are shared). Yellow lines 498 link cheater to mutualistic plants; light grey lines link mutualistic to mutualistic plants; and 499 dark grey lines link cheater to cheater plants. The closer plants are together in the graph, the 500 higher their interactions strength.

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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.

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