# Fungal phylogenies and plant functional traits structure root associated fungal networks in a subtropical forest

CHUNCHAO ZHU,*† ZIHUI WANG,† WENQI LUO,† JIAYI FENG,† YONGFA CHEN,† DONG HE,† M. D. FARNON ELLWOOD,‡ CHENGJIN CHU† and YUANZHI LI†

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

† *State Key Laboratory of Biocontrol and School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China*

‡ *20 Baily Place, Cheswick, Bristol, BS16 1BG, UK*

*Correspondence*

*Yuanzhi Li*

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

## Abstract

Rhizosphere fungi are essential for plant survival and ecosystem functioning, but the processes structuring plant-fungal interactions remain largely unknown. We constructed association networks between 43 plant species and two groups of root-associated fungi (mycorrhizal and pathogenic) using sequence data. We revealed modularity within the association networks using network analysis, and correlated this modular structure with functional traits and phylogenetic history driving plant-fungal interactions. We observed strong modularity in both plant-mycorrhizal fungal and plant-pathogenic fungal association networks. Plant functional traits and fungal phylogeny clustered within modules. Host plants of mycorrhizal fungi differed significantly between modules in terms of their leaf dry matter content, photosynthetic traits and root tissue density. Host plants of pathogenic fungi differed significantly between modules in terms of their dark respiration rate, light compensation point and root morphology. Modularity within fungi was a product of fungal phylogeny, whereas host plant modularity was a product of functional traits (leaf morphology, photosynthetic rate and root morphology). Our study illustrates the link between plant functional traits and fungal assembly, and highlights the importance of niche-based processes in shaping plant-fungus association networks. Our results suggest that plant traits may be instrumental in managing the composition of belowground fungal communities.

**Keywords:** host plant, fungus, pathogen, rhizosphere, specificity, photosynthetic traits, root traits

## Introduction

Plants and fungi interact in mutualistic and antagonistic ways, both of which are important to community assembly and ecosystem function (Bennett & Klironomos, 2018, 2019; Lei Chen et al., 2019; Connell, 1971). Specifically, pathogenic fungi with high specialization, and mutualistic fungi with low specialization, can commonly form antibiosis and symbiosis on the same set of plant roots (Wang et al., 2019). Pathogenic fungi maintain tree community diversity by reducing the recruitment and survival of dominant species (Bagchi et al., 2014; Lei Chen et al., 2019). Mutualistic mycorrhizal fungi enhance nutrient uptake and pathogen resistance of host plants (Bennett & Klironomos, 2018, 2019), generating positive plant-soil feedback loops and monodominance (McGuire, 2007). In turn, host plants invest in physical and chemical defenses to resist pathogenic fungi, ultimately influencing the composition and diversity of fungal communities (Wang et al., 2019). Host plants also share up to 20% of their net photosynthetic carbon with mycorrhizal fungi (Högberg & Högberg, 2002). To understand the assembly rules acting on communities of plants and fungi, it is therefore necessary to consider simultaneous interactions between various plant communities and the two major functional groups of fungi.

An effective way of studying the interactions between host plants and their root-associated fungal communities is via their network topology, which can also

reveal the vulnerability of the ecological networks to disturbance (Montoya, Pimm, & Solé, 2006). Modularity describes the non-random organization of a network into different modules (Olesen, Bascompte, Dupont, & Jordano, 2007). Modularity maintains species diversity and community stability, by confining the cascading effects of species extinction or environmental perturbation within a module, rather than allowing ripple effects to spread to other modules (Olesen et al., 2007). Mutualistic plant-mycorrhizal fungus networks and antagonistic plant-pathogenic fungus networks often exhibit modularity (Toju, Guimarães, Olesen, & Thompson, 2014; Vacher, Piou, & Desprez-Loustau, 2008). Plant-mycorrhizal fungus networks exhibit low levels of modularity (Toju et al., 2014), whereas plant-pathogenic fungus networks exhibit high levels of modularity (Vacher et al., 2008).

Studies of modularity have contributed much to descriptions of the topological structure of plant-root fungus networks (Barrett, Encinas-viso, Burdon, & Thrall, 2015; Chen et al., 2017; Dickie, Cooper, Bufford, Hulme, & Bates, 2017). Recent studies emphasize the need to test host plant species traits as well as phylogeny (Brousseau, Gravel, & Handa, 2018; Olito & Fox, 2015), in order to understand the ecological and evolutionary consequences of habitat fragmentation and climate change. Given the tight co-evolutionary relationships between plants and mycorrhizal fungi, arbuscular mycorrhizal fungi (AM) should be more phylogenetically related within modules than between modules (Chagnon, Bradley, & Klironomos, 2015). Whether modularity in the form of phylogenetic clustering is detectable in root pathogenic fungi remains unknown. It is possible that pathogenic

fungi and mutualistic mycorrhizal fungi, when competing for plant root resources, employ different host use strategies. A growing body of evidence supports the theory that host-plant phylogenies structure pathogenic and mycorrhizal fungal communities, rather than fungal phylogenies structuring host plant communities (Chagnon et al., 2015; Davison et al., 2020; Vacher et al., 2008). For example, species in the closely related *Orchis* genus were observed to symbiosis by similar root-associated Tullasnelaceae fungi, whereas phylogenetically related fungi did not share similar host plants (Jacquemyn et al., 2011). This highlights the potential role of plant phylogenies in shaping the module structure of root fungal association networks.

Direct measurements of functional traits are more informative than phylogenies for understanding the modular structure of plant-fungus association networks, but phylogenies can predict phenotypic traits. For example, Chagnon et al. (2015) showed that in a small mutualistic network comprising 8 species of herbaceous plants and 25 OTUs of AM fungus, three plant traits (specific leaf area, leaf dry matter content and specific root length) significantly affected module composition. In particular, leaf dry matter content (representing a plant's investment in leaf structural tissues) affected the assignment of plant species to modules (Chagnon et al., 2015), suggesting that plant life history affects module composition by regulating host/habitat preferences of mutualistic root-associated fungi. Elsewhere, plant nutrient absorption efficiency related to root morphology, and carbohydrate accumulation related to leaf photosynthesis and respiration rate have been reported to change habitat use and host preference of fungal communities (Davison et al., 2020; Koorem et al.,

2017; Sepp et al., 2019). Furthermore, leaf macroelements have been shown to mediate community assembly of root-associated pathogenic and mycorrhizal fungi (Wang et al., 2019). Nevertheless, it remains unclear whether these plant traits and the host preferences of fungi can further regulate network modularity in a subtropical forest community.

In this study, we compiled 519 root-associated fungi samples, and collected 17 functional traits from 45 plant species in a 50 ha stem-mapped subtropical forest. We then constructed plant-mycorrhizal and plant-pathogenic fungus association networks and tested whether there was non-random modularity within the plant-pathogenic and plant-mycorrhizal association networks. Specifically, we tested the following three hypotheses: (1) plant-pathogenic and plant-mycorrhizal association networks both exhibit non-random modularity; the antagonistic plant-pathogenic network should exhibit stronger modularity than the mutualistic plant-mycorrhizal network; (2) phylogenetic relatedness between host plants and pathogenic/mycorrhizal fungi is greater within modules; (3) functional traits of host plants should be more similar within than between modules.

## Materials and methods

### *Study site*

This study was conducted in a 50-ha subtropical forest plot in Heishiding nature reserve, Southern China (23°25′~23°29′N，111°49′~111°55′ E). Mean annual

temperature is 19.7 °C and annual precipitation is 1750 mm. The total area of this nature reserve is 4200 ha, with 2202 ha core area and 1660 ha experimental area. We established the 50-ha forest plot in 2012, and identified all trees with dbh (1.3 m) > 1m were identified to species. In total, this plot included about 269000 stems of 213 woody plant species.

***Root sampling and molecular identification***

We compiled the dataset of mycorrhizal and pathogenic fungal communities from 519 root samples of 45 plant species in a subtropical forest published in a previous study (Wang et al., 2019). The fine roots of 3~15 individuals for each host plant species were sampled randomly (Wang et al., 2019). At least three samples from each individual tree were collected in different directions and then pooled to create a single sample (Wang et al., 2019). DNA barcoding of *rbcLa* correctly identified 97 out of 100 fine-root samples. Root-associated fungi were identified by the interval transcribed spacer (ITS) region of fungal rDNA. After removing chimeric quality sequences, 11 million high-quality reads of internal transcribed spacer (ITS) region of rDNA were obtained. The operational taxonomic units (OTUs) of root fungi were discriminated using a threshold of 97% sequence identity. Each sequence was assigned to a taxonomic label based on the UNITE database using the Ribosomal Database Project Classifier (RDP) (Wang, Garrity, Tiedje, & Cole, 2007). Each fungal genus was then assigned into functional categories. The ectomycorrhizal fungi (EM) were identified based on a database of EM taxa and lineages (Tedersoo & Smith, 2013). AM fungi were identified by including all OTUs from Glomeromycota. EM

147 and AM fungi were pooled together to represent the mycorrhizal fungal guild.
148 Identifying fungal plant pathogens is challenging because identification can only take
149 place after plants are diseased. Pathogenic genera were firstly identified using the
150 Funguild database (Nguyen et al., 2016). We then referred to the literature, retaining
151 only potential pathogens (OTUs) which were identified to the species level and have
152 been found to be pathogenic to woody plants. Details of the molecular identification
153 of plants and fungi above are published elsewhere (Wang et al., 2019).
154 We removed two plant species (*Artocarpus hypargyreus* and *Ormosia*
155 *glaberrima*) with less than three sampled individuals. The plant-mycorrhizal fungus
156 (plant-MF) association network included 883 mycorrhizal fungal (AM and EM) and
157 43 plant species, and the plant-pathogenic fungus (plant-PF) association network
158 included113 fungal plant pathogens and 43 plant species.
159 ***Measuring plant traits***
160 We measured 17 functional traits of the 43 host plants, including 2 leaf morphological
161 traits, 3 leaf chemical traits, 8 photosynthetic traits and 4 root traits (Table S1). Details
162 about how these traits were sampled and measured can be found in previous studies
163 (Feng et al., 2018; He, Chen, Zhao, Cornelissen, & Chu, 2018; Luo et al., 2020; Wang
164 et al., 2019). The photosynthetic traits of 7 plant species and the root traits of 14 plant
165 species were unavailable and therefore removed in relevant analyses.
166 ***Reconstructing phylogeny of plant and fungal species***
167 We used four plant DNA barcodes (*rbcLa*, *matK*, *trnL* and ITS2) to reconstruct the
168 phylogenetic relationships between the two host species (Zhouwen unpublished

work). We used ITS sequences to reconstruct the root-associated fungus phylogenies. Multiple sequence alignments were obtained using Clustal Omega (https://www.ebi.ac.uk/). We used RAxML software (Stamatakis, 2014) to reconstruct the phylogenetic trees of plant and fungal species. Using the GTR + G model with default settings (Stamatakis, 2014), we inferred the best maximum likelihood phylogenies for 43 host plants, 883 mycorrhizal fungi and 113 pathogenic fungi (Figs S1-S3).

***Detecting modular structure of the plant-fungus association network***

We estimated modularity (M) between 0 (low modularity) to 1 (high modularity) in both the plant-MF and plant-PF association networks. Modularity and number of modules (Beckett, 2016), were calculated in the R package BIPARTITE (Dormann, Fründ, Blüthgen, & Gruber, 2009). We compared the observed modularity with that calculated from 1000 null networks with constant marginal totals and connectance, constructed using the "swap" method in the R package BIPARTITE (Artzy-randrup & Stone, 2005; Dormann et al., 2009). We used relative modularity

$RM = (M - \overline{M}¿¿random)/\overline{M}_{random}¿$ (Olesen et al., 2007) to compare the degree of modularity of plant-PF and plant-MF association networks, where $\overline{M}_{random}$ was the average modularity of the 1000 randomized networks.

***Detecting the constraints of phylogeny and traits on network modularity***

To understand the relationship between phylogeny and modularity, we correlated the

phylogenetic relatedness of each pair of species and their degree of co-occurrence within modules. The co-occurrence of a pair of species within modules was measured with the Jaccard index. To study the effects on functional traits on modularity, we first tested whether host plants were functionally different between modules in each of measured traits using one-way Type II ANOVA. We further tested whether plant traits were more similar (chance corrected within-group agreements (A) based on Euclidean distance) within modules than between modules in the observed network using a multiple response permutation procedure (MRPP) in the R VEGAN package (Oksanen et al., 2019). All analyses were conducted in R version 3.5.1 (R Core Team, 2015).

***Analyzing the drivers of module composition***

We assigned plants to modules using traits and plant phylogeny, and fungi to modules based on fungal phylogeny, using random forest models implemented in the R RANDOMFOREST package (Liaw & Wiener, 2002). We calculated phylogenetic eigenvectors to reveal phylogenetic relationships between plants or fungi (Diniz-Filho, de Sant'Ana, & Bini, 1998). We constructed a full model for each host plant, including 17 plant traits and the first 10 eigenvectors of the plant phylogeny. We did the same for each type of root fungus (MF/PF), including the first 10 eigenvectors of the fungal phylogeny. Finally, we fitted the reduced random forest models to explain the modular memberships for plants and fungi, selecting variables for each full model using the smallest OOB error (Evans & Murphy, 2019). Model accuracy was defined as the amount of variation explained by each random forest model in predicting the

assignment of plants and fungi to the observed modules. We fitted another two models containing phylogenetic and trait variables, to further partition the variance into unique and shared components when assigning plants to modules.

## Results

### *Structural properties of plant-MF and plant-PF association networks*

We divided the plant-MF association network into 14 modules (Fig. 1a) and the plant-PF association network into 9 modules (Fig. 1b). We observed significantly higher modularity relative to randomized networks in both the observed plant-MF association network (M = 0.467, CI null models [0.095, 0.108]) and in the plant-PF association network (M = 0.414, CI null models [0.044, 0.065]) (Fig. 1a, b). Moreover, relative modularity was higher in the plant-PF association network (9.17, Fig. 1b) than in the plant-MF association network (7.33, Fig. 1a).

### *The constraints of phylogenetic history and functional traits on modularity*

Distant fungal relatives were less likely to co-occur in modules of both the plant -MF and the -PF association networks (Table 1). Phylogenetic relatedness between plant species did not significantly affect distribution across modules (Table 1). Certain functional traits of host plants varied between modules in both the plant-PF and -MF association networks (Fig. 2). Specifically, leaf dry matter content (LDMC), maximum photosynthesis rate (Am), light saturation point (LSP) and root tissue density (RTD) differed significantly between modules in plant-MF association networks, while leaf dark respiration rate (Rd) and light compensation point (LCP),

specific root length (SRT) and specific root area (SRA) significantly differed between modules in plant-PF association networks (Fig. 2). In addition, Am, LDMC and LSP were more similar for host plants within modules than between modules in the plant-MF network (Table 2), while LCP, Rd, SRL and SRA were more similar for host plants within modules than between modules in the plant-PF network (Table 2).

***Drivers of module composition***

In assigning host plants of MF and PF to modules in observed networks, the reduced random forest models, including the traits and phylogeny of host plant species, gave correct prediction rates of 27.4% and 31.0% respectively (Fig. 3a, b). Plant traits were more accurate in predicting host-plant module memberships (22.7% and 20.7% for plant-MF and -PF networks) than plant phylogenies (3.3% and 3.4% for plant-MF and -PF networks) (Fig. 3a, b). Fungal phylogenies of both mycorrhizal and pathogenic fungi had accuracy rates of 28.8% and 32.7% in assigning MF and PF to observed modules (Fig. 3c, d).

## Discussion

Our study reveals the underlying phylogenetic patterns and functional processes generating modularity in antagonistic and mutualistic root-associated fungal networks. We found that, belowground, antagonistic plant-PF association networks exhibit stronger modularity than plant-MF networks. Our results concur with aboveground studies showing that modularity is more pronounced in antagonistic than in mutualistic networks (Thébault & Fontaine, 2010). Species traits most accurately

predicted module assignments for plants, whereas fungal phylogeny most accurately predicted module assignments for fungi. We also found a set of phylogenetically related root-associated fungi that tended to form symbiotic relationships with plants in the same module. These module plants shared similar traits, suggesting that trait-based selection drives the non-random assembly of plant-fungus networks (Chagnon et al., 2015). Our study illustrates the importance of fungus evolutionary history and plant functional traits in driving the network assembly of plant-root fungi.

***Greater modularity in the plant-PF association network***

Both mutualistic plant-MF and antagonistic plant-PF association networks exhibited modularity in the study forest, as found in some plant-fungus association networks in harsh alpine and subalpine habitat (Toju, Tanabe, & Ishii, 2016), and semi-natural grassland (Sepp et al., 2019). In our local plant-fungus association network, root-associated fungi (883 MF OTUs and 113 PF OTUs) were diverse, while host plants (43 species) were scarce. High partner selectivity of fungal species can account for modularity within a plant-fungus association network (Chagnon et al., 2015). This would result from competitive exclusion of root fungi for limited plant resources, causing niche partitioning and thus modularity.

Previous studies observed that mutualistic MF fungi had relatively low levels of host preference (Muneer et al., 2019; Roy-Bolduc, Laliberté, & Hijri, 2016). Accordingly, we found much stronger host specificity in antagonistic pathogenic fungi (Wang et al., 2019). This could explain the more pronounced modularity in our plant-PF association network (RM = 9.17) than in our plant-MF association network

(RM= 7.33). Elsewhere, antagonistic plant–herbivore interactions have been shown to exhibit greater modularity than mutualistic plant-pollinator interactions (Thébault & Fontaine, 2010).

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

Long term, stable symbiosis between host plants and AM fungi creates a situation where host plants within the same modules are exploited by phylogenetically related AM fungal assemblages (Chagnon et al., 2015). Root mycorrhizal fungi in our study forest included many species of EM (862 OTUs), and few species of AM (21 OTUs). Although the symbiotic relationship between plants and EM (hyphae surrounding host cells) are not as intimate as that of plants and AM (hyphae penetrating host cells), we found that phylogenetically related species of root mycorrhizal fungi tended to occur in the same modules as pathogenic fungi (Tables 1, S2 and S3). This result suggests that the modular organization of plant and root fungus networks generally reflect the main split in the fungal phylogeny. Phylogenetically related plants have been detected in the modules of mutualistic plant-AM networks, and in the compartments of plant-antagonistic fungus networks (including leaf and root decay fungi and parasitic fungi) (Chagnon et al., 2015; Davison et al., 2020; Vacher et al., 2008). In our study, however we found host plants interacted with mycorrhizal and pathogenic fungi in the same module were not phylogenetically related (Tables 1, S2 and S3). It is probable that the evolutionary history of plant-root fungus interaction networks depends upon the functional types of fungi and environmental conditions. To some extent, our results may also be constrained by statistical power, stemming from the relatively low

number of plant species that associate with MF and PF symbionts. Shared evolutionary history only partially explains the modular patterns observed in our two plant-fungus networks.

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

Host plants cannot acquire equal benefits from all fungal species or populations. Thus, to ensure optimal fitness alignments between hosts and symbionts, host selectivity may contribute to the shaping and evolution of host-fungus symbioses. For instance, previous studies have suggested that stress tolerant plants (assessed by carbon-use efficiency) might preferentially associate with AM fungi that enabled them to complete their life cycle with low biomass or reduced species turnover rates (Chagnon et al., 2015; Chagnon, Bradley, Maherali, & Klironomos, 2013). We found that mycorrhizal fungi associated with host plants that shared similar photosynthetic and morphological traits within a module, and with distinct traits between modules (Fig. 2 and Table 2). This could result from specialization of mycorrhizal fungi for a suite of plant traits, enabling the fungi to improve their fitness from interacting host plants. Significant differences in AM fungal community composition between C4 and C3 plants (Davison et al., 2020) suggests that plant photosynthetic capacity is regulated by fungi via host use of available carbon rewards. LDMC and RTD represent a plant's investment in leaf structure and root structures, which relate to the physical defenses and resource use efficiency of plants. In our plant-MF networks, plants in different modules exhibited different photosynthetic capacity (Am and LSP) and morphological structure (LDMC and RTD) (see Fig. 2a). These observations accord

with earlier observations of a distinct morphology (LDMC) in modules of a plant-AM network (Chagnon et al., 2015), further supporting the notion that module assembly of plant-MF association networks is regulated by plant ecological strategies (Chagnon et al., 2015). Specifically, we found that host plants with high photosynthetic capacity (high Am) in modules 1 and 9 attracted mycorrhizal fungi (Fig. 2a), which in turn boosted the root defense ability (RTD) of the host plants, thus helping the mycorrhizal fungi resist the symbiosis of other parasitic fungi. In contrast, as a result of interspecific competition, mycorrhizal fungi in modules 11 and 12 associated with plant roots with low photosynthetic capacity (indicated by low Am and LSP) and high physical defenses (RTD) (Fig. 2a). This example illustrates how evolutionary convergence of plant traits, leading to functional complementarity between host plants, would result in modularity between mycorrhizal fungi.

To resist attack from root pathogens, whilst overcoming the effects of pathogenic diseases, host plants need to consume vast amounts of energy. Respiration allows plants to provide timely responses to attack from pathogens by releasing energy from accumulated nutrients. Energy storage and release strategies of host plants likely regulate the distribution of pathogens across modules. Our results support this notion, in terms of the remarkable differences we observed in photosynthesis and dark respiration traits (Rd and LCP) and root nutrient use traits (SRL and SRA) of PF host plants between modules (Fig. 2b). Traits associated with nutrient use in roots can also impact non-random interactions between host plants and pathogenic fungi; plants with ramified root systems (low nutrient use efficiency), for

example, were less likely to be attacked by pathogens thanks to protection derived from their AM fungal symbionts (Sikes, Cottenie, & Klironomos, 2009). These results show clearly that trait-based plant ecological strategies are able to dictate the host selectivity of root fungi and ultimately modular structure.

***Drivers of modularity***

Our findings confirm that plant traits and fungal phylogeny play important roles in predicting the assignment of host plants and fungi to network modules, revealing that niche-based processes might be the main drivers of plant-fungus association networks. Nevertheless, the predictors we used (leaf morphology, photosynthesis and root morphology, see Fig. 3), failed to explain a substantial amount of the variation in assigning plants and fungi to modules. Incorporating the functional traits of fungi, and especially root chemical defense traits, will help to elucidate the assembly rules of plant-fungus association networks.

Use of the network approach has revealed important insights into the topological structure of a highly diverse plant root-fungus association network. Analyzing the constraints of host plant phylogeny and species traits on module composition enabled us to elucidate the ecological and evolutionary processes driving plant–fungus interactions. Plant distributions and soil environments colonized by root fungi can affect plant-fungus interaction patterns (Burke, López-Gutiérrez, Smemo, & Chan, 2009). Future work on the non-random topological structure of plant-fungus networks under various habitats will reveal how fungus networks are responding to environmental change, and the consequences for species diversity and community

stability.

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## Authors' contribution

CZ analyzed the data and led the writing. ZW, WL, JF, YC and DH collected plant-fungus association data, root trait data, photosynthetic trait data, leaf stoichiometric and leaf morphological trait dataset, respectively. MDFE, CC and YL revised the manuscript.

## Data availability statement

Taxonomic information of root fungal OTUs, interaction matrices of plant-mycorrhizal fungus and plant-pathogenic fungus can be available from a previously published study (https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.15786).

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**Table 1** The relationships between phylogenetic relatedness between pairs of host plants (or fungi) and their co-occurrence in a same module in plant-MF and plant-PF networks. Phylogenetic relatedness did not correlate significantly with pairwise co-occurrence of host plant species within modules (MF hosts: $r = 0.045$, $p = 0.172$ and PF hosts: $r = -0.049$, $p = 0.140$). However, decreasing phylogenetic relatedness reduced pairwise co-occurrence of fungal species within modules (MF: $r = -0.007$, $p < 0.001$ and PF: $r = -0.047$, $p < 0.001$).

| **Networks** | **Module composition** | ***r*** | ***p* value** |
|---|---|---|---|
| Plant-MF network | MF hosts | 0.045 | 0.172 |
| | **MF** | -0.007 | **<0.001** |
| Plant-PF network | PF hosts | -0.049 | 0.140 |
| | **PF** | -0.047 | **<0.001** |

**Table 2** Results of a multiple response permutation procedure (MRPP) test of dissimilarity for host plant trait composition. Traits with a *delta* value smaller than 0.05 were significantly more similar within modules than between modules.

| | **plant-MF association network** | | **plant-PF association network** | |
|---|---|---|---|---|
| **Traits** | *A statistics* | *delta* | *A statistics* | *delta* |
| SLA | -0.043 | 0.646 | 0.03674 | 0.247 |
| LDMC | 0.158 | **0.036** | 0.026 | 0.320 |
| LCC | -0.005 | 0.497 | 0.067 | 0.144 |
| LNC | -0.043 | 0.683 | 0.004 | 0.439 |
| LPC | -0.067 | 0.773 | 0.084 | 0.116 |
| Am | 0.263 | **0.017** | -0.072 | 0.837 |
| IQE | -0.060 | 0.655 | -0.052 | 0.706 |
| LCP | -0.063 | 0.703 | 0.271 | **0.003** |
| Rd | -0.090 | 0.790 | 0.242 | **0.002** |
| LSP | 0.185 | **0.048** | -0.077 | 0.852 |
| Jm | 0.096 | 0.155 | -0.069 | 0.812 |
| Im | 0.062 | 0.279 | -0.054 | 0.718 |
| RDM | 0.013 | 0.458 | 0.101 | 0.122 |
| AD | -0.045 | 0.636 | -0.019 | 0.565 |
| SRL | 0.051 | 0.303 | 0.146 | **0.049** |
| RTD | 0.150 | 0.102 | -0.085 | 0.858 |
| SRA | 0.050 | 0.330 | 0.156 | **0.044** |

**Figure legends:**

**Fig. 1** Network modularity of plant-mycorrhizal fungus (plant-MF, a) and plant-pathogenic fungus (plant-PF, b) association networks. (a) The plant-MF association network contained 14 modules; (b) the plant-PF association network contained 9 modules (b). Plant species are denoted by triangles and fungal species are denoted by circles. Lines connect plant and fungal species in different modules. Relative modularity values of observed plant-MF and plant-PF networks were calculated using randomized association networks.

**Fig. 2** Differences in plant leaf, photosynthetic efficiency and root functional traits between modules in (a) plant- MF and (b) plant- PF association networks. Of the 17 traits tested, LDMC, AM, LSP and RTD differed significantly between modules of the plant-MF association network, whereas Rd, LCP, SRL and SRA differed significantly between modules of the plant-PF association network. Significance levels are as follows: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

**Fig. 3** Venn diagrams partitioning the variation explained by species traits and phylogeny in assigning species of host plants and fungi to modules. In the association networks of (a) plant-MF and (b) plant-PF, variation in assigning plants to modules is partitioned into plant traits and phylogeny. In the association networks of (c) plant-MF and (d) plant-PF, variation in assigning fungi to modules is uniquely explained by fungus phylogeny.

**Supplements information:**

**Fig. S1** Phylogenetic tree of 43 plant species in a 50-ha subtropical forest plot in Heishiding nature reserve of Southern China.

**Fig. S2** Phylogenetic tree of 883 mycorrhizal fungal species in a 50-ha subtropical forest plot in Heishiding nature reserve of Southern China.

**Fig. S3** Phylogenetic tree of 113 pathogenic fungal species in a 50-ha subtropical forest plot in Heishiding nature reserve of Southern China.

**Table S1** The details of 17 functional traits used in our study. Leaf traits and root traits are listed.

**Table S2** Standardized mean nearest taxon distance (MNTD) of species composition of mycorrhizal fungi (MF, above) and plants (below) in the modules of the plant-MF association network.

**Table S3** Standardized mean nearest taxon distance MNTD of species composition of pathogenic fungi (PF, above) and plants (below) in the modules of the plant-PF association network.

**Method S1** Phylogenetic constraints of plants and fungi on module composition in the plant-fungus association network.