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ARTICLE

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Plant functional traits and abundance jointly shape keystone plant species in a plant–ectomycorrhizal fungus network

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Abstract

Keystone species are more important than others for community dynamics and stability. Keystone species can be identified and evaluated by their centrality (i.e., a relative ranking of the topological positional importance of a species) in ecological networks. Studies of node centrality of plant–fungus bipartite networks, for example, have identified the keystone species that are important for maintaining network structure and stability. However, the underlying drivers of the importance of species in a network have rarely been examined. We assessed the centrality (degree, closeness, and betweenness) of plant and fungal species in a plant–ectomycorrhizal fungus network in a subtropical forest in southern China. Based on the phylogenies of plants and fungi and plant traits, we explored ecological factors that led to a species taking a central position or not. We found one plant species (*Ternstroemia gymnanthera*) and four species of ectomycorrhizal fungi (*Russula citrina*, *Scleroderma* sp., and two *Cenococcum* sp.) were characterized by the highest centrality of degree, closeness, and betweenness among the bipartite network nodes and thus played key roles in maintaining network structure. Centrality for fungi (not for plants) was phylogenetically constrained. Plant traits and abundance together explained 46.36%, 46.0%, and 43.7% of variation in the centrality of degree, closeness, and betweenness of plant species in the bipartite network, respectively. When plant or fungal species were sequentially removed on the order of higher to lower centrality, network was less stable than randomly removed. We suggest that abundance and traits determine the positional importance of plant species in a network. This work helps understand how plant–fungus association networks will respond to species extinction and changes in species abundance and functional traits due to habitat fragmentation and human activities.

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KEYWORDS
degree centrality, keystone species, mycorrhizal fungi, network property, network robustness, plant–fungus network, rhizosphere, subtropical forest, symbiosis, topological roles

INTRODUCTION

The topological structure of complex ecological networks strongly determines their dynamics and stability (Allesina & Pascual, 2008; Robinson & Strauss, 2020; Thébault & Fontaine, 2010). However, not all nodes in a network are equally important for the dynamics and stability of the system. For example, in conservation biology, it is crucial to identify keystone species, that is, relatively more important nodes than others for maintaining community structure and ecosystem functioning. Identification of keystone species can increase predictability of the persistence of species-rich communities and improve the efficiency of management efforts for species with high extinction risk (Capocefalo et al., 2018).

Complex network theory has been applied as a robust theoretical framework to assess and identify the keystone species. From a graph theoretical perspective, we can identify keystone species by ranking species centrality which quantifies the topological positional importance of a species to the community structuring (Lewinsohn & Cagnolo, 2012). Some nodes have high centrality (central positions) in a network and connect to most other nodes, while many other nodes have low centrality (peripheral positions). The top-ranked nodes by centrality (most central nodes) are often considered as the keystone species in the community (Jordán et al., 2006; Mello et al., 2015). The positional importance of these central nodes in a network is illustrated by a faster breakdown of the whole network structure and stability when nodes are selectively removed on the order of higher to lower centrality than when species are randomly removed from a network (Albert et al., 2000; Dunne et al., 2002; Stouffer et al., 2012). Identifying the keystone (central) species in the network and illustrating the underlying ecological factors affecting network centrality of species thereby could substantially improve understanding network assembly.

Many studies have evaluated the positional importance of species in aboveground ecological networks and demonstrated that the centrality was regulated by abundance (positive effect on centrality) (Hervías-Parejo et al., 2020; Montoya-Arango et al., 2019), functional traits (Gómez & Perfectti, 2012), and evolutionary history (phylogenetic relatedness) (Dallas et al., 2019). Network-based tools are being increasingly applied in belowground fungal networks (Chagnon et al., 2013; Chen et al., 2017; Montesinos-Navarro et al., 2012; Toju et al., 2015). Few studies have evaluated the positional importance of particular plant and fungal species in plant–fungus networks (Argüelles-Moyao et al., 2022; Fodor, 2013; Tylianakis et al., 2018). However, little information exists about the complex relationships between species centrality and other ecological characteristics (traits and environments) in plant–fungus networks. A lack of such information has substantially hindered our understanding of the community-scale processes of belowground plant–fungus symbioses. Specifically, the information on the network centrality of a species will entail the crucial consequences for the performance of the species and offer insights into the function of the whole ecosystem and complex ecosystem stability.

For the plant–root fungus symbioses, a growing number of studies make efforts to explore the underlying drivers of species specialization (negatively related to the centrality of degree) and assembly rules of the fungal community (Gilbert et al., 2007; Sepp et al., 2019; Wang et al., 2019). By analyzing the fungal community on each plant (plant individual × fungal species matrix), studies have illustrated that the species richness of root fungal community can be regulated by plant traits (Sepp et al., 2019), abundance (Gilbert et al., 2007), and phylogenetic relatedness (Wang et al., 2019). For instance, root fungal richness is related to increasing leaf carbon concentration, apparent quantum yield (AQY), root diameter, root nitrogen, and specific root area while decreasing leaf nitrogen concentration and specific root length (SRL) (Sweeney et al., 2021; Wang et al., 2019). Specifically, plant species with thin root diameter and long SRL tend to be colonized by a low richness of ectomycorrhizal (EM) fungi (Comas et al., 2014). Furthermore, fungal richness on host plant (Tedersoo et al., 2013) and host range of fungi (Gilbert & Webb, 2007) are often phylogenetically conserved. Thus, ecological traits and phylogenies are expected to impact the positional importance of a species in plant–fungus network structure. A correlation has been reported between species abundance and node centrality (closeness) in a plant–arbuscular mycorrhizal (AM) fungus network (Tylianakis et al., 2018). Nevertheless, no evidence illustrates how traits and phylogeny regulate the species centrality in plant–fungus networks.

To fill this knowledge gap about what drives species centrality, we compiled a highly diverse plant–mycorrhizal fungus interaction matrix previously constructed in a 50-ha subtropical forest plot in South China (Wang et al., 2019). We quantified the network centrality of species using three commonly used metrics of centrality (degree, closeness, and betweenness) because there is no unifying approach to evaluate the network centrality of species (Lai et al., 2012). We explored which plant and mycorrhizal fungal species showed the highest centralities in the bipartite plant–mycorrhizal fungus network. We further collected plant–mycorrhizal fungal phylogeny and plant functional traits (photosynthesis and root structure traits) to understand the ecological attributes leading a species become central in a network. We hypothesize that (1) there exists a phylogenetic constraint in the centrality of fungal and plant species and (2) abundance, phylogeny, and traits of the plants together determine the centrality of plant species. Specifically, we expect that plant abundance has a positive effect on the centrality of plant species due to that fungi have a higher probability to encounter abundant plants than rare plants. Also, plants with high nutrient reward potential (such as high leaf photosynthesis efficiency and root nutrient-using efficiency) are expected to exhibit high centrality by attracting the occupancy of EM fungi. Lastly, to verify the importance of species with high centrality to network stability, we compared the impact of the removal of plant and fungal species from most to least centrality with random removal on the robustness of network.

METHODS

Study area

This study was conducted in a 50-ha subtropical forest plot in the Heishiding Natural Reserve of Southern China, which is located in the 23°25′–23°29′ N, 111°49′–111°55′ E with the Tropic of Cancer going through its center. In this region, the mean annual temperature is 19.7°C and annual precipitation is 1750 mm. The 50-ha forest plot was established in 2012. All the trees with a diameter >1 cm (at 1.3 m breast height) were identified as species. This plot included about 269,000 stems of 213 woody plant species.

Data collection

The dataset of root-associated fungus community on 512 root samples of 43 tree species was previously collected in the 50-ha forest plot (Wang et al., 2019). These trees belonged to 21 families and 37 genera (Appendix S1: Table S1). These focal plants were composed of seven abundant species (>1000), 19 rare species (<50 individuals in the plot), and 17 common species (Appendix S1: Table S1). Fine roots were excavated by tracing from the thick roots of each tree (5–19 individuals for each plant species; Appendix S1: Table S1). The discrepancy in sampling individuals among tree species resulted from (1) the rarity and root structure of some tree species that limited the collection of fine roots and (2) failure to extract high-quality DNA. At least three samples from each tree were collected in different directions and then pooled to create a single sample (Wang et al., 2019). Out of 100 fine-root samples of host plants (randomly selected from all root samples) we traced, 97 were correctly validated by DNA fragment *rbcL*a (Wang et al., 2019). Thus, we considered that the tracing method could reliably construct the associations between plant roots and fungi.

The root-associated fungi were identified by the internal transcribed spacer (ITS) region of fungal rDNA in a previous study (Wang et al., 2019). Polymerase chain reaction production of ITS region was sequenced using Roche 454 sequencing technology. The sequencing depth observed for the root fungal community on each plant individual ranged from 3217 to 66,056, with more than 5000 sequencing depth for 91% root sample (Appendix S1: Figure S1). Rarefaction curve showed that the number of fungal operational taxonomic units (OTUs) increased slowly when the number of sequences reached 5000 (Appendix S1: Figure S1). Thus, the coverage of fungal OTUs is considered to be sufficiently high to reflect fungal diversity associated with plant species and conduct network analyses of plant–fungus. To mitigate the false-positive association from high-throughput sequencing, each root sample was subsampled to the minimum number of reads (Wang et al., 2019).

The OTUs of root fungi were distinguished using a threshold of 97% sequence identity (Wang et al., 2019). The OTUs with less than five sequences were removed to delineate the impact of tag switching, cross-contamination, and sequencing errors (Wang et al., 2019). Each sequence was assigned to a taxonomic label based on UNITE database using the Ribosomal Database Project (RDP) Classifier (Wang et al., 2007). Afterward, EM fungi were assigned taxonomic information using a database of EM fungal taxa and lineages (Tedersoo & Smith, 2013) while AM fungi were assigned if they belonged to Glomeromycota (Schüßler, 2002). As 22 AM and 862 EM fungal OTUs were identified, this study only focused on analyzing the network centrality of EM fungi. These EM fungi belonged to 17 families and 23 genera of the root fungal microbiota, which

included five most abundant families Russulaceae (58.27%), Sclerodermataceae (13.31%), Incertae_sedis (9.14%), Clavulinaceae (6.97%), and Cortinariaceae (6.45%) and five most abundant genera *Russula* (51.22%), *Scleroderma* (13.31%), *Cenococcum* (9.14%), *Lactarius* (7.05%), and *Clavulina* (6.97%). The most common EM fungi were *Scleroderma* sp. AWW260 (131.558 ± 179.03 OTUs per plant species), *Russula* sp. B220 (106.279 ± 300.439 OTUs per plant species), and *Inocybe* sp. (73.418 ± 293.879 OTUs per plant species). There were four shared OTUs (two species of *Cenococcum*, *Russula citrina*, *Scleroderma* sp. AWW260) among 43 host plant species. Finally, we used the data collected for 43 plant species and 862 EM fungal OTUs to construct one plant–EM fungus network matrix (43 rows and 862 columns) based on binary (presence/absence) link information.

Evaluating the species centrality in a plant–EM fungus network

Weighted centrality metrics can be evaluated based on quantitative link information, but few biological interpretations have been made on the use of weighted centrality metrics (e.g., weighted betweenness) in analyses of plant–microbe associations (Toju et al., 2018). Thus, to evaluate the positional importance of a given species in network organization, we calculated three centrality metrics based on binary (presence/absence) link information, instead of quantitative (frequency) link information (i.e., the mean abundance of each fungal species on each plant individual). The three centrality metrics measure the structural importance of a given node from different aspects. Degree centrality (DC) evaluates the importance of a species according to the summed number of links per node in the network (i.e., the level of generalization) (Freeman, 1978). DC of a node i is $DC_i = E_i$, where E_i is the number of edges connected to node i. A species with high DC can be important because it can directly affect other interacting partners (e.g., via cascading extinction effect). Betweenness centrality (BC) is defined as the proportion of shortest paths between all possible node pairs that pass through a determined node (Newman, 2003). BC for a node i is $BC_i = \frac{2\sum_{i \neq j, j<k} g_{jk}(i)/g_{jk}}{(N-1)(N-2)}$, where N is the number of species in the network, g_{ik} is the number of shortest paths connecting any two species, and $g_{ik}(i)$ is the number of shortest paths among g_{ik} that go through node i. Thus, a species with high BC can be important because it mediates many indirect interactions between species (Lai et al., 2012) and acts as connector between different parts of the ecological networks (Anderson & Sukhdeo, 2011). Closeness centrality (CC) measures proximity of a node to all other nodes in the network, that is, how many steps away one species is from all others in the network (and then inverses it) (Poulin et al., 2013). CC for a node i is $CC_i = \frac{N-1}{\sum_{j=1;\, i \neq j}^{N} d_{ij}}$, where N is the number of species and d_{ij} is the length of the shortest paths between nodes i and j in the network. A species with high CC can be important because its population fluctuation can affect others more rapidly (Lai et al., 2012). All three centrality metrics (i.e., DC, CC, and BC) for each species were calculated using the R package *igraph* (Csardi & Nepusz, 2006). For each centrality index, we identified and pooled the top-ranked species by centrality and considered them as keystone species (Jordán et al., 2006; Mello et al., 2015).

To evaluate the impact of unequal sampling effort among plant species on network centrality of plant species in our plant–EM fungus network, we checked how centrality metrics varied with the number of sampled individuals of each plant species. For each plant species, multiple root fungal samples with unequal sampling effort (1–18 individuals) were sampled with replacement from its empirical sample and then used to reconstruct new plant–EM fungus networks with the empirical root fungal samples of other plant species in our data. For each sampling effort of each focal plant species, 100 new plant–EM fungus networks which were generated from 100 runs of sampling with replacement were used to calculate the mean and SD of network centrality.

Detecting the phylogenetic constraint of species centrality

To evaluate phylogenetic signals for three centrality metrics of plants and EM fungi, two of the most widely used statistics, Pagel's λ (Pagel, 1999) and Blomberg's K (Blomberg et al., 2003), were implemented within the *phylosig* function in the R package *phytools* (Revell, 2012). The maximum likelihood phylogenetic tree of plants was previously reconstructed using four plant DNA barcodes (*rbcL*a, *matK*, *trnL*, and ITS2) (Zhu, Wang, Luo, et al., 2022). The phylogeny for the EM fungi was previously reconstructed using ITS sequences with a constraint backbone phylogeny (Zhu, Wang, Deane, et al., 2022; Zhu, Wang, Luo, et al., 2022), which was generated based on fungal taxonomic ranks using a Perl script (Tedersoo et al., 2018). The two phylogenies were used to test phylogenetic signals of centrality metrics. Both λ and K statistics assume a Brownian motion model of the evolution, and their values vary from 0 to 1 for λ and from 0 to ≫1 for K. The statistical significance of K was measured by comparing the observed variance of independent contrast of a metric of centrality to that in the 999 null models by shuffling taxa labels across tips on the phylogenetic tree (Blomberg et al., 2003), whereas the

statistical significance of λ was assessed based on a comparison of the likelihood of a model accounting for the observed λ with the likelihood of a model that assumed complete phylogenetic independence (Pagel, 1999). All calculations were performed using the R software (R Core Team, 2019).

The effects of plant abundance and traits on the centrality of fungal host plants

Plant abundance was calculated as the sum of all individuals of each plant species in the 50-ha plot. Out of 43 plant species, we collected 11 functional traits of 29 plant species (six photosynthesis traits and five root traits; Appendix S1: Table S2) from trait database in the 50-ha forest plot (see Feng et al., 2018; He et al., 2018; Luo et al., 2021). Further, we constructed multivariate linear regression models to reveal how plant abundance, photosynthesis, and root traits determined the centrality of host plant species in the bipartite plant–EM fungus network. In the regression model, each of three centrality metrics (DC, CC, and BC) of plant species was taken as a response variable and 11 plant traits and plant abundance were taken as explanatory variables. All three regression models had normal residuals; thus, we did not conduct variable transformation. Centrality metrics and explanatory variables were standardized, ranging from 0 to 1 in all three regression models. Finally, a reduced model for each full linear regression was selected based on a minimum Akaike information criterion (AIC) (Burnham & Anderson, 2004).

The effects of plant and fungal species extinction on network robustness

To test the importance of central species in maintaining community stability, we estimated network robustness by assessing the vulnerabilities of fungal and plant communities to the primary extinctions of their plant and fungal partners. We simulated extinction cascades in fungal and plant communities by removing their host plants and associated fungi in descending order from their centrality in four ways: systematic removals of host plants and fungi from strong to weak DC, from strong to weak CC, from strong to weak BC, and random removal of host plants and fungi without replacement. Random removal represented a null model with which to compare three types of systematic removal. A fungal or plant species was considered to be extinct if it was left without an associated partner. The curve of secondary extinction of root fungal and plant communities was fitted with an exponential regression model $y \sim 1 - x^a$ (Burgos et al., 2007; Memmott et al., 2004). In the model, x represented the proportion of species removed (primary extinction) in one trophic level and y represented the proportion of species that did not go secondarily extinct in the other trophic level. The power of secondary extinction curve (a, representing extinction vulnerability) and the area below the curve (R, representing network robustness) were used to quantify the tolerance of a system to the extinction of its component species (Burgos et al., 2007; Memmott et al., 2004). Above statistical analyses were performed in the R package *bipartite* (Dormann et al., 2009).

RESULTS

Species centrality and keystone species in the bipartite plant–EM fungus network

When the number of sampled individuals of each plant species reached 10, centrality metrics (degree, closeness, and betweenness) of plant species in the bipartite plant–EM fungus networks were approximately stable (Appendix S1: Figures S2–S4). This indicated that unequal sampling effort among species should have a minor effect on centrality metrics of plant species. Furthermore, by pooling the results from the top rank for each centrality index, we identified one most central plant and four most central EM fungi (Figure 1). For plants, *Ternstroemia gymnanthera* established symbolic relationships with the high richness of EM fungal community along with the highest degree centrality (DC = 193) in the bipartite plant–EM fungus network (Figure 1; Appendix S1: Figure S5). Besides, the species had the highest closeness (CC = 0.396; Appendix S1: Figure S5) and betweenness ($BC = 4.723 \times 10^4$; Appendix S1: Figure S5) in the bipartite network, suggesting its importance in maintaining network cohesiveness and mediating population dynamics. For fungi, four EM fungal species (*R. citrina* OTU123, *Scleroderma* sp. OTU153, *Cenococcum* sp. OTU2423, and *Cenococcum* sp. OTU3918) were symbiotic on all host plants (Figure 1) and had the equally highest degree (DC = 43; Appendix S1: Figure S6), closeness (CC = 0.512; Appendix S1: Figure S7), and betweenness ($BC = 7.143 \times 10^3$; Appendix S1: Figure S8) in the network.

The phylogenetic constraint of species centrality

We found no significant phylogenetic signals on all three centrality metrics (DC, CC, and BC) for EM host plants (all $p > 0.05$; Table 1). But for EM fungi, results were metric-specific; Pagel's λ showed significant but weak

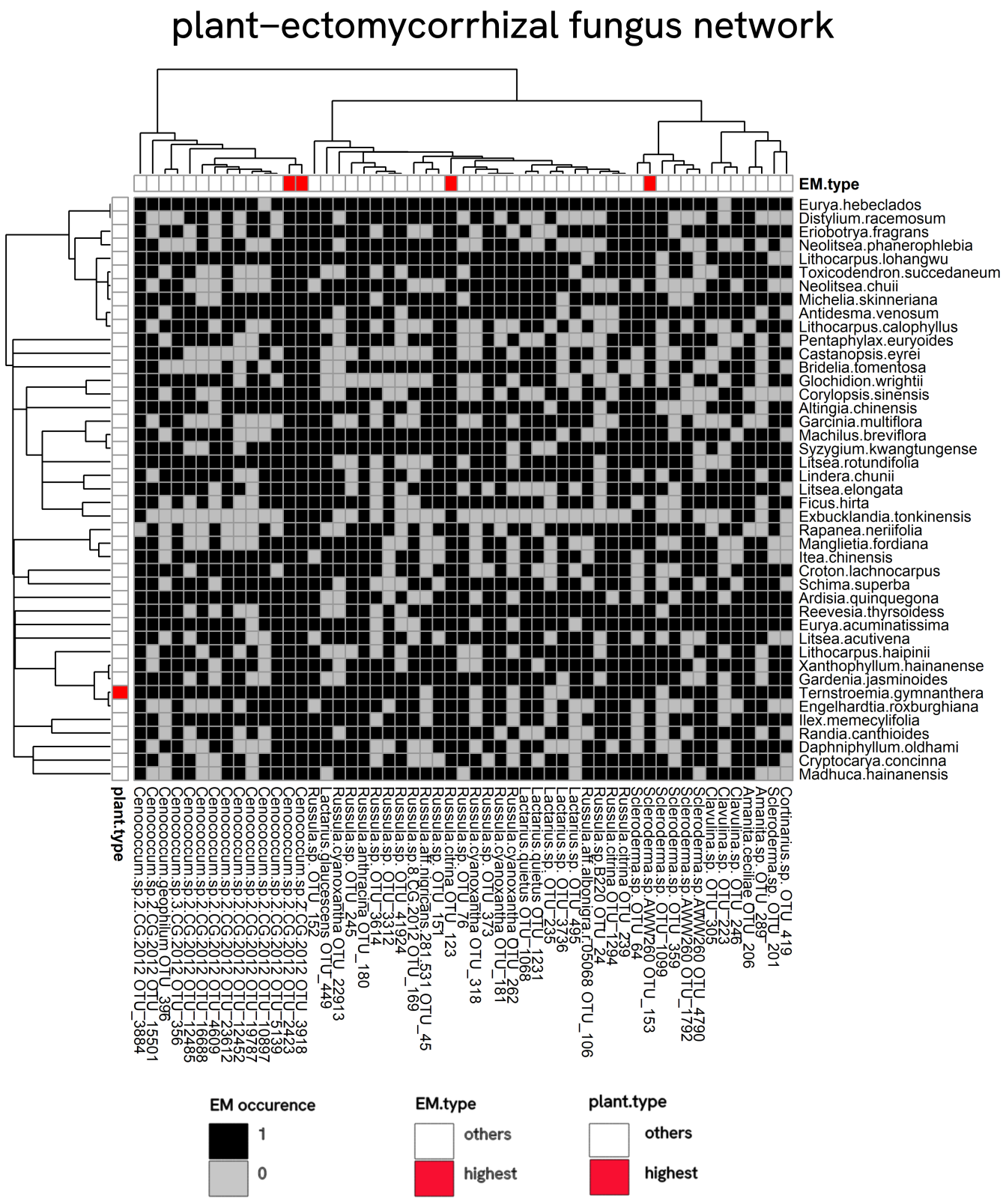


FIGURE 1 A visual plot of qualitative plant–ectomycorrhizal (EM) fungus network. All plant species are denoted in rows and only partial fungal species are shown in columns. In the heatmap, black is filled if there is link information (presence) between plant species and fungal species, gray is filled if there is no link information (absence). The dendrogram trees for plant species (left) and EM fungal species (top) are also shown. The four fungal species with highest centrality (EM.type: highest) and one plant species with highest centrality (plant.type: highest) are highlighted in red, while the other species are highlighted in white (EM.type: others and plant.type: others).

TABLE 1 Phylogenetic signals of species centrality in the bipartite plant–ectomycorrhizal (EM) fungus network.

Plant–EM fungus network	Centrality metric	*K*	*p*	λ	*p*
Plants	Degree	0.000	0.765	0.000	0.349
	Closeness	0.000	0.747	0.000	0.34
	Betweenness	0.000	0.81	0.000	0.483
EM fungi	Degree	0.000	1.00	0.138	<0.001
	Closeness	0.000	1.00	0.190	<0.001
	Betweenness	0.000	1.00	0.100	<0.001

Note: *K* and λ statistics indicate the strength of the phylogenetic signal in the centrality metrics. The value of *p* denotes the probability that centrality metrics deviate from the expectations of a Brownian motion evolution model.

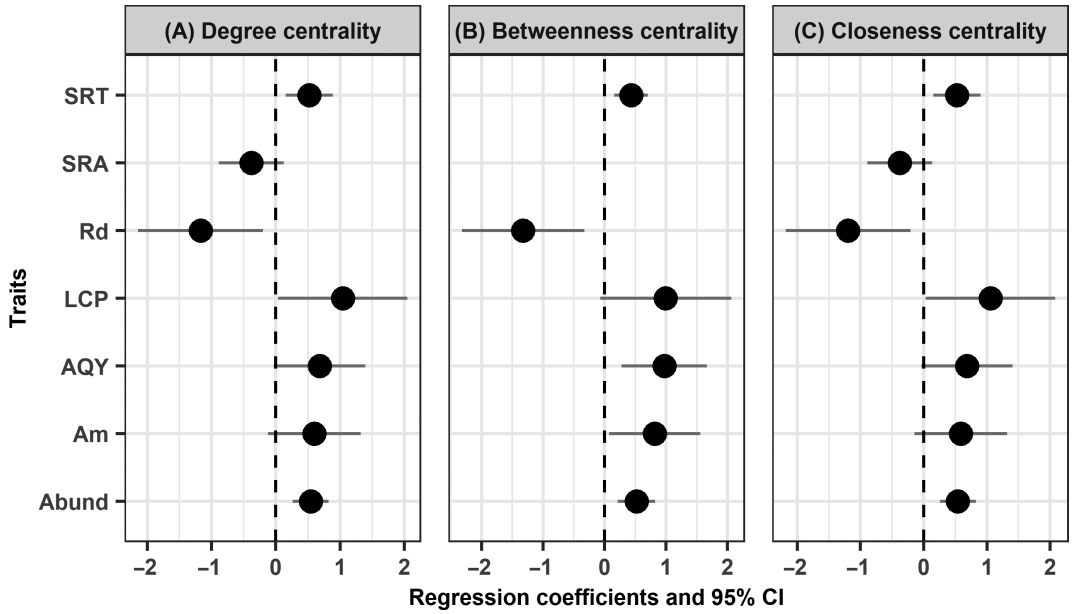


FIGURE 2 Multiple linear model evaluating the relative importance of plant abundance, photosynthetic and root variables on degree centrality (A), closeness centrality (B), and betweenness centrality (C) in the plant–ectomycorrhizal fungus network. The *x*-axis shows the regression coefficients and 95% CIs and the *y*-axis shows plant variables. In the figure, those variables whose 95% CI of regression coefficient does not overlap with zero are statistically significant. Root traits: SRT, specific root tips; SRA, specific root area. Photosynthesis traits: Rd, dark respiration rate; Am, maximum photosynthesis rate; LCP, light compensation point; AQY, apparent quantum yield. Abund, plant abundance.

phylogenetic signals in all three centrality metrics (all $p < 0.05$; Table 1) but this was not evident using Blomberg's *K* (all $p > 0.3$; Table 1).

The effects of functional traits and abundance on the centrality of host plants

Regression analysis showed that three reduced regression models explained 46.36%, 46.0%, and 43.7% of variation in the centrality (DC, CC, and BC) for fungal host plants, respectively. The three network centrality indices (DC, CC, and BC) of fungal host plants significantly increased with specific root tips (SRT) and plant abundance, while significantly reduced with leaf dark respiration rate (Rd) (Figure 2). In addition, the BC of fungal host plants significantly increased with AQY (Figure 2B).

The effects of plant and fungal species extinction on network robustness

Exponential regressions showed that the secondary extinction of root fungal community for random removal of the plants (random: $a = 2.179$; Table 2) was slower than for the removal of plants with decreasing DD, BC, and CC ($a = 1.197$, 1.130, and 1.197; Table 2). Similarly, the secondary extinction of plant community for random

TABLE 2 Proportion of species that are still alive in one trophic level to secondary extinctions as an exponential function of proportion of species removed (primary extinction) in the other trophic level in a bipartite plant–ectomycorrhizal (EM) fungus network.

Trophic level of primary extinction	Scenario of primary extinction	Regression coefficient *a*	*t*-statistic	*p* value
Plants	Decreasing degree	1.197	51.16	<0.001
	Decreasing betweenness	1.130	57.69	<0.001
	Decreasing closeness	1.197	51.16	<0.001
	Random	2.179	28.77	<0.001
EM fungi	Decreasing degree	15.510	142.90	<0.001
	Decreasing betweenness	15.511	154.50	<0.001
	Decreasing closeness	4.829	595.10	<0.001
	Random	101.525	363.80	<0.001

Note: Four different scenarios of primary extinction included systematic removals of species in a trophic level with decreasing degree, closeness, betweenness, and random removal of species without replacement. In the fitted models of exponential regressions, regression coefficient *a*, *t*-statistic, and *p*-value are shown in the table.

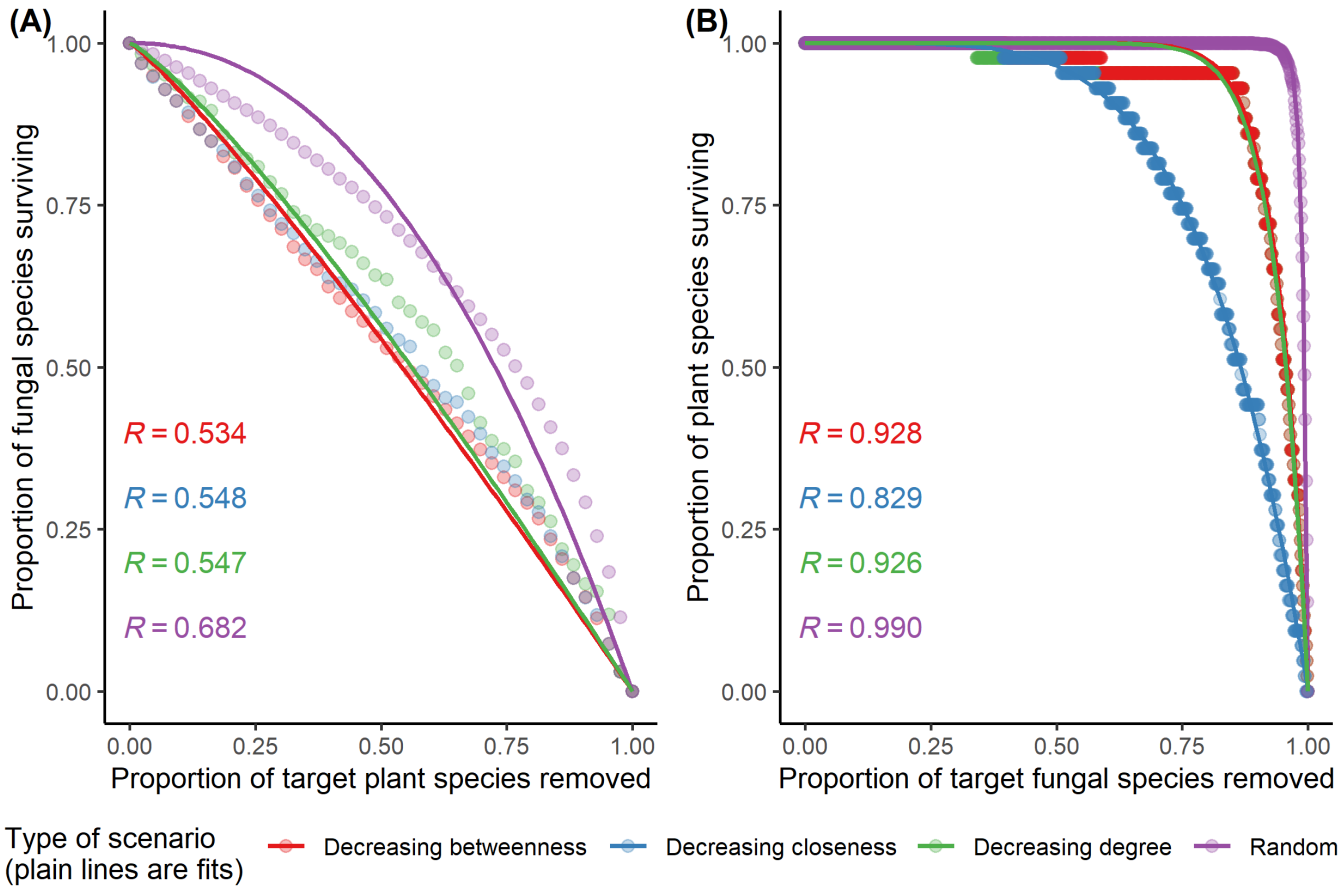


FIGURE 3 Simulation of network robustness in terms of four different scenarios of primary extinction of plants (A) and fungi (B) in a bipartite plant–ectomycorrhizal fungus network. The four scenarios of primary extinction included systematic removal of species with decreasing degree, closeness, and betweenness and random removal of species without replacement. Network robustness (*R*) under four scenarios of primary extinction of plants and fungi are denoted in different colors. The solid lines are the fitted curve of the proportion of surviving species in one trophic level against the proportion of species removed (primary extinction) in the other trophic level as an exponential regression model. In (A), two secondary extinction curves of fungal species are overlapping under the removal of plant species with decreasing betweenness and closeness (see regression coefficients in Table 2).

removal of fungal species ($a = 101.525$; Table 2) was slower than for the removal of fungal species with decreasing DC, BC, and CC (a = 15.510, 15.511, and 4.829; Table 2).

The network robustness was higher for the random removal of plant species ($R = 0.682$; Figure 3A) than for the removal of plant species with decreasing DC, BC, and CC ($R = 0.547$, 0.534, and 0.548; Figure 3A). Similarly, the network robustness was higher for the random removal of fungal species ($R = 0.990$; Figure 3B) than for the removal of fungal species with decreasing DC, BC, and CC ($R = 0.926$, 0.928, and 0.829; Figure 3B).

DISCUSSION

Keystone species and their roles in maintaining network robustness

For plants, *T. gymnanthera* is characterized by the highest DD, CC, and BC among plant nodes in the bipartite plant–EM fungus network, which suggests that this species should play a keystone role in maintaining network structure and stability. A positive influence of keystone species on the EM fungi, biomass production, carbon stock, and nutrient cycling (Li et al., 2020) may explain central placement of this species in the bipartite network of plant–EM fungus. For EM fungi, previous studies found that *Scleroderma* spp. and *Cenococcum* genus were broad-host-range colonizers with a high number of direct interaction partners at the genus level (Chen, 2006; Dickie, 2007). Similarly, we further found that *R. citrina*, *Scleroderma* sp., and two unresolved species of *Cenococcum* genus had the highest DC, CC, and BC among the EM fungal nodes of the bipartite network. The observed pattern that multiple fungal species occupy central positions in the network may ensure biomass flow across trophic levels if one keystone (central) species is suddenly removed or destructed (Lai et al., 2012). In addition, multiple species taking central placement in our plant–EM fungus network can guarantee the functionality of ecosystem due to differential responses of different species to environmental fluctuations which is proposed by the insurance hypothesis (Hooper et al., 2005).

Indeed, we found that network robustness decreased faster under removal of plants and fungi with decreasing centrality than random removal of plants and fungi. Moreover, the robustness of plant community under removal of fungi with decreasing centrality was particularly higher than that of fungal community under removal of plants with decreasing centrality (see Figure 3). Assuming that there is a chance to establish new interaction relationships between fungal OTUs present in the removed plant and other plant species, this rewiring tends to occur with plant species that are close relatives of the preferred hosts (Montesinos-navarro et al., 2019). Thus, phylogenetic relatedness of the fungal and plant community may associate with network stability. Taken together, to maintain ecosystem functioning and stability, it is thus advisable to conserve these keystone plant and fungal species.

The phylogenetic constraint of centrality of EM fungi

Previous studies showed that the architectures of a plant–fungus network were largely constrained by plant phylogeny rather than fungal phylogeny (Chen et al., 2017; Jacquemyn et al., 2011). In contrast, we found that network centrality for EM fungi was significantly phylogenetically constrained (only supported by λ statistics, Table 1), while the network centrality of host plants was not phylogenetically constrained (supported by λ and *K* statistics, Table 1). *K* statistics did not support phylogenetic constraint in the network centrality of EM fungi (Table 1), which might be due to the weak statistical power and skewed distribution of fungal centrality (Appendix S1: Figures S6–S8). Nonetheless, there are some indications that fungal phylogeny and phylogenetically conserved fungal traits may substantially contribute to the community assembly of plant–EM fungus networks.

However, no phylogenetic signal in the network centrality of host plants (Table 1) suggests that strong interspecific competition for plants may shape the plant–EM fungus association network. As well, competition can enhance the functional complementarity of phylogenetically related plant species and thus promote their coexistence in a community. A measure of phylogenetic signal, representative of the entire community, ideally requires sampling a large number of species that are broadly distributed across the phylogeny for the community (Rezende et al., 2007). Though a low number of plant species are collected in our study, our focal plants cover 21 families and are phylogenetically diverse (Faith's PD = 2.109 for 43 focal plant species; Faith's PD = 5.796 for 231 plant species investigated in the plot). These may lead to the network centrality of host plants being not strongly phylogenetically conserved (Table 1).

The effects of plant abundance and functional traits on the centrality of plant species

Consistent with our hypothesis, abundant plants tended to take centrality placement in our plant–EM fungus

network (Figure 2). This supports that abundant plant species are more generalized than less abundant plant species in their partner associations in a network (Sepp et al., 2019). As no other species are likely to take the placement of central plant species in a network structuring, the removal of central plants (with high abundance) may have a large effect on the function and dynamics of a subtropical forest ecosystem.

The central placement of a plant species in a network is affected not only by plant abundance, but also by other plant traits such as plant photosynthesis and root traits (Figure 2). Plant photosynthesis traits can significantly affect the topological position of plants in the plant–EM fungus network (Figure 2), probably via regulating the selectivity of plants for EM fungi. EM fungi have been reported to improve photosynthetic rates and growth of shaded plant seedlings as well as reduce the light compensation point (Shi et al., 2017). This finding indicates that EM fungi can enhance the survival and competitiveness of host seedlings under low light (Shi et al., 2017). Furthermore, optimal light availability of shade-tolerant plants has been found to reduce plants' selectivity for specific AM fungi and promote compatibility with a larger number of AM fungal taxa (Neuenkamp et al., 2021). Therefore, the high network centrality of shade-tolerant plant species in our forest plot is probably ascribed to their high light availability reducing plants' selectivity for specific EM fungi. Further, higher respiratory rates in the soil (including plant fine roots and mycorrhizal fungal respiration) are exhibited by mycorrhizal plants than non-mycorrhizal plants (Neumann & Matzner, 2014). As the energy and carbohydrates required by EM fungi are supplied mainly by host plants, reduced leaf dark Rd may benefit plant species with high centrality to accumulate more carbohydrates to support diverse EM fungi. These suggest that plant photosynthetic-derived carbon rewards on EM fungi may increase centrality of plant species and make host plants achieve beneficial responses from a variety of EM symbiosis.

In addition, root traits were found to have a strong determinant effect on the structure of the rhizosphere community of plant species (Sweeney et al., 2021). Similarly, our results showed a positive association between network centrality of plant species and SRT in our forest plot (Figure 2). This may be attributed to that plants with high SRT can attract and support rich EM fungal community form sheaths or mantles on the root tips (along with a network of hyphae). Thus, this result supports our hypothesis that centrality of plants is positively related to root nutrient-using efficiency (indicated by high SRT). In addition, a strong negative correlation between average root diameter (AD) and SRT (Pearson $r = -0.73$, $p < 0.001$) and positive correlation between SRL and SRT (Pearson $r = 0.94$, $p < 0.001$) may make AD and SRL not be retained in the reduced regression model of centrality of host plant (see Figure 2). This implies that richness of EM fungi tends to decrease with AD but increase with SRL, in contrast to previous findings (Comas et al., 2014). Nevertheless, our results still suggest the functional roles of host plants in a network such as network hubs and connectors are contingent on root traits.

Taken together, plant functional traits (photosynthesis and root traits) and abundance regulated centrality of fungal host plants and explained almost half of the variation in network centrality for EM host plants in our forest plot. This result should be slightly biased by selection effect of host plants because no constant lower functional, phylogenetic diversity and evenness were observed for our focal plant taxa than all plant taxa with measured traits in the plot (Appendix S1: Figure S9). Furthermore, the plants with the highest centrality did not exhibit extreme values in species abundance and functional traits (Appendix S1: Figure S9), indicating that centrality should be driven by the combined effect of abundance and traits of a variety of plant species. To sum up, our conclusion should be robust and supports that the centrality of fungal hosts in network organization may be driven by their ecological attributes (Stouffer et al., 2012).

CONCLUSIONS

The present study is the first attempt to estimate the centrality of plant and EM fungal species and identify keystone species in a bipartite plant–EM fungus network in a subtropical forest. Especially, we explore the ecological factors of the network centrality of EM fungal host plants from phylogenetic and functional perspectives. In contrast to previous studies, we found phylogenetic constraint in network centrality of EM fungi but not for host plants in the network. Our findings suggest that leaf photosynthesis and root morphological traits may drive the topological structure (e.g., centrality) of plant–fungus association networks. Moreover, abundant plants may play an important role as reservoirs of symbiotic fungal diversity and thus contribute to maintaining ecosystem functions. We highlight the importance of central species in maintaining network structure and community stability. These findings provide valuable recommendations for making strategies to conserve keystone species and restore the function and stability of subtropical forest ecosystems in a changing world.

AUTHOR CONTRIBUTIONS

Chunchao Zhu and Chengjin Chu designed the research and led the writing. Minhua Zhang, Zheyi Liu, and

Wenqi Luo improved and revised the manuscript. Zihui Wang provided plant–fungus interaction data.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw data on plant–fungal interaction and taxonomic information of root fungi are available at https://doi.org/10.1111/nph.15786 (Wang et al., 2019: supplementary tables S2 and S3).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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