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Research

Effects of host phylogeny, habitat and spatial proximity on host specificity and diversity of pathogenic and mycorrhizal fungi in a subtropical forest

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Summary

- Soil plant-pathogenic (PF) and mycorrhizal fungi (MF) are both important in maintaining plant diversity, for example via host-specialized effects. However, empirical knowledge on the degree of host specificity and possible factors affecting the fungal assemblages is lacking.
- We identified PF and MF in fine roots of 519 individuals across 45 subtropical tree species in southern China in order to quantify the importance of host phylogeny (including via its effects on functional traits), habitat and space in determining fungal communities. We also compared host specificity in PF and MF at different host-phylogenetic scales.
- In both PF and MF, host phylogeny independently accounted for > 19% of the variation in fungal richness and composition, whereas environmental and spatial factors each explained no more than 4% of the variation. Over 77% of the variation explained by phylogeny was attributable to covariation in plant functional traits. Host specificity was phylogenetically scale-dependent, being stronger in PF than in MF at low host-phylogenetic scales (e.g. within genus) but similar at larger scales.
- Our study suggests that host-phylogenetic effects dominate the assembly of both PF and MF communities, resulting from phylogenetically clustered plant traits. The scale-dependent host specificity implies that PF were specialized at lower-level and MF at higher-level host taxa.

Introduction

Among the most important biotic interactions are those that occur between plant roots and soil fungi (Wardle, 2004). Root–fungi associations can be pathogenic, beneficial or neutral, and they contribute to the survival and growth of host individuals (Berendsen *et al.*, 2012; Philippot *et al.*, 2013). Soil plant-pathogenic fungi (PF) can cause tissue damage such as root necrosis, whereas mycorrhizal fungi (MF) are commonly beneficial by facilitating plant nutrient uptake and improving plant resistance to disease (Van Der Heijden *et al.*, 2006). Despite these opposing effects, both fungal groups are considered to be important in regulating the biodiversity of plant communities (Connell, 1971; Hart *et al.*, 2003; Laliberté *et al.*, 2015). For soil plant pathogens, a limited dispersal ability and high host specificity can result in conspecific negative distance dependence on recruitments, as suggested by the Janzen–Connell hypothesis (Janzen, 1970; Connell, 1971), which is thought to make room for heterospecific species and to promote plant species coexistence as a stabilizing force (Bagchi *et al.*, 2010; Liu *et al.*, 2015). MF have been hypothesized, though not confirmed, to maintain plant diversity by reducing plant resource competition (Jiang *et al.*, 2017), based on observations that plant–mycorrhizal interactions are stronger where soil nutrients are limiting the growth of host trees (Cox *et al.*, 2010; Yang *et al.*, 2014). Moreover, it has been proposed that MF counteract the influence of PF on the maintenance of plant diversity by host-specific positive effects (Liang *et al.*, 2015; Bennett & Klironomos, 2018, 2019).

Underlying these proposed diversity maintenance mechanisms are prerequisite assumptions that PF are host-specialized and dispersal-limited, while the effects of MF are soil nutrient-related. Although each of these mechanisms and their required assumptions have been explored separately (Konno *et al.*, 2011; Liu *et al.*, 2012; Yang *et al.*, 2014), the relative contribution of host identity, soil nutrients and space to fungal assemblages and the degree of host specificity in the two fungal guilds is little understood (van der Linde *et al.*, 2018; Yang *et al.*, 2019). Here we address this problem by quantifying and comparing the relative importance of various factors that could be structuring the community composition of soil PF and MF in a subtropical forest.

Phylogenetic relatedness among hosts has been shown to influence the richness and community composition of both mutualists

and antagonists, implying a certain specificity of such organisms among phylogenetically related hosts. Examples include fish parasites (Poulin, 2010), insect herbivores (Ødegaard *et al.*, 2005) and plant-associated fungi, both pathogenic (Gilbert & Webb, 2007) and ectomycorrhizal (Bahram *et al.*, 2012; Tedersoo *et al.*, 2013). These host phylogenetic effects may result from coevolution between plants and their dependent partners, or, if plant functional traits are phylogenetically conserved, the host relatedness can directly affect the community assembly of their dependents (De Deyn & Van Der Putten, 2005). It has been suggested that it is plant functional traits, which are often phylogenetically conserved, that lead to the observed host phylogenetic effects (Wardle, 2004). Although plant traits have been used to predict the plant–soil microbe associations in previous studies, the effects could vary across studies as a result of the use of different sets of traits (Guo & Gong, 2014; Legay *et al.*, 2014; López-García *et al.*, 2017). For example, most previous studies have focused on above-ground traits, whereas there is increasing evidence that root traits probably play a more important role in structuring below-ground communities (Bardgett *et al.*, 2014). But because of the lack of data on plant root traits, there is little understanding about whether and how much the observed host phylogenetic effect is caused by phylogeny-related plant traits.

Although the effects of host phylogeny on fungal community are widely documented, the magnitude of any host-phylogenetic effects could differ among fungal guilds as a result of factors such as coevolutionary history, ecological specificity, as well as interactions between ecological and evolutionary processes (Molina & Horton, 2015; Heilmann-Clausen *et al.*, 2016). Generally, a stronger host-phylogenetic constraint is expected on community composition of parasitic organisms than of mutualists, owing to the need to avoid host defenses (Antonovics *et al.*, 2013). However, empirical evidence for this is lacking, and there are at least two challenges to garner this evidence. First, heterogeneity in abiotic conditions and spatial autocorrelation arising from the similar niche requirements of phylogenetically related hosts could confound inference of any host-phylogenetic effect (Kühn *et al.*, 2009; Weber & Agrawal, 2012). Second, the host-phylogenetic scale considered could be too narrow to reveal any effect of host phylogeny on fungal community composition (Ødegaard, 2000; Ødegaard *et al.*, 2005; Tedersoo *et al.*, 2014). In the latter case, it would be important to take the range in host phylogeny into account when comparing mutualists and antagonists.

Abiotic conditions not only affect phylogenetic relatedness of host plants resulting from environmental filtering but also influence fungal communities as a result of variations in, for example, soil moisture, nutrients and pH (Kernaghan, 2005; Cox *et al.*, 2010; Yang *et al.*, 2014). Host plants can enhance their carbon allocation to mutualistic MF in nutrient-poor conditions to facilitate greater exchange of a limited resource (Johnson *et al.*, 2003; Treseder, 2004). This has been proposed as a major mechanism by which soil nutrient availability affects the species richness and composition of mycorrhizal communities. PF and their pathogenicity, however, are more sensitive to variations in soil moisture, light conditions and temperature (Ichihara & Yamaji, 2009; Tack *et al.*, 2012). In some cases, abiotic conditions can have greater influence on fungal community composition (e.g. arbuscular mycorrhizal and saprotrophic fungi) than on host identity (Van Geel *et al.*, 2017; Yang *et al.*, 2019). Therefore, not accounting for abiotic variables can leave much of the variation in fungal species richness and community composition unexplained (Weber & Agrawal, 2012). Spatial autocorrelation, arising from both abiotic and biotic processes (e.g. dispersal limitation), is a nuisance in inferring environmental influences on fungal communities. The presence of spatial autocorrelation can potentially confound the effects of habitat and host identity on fungal communities (Peay *et al.*, 2010); for example, without considering the effects of spatial autocorrelation, a higher host-phylogenetic effect could be observed. Therefore, it should be properly controlled for.

In this study, we aimed to quantify the variation in local species richness and community composition of PF and MF caused by host phylogeny, habitat and spatial autocorrelation. Specifically, we focused on addressing the following questions: (1) how much do each of the three components (host phylogeny, habitat and spatial processes) contribute to the assembly of soil PF and MF, and are host phylogenetic effects due to phylogenetically conserved plant functional traits; and (2) is there a difference in host specificity between PF and MF, and does this depend on the host-phylogenetic scale considered?

Materials and Methods

Study site

Our field site was a 50 ha stem-mapped forest plot located in the Heishiding Nature Reserve, Guangdong Province, China (centered on 111°53′E, 23°27′N). The reserve consists of *c.* 4200 ha of subtropical forest, providing habitat for over 1600 seed plant species belonging to 669 genera and 188 families. The region is located on the Tropic of Cancer and has a subtropical humid-monsoon climate with mean annual temperature of 19.7°C, and annual precipitation of 1750 mm. The topography varies, but is generally steep, with slopes between 20° and 40°. Site elevation ranges from 150 to 700 m above sea level. The 50 ha study plot was stem-mapped according to the Center for Tropical Forest Science (http://www.forestgeo.si.edu/) protocols in 2011, and in this way trees/shrubs with diameter at breast height (DBH) ≥ 1 cm were mapped (there were over 269 000 individual stems).

Sampling and data collection

A total of 45 tree species were selected based on the phylogeny and their abundance in the stem-mapped plot, where the phylogenetic distance among the most distantly related taxa is greater than that in most previous studies (Morris *et al.*, 2008; Tedersoo *et al.*, 2013; Glassman *et al.*, 2017). We first chose three species from each of *Litsea* and *Lithocarpus*, two of the most abundant genera in the plot, so that the three species were from the same genus (cogeneric, the finest phylogenetic scale). The remaining 39 species were selected from a larger set of genera, with the

condition that some of the genera were from the same families as *Litsea* (*Lithocarpus*) and the others were from different families. Six species were then selected from the confamilies of *Listea* and *Lithocarpus* to form the intermediate phylogenetic scale (the confamilial level). The rest of the 33 species were selected from other families to form the coarsest phylogenetic scale (i.e. species of different families). The phylogenetic tree of the 45 species is shown in Supporting information Fig. S1. Fieldwork of fine root sampling was performed from February to May 2014. For each species, fine roots of five to 15 individual stems were sampled. Fine roots were excavated by meticulous tracing from thick roots of each stem. At least three replicate root fragments, each *c.* 2 cm long, were traced from different directions around a stem. Samples from each stem were then pooled to create a single sample. Fine root samples were immediately cooled in the field and stored at −20°C in a refrigerator until processing. In total, 519 fine root samples were collected. To ensure the accuracy of species identification of the sampled fine roots using the tracing method, we compared the species of the fine roots identified from molecular markers (*rbcLa* and *matK*, enough for distinguishing 45 species) for 100 randomly selected root samples against the expected host species from which the roots were supposed to be traced in the field. Of these, 97 samples were correct, which we considered to be an acceptable sampling error. To acquire the phylogenetic relationships among host species, fresh leaves from three adult individuals for each species were collected and preserved in mesh bags with desiccant silica gel. For each species, sequence information of four general plant DNA barcodes (*rbcLa*, *matK*, *trnH-psbA* and *trnLc-trnLd*) was obtained after a series of established laboratory processes to construct phylogenetic trees (Jurado-Rivera *et al.*, 2009; Amani *et al.*, 2011). Habitat conditions at the location of each host stem were described using light, five topographic variables and 28 edaphic variables (Table S1). Data of these environmental variables were derived from the Heishiding database (sampled from 625 soil cores randomly distributed over the plot; C. Chu & F. He, unpublished) using Kriging interpolation. To test for any association between host plant functional traits and variations in fungal species richness and community composition, we compiled a database of 24 leaf, stem and root traits (Table S1). This analysis was limited to 31 of the 45 host species, for which trait data were available.

Molecular identification of root-associated fungi

Molecular analyses consisted of five stages: DNA extraction, PCR, high-throughput sequencing, reads assembly and authentication of operational taxonomic units (OTUs). Before analysis, roots were gently washed with cold deionized water to remove adhering soil particles and then immersed in 75% ethanol for 5 min for surface sterilization and to remove any surficial fungal hyphae which could contaminate genuine root fungi. Root samples of *c.* 150 mg were then carefully ground with liquid nitrogen. Afterwards, DNA were extracted using the High Performance (HP) Plant DNA Kit (Omega Bio-tek; Feiyang Inc., Guangzhou, China) following the manufacturer's recommendations. To ensure comparable results, extracted DNA was quantified using a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and diluted to the same concentration. A nested PCR was conducted targeting the internal transcribed spacer (ITS) region of rDNA, which is a standard barcoding marker for fungal identification (Schoch *et al.*, 2012). For each DNA product, three replicates were performed, with each 20 μl reaction including 1 μl template DNA, 0.4 μl of each primer (ITS1F, ITS4) in 10 μM ml^{-1}, 1.6 μl of deoxynucleoside triphosphate (dNTP), 0.4 μl BSA, 2 μl PCR buffer, 0.2 μl Taq DNA (Takara Biotechnology, Dalian Co. Ltd, Dalian, China) and 14 μl H_2O. The PCR temperature profile consisted of an initial cycle of 30 s at 98°C, followed by 30 cycles of 98°C for 10 s, 53°C for 20 s and 72°C for 10s, then a final cycle of 7 min at 72°C. The primer set of ITS3/ITS4 with a sample-specific, 8 bp barcode on the reverse primer was used in the second step PCR. The temperature conditions for the second step were the same as for the first, but the reactions consisted of 1 μl template PCR products, 0.6 μl of each primer in 10 μM ml^{-1}, 2.4 μl of dNTP and 0.6 μl BSA, 3 μl PCR buffer, 0.2 μl Taq DNA and 21.6 μl H_2O. Final amplification products were pooled per individual tree and detected by agarose gel electrophoresis. A composite DNA sample for sequencing was created by combining equimolar ratios of amplification products from individual subsamples as described previously (Fierer *et al.*, 2008). The composite DNA was gel-purified and sequenced using a Illumina Hiseq 2500 system (Illumina, San Diego, CA, USA).

Raw sequences were denoised using the command 'shhh.flows' (Quince *et al.*, 2009) and 'pre.cluster' (Huse *et al.*, 2010) in the MOTHUR software (v.1.26; Schloss *et al.*, 2009), and then chimeric sequences were identified and removed using UCHIME in *de novo* mode (Edgar *et al.*, 2011). About 11 million quality filter reads were retained, which were then identified to operational taxonomic units (OTUs) at a 97% sequence similarity level, and later assigned to taxonomy based on the UNITE database using the Ribosomal Database Project Classifier (Wang *et al.*, 2007), with a minimum confidence of 80%. OTUs with fewer than five sequences were excluded to minimize the impact of tag switching, cross-contamination, and sequencing errors (Dickie, 2010; Lindahl *et al.*, 2013). We then subsampled each sample to 3000 reads using the bootstrapping method (Smith & Van Belle, 1984; Muller, 1992) to eliminate the effects of sample size.

We then assigned fungal OTUs to different functional categories. The ectomycorrhizal (ECM) fungi were identified based on a database of ECM taxa and lineages (Tedersoo & Smith, 2013), and arbuscular mycorrhizal (AM) fungi were identified by including all OTUs from *Glomeromycota*. As there is a much larger difference in physiology and ecology between pathogenic and mycorrhizal associations than within mycorrhizas (Bonfante-Fasolo & Perotto, 1991), ECM and AM were pooled together to represent the mycorrhizal fungal guild. We also conducted a separate analysis on ECM and AM because of the documented important differences between these two guilds (Bennett *et al.*, 2017). As yet, relatively few studies have investigated the mechanisms underlying community assembly of PF communities. This is perhaps a result of the challenge involved in the authentication of pathogens, which is problematic because the definition of

'plant pathogens' is based on the occurrence of plant disease that is highly complicated and is subject to the effects of many factors (Benítez *et al.*, 2013). In line with previous studies, we selected a group of fungi known to infect woody plants and regarded as having a higher probability of causing disease than other fungi (Tedersoo *et al.*, 2014; Nguyen *et al.*, 2016). We first identified pathogenic genera according to the Funguild database (Nguyen *et al.*, 2016), and because these genera still include non-pathogenic species, we then searched each potential PF species in the literature. We retained only potential PF (OTUs) that were identified to the species level and had been clearly found to be pathogenic to woody plants (Tables S2, S3). Although only a subset of the present pathogens was identified, we considered all of the common pathogens that have been discovered in previous studies in order to provide a reliable and representative set of pathogens.

Statistical analyses

Phylogenetic trees of the 45 species were generated in three steps:

First, NA sequences were checked against online databases in GenBank using BLAST to assess the accuracy of sampling. These were then aligned using MUSCLE (Edgar, 2004) and joined to build a super dataframe using the R package PHYLOTOOLS (Kress *et al.*, 2009). Second, The JMODELTEST program was used to select the best nucleotide substitution model (Posada, 2008). Finally, the super dataframe, together with a phylogenetic tree obtained from APG III (Reveal & Chase, 2011) as the constrained tree, was loaded into the PHYLOGENERATOR program, which reconstructed a maximum likelihood tree (Stamatakis *et al.*, 2008).

We used Moran's eigenvector maps (MEMs) to decompose phylogenetic and geographic distance matrices into a series of orthogonal eigenvectors representing the phylogenetic and spatial relationships among hosts. MEMs provide a general means to generate orthogonal variables such as principal coordinates of neighbor matrices and we used the eigenvectors of both phylogenetic and spatial MEMs as predictors in variation partitioning and multivariate regression (Dray *et al.*, 2006). To detect any autocorrelation in phylogenetic and spatial distance matrices for species richness and community composition we used the Moran's I and Mantel's r statistics, respectively.

The fungal species richness of each guild on each host tree was regressed on predictor variables for host phylogeny, environmental and spatial factors using generalized linear models (GLMs). As raw data followed a negative binomial distribution (NBD) (Kolmogorov–Smirnov, $D = 0.04$, $P = 0.87$), we used the NBD as our GLM error structure, implemented with function 'glm.nb' in the R package MASS (Venables & Ripley, 2003). Abiotic environmental predictors were rescaled using the formula $(x - x_{min})/(x_{max} - x_{min})$ to standardize the effects of the variables. Where predictors had pairwise Pearson's correlation coefficients > 0.8, one predictor was removed to reduce multicollinearity, resulting in a total of 187 predictors (43 host phylogenetic, 34 environmental and 110 spatial; Table S1). We built a candidate set of seven models representing the individual and joint linear contributions for the three types of mechanism (host phylogeny, environment and spatial proximity) using all predictors for each mechanism (Table 1). A stepwise selection procedure was used for model selection based on Akaike's information criterion corrected for small sample size (Burnham & Anderson, 2004). The pure effect of one factor was evaluated as the difference in R^2 between models with and without this factor. We also regressed fungal species richness on predictors for host plant functional traits and host phylogeny in the same way as described earlier but using a smaller dataset for which plant functional traits were available.

To quantify fungal compositional heterogeneity among individual host trees, we used the total variance in fungal community

Table 1 Model selection for negative binomial generalized linear models for species richness in plant-pathogenic and mycorrhizal fungi.

Model structure	LL	n	AIC_c	ΔAIC_c	$wAIC_c$	Pseudo-R^2
Plant-pathogenic fungi						
Environmental	−1290.5	4	2592.9	177	< 0.001	0.052
Spatialial	−1266.4	12	2560.7	144.8	< 0.001	0.134
Environmental + spatial	−1257.3	14	2546.7	130.8	< 0.001	0.163
Host phylogeny	−1194.7	20	2433.4	17.5	< 0.001	0.361
Host phylogeny + environmental	−1192.3	20	2428.6	12.7	< 0.001	0.369
Host phylogeny + spatial	−1183.3	24	2418.7	2.8	0.198	0.397
Host phylogeny + environmental + spatial	−1180.9	25	2415.9	0	0.801	0.405
Mycorrhizal fungi						
Environmental	−1821.2	7	3655.9	125.0	< 0.001	0.072
Spatialial	−1821.9	6	3653.2	122.3	< 0.001	0.073
Environmental + spatial	−1810.1	12	3632.6	101.7	< 0.001	0.129
Host phylogeny	−1758.3	19	3552.3	19.9	< 0.001	0.276
Host phylogeny + environmental	−1757.2	26	3539.8	8.9	< 0.001	0.312
Host phylogeny + spatial	−1747.6	20	3539.3	8.4	< 0.001	0.297
Host phylogeny + spatial + environmental	−1733.4	30	3530.9	0	0.999	0.335

In both cases, the top-ranked model combined all three predictors. LL, log-likelihood; n, number of predictors; $wAIC_c$, probability that the selected model is the best among the candidate set given the data; AICc, Akaike information criterion corrected for small sample size; pseudo-R^2, amount of explained deviance in the model.

matrices between two hosts. This then allowed us to use variance partitioning methods based on redundancy analysis (RDA) to quantify the separate and combined contributions of host phylogeny, environmental and spatial factors on fungal composition (Borcard *et al.*, 1992; Peres-Neto *et al.*, 2006). We used the Hellinger-transformed species abundance data for PF and MF communities as our two response matrices, with three explanatory matrices comprising all phylogenetic, environmental and spatial predictors, respectively (Table S1). Following Blanchet *et al.* (2008) we first tested the significance of the global model using all predictors. Variable selection was then done using forward selection implemented with function *forward.sel* in the R package PACKFOR (Dray *et al.*, 2011) following the recommended stopping rules in Blanchet *et al.* (2008). The variation partitioning was conducted using the *varpart* function in the VEGAN package (Oksanen, 2008). We also used variation partitioning to elaborate any independent and combined effects of host functional traits and phylogeny in structuring fungal communities. Response variables were matrices of composition of fungal community, and predictors comprised the host phylogenetic and plant functional trait matrices.

We used the degree to which host phylogeny can explain fungal community composition in RDA to represent the strength of host specialization and compared this between the two guilds at different host-phylogenetic scales. To test sensitivity of the results to the definition of phylogenetic scale, we used both ordinal and continuous measures of the scale. The ordinal scale had four phylogenetic levels with the minimum including species only from the genus *Listea* (the cogeneric level), the second including species from within the Lauraceae family (the confamilial level), and the next two scales including species from families at small and large phylogenetic distances, respectively. No ordinal scale could be set for *Lithocarpus* because of the lack of species from the same family. As a continuous (numeric) measure of phylogenetic scale, we chose species from the focal genera as an initial scale and added species one at a time in order of increasing phylogenetic relatedness. The phylogenetic distance between the focal genus and the added species was then regarded as the phylogenetic scale. In both measures, for each scale we repeated the RDA analysis, with fungal community composition as response variables and host phylogeny as predictors.

Finally, we constructed plant–fungal interaction networks to evaluate any differences in host specialization between the two fungal guilds and among host-phylogenetic scales. To account for the sampling inequality, the average abundance of OTUs per host individual were used as interaction frequencies. As the algorithms require interaction frequencies to be integers, we multiplied the interaction matrix elements by 1000 and rounded them to the nearest integer. For the two resulting networks (plant–PF, plant–MF), we calculated d' and H_2' indices to quantify specialization. Additionally, we conducted separate analyses on ECM and AM fungi to test whether there were statistically significant differences in their host specificity. The index d' indicates interaction specialization at the OTU level and takes values from 0 to 1, indicating the lowest to highest specialization, respectively (Blüthgen *et al.*, 2006). We calculated d' for each fungal OTU and used Kruskal–Wallis tests to detect whether the d' index differed between PF and MF networks at each phylogenetic scale. The index H_2' characterizes specialization at the network level and can be used to compare between networks (Blüthgen *et al.*, 2006). The value of H_2' also falls between 0 (lowest specialization) and 1 (highest). To test the statistical significance of the observed H_2' for each network we used a null model approach, generating 1000 random networks, with fixed row and column totals equal to the corresponding row and column sums of the raw networks (Dormann *et al.*, 2008). We then compared the observed H_2' with the distribution of values from the null models. Network analyses were conducted at each of the four host-phylogenetic scales using the BIPARTITE package in R (Dormann *et al.*, 2009; Dormann, 2011).

Results

A total of 8.3 million sequence reads were revealed from the 519 root samples, which were assigned to 18 103 OTUs. After rarefying, 12 880 OTUs remained, with 44.4% classified to 145 fungal families and 32% to 368 fungal genera. Among those 4124 OTUs that were successfully classified to fungal genera, 114 (2.8%) were categorized as PF and 889 (21.6%) as MF (868 as ECM and 21 as AM fungi). The abundances of PF and MF accounted for 1.6% and 4.7% of all fungi, respectively, and 4.7% and 13.8% of identified-to-genera fungi. The three most abundant genera among PF were *Cylindrocarpon*, *Mycoleptodiscus* and *Calonectria* (19.6%, 18.8% and 18.6% of PF abundance) and among MF were *Russula*, *Scleroderma* and *Cenococcum* (50.7%, 13.2% and 9.1% of MF abundance; Fig. S2). ECM fungi were preponderant in the MF community, which we ascribe to the abundance of their host trees (e.g. *Fagaceae*) in our study forest (Fig. S3), their inherently higher diversity compared with AM fungi, and possibly the low generality of our primers for AM fungi (Redecker, 2000). Although sample-based species accumulation curves for MF and PF did not reach asymptotes, suggesting that neither group was exhaustively sampled, the accumulation was decelerated (Fig. S4a,b). This is typical of microbial species accumulation (Kembel *et al.*, 2014). A separate analysis showed that AM seemed to reach asymptotes faster than ECM (Fig. S4c,d). The observation that there was no major difference in the shape of the curves among guilds suggests that the sampling intensity of the guild groups was comparable and would not create systematic bias in the results, although the primers we used only detected a subset of the AM fungi.

Fungal species richness

Host phylogeny explained 36.1% of total variation in PF richness and 27.6% in MF richness (Table 1). Environmental predictors explained a much smaller amount of variation in PF (5.2%) and MF (7.2%) richness but with different explanatory variables; for example, PF was more affected by light, whereas MF correlated best with topography (Tables 1, S4). For spatial variables, the explained variances in species richness were 13.4% for PF and 7.3% for MF (Table 1). Fungal species richness exhibited a

positive spatial autocorrelation at distances up to 200 m for PF (Moran's $I = 0.042$, $P < 0.001$) but showed no obvious autocorrelation at any distance for MF (Moran's $I = 0.016$, $P = 0.28$; Fig. S5).

Host phylogeny, environment and spatial variables and their interactions together explained 40.5% and 33.4% of the total variation in species richness of PF and MF, respectively (Fig. 1a, b). Among the explained variation, host phylogeny independently accounted for 60%. The pure spatial effects account for a larger proportion than the environment in explaining the variation of PF richness, but a smaller proportion than the environment in the case of MF (Fig. 1a,b). There was also little evidence of any interaction in the effect of the three explanatory factors, with none of the joint effects accounting for > 6% of the total variation (Fig. 1).

Although host phylogeny dominated in explaining variation in richness, this was largely attributable to its effects on plant functional traits. Over 77% of variation explained by host phylogeny was attributable to its combined influence with plant functional traits in both PF and MF (Fig. S6). The plant functional trait model for species richness in PF (pseudo-$R^2 = 0.29$) and MF (pseudo-$R^2 = 0.23$) included, respectively, 11 and 13 plant leaf,

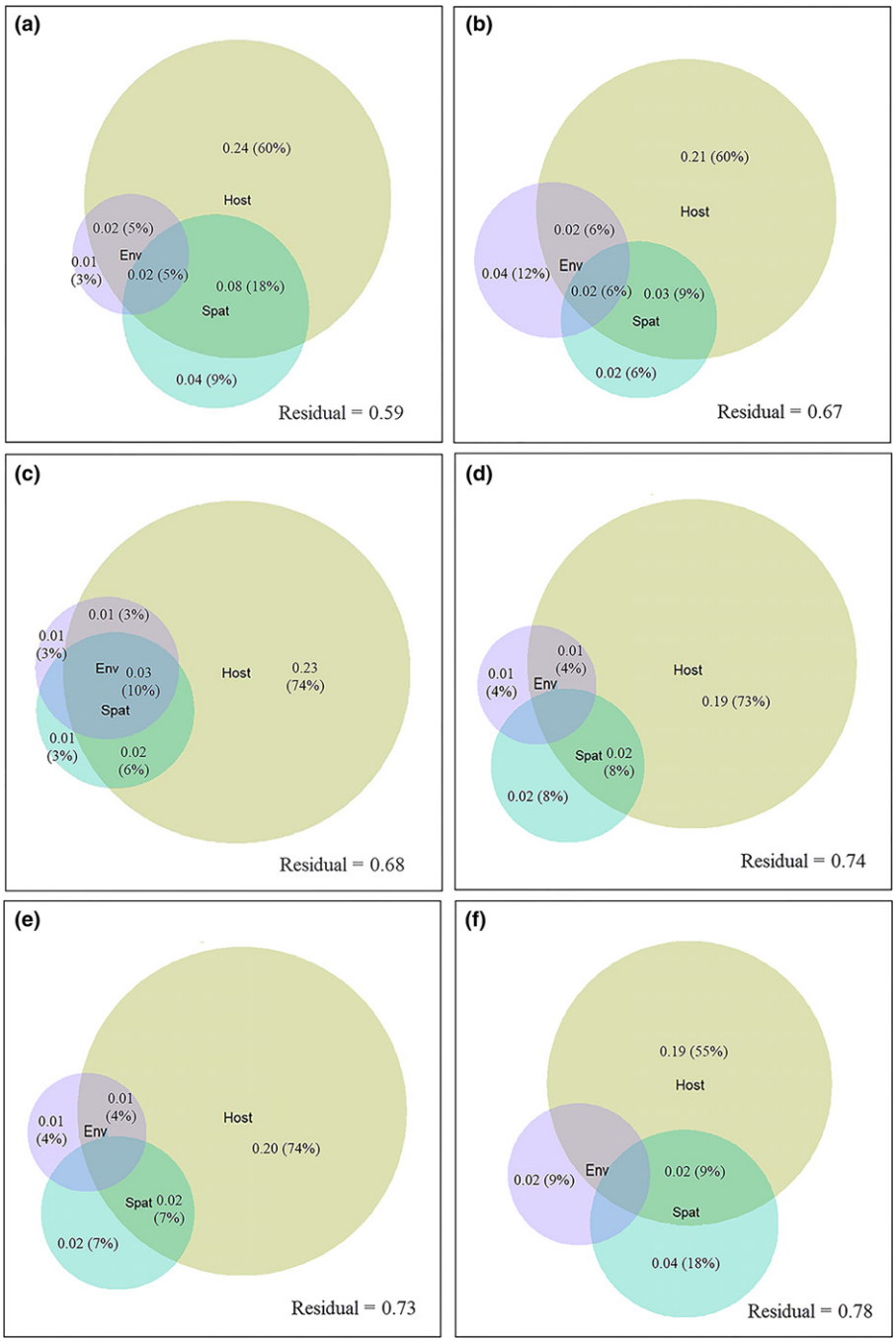

Fig. 1 Variation partitioning for: (a) species richness of plant-pathogenic fungi; (b) species richness of mycorrhizal fungi; (c) community composition of plant-pathogenic fungi; (d) community composition of mycorrhizal fungi; (e) community composition of ectomycorrhizal fungi; and (f) community composition of arbuscular mycorrhizal fungi. Venn diagrams in each panel show total variation partitioned into independent and joint components of the three explanatory factors: host phylogeny (Host, yellow), environment (Env, purple) and space (Spat, blue). Overlapping circles represent the variation explained by interactions of those predictors. The unexplained variation is represented by the area outside of the circles (denoted 'Residual').

photosynthetic, stem and root functional traits (Fig. 2). In the model of PF richness, the three root-related traits clearly had the largest effect size, whereas effects were more evenly distributed among the different trait classes for MF (Fig. 2). Of nine functional traits common to both models, eight were opposite in their effects on PF and MF richness. Specifically, increasing leaf pH, leaf carbon concentration, apparent quantum yield (*b*), wood density (WD), root branching (BRAN) and specific root area (SRA) were all associated with decreased PF richness and increased MF richness. Similarly, light compensation point and specific root tips (SRT) had positive effects on PF richness but were negative for MF (Fig. 2).

Community composition

Host phylogeny, environment and space and their interaction terms together explained 32% of total variance in community composition for PF and 26% for MF (Fig. 1c,d). The independent effect of host phylogeny accounted for the great majority of these variations. Except for their three-way interaction (explaining 10% of variation in PF composition), no other independent or shared fraction accounted for > 8% of variation for either fungal guild (Fig. 1c,d). A similar pattern in community composition was found for AM and ECM fungi where host phylogeny independently accounted for 55–74% of the variation, followed by pure spatial effects (explaining 7–18% of variation) and with environment explaining the least (Fig. 1e,f). At a phylogenetic distance < 0.2 between host neighbors, there was a weak but significant positive autocorrelation in fungal composition across host-phylogenetic distance (Mantel's $r = 0.089$, $P = 0.001$ for PF; $r = 0.076$, $P = 0.001$ for MF), which became negative at larger host-phylogenetic distances (Fig. S7). The strength of the host-phylogenetic effect on fungal community composition changed with host-phylogenetic scale, particularly for MF (Fig. 3). For example, at the smallest scale focused on *Listea*, host phylogeny explained 33.0% of the variation in community composition for PF but only 11.0% for ECM, even less for AM fungi (Fig. 3a,b). With increasing host-phylogenetic scale, the explained fraction for PF remained approximately constant, but consistently increased for both ECM and AM fungi. The host phylogeny has stronger effects on PF than on ECM and then AM fungi. These differentiated effects are particularly evident at small host-phylogenetic scales. The same results were obtained in the analysis but focusing on *Lithocarpus* (Fig. 3c). Host functional traits predicted 25% and 17% of the community composition in PF and MF, respectively, and these effects were largely attributable to host phylogeny, which explained 29% and 22% for the two guilds (Fig. S6).

Host specialization

We found that the observed network-level specialization index H_2' was significantly higher than null models for both PF–plant and MF–plant networks at all scales (Fig. S8a), indicating a structure that was more specialized than random. Heat maps of the abundance of fungal genera in each host species indicate that this host specificity is widespread among taxa and not the result of strong interactions among a few hosts or fungal genera (Fig. S9). The relative degree of specialization between PF and MF depended on host-phylogenetic scale, consistent with the host-phylogenetic effects on fungal community composition (Figs 3, S8). At the smallest scale (i.e. species within genus), the degree of host specialization was higher in PF than in MF both at the OTU (species) level (PF, $d' = 0.16 \pm 0.12$; MF, $d' = 0.10 \pm 0.06$; Kruskal–Wallis $\chi^2 = 6.5$, $P = 0.01$) and at the network level (PF, $H_2' = 0.53$; MF, $H_2' = 0.26$). However, with increasing host-phylogenetic scale, differences in specialization between PF and MF decreased, becoming statistically undetectable at coarse scales (PF, $d' = 0.31 \pm 0.12$; MF, $d' = 0.29 \pm 0.09$ (Kruskal–Wallis, $\chi^2 = 0.63$, $P = 0.43$); PF, $H_2' = 0.38$; MF, $H_2' = 0.43$; Fig. S8).

It was found that there was little difference in host specificity between AM and ECM at both small and large phylogenetic scales (e.g. at the smallest scale: AM, $H_2' = 0.25$; ECM, $H_2' = 0.28$; and at the largest scale: AM, $H_2' = 0.47$, ECM, $H_2' = 0.43$). More importantly, the host specificity of both AM and ECM fungi increased with larger host-phylogenetic scales (Fig. S10).

Discussion

The role of soil pathogenic and mutualistic fungi in promoting host plant species coexistence (e.g. via host-specialized effects) is widely recognized but there is a lack of understanding regarding the degree of host specificity and how different factors contribute to patterns of host specificity (Janzen, 1970; Connell, 1971; Chesson & Kuang, 2008; Laliberté *et al.*, 2015). In this study, we first found a consistent pattern in PF and MF that host phylogeny explained most of the variation in both fungal species richness and community composition, overwhelming the effects of environmental and spatial variables, suggesting that host specialization rather than habitat filtering or spatial autocorrelation determines fungal communities of these two guilds. Second, we found that the observed host-phylogenetic effect was mainly caused by plant functional traits which are often phylogenetically related. Lastly, the relative degree of host specificity in PF and MF was phylogenetic scale-dependent, being clearly higher in PF than in MF at low scales but more similar as the phylogenetic scale increased. Thus our study illustrates the importance of different factors affecting the assembly of these two important fungal guilds and shows for the first time that PF are more host-specialized than MF, especially at lower phylogenetic levels (i.e. among phylogenetically closely related host species, e.g. cogeneric species).

Host phylogeny dominates the variation in fungal community richness and composition

It is widely accepted that phylogenetic relatedness among hosts has a critical influence on the community assembly of dependent species (Gilbert & Webb, 2007; Tedersoo *et al.*, 2013; van der Linde *et al.*, 2018; Yang *et al.*, 2019). However, the contribution of host phylogeny relative to other important factors (e.g.

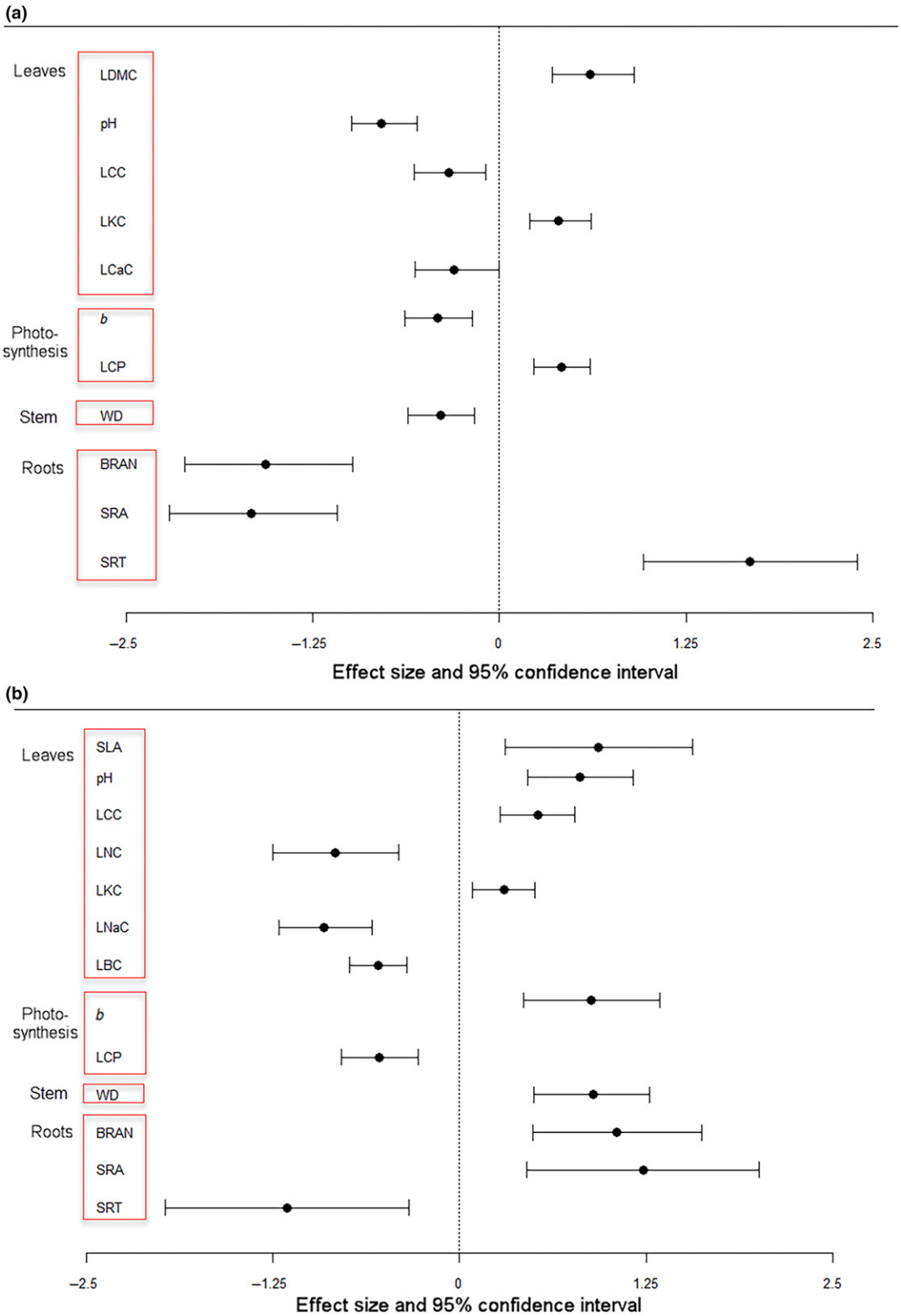

Fig. 2 Influence of plant leaf, photosynthetic efficiency, stem and root functional traits on the species richness of pathogenic (a) and mycorrhizal (b) fungi. Each plot shows standardized coefficient estimates for negative binomial generalized linear models, representing effect size and direction for each trait with error bars indicating a 95% confidence interval in the estimate. LDMC, leaf dry matter content; LCC, leaf carbon concentration; LKC, leaf kalium concentration; LCaC, leaf calcium concentration; *b*, apparent quantum yield; LCP, light compensation point; WD, wood density; BRAN, root branching; SRA, specific root area; SRT, specific root tips.

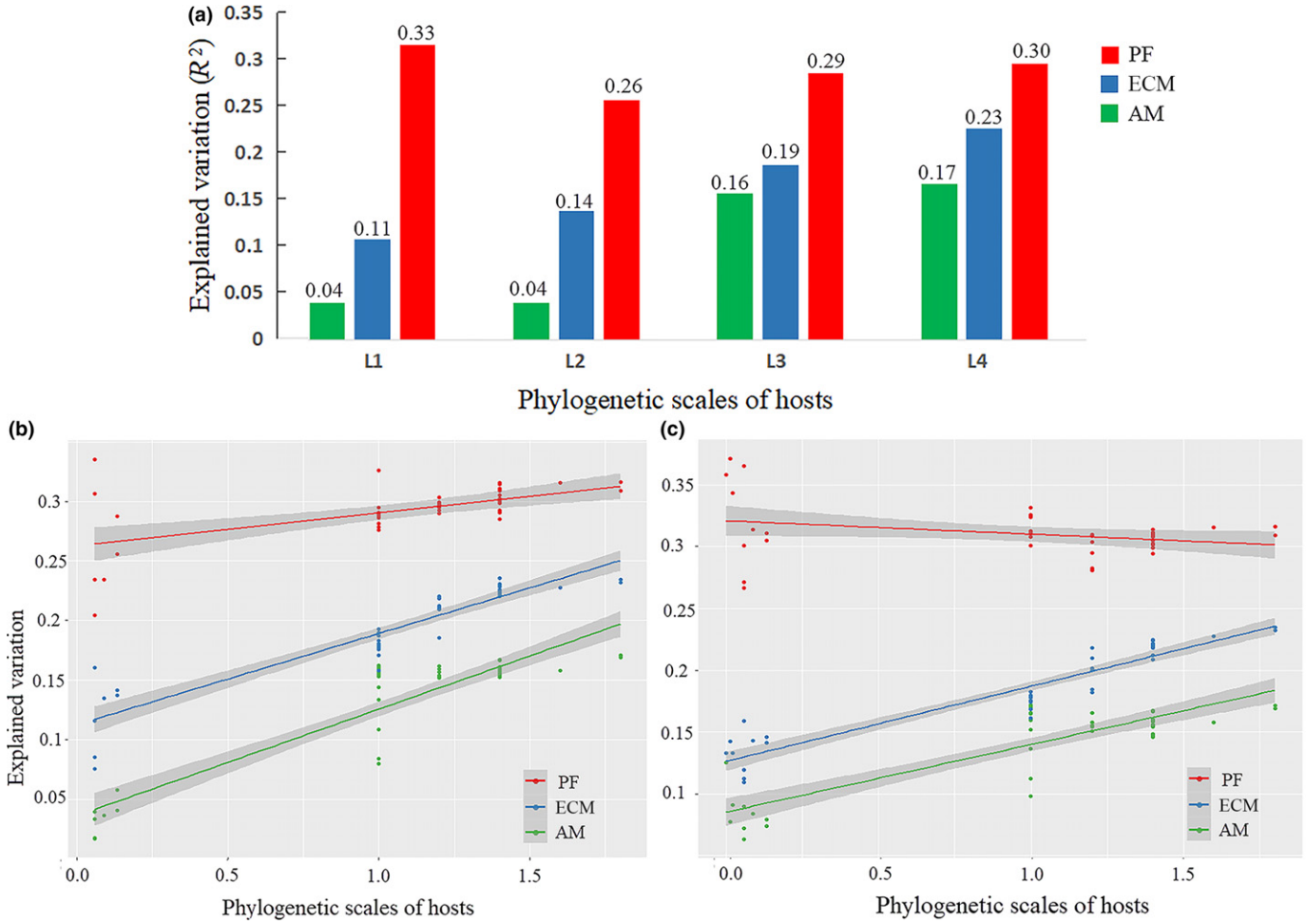

Fig. 3 Explained variation (R^2) of redundancy analysis (RDA) models of the community composition of pathogenic (PF), ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi (MF) as a function of host phylogeny at different host-phylogenetic scales, including a four-level ordinal scale (L1–L4) based on *Listea* (a) and numerical scales focused on *Listea* (b) and *Lithocarpus* (c). In all RDA analyses, host phylogeny was the predictor and community composition the response. For the ordinal scale, levels L1–L4 represent increasing phylogenetic distance of host species: L1, same genera; L2, same family; L3, different families with small phylogenetic distance; and L4, large phylogenetic distance. For numerical scales (b, c), phylogenetic distance between focal genera was increased with the addition of species (see the Materials and Methods section).

environment and space) is poorly known. Most previous studies have focused on mycorrhizal fungi (Bahram *et al.*, 2012; Tedersoo *et al.*, 2013), with little emphasis on plant pathogens, and even less on PF and MF within the same study system (Bennett & Klironomos, 2018; Yang *et al.*, 2019). This leaves little understanding on how these two functionally opposing guilds may coevolve with specific hosts and how this specificity might be affected by environment and space. By disentangling the individual and joint influences of host phylogeny, abiotic conditions and spatial proximity on the assembly of PF and MF communities, we found that the independent effects of host phylogeny accounted for > 60% of the explained variation in richness and composition for both fungal guilds – at least three times that of any other single factor, or their interactions.

The strong effects of host phylogeny on MF relative to other factors coincides with previous studies conducted on a wide phylogenetic range of plants (Tedersoo *et al.*, 2013; van der Linde *et al.*, 2018; Yang *et al.*, 2019), yet conflicts with studies involving more closely related plant species (Morris *et al.*, 2008; Glassman *et al.*, 2017). Our finding that the effect of host phylogeny on MF increased at a larger phylogenetic scale to a greater extent than in PF (Fig. 3) could help to explain these contradictory results. The pure effect of host phylogeny on PF is similar to that on MF, suggesting that the same processes involving host phylogeny structure these two functionally distinct fungal guilds. The dominant contribution of host phylogeny to fungal compositional variation indicate that host-fungal coevolution is central in structuring fungal communities (Heilmann-Clausen *et al.*, 2016).

One of the main mechanisms by which host-phylogenetic effects can be manifested is where plant functional traits are phylogenetically clustered, as we found. However, most studies linking host functional traits to communities of soil microorganisms have focused either on leaf traits alone (de Vries *et al.*, 2012) or on root traits exclusively (Valé *et al.*, 2005). Few studies have simultaneously considered both above- and below-ground plant traits (Legay *et al.*, 2014). In this study, plant traits related to leaves, photosynthesis, stems and roots were all correlated to the assembly of root fungal communities. Some of the traits that

were significantly correlated with fungal communities in this study were also reported in previous studies (e.g. leaf dry matter content, specific leaf area and leaf N content (de Vries *et al.*, 2012; Legay *et al.*, 2014), but most were new to the literature (e.g. leaf pH, WD, BRAN, SRA and SRT). We also demonstrated a differential importance of these trait types between the fungal guilds, where root-related traits were best correlated with PF. Together, these functional traits explained almost the same variation in fungal richness and community composition as that attributable to host phylogeny, and the effects of host phylogeny and plant functional traits overlapped strongly with each other. This result implies that phylogenetically conserved functional traits are largely behind the observed host-phylogenetic effects. Thus, host-phylogenetic relatedness seems to be a good proxy for functional similarity when trait data are not available.

Host specificity in PF and MF changes with phylogenetic scale

Our study showed for the first time that the degree of host specialization between PF and MF was dependent on the phylogenetic scale considered. The effect of host phylogeny on fungal community composition was stronger on PF than on MF at fine phylogenetic scales but similar at large scales. This is consistent with the results of the network analysis where the specialization indices d' and H_2' differed significantly between PF and MF at low, but not high, phylogenetic scales. Both lines of evidence indicate higher host specificity in PF among closely related host species, but the degree of host specificity in PF and MF becomes similar with increasing phylogenetic scale (i.e. distance) (Fig. 3). This suggests that MF could be specialized on high-order host taxa as a result of their long evolutionary history, whereas PF are necessarily specialized on host species because of intensified antagonistic evolution to avoid host defenses (Antonovics *et al.*, 2013).

Phylogenetic scale-dependent host specificity in PF and MF provides insights into their roles in maintaining plant diversity. Strong host specificity in PF could increase seedling mortality of conspecifics in the proximity of parent trees while leaving heterospecifics unaffected (i.e. the Janzen–Connell effect, promoting plant diversity (Janzen, 1970; Connell, 1971)), whereas for MF, which are traditionally thought of as being relatively general in their colonization of plants (Smith & Read, 2008), we found a significant degree of host specificity at high-level host taxa. This result suggests that mycorrhizal fungi could counteract the Janzen–Connell effect by facilitating the establishment of conspecific and phylogenetically related seedlings that might reduce the diversity of more phylogenetically distant plant species (Laliberté *et al.*, 2015; Liang *et al.*, 2015; Bennett *et al.*, 2017; Bennett & Klironomos, 2018).

Effects of environmental conditions and space on fungal richness and composition

Although abiotic conditions and spatial autocorrelation have been found to affect local fungal community composition (Cox *et al.*, 2010; Peay *et al.*, 2010), their contribution relative to host identity could vary among fungal guilds and among studies. For example, the community composition of AM can be determined mostly by abiotic factors in one system (Van Geel *et al.*, 2017), while being strongly related to hosts in another (Neuenkamp *et al.*, 2018). Differences in the factors determining ECM fungal communities have also been demonstrated (e.g. Tedersoo *et al.*, 2013; Yang *et al.*, 2019 vs Morris *et al.*, 2008; and Glassman *et al.*, 2017). In our study, the total contribution of the environment and space did not account for > 27% of explained variation in the richness or community composition of either guild, consistent with Tedersoo *et al.* (2013) but lower than that found in Morris *et al.* (2008) and Glassman *et al.* (2017). However, both studies investigated host–fungal associations for hosts sampled from only narrow phylogenies, which should be expected to lead to a low variation in fungal diversity compared with studies that include phylogenetically distant hosts (Tedersoo *et al.*, 2013). Therefore, the comparison among different fungal guilds or studies should take both host-phylogenetic and spatial scales into account in a study (van der Linde *et al.*, 2018).

Despite their limited influence, differences in the relative contribution of environmental and spatial interactions between fungal guilds were generally in agreement with their established roles in species coexistence theory. For example, stronger spatial autocorrelation signal in both species richness and community composition for PF was found (Fig. S5), probably arising from their limited dispersal ability. The spatial aggregation of PF in soil adjacent to conspecific adults produces high seedling mortality and thus promotes plant diversity (Bagchi *et al.*, 2010). Underlying this observation is an untested assumption that PF abundance increases with host density, and *post hoc* analysis of our data provides some support for this (Notes S1).

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Author contributions

ZW, WS and YL conceived the study. ZW collected the data. ZW and YJ analyzed the data. FH, WS, ZW and YL interpreted the results. ZW, DCD and FH wrote the manuscript with input from all authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Phylogenetic tree of the 45 host plant species included in this study.

Fig. S2 Relative abundance of genera in plant-pathogenic and mycorrhizal fungi.

Fig. S3 Community composition of tree families in the 50 ha forest plot where this study was conducted.

Fig. S4 Species accumulation curves (SAC) of root-associated pathogenic fungi, mycorrhizal fungi, arbuscular mycorrhizal and ectomycorrhizal fungi.

Fig. S5 Spatial autocorrelation at different distance classes. Shown are Moran's *I* and Mantel's *r* for plant-pathogenic and mycorrhizal fungal richness and community composition, respectively.

Fig. S6 Variation partitioning in species richness and community composition of mycorrhizal and pathogenic fungi, using host phylogeny and host functional traits as variables.

Fig. S7 Mantel correlograms of the community composition of pathogens and mycorrhizal fungi at different host-phylogenetic distances.

Fig. S8 Variations in host specialization in plant pathogenic and mycorrhizal fungal guilds at four host-phylogenetic scales.

Fig. S9 Heatmap of the abundance of pathogenic and mycorrhizal fungal genera in 45 plant species.

Fig. S10 Variations in host specialization index H_2' in arbuscular mycorrhizal and ectomycorrhizal fungi at four host-phylogenetic scales.

Notes S1 Additional details on testing the spatial aggregation of plant pathogens.

Table S1 Summary of all variables applied in this study.

Table S2 Characteristics and frequency of mycorrhizal and plant-pathogenetic fungal OTUs across plant individuals.

Table S3 Identification of plant pathogens and mycorrhizal fungi.

Table S4 Coefficient estimates, standard errors, *z*-statistics and *P*-values of the 'best' models for plant-pathogenic fungal species richness and mycorrhizal fungal richness.

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