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Zhu, Chuchao; Wang, Zihui; Luo, Wenqi; Feng, Jiayi; Chen, Yongfa; He, Dong; Ellwood, M. D. Farnon; Chu, Chengjin; Li, Yuanzhi

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Chuchao Zhu [1, 2], Zihui Wang [2], Wenqi Luo [2], Jiayi Feng [2], Yongfa Chen [2], Dong He [2], M. D. Farnon Ellwood [3], Chengjin Chu [2] and Yuanzhi Li [2]

[1] *Department of Bioengineering, Zhuhai campus of Zunyi Medical University, Zhuhai, 519041, China*

[2] *State Key Laboratory of Biocontrol, School of Ecology and School of Life Sciences, Sun Yat-sen University, 510275 Guangzhou, China*

[3] *School of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2DG, United Kingdom*

Correspondence

Yuanzhi Li

Email: liyuanzhi@mail.sysu.edu.cn, Tel: (+86) 2084111541

Abstract

Although rhizosphere fungi are essential for plant survival and ecosystem functioning, little is known about the processes that structure plant–fungal association networks. In this study, we constructed association networks between 43 plant species and two groups of root-associated fungi (mycorrhizal and pathogenic fungi; MF and PF, respectively) in a diverse subtropical forest. We then evaluated the modularity of plant–MF and plant–PF networks and linked them to the functional traits and phylogenies of both plants and fungi. We observed strong modularity in both plant–MF and plant–PF networks. Phylogenetically related fungi tended to emerge in the same modules. MF from distinct modules associated with plants with different specific root length and specific root area in plant–MF networks. PF from distinct modules associated with plants with different dark respiration rate and light compensation point in plant–PF networks. Plant affiliation to modules was explained by both plant traits and phylogeny (22% for plant–MF and 37% for plant–PF networks). In contrast, fungal affiliation to modules was explained by fungal phylogeny (16% for plant–MF and 29% for plant–PF networks). Our results elucidate the link between modularity in plant–root fungal networks and the functional traits and phylogeny of the plants and fungi. Our study highlights the importance of traits and phylogeny in governing root fungal community assembly from network perspective.

Keywords: network modules, mycorrhizal fungi, root microorganisms, pathogenic fungi, photosynthetic and root traits, network assembly

Introduction

Plants and fungi can associate in mutualistic and antagonistic ways, both of which are important for community assembly and ecosystem functioning (Connell 1971, Bennett and Klironomos 2018, 2019, Chen et al. 2019). Pathogenic fungi (PF) help maintain species diversity by reducing the recruitment and survival of dominant species (Bagchi et al., 2014; Chen et al., 2019), whereas mutualistic ectomycorrhizal fungi (EM) and arbuscular mycorrhizal fungi (AM) can maintain species diversity through either positive or negative plant–soil feedbacks (Bennett et al. 2017, Toju et al. 2018). In turn, plants may also influence the composition and diversity of soil fungal communities (Dassen et al. 2017, Wang et al. 2019, Schmid et al. 2021)—for example, by changing carbon availability and/or using physical or chemical defences against different types of fungal symbionts (Högberg and Högberg 2002). Therefore, elucidating the structure and influencing factors of plant–mycorrhizal fungal (MF) and plant–pathogenic fungal (PF) networks may provide more insights into the mechanisms governing species coexistence and community assembly.

Network modularity is an important topological feature of networks that describes the organisation of a network into different groups (modules), where species within groups associate more intensely than species between groups (Olesen et al. 2007). A modular structure may reflect the existence of strong preferences or selective processes that shape trophic associations between fungi and their hosts (Chagnon et al. 2018). Modularity maintains species diversity and community stability by confining the cascading effects of species extinction or environmental perturbation within a module and preventing ripple effects from spreading to other modules (Olesen et al. 2007). Investigating the modular structure can thus reveal the underlying non-random ecological processes

of community assembly (e.g., local adaptation and resource competition) (Valverde et al. 2020) and reveal the topological vulnerability of ecological networks to disturbances (e.g., simulated species extinction cascades) (Montoya et al. 2006).

Network modularity is often reported in macro-organisms, including food webs (Montoya et al. 2015) and mutualistic pollinator and seed-dispersal networks (Donatti et al. 2011, Morente-López et al. 2018). However, there is no consensus regarding the modular structure of belowground plant–root fungal networks. Mixed evidence for modularity (significantly and non-significantly strong modularity compared to 1000 randomised networks) was reported in mutualistic plant–MF networks (Montesinos-Navarro et al. 2012, Bahram et al. 2014, Toju et al. 2014). This inconsistency may be due to low sampling efforts for root samples, resulting in a limited number of root fungal groups (Bahram et al. 2014). Nevertheless, strong modularity is frequently reported in antagonistic tree-parasitic fungus and plant–pathogen networks (considering root PF as one component) (Vacher et al. 2008, Bufford et al. 2020). However, no previous studies have independently illustrated a modular structure in plant–root PF networks. Therefore, sufficient samples from diverse communities are necessary to explore modularity in plant–MF and plant–root PF networks.

To reveal the mechanisms underlying a modular structure, a growing number of studies account for module division and species composition in a module (Donatti et al. 2011, Torrecillas et al. 2014, Robinson et al. 2015). For instance, the modules of aboveground mutualistic pollinator and seed-dispersal networks have been reported to show convergence in functional traits and toward syndromes, respectively (Donatti et al. 2011, Robinson et al. 2015). However, to our knowledge, only three plant traits (plant specific root length, specific leaf area, and leaf dry matter content) have

been used to explore module assembly in plant–MF networks (Chagnon et al. 2015). Of these traits, only leaf dry matter content (representing a plant's investment in leaf structural tissues) has been reported to show convergence in modules and significantly affect the association of AM host plants with modules (Chagnon et al. 2015). This finding highlights the importance of plant ecological strategies in module composition and assembly (Torrecillas et al. 2014).

Ecological strategies are represented by the traits of both plant and fungal components, and are important in explaining fungal community assembly. Plant traits—including root morphology (related to plant nutrient acquisition efficiency) and the photosynthesis and respiration rates of leaves (related to carbohydrate accumulation)—are key predictors of root fungal communities (Koorem et al. 2017, Sepp et al. 2019, Davison et al. 2020). For instance, plants with high specific root area tend to be colonised by MF, whereas those with low specific root area tend to be colonised by PF (Wang et al. 2019). Fungal traits—such as fruit body size (Abrego et al. 2017) and hyphal exploration type (Olchowik et al. 2021)—are also critical in determining the colonisation success and community assemblages of root fungal communities. Despite the importance of plant and fungal traits in community assembly, there is a lack of evidence about their effects on module assembly. Incorporating plant and fungal traits to explain module composition can provide more insights into the mechanism of assembly of plant–root fungal networks.

As a proxy for their respective functional traits, plant and fungal phylogenies have also been used to explore the mechanisms of non-random organisation of network modularity (Chagnon et al. 2013). Previous studies have shown that modules are clustered by host phylogeny in plant–PF networks (Bufford et al. 2020), and by fungal phylogeny in plant–AM networks (Chagnon et al.

2015). Whether modularity in the form of fungal phylogenetic clustering is detectable in root PF networks remains unknown. This is important to elucidate, as it is possible that when competing for plant root resources, root PF and mutualistic MF exhibit different patterns of phylogenetic conservatism in host use.

To better understand the structure and assembly of plant–root fungal networks, it is essential to simultaneously explore plant–MF and plant–PF networks in diverse communities and link them to the phylogeny and functional traits of both host plants and root fungi. In this study, we complied two datasets of plant–MF and plant–PF networks comprising of 43 plant species, 883 MF, and 113 PF (Wang et al. 2019). We measured 17 functional traits (including root and photosynthesis traits) of the 43 host plant species in a 50-ha plot in a subtropical forest. After synthesising these data, we tested the following hypotheses: (i) there is strong modularity in the plant–MF and plant–PF networks in a highly diverse subtropical forest community; (ii) as in plant–seed dispersal and plant–pollinator networks, module composition is expected to be constrained by functional traits and phylogeny of plants and fungi; and (iii) the relative importance of functional traits and phylogeny may be different in predicting module composition in these networks.

Materials and methods

Study site

This study was conducted in a 50-ha plot in a subtropical forest in Heishiding Nature Reserve, Southern China (23°25′–23°29′ N， 111°49′–111°55′ E). The mean annual temperature of this area is 19.7 °C, and the annual precipitation is 1,750 mm. The total area of this nature reserve is 4,200

ha, including a 2,202 ha core area and a 1,660 ha experimental area. We established the 50-ha forest plot in 2012, and identified all trees with a diameter at breast height (DBH) >1 cm. In total, this plot included approximately 2,69,000 stems of 213 woody plant species (Wang et al. 2019).

Root sampling and molecular identification

We compiled the dataset of fungal communities from 512 root samples of the 43 plant species (no less than 5 sampled individuals for each plant species) in the Heishiding plot. These plant species were selected based on their taxonomic placement and abundance. Specifically, we selected three *Litsea* spp. and three *Lithocarpus* spp. (the two most abundant genera), along with species from other genera (including some genera that were in the same families as *Litsea* and *Lithocarpus*). For each plant species, we randomly selected 5–15 individuals for fine root sampling. The unequal sampling efforts for each species tree resulted from: (i) the rarity of some tree species that limited the collection of sufficient root samples and (ii) failure in extracting high-quality DNA. At least three root fragments (each approximately 2 cm in length) around an individual tree were traced from different directions and then pooled to create a single sample. The fine root samples were immediately cooled on ice in the field and stored at -20 °C in a refrigerator until processing. More sampling details can be found in a previously published paper (Wang et al. 2019).

Of 100 fine-root samples (randomly selected from all root samples), the tree species of 97 root samples traced in the field were correctly confirmed by *rbcLa* sequences obtained from a Sanger sequencing platform. Thus, we considered the tracing method as an accurate strategy to capture the taxonomic (species level) information of sampled fine roots. Root-associated fungi were identified by the internal transcribed spacer (ITS) region of fungal rDNA. After removing chimeric sequences,

144 we obtained 11,000,000 high-quality reads of the ITS region of fungal rDNA. The operational
145 taxonomic units (OTUs) of root fungi were discriminated using a threshold of 97% sequence
146 identity. Each sequence was assigned to a taxonomic label based on the UNITE database using the
147 Ribosomal Database Project (RDP) classifier (Wang, Garrity, Tiedje, & Cole, 2007). Each fungal
148 genus was then assigned into functional categories. We identified EM fungi by blasting our fungal
149 genera against the fungal genera in a database of EM taxa and lineages (Tedersoo and Smith 2013).
150 We assigned all OTUs in Glomeromycota to AM fungi (Schüßler 2002). Because we could only
151 identify 21 OTUs of AM, EM and AM were pooled to represent the MF guild. Identifying fungal
152 plant pathogens is challenging, because identification can only take place after the plants are
153 diseased. Therefore, pathogenic genera were initially identified using the FUNGuild database
154 (Nguyen et al. 2016). We then consulted the literature and retained only potential pathogens (OTUs)
155 that had been identified to the species level and are known to be pathogenic to woody plants.
156 To evaluate network modularity, we constructed a plant–PF association network including 113
157 fungal plant pathogens and 43 plant species, as well as a plant–MF association network including 883
158 mycorrhizal fungi (862 EM and 21 AM) and 43 plant species. To account for the sampling inequality,
159 each cell in each network matrix was filled with the mean abundance (sequenced reads) of each fungal
160 OTU (species) on each sampled tree, and the numbers were rounded to the nearest integer. Abundance
161 of fungal OTUs on each sampled tree was calculated after subsampling each sample to 3000 sequence
162 reads to eliminate the effects of sample size (Wang et al. 2019).
163 **Functional traits**
164 We recorded and analysed the data of 17 functional traits of the 43 host plants, including 2 leaf

165 morphological traits, 3 leaf chemical traits, 8 photosynthetic traits, and 4 root traits (Table S1).
166 Details about how these traits were sampled and measured can be found in previous studies (Feng et
167 al. 2018, He et al. 2018, Wang et al. 2019, Luo et al. 2020). The photosynthetic traits of 7 plant
168 species and the root traits of 11 plant species were unavailable, and these species were removed
169 from relevant analyses. We collected the data of 4 functional traits of fungi (growth form, fruitbody
170 type, hymenium type, and hyphal exploration type) from the FungalTraits database (Põlme et al.
171 2020). There was no variation in the four fungal traits for AM fungi (21 OTUs). For the other fungi
172 (PF, MF, and EM), the fungal traits available for statistical analysis are listed in Table S2.

173 **Statistical analysis**

174 ***Reconstructing phylogeny of plant and fungal species***

175 We used four plant DNA barcodes (*rbcLa*, *matK*, *trnL*, and ITS2) to reconstruct the phylogenetic
176 relationships between all local plant species using the RAxML software (Stamatakis 2014). The
177 sequences were aligned in the Clustal Omega software using the default Gonnet transition matrix,
178 with 6 bits of gap opening penalty and 1 bit of gap extension penalty. The best maximum likelihood
179 phylogeny for plants (Fig. S1) was inferred using the GTR + GAMMA evolutionary model and
180 1000 fast bootstrap replicates in the RAxML software (Stamatakis 2014).

181 We used the taxonomic rank information of local root MF and PF (996 OTUs) and one
182 outgroup (*Olpidium brassicae*) to reconstruct the phylogeny of root-associated fungi using a Perl
183 script (taxonomy_to_tree.pl script of Tedersoo et al. 2018). By incorporating a fungal taxonomic
184 backbone, we converted the hierarchical classification of our focal fungal taxa to a Newick-
185 formatted phylogeny (Fig. S2). In the reconstructed fungal phylogeny (Fig. S2), we assigned a

branch length of 60 between each of the taxonomic ranks (e.g. species, genus, family).

Detecting the modular structure of plant-root fungal association networks

We first estimated modularity (M)—ranging from 0 (low modularity) to 1 (high modularity)—in abundance-weighted plant–MF and plant–PF networks. A LPAwb+ algorithm was used to search the module divisions that maximised weighted modularity (Beckett 2016), such that maximal modularity was obtained when no better division into modules could be detected (Beckett 2016). To stabilise this computation, we re-ran the algorithm 50 times and reported the most modular result (i.e., the maximum value of modularity and module divisions). As modularity vary with network size and connectivity, we used the z-score of network modularity $z = (M - \bar{M}_{random})/\bar{M}_{random}$ (Olesen et al. 2007) to quantify the degree of network modularity among networks. We generated 1000 randomised null networks with constant marginal totals and connectance using the "swap" method (Artzy-randrup and Stone 2005, Dormann et al. 2009). From these randomised networks, we calculated $\bar{M}_{random}$ as the average modularity. The above calculations were conducted in the R package *bipartite* (Dormann et al. 2009).

Detecting the constraints of phylogeny and functional traits on network modularity

To understand the relationship between phylogeny and network modularity, we evaluated the correlation between plant (and fungus) co-occurrence in modules and their phylogenetic proximity. To evaluate the significance of the correlation, we compared the correlation coefficient calculated from the matrix of observed species-module data to those calculated from 999 randomised matrices. This comparison was performed using the "comm.phylo.cor" function in the R package *picante*

(Kembel et al. 2010). The co-occurrence of a pair of species within modules was measured using Schoener's index of co-occurrence (Hardy 2008). To test whether some plant traits (Table S1) differed significantly between modules in the observed network, we conducted one-way Type II ANOVA and kruskal–Wallis rank sum test. Some trait variables were log-transformed to meet the assumption of homogeneity of variance and normality of error distribution (Table S3). To detect the impact of fungal traits on the fungal composition of modules, we performed Fisher's exact tests for a series of matrices (module-fungal trait) containing the richness or abundance data of fungal species belonging to each trait category in modules. Five fungal traits (growth form, fruit body type, and hymenium type, hyphal exploration type and mycorrhiza type) were used to explain the fungal composition of modules. Although the morphological traits for some EM fungal groups (e.g., *Cenococcum*) were incomplete in the fungal trait database (Põlme et al. 2020), we assumed that these incomplete traits are sufficient for explaining network assembly. All these analyses were conducted in R version 3.5.1 (R Core Team 2015).

Analysing the drivers of module composition

To explain the module membership of fungi and their host species from their traits and phylogeny, we used random forest models implemented in the R package *randomForest* (Liaw and Wiener 2002). To explain the module membership of the plant host species, we constructed a full random forest model with 17 plant traits and the first 10 eigenvectors of plant phylogeny (Fig. S1) as explanatory variables. To explain the module membership of root MF/PF/EM/AM fungi, we used 5 fungal traits (fruit body type, hymenium type, growth form, hyphal exploration type, and mycorrhiza type, or the available trait combination for each group of fungi; see Table S2) and the

first 10 eigenvectors of the MF/PF/EM/AM phylogeny as explanatory variables (see Fig. S2). Phylogenetic eigenvectors were calculated from principle coordinate analysis (PCoA) of the phylogenetic distance matrix of plants/fungi (Diniz-Filho et al. 1998). Finally, we used reduced random forest models to explain the modular memberships for plants and fungi, with selected explanatory variables based on the smallest out-of-bag error (Evans and Murphy 2019). Model accuracy was defined as the amount of variation explained by each reduced random forest model in allocating plants and fungi to the observed modules. Finally, we partitioned the variance explained by the reduced random forest models into unique and shared components of traits and phylogeny.

Results

Structural properties of plant–MF and plant–PF association networks

We observed significantly higher modularity (relative to those in randomised networks) in the observed plant–PF (z = 7.82, M = 0.431, confidence interval [CI] of null models [0.043, 0.062]) and plant–MF (z = 6.95, M = 0.487, CI of null models [0.056, 0.067]) association networks. The plant–MF association network was divided into 16 distinct modules (Fig. 1a), with EM fungi in all modules and AM fungi in 9 modules (Fig. 1a). The plant–PF association network was divided into 9 distinct modules (Fig. 1b).

The constraints of phylogenetic history and functional traits on modularity

Excluding AM fungi, closely related fungi in other functional groups were more likely to co-occur in modules of the plant–root fungal association networks (Table 1). However, phylogenetic relatedness between plant species did not significantly affect plant distribution across modules

247 (Table 1). In plant-MF network (Table S4), the mean nearest taxon distances (MNTD) were
248 significantly lower than null expectation in 3 modules of MF (modules 7, 9 and 16) and in 2
249 modules of plants (modules 9 and 11). In plant-PF network (Table S5), MNTD were significantly
250 lower than null expectation in only one module of PF (module 4) and plants (module 1),
251 respectively.

252 Certain functional traits of host plants varied between modules in the plant–PF and plant–MF
253 association networks (Fig. 2 and Table S3). Specifically, leaf dry matter content, root tissue density,
254 specific root length (SRL), and specific root area (SRA) differed significantly between modules in
255 the plant–MF association network (Fig. 2 and Table S3). Moreover, leaf dark respiration rate (Rd)
256 and light compensation point (LCP) differed significantly between modules in the plant–PF
257 association network (Fig. 2 and Table S3). The richness and abundance of MF across modules were
258 significantly affected by fruit body type, hymenium type, hyphal exploration type, and mycorrhizal
259 type (Table S2). The abundance (but not richness) of PF across modules was significantly affected
260 by growth form, fruit body type, and hymenium type (Table S2).

261 **Drivers of module composition**

262 In assigning fungal host plants to modules in the two observed networks, the reduced random forest
263 models provided correct prediction rates of 22–38% (Fig. 3a, c, e, g). Compared to plant
264 phylogenies, plant traits were more accurate in predicting the module memberships of plants hosts
265 for MF and AM and EM (Fig. 3a, e, g). Compared to the independent effects of plant traits and
266 phylogeny, their interactions were more accurate in predicting the module memberships of PF host
267 plants (Fig. 3c). In assigning root fungi to network modules, only fungal phylogeny was retained

268 (and fungal traits were removed) during model selection. As a result, the reduced models had
269 accuracy rates of 16–38% in assigning root fungi to the observed modules (Fig. 3b, d, f, h).

270 **Discussion**

271 **Non-random modularity in plant–root fungal association network*s***

272 We found that plant–MF and plant–PF networks in a subtropical forest tended to exhibit significant
273 modularity (Fig. 1). This is similar to the patterns observed in some plant–fungus association
274 networks in harsh alpine and subalpine habitats (Toju et al. 2016) and semi-natural grasslands (Sepp
275 et al. 2019). In the forest plot, plant–fungus networks may show a modular structure due to the
276 similar host habitat preferences and spatial distribution among phylogenetically closely related
277 fungi. The fungal composition of each module of the plant–MF network was dominated by EM
278 instead of AM (Fig. 1a and Table S6). This implies that the strong modular structure of the plant–
279 MF fungal network may be primarily determined by relatively higher host specialization in EM than
280 AM (Van Der Heijden et al. 2015). Similarly, stronger host specificity of antagonistic PF (Wang et
281 al. 2019) may result in modularity in the plant–PF network. Moreover, network size (Olesen et al.
282 2007), asymmetry in species number (Põlme et al. 2018), and connectivity (Thébault and Fontaine
283 2010) may also affect the detection of modularity. Therefore, further studies focusing on the
284 ecological and evolutionary factors shaping network patterns are warranted to better predict the
285 modular pattern of antagonistic and mutualistic fungal networks.

286 **Phylogenetic clustering of pathogenic and mycorrhizal fungi within modules**

287 We found that fungal species that were phylogenetically closely related tended to emerge in the

288 same modules of plant–root fungal networks (plant–MF and plant–PF networks; Tables 1, S4, S5),
289 which is consistent with the findings of a previous study (Chagnon et al. 2015). These results
290 suggest that the modular organisation of plant and mycorrhizal fungal networks generally reflects
291 the main split in the fungal phylogeny. Phylogenetically related plants were also found in the
292 networks of plants with antagonistic fungi (including leaf and root decay fungi and parasitic fungi)
293 (Vacher et al. 2008, Bufford et al. 2020). Consistent with a previous study (Chagnon et al. 2013), a
294 lack of phylogenetic relatedness among plant hosts associating with root fungi in the modules
295 (Table 1) may be ascribed to low plant species richness in each module and insufficient sampling
296 efforts. We found a significant effect of fungal phylogeny, but not of host phylogeny, on plant–root
297 fungal networks (Table 1), which is not in agreement with the findings of previous studies (Davison
298 et al. 2020). This inconsistency in the effects of evolutionary history may be due to differences in
299 the functional groups of fungi and environmental conditions. Due to the relatively low number of
300 plant species that associated with fungal symbionts, our results may also be constrained by
301 statistical power. Taken together, our results suggest that the evolutionary history of plants and fungi
302 only partially explains the modular patterns observed in our plant–fungus networks.

303 **Module-level trait convergence in plant–MF and plant–PF association networks**

304 To resist attacks from root pathogens and improve the acquisition efficiency of water and nutrients
305 from mycorrhizal fungi, host plants need to allocate vast amounts of material and energy resources
306 to root fungi. Thus, the resource acquisition and allocation strategies of host plants likely regulate
307 fungal selectivity (Sachs et al. 2004) and affect the shaping and evolution of plant–fungal
308 symbioses. Here, we show that plant and fungal traits varied across the modules in plant–fungal

309 networks, potentially driving the plant-fungal associations. The specific plant traits that structure
310 network modules differed between plant–MF and plant–PF networks (Fig. 2 and Table S3).
311 In the plant–MF network in our subtropical forest, MF fungi from distinct modules varied in
312 fungal exploration traits (Table S2) and tended to associate with plants with different root traits
313 (Figs. 2, S3 and S4, Table S3). For example, *Scleroderma* spp. fungi (EM) with long-distance
314 exploration dominated in module 8 (Table S6), and *Russula* spp. fungi (EM) with contact hyphal
315 exploration dominated in module 11 (Table S6). This suggests that module membership in these
316 fungi may be driven by the high and low efficiency (respectively) of nutrient acquisition by the fine
317 roots of host plants (indicated by SRL, SRA, and specific root tips; Figs. 2 and S3, Table S3). Thus,
318 fine roots with low (or high) investment in foraging tend to shape modules containing EM fungi
319 with low (or high) investment in foraging. This is opposite to the general expectation of
320 complementary relationship between root and mycorrhizal fungi but supporting a matching strategy
321 where longer and thinner roots associated with more mycorrhizal hyphae biomass (Chen et al.,
322 2016). These results support the notion that the module assembly of plant–MF association networks
323 can mainly be ascribed to ecological strategies based on plant (Chagnon et al. 2015) and fungal
324 traits.
325 Similarly, PF from distinct modules differed in functional traits (hymenium type, growth form,
326 and fruitbody type, Table S2) and tended to associate with plants with differing photosynthetic traits
327 (Rd and LCP; see Fig. 2 and Table S3). The two most abundant genera of PF (*Cylindrocarpon* and
328 *Mycoleptodiscus*) dominated in module 4 (Table S7), which may be driven by their preference for
329 host plants with high shade-tolerance (lowest LCP and Rd; Fig. 2c, d). The other two PF genera

330 (*Colletotrichum* and *Pestalotiopsis*) were dominant in module 1 (Table S7), which may be due to
331 selection of light-dependence plants (highest LCP and Rd; Fig. 2c, d). These results suggest that
332 plant photosynthesis traits, important indicators of plant allocation on growth and defense, can
333 dictate the host selectivity on PF (García-Guzmán and Heil 2014) , while root traits, as the basis of
334 plant acquisition for water and nutrients, affect the structure of network modules for MF.

335 **The contribution of functional traits and phylogeny to a modular structure**

336 Our results show that plant traits were more important than plant phylogeny in predicting module
337 composition of MF host plants while plant phylogeny was more important for PF host plants (Fig.
338 3). This implies that PF were sharing more coevolutionary history with plant host than MF, while
339 MF were more likely selected by the ecological traits of plants. The low accuracy of fungal traits in
340 predicting module fungal composition (Fig. 3) may be due to the low variation in the fungal traits
341 database (e.g., fungal morphology did not vary within a genus) (Põlme et al. 2020). Other
342 processes—such as environmental filtering (e.g., soil types and soil properties) (Torrecillas et al.
343 2014, Arraiano-Castilho et al. 2020) and stochastic effects—may jointly drive plant–fungal network
344 assembly by affecting the co-occurrence of plants and fungi. However, the accuracy of predicting
345 module composition was not substantially improved by incorporating functional traits, phylogeny,
346 and soil environmental variables in the model (Fig. S5). Soil environmental variables (obtained
347 from 625 soil cores sampled randomly in a forest plot) (Luo et al. 2021) as well as plant functional
348 traits (at the species level) may reduce the accuracy in predicting module composition (Fig. S5).
349 This suggests that functional traits at the individual level (including intraspecific trait variability)
350 and soil environmental variables around each plant stem should also be used to investigate modular

assembly. Incorporating these data may also shed light on the dynamics of root–fungal networks along environmental gradients.

Conclusions

Analysis of plant–root fungal symbiont network is crucial in finding ecological variables, which regulates host selectivity of fungi and organizes root fungal symbiont community structures. We illustrate that plant nutrient acquisition efficiency indicated by root traits may regulate host selectivity of MF, while plant carbohydrate accumulation and shade tolerance indicated by leaf photosynthesis traits mainly regulate host selectivity of PF. Such host selectivity may be also constrained by phylogenetically conservative fungal traits. These results suggest that trait and phylogeny based host selectivity enhance to shape a series of network modules (groups). Overall, our study contributes to elucidating the ecological and evolutionary factors shaping modular structure of plant–fungal association network and reveal the mechanisms governing species coexistence and community assembly.

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.kwh70rz5z> (Zhu et al. 2022).

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Figure legends

Figure 1. Modularity of plant–mycorrhizal fungi (plant–MF, a) and plant–pathogenic fungi (plant–PF, b) networks. The plant–MF network contains 16 modules, where plants, ectomycorrhizal fungi (EM), and arbuscular mycorrhizal fungi (AM) are indicated by red triangles, blue circles, and cyan rectangles, respectively. The plant–PF association network contains 9 modules, where plants and PF are indicated by red triangles and blue circles, respectively. The *z*-score of modularity has been calculated using the modularity indices of the observed plant–fungal networks and the 1000 randomly organised networks.

Figure 2. Differences in plant photosynthetic and root functional traits between modules in plant–mycorrhizal fungal (plant–MF) (a, b) and plant– pathogenic fungal (plant–PF) (c, d) association networks. Bar plots only show partial plant variables at species level that are significantly different among modules, as identified by analysis of variance (ANOVA, see Table S3). These variables include log-transformed specific root length, log-transformed specific root area, log-transformed dark respiration rate and light compensation point. Significance levels are as follows: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). For modules including > 2 species with available traits, we calculate the standard error of the traits with the error bar shown on the bar. For modules including only one species with available trait, only the mean of the traits is shown. Number of plant species with available functional traits in each module is denoted on the top of a bar.

Figure 3. Venn diagrams illustrating the variation explained by species traits and phylogeny in assigning root fungi and their host plants to modules. In the association networks of plant–mycorrhizal fungi (MF) and plant–pathogenic fungi (PF), the variation in assigning host plants of

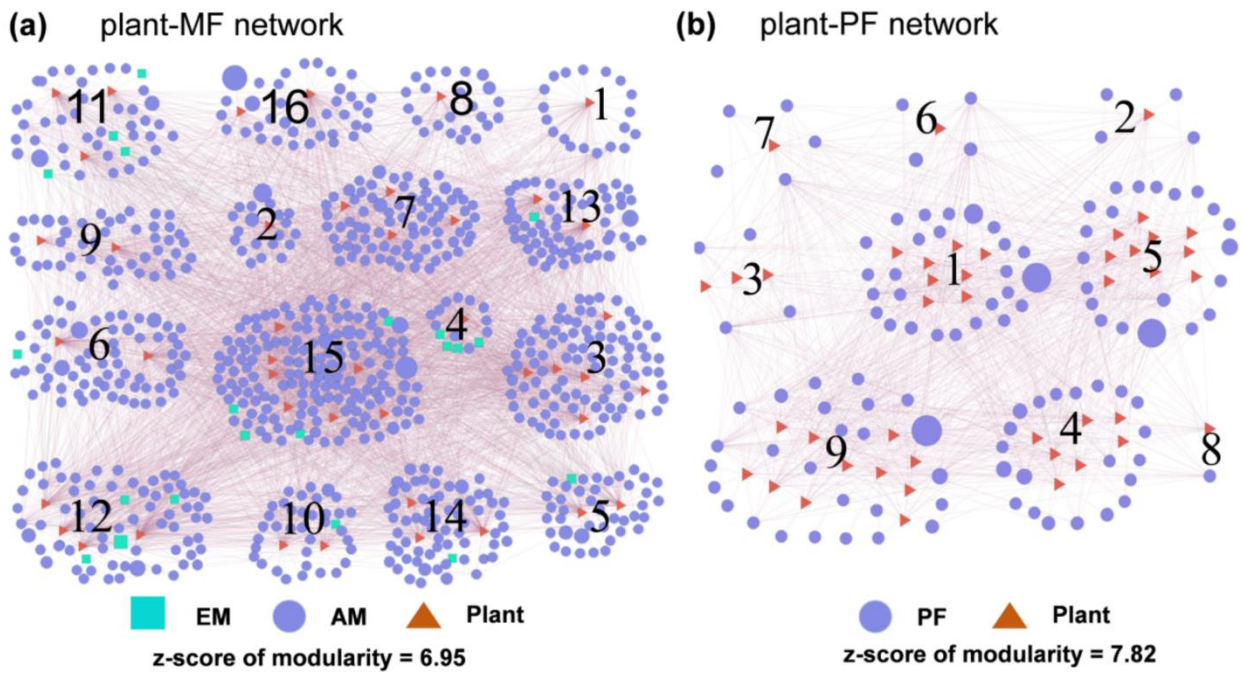
fungal MF and PF to modules is partitioned into plant traits and phylogeny (a, c), whereas the variation in assigning host plants for ectomycorrhizal fungi (EM) and arbuscular mycorrhizal fungi (AM) to modules is uniquely explained by plant traits (e, g). The variation in assigning fungi to modules is uniquely explained by the fungal phylogeny for all fungi (b, d, f, h).

524 **Table**

525 **Table 1.** The relationships between phylogenetic relatedness between pairs of host plants (or fungi)
526 and their co-occurrence in a same module in plant-pathogenic fungal (PF) and plant-mycorrhizal
527 fungal (MF) association networks. Significance effects ($p < 0.05$) are shown in bold type.

Networks	**Module composition**	**No. of species**	***r***	***p* value** 528
Plant-PF network	PF hosts	43	-0.034	0.309 529
	PF	113	-0.044	**<0.001**
Plant-MF network	MF hosts	43	0.014	0.681 530
	MF	883	-0.073	**<0.001**
	EM hosts	43	0.014	0.681 531
	EM	881	-0.075	**<0.001** 532
	AM hosts	43	0.105	0.148
	AM	21	-0.061	0.381

533 **Figure 1.**



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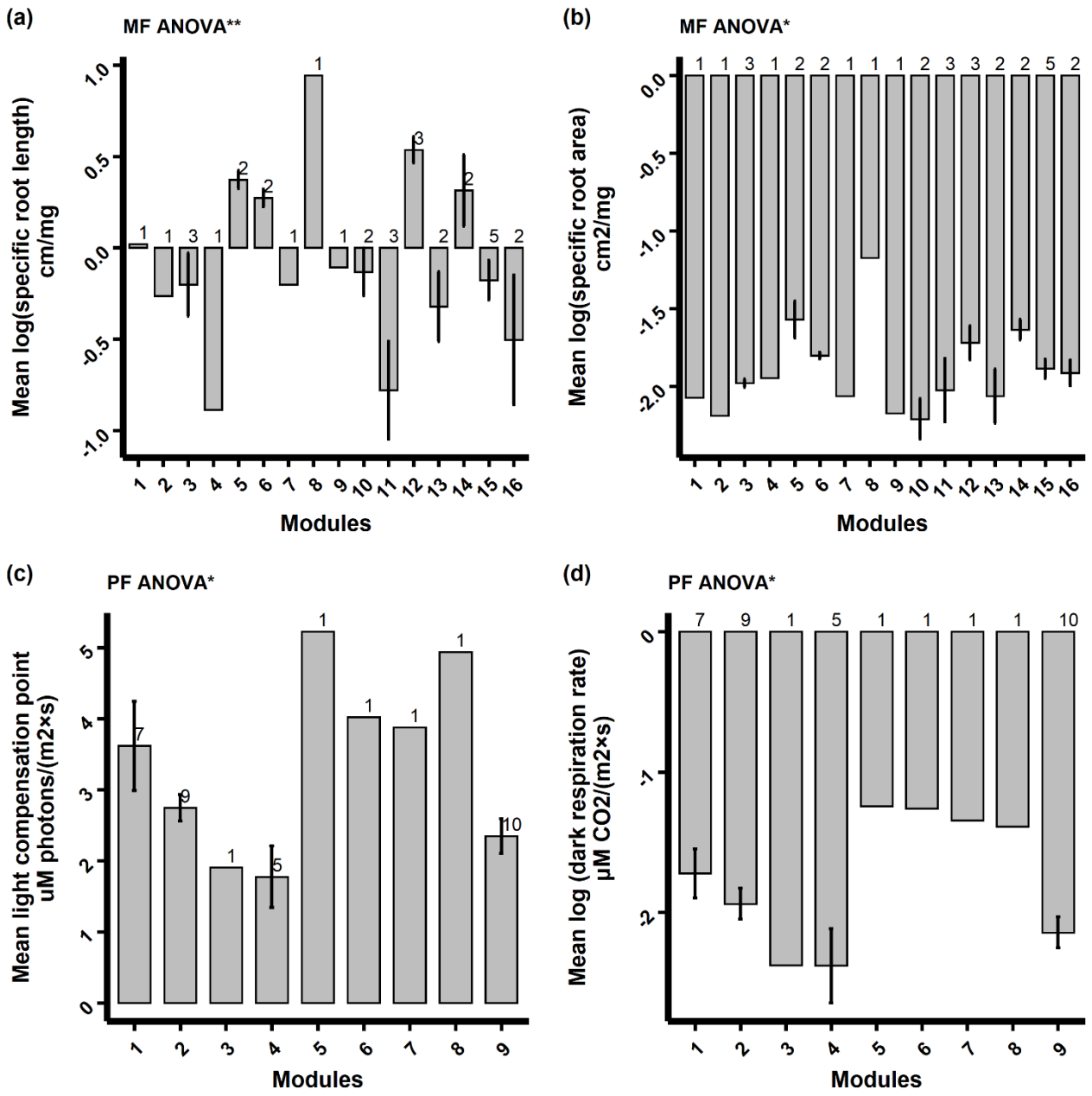
536 **Figure 2.**



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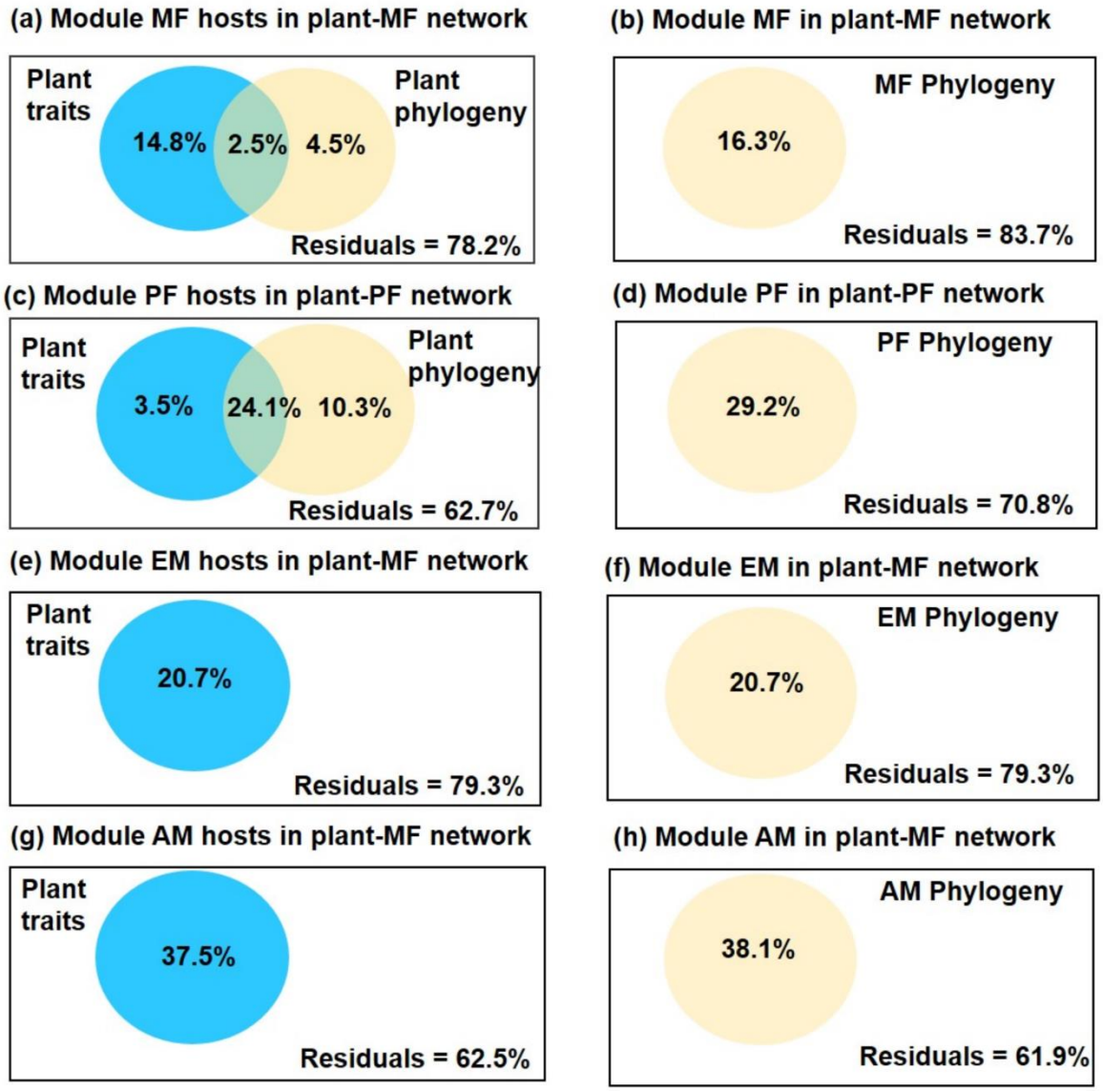
539 **Figure 3.**



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