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Correlation between fine root traits and pathogen richness depends on plant mycorrhizal types

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Root uptake strategies are associated with the strength of negative plant–soil feedback (PSF) induced by soil pathogens. Given the intensified effect of pathogen richness in fine roots on the strength of negative PSF through the synergistic effects of multiple pathogens, researchers have proposed a trade-off between nutrient acquisition and pathogen defence in roots. However, empirical evidence is lacking. In addition, because the interaction between pathogens and fine roots depends on the mycorrhizal types of tree species, both fine root traits and mycorrhizal types should be incorporated to reveal covariation in pathogen richness and the strength of negative PSF. In this study, we selected 50 arbuscular mycorrhizal (AM) tree species and 7 ectomycorrhizal (ECM) tree species in a subtropical forest to investigate the relationships between fine root traits and pathogen richness in fine roots and determined whether their relationships depended on plant mycorrhizal types. Our results showed that pathogen richness was negatively correlated with fine root diameter but was positively correlated with specific root length for the AM-associated species, while for the ECM-associated species, the pathogen richness was only found to have a significant negative relationship with the relative abundance of ECM fungi. These findings highlight the difference between AM- and ECM-associated species in pathogen defence and bridge the gap between root traits and pathogen richness, which is significant for improving our understanding of the potential factors mediating the strength of PSF and thus maintaining tree species diversity.

Keywords: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, fine root traits, pathogen richness, plant–soil feedback, root economics space

Introduction

Negative plant–soil feedback (PSF), a process in which plant survival/growth is negatively affected by alterations in soil due to the presence of plants (Bever et al. 1997), is a key driver underlying conspecific negative density dependence (CNDD), which maintains tree species diversity in forest ecosystems (Packer and Clay 2000, Bever et al.

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2010, Mangan et al. 2010, Liu et al. 2012, Bagchi et al. 2014). The accumulation of soil pathogens (e.g. fungal pathogens) around adult trees, which reduces the survival and growth of conspecific seedlings, has been recognised as one main factor inducing negative PSF (Packer and Clay 2000, Liu et al. 2012). Under the framework of the plant disease triangle (Liu and He 2019), the severity of plant diseases depends on not only the environmental conditions but also root traits and mycorrhizal types and species, whereby the strength of negative PSF may vary among plant species with different root traits and mycorrhizae. Moreover, the variation in the magnitude of negative PSF has been proposed to depend on the trade-off between nutrient acquisition of roots and pathogen defence of roots (Laliberté et al. 2015). Nevertheless, to date, no studies have quantified how root traits are related to pathogens in natural ecosystems (Newsham et al. 1995, Laliberté et al. 2015). Therefore, exploring the relationships between fine root traits and pathogens, together with identifying the traits associated with the susceptibility of roots to pathogens, is needed to improve the understanding of the drivers maintaining plant biodiversity in natural ecosystems.

Early studies proposed a root economics spectrum to represent the variation of fine root traits (Freschet et al. 2010, Reich 2014). With rapid progress in fine root research, the root economics spectrum has been remarkably broadened to reflect variations in fine root traits due to multiple evolutionary pressures and tradeoffs (Kong et al. 2014, Weemstra et al. 2016, Ma et al. 2018). Furthermore, one recent study shows the variation of fine root traits does not change along a single-dimensional root economics spectrum but along a collaboration gradient and a conservation gradient (i.e. root economics space) (Bergmann et al. 2020). The root collaboration gradient, covering the covariation in specific root length (SRL, root length per unit root dry mass) and root diameter (RD), is the main dimension for explaining root resource acquisition strategies (Bergmann et al. 2020) and reflects a tradeoff between plant species relying on their own roots (i.e. the 'do-it-yourself' strategy) and on mycorrhizal fungi (i.e. the 'outsourcing' strategy). In contrast, the conservation gradient, which is orthogonal to the collaboration gradient, represents a tradeoff between a fast and slow return on investment. In addition, plants with a higher SRL (relatively larger surface area and faster growth rates) are assumed to be associated with higher susceptibility to soil pathogens, possibly due to the increased chances of encountering soil pathogens while exploring larger soil volumes (Xia et al. 2021) and the decreased protection provided by mycorrhizal fungi (Cortois et al. 2016). Nevertheless, how root susceptibility to soil pathogens varies under the RES framework remains unclear.

In the natural environment, plants are generally confronted with multiple pathogens with diverse modes of action (Abdullah et al. 2017), and synergistic pathogenicity in plants has been widely detected (López-Villavicencio et al. 2011, Tollenaere et al. 2012, Zhang et al. 2018, Murtza et al. 2022). As a higher richness of soil pathogens may mean a greater possibility of pathogen co-infection, a stronger negative PSF may be observed (Semchenko et al. 2018). Co-infection induced by multiple pathogens can cause stronger negative impacts on their hosts in different ways, such as promoting pathogen reproduction, burdening plant immune systems, increasing overall infection risk and aggravating plant diseases (Cressler et al. 2014, Tollenaere et al. 2016, Zhang et al. 2018). In contrast, mycorrhizal fungi, such as arbuscular mycorrhizal (AM) fungi and ectomycorrhizal (ECM) fungi, not only facilitate nutrient uptake but also protect host roots from infection of soil pathogens, thereby alleviating pathogen-induced negative PSF (Veresoglou and Rillig 2012, Liang et al. 2015, Segnitz et al. 2020). Generally, ECM fungi provide better protection to their hosts than AM fungi because mycorrhizal sheaths and Hartig nets formed by ECM fungi would prevent soil pathogens from attacking fine roots (Bennett et al. 2017, Kadowaki et al. 2018). Common networks of ECM fungi have been observed to greatly improve the seedling survival of ECM-associated species (Marx 1972, McGuire 2007, Liang et al. 2020). By contrast, AM-associated species usually have higher pathogen loads and acquire protection from AM fungi in indirect ways, such as through competition of AM fungi with pathogens and activation of immune responses of host plants by AM fungi, whereby resistance of AM-associated species to pathogens is relatively low compared with ECM-associated species (Wehner et al. 2010, Cameron et al 2013, Chen et al. 2019). Thus, tree mycorrhizal types should be considered when investigating the relationship between root traits and pathogen richness.

The order-based classification of fine roots (i.e. the most distal root tip is classified as the first order, which grows on the second-order root, and so on) has greatly improved the comparison of fine root traits between plant species (McCormack et al. 2015, Freschet and Roumet 2017). The first three orders have been generally classified as absorptive fine roots, as they have similar structures and functions (McCormack et al. 2015). However, being distinct from the first two orders, the third-order roots are more lignified and harder to be colonised by mycorrhizal fungi (Guo et al. 2008), which are assumed to be less involved in resource uptake and less subjected to environmental control. Moreover, high lignification can help root defence against soil pathogens (Vance et al. 1980); therefore, the relationship between pathogens and the traits of the highly lignified third-order roots is presumably weaker than that of the first two orders. It is thus worthwhile investigating whether the root traits of the first two orders can better explain pathogen richness than those of the first three orders.

In this study, we selected 57 tree species in a subtropical forest (including 50 AM-associated species and 7 ECM-associated species), aiming to test the relationships between fine root traits and pathogen richness in fine roots. Specifically, we hypothesised that 1) under the framework of the root economics space (RES), the fine root traits along the collaboration gradient (i.e. RD and SRL) were correlated with pathogen richness, and more specifically, fine roots with the 'do-it-yourself' strategy (i.e. higher SRL and smaller RD)

tended to accumulate a higher richness of pathogens than the 'outsourcing' roots; 2) there were significant (negative/positive) relationships between fine root traits and pathogen richness for AM-associated species, while there was a significant negative relationship between the relative abundance of ECM (EMRA) and pathogen richness for ECM-associated species, possibly due to the strong protection of ECM fungi against soil pathogens for their hosts; 3) root traits of the first two orders better explained pathogen richness than those of the first three orders (which have been widely used at present).

Methods

Study site

Our study was performed in a 25 ha stem-mapped forest plot located in Baishanzu Nature Reserve, Zhejiang Province, China (centred on 27°45′43″N, 119°11′53″E, <https://forestgeo.si.edu/sites/asia/baishanzu>). This reserve is a mid-subtropical evergreen broad-leaved forest with a humid monsoon climate. The annual mean temperature is 12.8°C and the annual mean precipitation is 2218 mm (Luo et al. 2012). The first census of this plot was completed in 2016, and a total of 204 038 free-standing individuals with diameters at breast height (DBH) ≥ 1 cm (excluding vine species), belonging to 42 families, 86 genera and 149 species, were tagged and mapped spatially. The plot was composed of a northern slope and a southern slope, and the altitude of the plots ranged from 1406 to 1646 m a.s.l.

Fine root sampling and trait measurement

In the 25 ha plot, 57 woody tree species were selected, considering their phylogeny and mycorrhizal types. For each selected tree species, three to seven individuals were sampled, totalling 277 individuals. Root sampling was conducted from July to October 2020.

The lateral roots were carefully excavated from soil at a 0–20 cm depth at 2–3 m from the trunk of each sampled individual, and their branches were cut from the main lateral roots (Guo et al. 2008, Corrales et al. 2021). For each sampled individual, at least six intact root branches with five orders were obtained. The fine root samples were placed into plastic valve bags and stored in an ice barrel before dissection in the laboratory. The fine root branches were washed gently under a hose with clean running water to clean away the heavy clay and coarse organic matter. They were then rinsed in double-distilled water in an enamel plate to remove fine particles. The washing processes followed the handbook by Pérez-Harguindeguy et al. (2013). Root branch orders were defined according to Pregitzer's study, with the most distal tips being the first order (Pregitzer 2002). Each root sample was divided into three subsamples after removing the fourth and higher orders. The first subsample (weighed 0.2 g) was stored in a −80°C refrigerator before the molecular characterisation of fungi. The second subsample was dissected into the first two orders and the third order for measuring root traits separately. The third subsample was used to inspect the mycorrhizal type.

To determine the difference in fine root traits between the first two orders and the third order and whether the third-order root was involved in root–pathogen interactions, we specifically divided the first three-order roots into the first two orders and the third order. Given that the fourth and higher orders of roots are highly lignified and mainly responsible for transport (with little interaction with soil microbes) (Guo et al. 2008, McCormack et al. 2015, Freschet and Roumet 2017), they were excluded from this study.

The functional traits of fine roots and mycorrhizal types of fine roots were measured within one day after sampling. To determine the relationship between root traits and pathogen defence, we selected healthy root fragments for measuring root traits (and for molecular analysis of pathogens in roots) and estimated the association between root traits and pathogen richness in roots. For those sick fine roots, their defence had been completely pierced, so it was no longer proper to study the relationship between root traits and pathogen defence. For each sampled individual, 20 fragments of the first two orders of roots (as a whole) and 15 fragments of the third-order roots were placed on a transparent plastic plate and scanned separately using a flat-bed scanner. Root length (RL), root diameter (RD), root surface area (RSA) and root volume (RV) were measured with WINRHIZO software (Regent Instruments Inc.). Root dry mass (RDM) was weighed on an electronic balance with a one in ten-thousandth accuracy after drying for 48 h at 70°C. The specific root length (SRL), specific root area (SRA) and root tissue density (RTD) were calculated as the ratios of RL/RDM, RSA/RDM and RV/RDM, respectively. The traits of the first two orders, the first three orders and the third order were calculated and marked as $trait_{1–2}$, $trait_{1–3}$ and $trait_3$, respectively (e.g. $RD_{1–2}$ for RD of the first two orders).

The root subsamples for inspecting mycorrhizal types were fixed in formalin-aceto-alcohol (FAA) solution (100% glacial acetic acid, 37% methanol and 50% ethanol at a ratio of 1:1:18) for temporary storage. Before inspecting the mycorrhizal types, the fixed root samples were removed from the FAA solution and washed in deionised water. Each root sample was cut into 1.0–1.5 cm fragments of roots and separated into two parts for the following inspection: one was inspected directly for ECM colonisation under a dissecting microscope, and the other was inspected for AM colonisation after a series of treatments. For ECM colonisation (after root clearing; see above description), 50 root fragments for each individual plant (including the first two orders and the third order) were counted, and ECM-colonised fragments were identified when mycorrhizal sheaths, hyphae and Hartig nets were detected under a dissecting microscope (Teste et al. 2006). ECM colonisation was calculated as the number of ECM-colonised fragments divided by the total number of root fragments (Teste et al. 2006). For AM colonisation, the presence of vesicles or arbuscules was considered evidence of AM colonisation (Brundrett 2004), and the fragments with

such structures were regarded as AM-colonised fragments. First, the root fragments were placed in a 10% (w/v) KOH solution for five h at 90°C in glass tubes, rinsed twice with deionised water and then bleached (with NH_4OH, H_2O_2 and deionised water at a ratio of 1:1:200) at room temperature until the colour of the roots faded. Second, the bleached root sample was then rinsed twice with deionised water and placed in ink vinegar solution (black ink 5% (v/v) and acetic acid at a ratio of 5:100) at 100°C for five min for staining. Lastly, the stained root sample was rinsed three times in deionised water and transferred to acidified water (pH ≈ 3) for 30 min. Finally, acidified roots were placed in a 50% (v/v) lactoglycerol (lactic acid, glycerol and H_2O at a ratio of 1:1:1) solution for de-staining and inspected under a microscope. The number of root fragments colonised by AM was divided by the total number of root fragments to estimate AM colonisation.

The mycorrhizal type of each root sample was determined based on the abovementioned estimation of colonisation rates. Only a small number of samples were found to be colonised by both ECM and AM fungi (i.e. dual-mycorrhizal associations in some plant species of Fagaceae). For these samples, we determined their dominant mycorrhizal types based on the colonisation rates of AM fungi versus ECM fungi.

High-throughput sequencing of fungi

The frozen root samples were sent to the laboratory on dry ice for high-throughput sequencing within one week after sampling. The surface of the root samples was sterilised with alcohol before DNA extraction. The genomic DNA of the root samples was extracted for DNA sequencing using an E.Z.N.A. Soil DNA Extraction Kit (Omega Bio-tek, GA, USA) following the manufacturer's instructions. The concentration of DNA was quantified using a Qubit 2.0 Fluorometer to ensure that adequate amounts of high-quality genomic DNA had been extracted.

The nuclear ribosomal internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) using the fungal primer set of ITS1 (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC). PCR was performed with standard protocols, and the reaction was set up as follows: 2 μl microbial DNA (10 ng μl^{-1}); 1 μl amplicon PCR forward primer (10 μM); 1 μl amplicon PCR reverse primer (10 μM); and 2 × 15 μl KAPA HiFi Hot Start Ready Mix (for a total of 30 μl). The plate was sealed, and PCR was performed in the GeneAmp PCR System 9700 with the following programme: denaturing at 95°C for 3 min, 5 denaturing cycles at 95°C for 30 s, annealing at 45°C for 30 s, elongation at 72°C for 30 s, then 20 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 30 s and a final extension at 72°C for 5 min. The PCR products were checked using electrophoresis in 1% (w/v) agarose gel in TBE buffer (Tris–Borate–EDTA) stained with ethidium bromide and visualised under UV light. AMPure XP beads were used to purify the free primers and primer dimers in the amplicon product.

The library was constructed using a universal Illumina adaptor and index. Before sequencing, the DNA concentration of each PCR product was determined using a Qubit 2.0 Green double-stranded DNA assay, and the quality was controlled by the Agilent 2100 Bioanalyzer system. The amplicons from each reaction mixture were pooled in equimolar ratios based on their concentrations. Sequencing was performed using the Illumina MiSeq PE300 system according to the manufacturer's instructions.

Raw reads were trimmed by removing adaptor sequences and low-quality bases, and then merged with paired-end reads following the Mothur pipeline (Schloss et al. 2009). Chimeric sequences were removed using UCHIME (Edgar et al. 2011). The qualified sequences were further clustered into operational taxonomic units (OTUs) at 97% sequence similarity using USEARCH (Edgar 2010). The taxonomic classification of each OTU was determined using the RDP classifier with a confidence interval of 80% trained on the UNITE database (Abarenkov et al. 2010). Sequences that were not assigned to the fungal kingdom and singletons were removed. Each sample was rarefied to 11 656 sequences to control for unequal sequencing depth among the samples. In total, there were 9661 fungal OTUs. Based on the FUNGuild database (Nguyen et al. 2016), the functional guilds of fungal OTUs were categorised. In this study, (putative) plant-pathogenic OTUs were only selected when they were assigned as 'plant pathogen' with 'highly probable' or 'probable' confidence rankings, and the number of plant-pathogenic OTUs was used to represent fungal pathogen richness. For AM-associated species, there were 187 pathogenic OTUs (Supporting information) and 86 pathogenic OTUs for the ECM-associated species (Supporting information). The Shapiro–Wilk normality test was applied to determine whether the pathogen richness data followed a normal distribution, and the log transformation was performed if needed. To estimate the relative abundance of AM (AMRA) and EMRA in each sample, OTUs assigned to 'Arbuscular mycorrhizal' or 'Ectomycorrhizal' with 'highly probable' or 'probable' confidence rankings were used.

In the models testing the effects of root traits on pathogen richness, we selected the relative abundance of mycorrhizal fungi rather than mycorrhizal colonisation rates as the independent variable because the former was estimated from the same root subsamples used for measuring pathogen richness (but mycorrhizal colonisation rate and pathogen richness were measured using different subsamples of one fine root sample, see the abovementioned description). In addition, microscopic estimates of the mycorrhizal colonisation rate usually have sampling biases (Kokkoris et al. 2019), so mycorrhizal colonisation rate was only used for identifying mycorrhizal types in this study. Although the FUNGuild database has been broadly used to classify fungal guilds at present (Chen et al. 2019, Corrales et al. 2021), it is a coarse functional classification of fungi. Considering that the presence of mycorrhizal structures (e.g. vesicles and arbuscules for AM-associated species, hyphae and Hartig nets for ECM-associated species) is more representative

for identifying mycorrhizal types of plants than the relative abundance of mycorrhizal fungi, we performed microscopic estimates of mycorrhizal colonisation rates. Moreover, the correlation analysis between the relative abundance of pathogenic fungi and EMRA showed that there was no correlation between them, although they came from the same sequencing sample (Supporting information).

It should be noted that not all putative pathogens detected in healthy roots might be pathogenic to the selected plants. Given the intensified negative PSF under a high diversity of putative fungal pathogens (Semchenko et al. 2018), fine roots with high pathogen richness are expected to experience increased disease risk. In addition, because most plant diseases are caused by fungal pathogens (70–80%; Dayarathne 2021), this study focused on fungi (without including oomycetes and bacteria, some of which can also induce plant diseases).

Plant phylogeny construction and phylogenetic signal detection

Based on the recently published phylogeny (plastid genomes) for the tree species of the Baishanzu 25 ha plot (Jin et al. 2022), the phylogenetic trees were constructed for the selected AM- and ECM-associated species, respectively, in this study. Based on Blomberg's *K* (Blomberg et al. 2003), we tested whether the pathogen richness in roots was independent of the phylogeny separately for AM- and ECM-associated plants.

Statistical analyses

All analyses in this study were conducted separately for AM- and ECM-associated species. To determine whether the data of fine root traits supported RES and how the measured root traits were correlated (including RD, SRL, SRA, RTD and relative abundance of mycorrhizal fungi), two phylogenetically informed principal component analyses (pPCA, which could better present the relationships among traits by removing their phylogenetic co-variance; Bergmann et al. 2020) were conducted at the species level for AM- and ECM-associated species, respectively. The significance of principal components was tested with the R package 'paran' and the first two PCs were retained.

We used linear mixed-effects models to model the effects of the root traits on the richness of fungal pathogens at the individual level of the selected plants. All root traits of the first two orders and first three orders (i.e. $RD_{1–2}$, $SRL_{1–2}$, $SRA_{1–2}$, $RTD_{1–2}$, $RD_{1–3}$, $SRL_{1–3}$, $SRA_{1–3}$ and $RTD_{1–3}$) and their corresponding relative abundance of mycorrhizal fungi (i.e. AMRA and EMRA) were treated as fixed effects, with the species identity as a random effect. The backward selection was used to determine the most parsimonious models based on Akaike's information criterion using the package 'buildmer' in R (Voeten 2021).

All statistical analyses were performed using R ver. 3.6.3 (<www.r-project.org>). pPCA and the trimming phylogenetic trees were conducted with the package 'phytools' (Revell 2012), and the phylogenetic signals were tested using the package 'ape' (Paradis and Schliep 2019). The linear mixed-effects models were conducted using the package 'lme4' (Bates et al. 2015). The relationships between selected variables and pathogen richness were estimated and visualised with the packages 'ggeffects' and 'ggplot2'.

Results

Root traits of AM- and ECM-associated species

In total, we investigated 235 individuals of 50 AM-associated species and 42 individuals of 7 ECM-associated species, representing a range of root trait characteristics covering the RES (Fig. 1). pPCA among the root traits indicated that the first two axes respectively accounted for 46.6% and 25.4% of the total variation in the AM-associated species (Fig. 1a), and the first two axes explained 44.6% and 38.5% of the total variation in the ECM-associated species (Fig. 1b). For the AM-associated species, the first dimension was dominated by $RD_{1–2}$, RD_3, $SRL_{1–2}$ and SRL_3, and the second dimension was dominated by $RTD_{1–2}$. $RD_{1–2}$, RD_3 and AMRA were positively correlated with PC1, while the remaining traits were negatively correlated with PC1; $RTD_{1–2}$ and RTD_3 were positively correlated with PC2, while the remaining traits were negatively correlated with PC2 (Supporting information). For the ECM-associated species, the first dimension was dominated by $RD_{1–2}$ and SRL_3, and the second dimension was dominated by $RTD_{1–2}$ and $SRA_{1–2}$. $SRL_{1–2}$, SRL_3, $SRA_{1–2}$, SRA_3, $RTD_{1–2}$ and RTD_3 were negatively correlated with PC1, while the remaining traits were positively correlated with PC1; $RTD_{1–2}$, RTD_3, EMRA, $SRL_{1–3}$ and $SRA_{1–3}$ were positively correlated with PC2, while the remaining traits were negatively correlated with PC2 (Supporting information). The directions of RD, SRL and RTD in the biplots and their loading scores on the principal components partially supported the framework of RES for both AM- and ECM-associated species.

Relationships between fine root traits and pathogen richness

For either AM- or ECM-associated species, there was no significant phylogenetic signal for root pathogen richness ($K = 0.284$, $p = 0.201$ and $K = 0.558$, $p = 0.815$, respectively; Fig. 2), indicating that pathogen richness was independent of host phylogeny. Variation in root pathogen richness for the AM-associated species was best explained by $RD_{1–2}$, $SRL_{1–3}$ and $RTD_{1–2}$ (Table 1). There was a significant negative effect of $RD_{1–2}$ (coef. = –0.251, $p = 0.002$; Table 1, Supporting information) and a significant positive effect of $SRL_{1–3}$ (coef. = 0.156, $p = 0.016$; Table 1, Supporting information) on pathogen richness. $RTD_{1–2}$ was not significantly related to variations in root pathogen richness (coef. = 0.189, $p = 0.112$; Table 1). The three fixed variables together explained 11.8% (marginal R^2) of the variation in pathogen richness. Together with the random effect (i.e. species identity), 13.2%

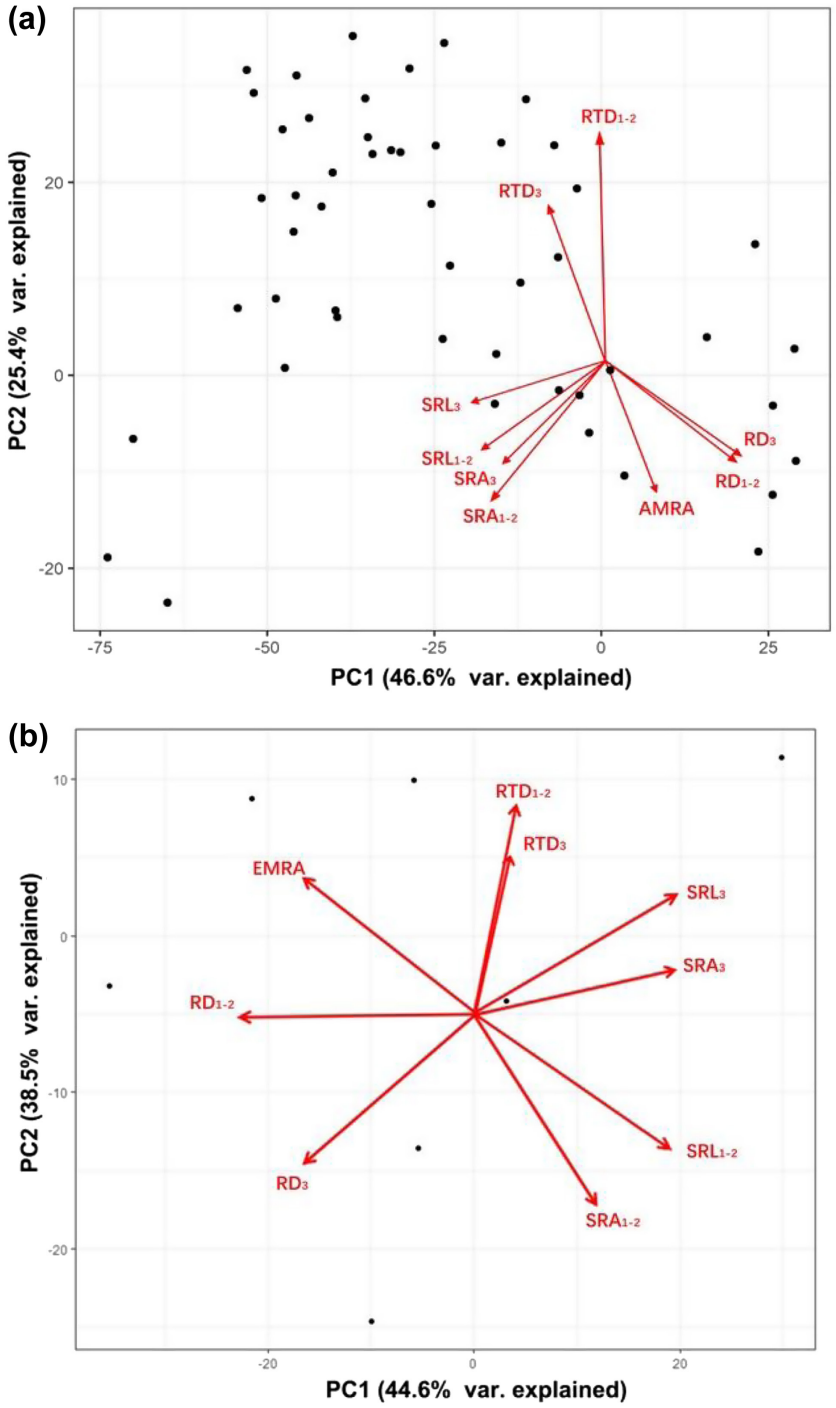

Figure 1. Phylogenetically informed principal component analyses (pPCA) for root traits for arbuscular mycorrhiza (AM)-associated species (a) and for ectomycorrhiza (ECM)-associated species (b). The subscript of each trait is the abbreviation for the root order ($trait_{1-2}$, trait of the first two orders; $trait_3$, the third-order trait). Each dot represents a plant species. RD, root diameter; SRL, specific root length; RTD, root tissue density; SRA, specific root area; AMRA, relative abundance of arbuscular mycorrhizal fungi; EMRA, relative abundance of ectomycorrhizal fungi.

(conditional R^2) of the variation were explained. In the case of the ECM-associated species, EMRA was only retained in the most parsimonious model, and showed a significantly negative effect on pathogen richness (coef. = −1.839, $p < 0.001$; Table 1, Supporting information). EMRA explained 36.3% (marginal R^2) of the variation in pathogen richness, and the fixed and random effects together explained 38.1% (conditional R^2) of the variation in pathogen richness.

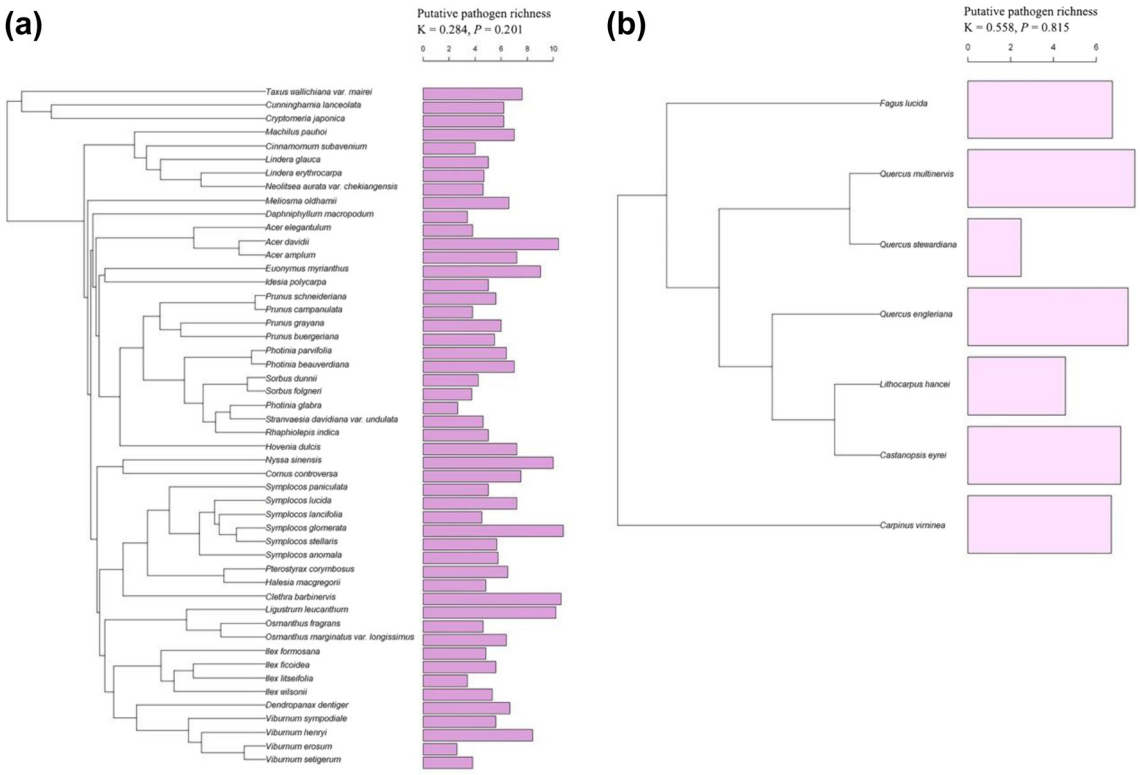

Figure 2. Richness of putative fungal pathogens as structures by plant phylogeny for arbuscular mycorrhiza (AM)-associated species (a) and for ectomycorrhiza (ECM)-associated species (b). The plots show pathogen richness at the plant species level with Blomberg's *K* measures of the phylogenetic signal.

Discussion

By measuring fine root traits of 57 tree species spanning major families across a 25-ha subtropical forest plot, we tested the RES and examined whether it was associated with pathogen richness in fine roots. In line with the RES, for both AM- and ECM-associated species, RD and SRL were negatively correlated and both dominated PC1, thus forming a collaboration gradient (Fig. 1). Under the RES framework, fine roots with an 'outsourcing' strategy benefit from a high collaboration with mycorrhizal fungi (Bergmann et al. 2020). However, AMRA was found to be more associated with the conservation gradient than the collaboration gradient for the AM-associated species, which disagrees with the RES framework. One possible explanation is that the increased relative abundance of AM fungi in this study might lead to an increase in root nitrogen (N) because of the high N content in the hyphae of AM (Hodge and Fitter 2010), but we did not measure root N %, and mycorrhizal colonisation rates did not correlate with root N in the study of Bergmann et al. (2020). The discrepancy between our AM results and the RES could be also ascribed to the usage of the relative abundance of AM fungi rather than the absolute colonization rate of AM fungi in this study. The latter showed a significant correlation with root diameter ($p < 0.001$; Supporting information) consistent with the previous findings (Kong et al. 2014), while the relative abundance of AM fungi was independent of the root diameter ($p = 0.053$; Supporting information).

Our first hypothesis that the do-it-yourself strategy is associated with higher root pathogen richness is supported by AM but not ECM root trait data. Specifically, RD was negatively related to pathogen richness, and SRL was positively related

Table 1. Model statistics of the most parsimonious models for arbuscular mycorrhiza (AM)-associated species and for ectomycorrhiza (ECM)-associated species. The relationship between each fixed effect kept in the most parsimonious model and the pathogen richness is presented in the Supporting information.

Model	Dependent variable	Fixed effect	Coef.	F-value	p
AM-associated species	Pathogen richness	RD_{1-2}	−0.251	9.73	0.002
		SRL_{1-3}	0.156	5.75	0.016
		RTD_{1-2}	0.189	2.72	0.11
ECM-associated species	Pathogen richness	EMRA	−1.839	23.40	< 0.001

Significance of the fixed effects was estimated by Satterthwaite's approximation. RD_{1-2}, root diameter of the first two orders; SRL_{1-3}, specific root length of the first three orders; RTD_{1-2}, root tissue density of the first two orders; EMRA, relative abundance of ECM fungi.

to pathogen richness for the AM-associated species, while none of the morphological traits was selected by the model for the pathogen richness in ECM species. EMRA had a significantly negative relationship with pathogen richness, supporting the second hypothesis. This relationship is in line with the defence function of ECM fungi against soil pathogens via forming sheaths and Hartig nets (acting as physical barriers from infection or pathogen spread; Marx 1972). Mutualisms of ECM fungi with plants can alleviate the strength of pathogen-induced PSF (Bennett et al. 2017) and generate positive PSF (Segnitz et al. 2020), thereby causing monodominance in forests (McGuire 2007). The strong negative relationship between EMRA and pathogen richness means that plants benefit from better protection under a higher EMRA, possibly due to the formation of more sheaths and Hartig nets. Meanwhile, the formation of sheaths and Hartig nets can increase the RD of ECM-associated species to some extent (Withington et al. 2006), which possibly causes an inaccurate measurement of root traits (e.g. RD), thereby obscuring the relationship between pathogen richness and root traits.

Given a recent study showing that fine roots with larger RD and lower SRL tend to maintain a lower level of defensive compounds compared with thinner roots with higher SRL (Xia et al. 2021), we suggest that the 'do-it-yourself' strategy is associated with an elevated pathogen pressure for the AM-associated species. Consequently, it is expected that fine roots of AM-associated species with smaller RD and higher SRL are more susceptible to soil pathogens, and these plants may require stronger chemical protection against soil pathogens. The findings of this study may expand the ecological significance of RES in the context of pathogen defence, especially for AM-associated hosts. Some protection of AM fungi against pathogens has been previously acknowledged (Wehner et al. 2010), but compared to ECM protection, it is usually weak and the underlying mechanisms are complicated. Therefore, it is hard to detect and understand the protection of AM fungi in natural ecosystems, especially in forests with high biodiversity (Laliberté et al. 2015), and this may be the reason why we did not observe a relationship between AMRA and pathogen richness in our model. In turn, the relationships between AMRA and individual root traits that were associated with pathogen richness (i.e. RD and SRL) and generally with negative PSF (Cortois et al. 2016, Semchenko et al. 2018) suggest that there may be undetected relationships that warrant further study.

The difference in the association of root traits with pathogen richness in roots between AM- and ECM-associated species highlighted their differences in pathogen defence. Compared with ECM-associated species, AM-associated species accumulate more pathogens and suffer much stronger pressure from soil fungal pathogens (Chen et al. 2019). In addition, AM fungi provide less protection for roots of AM-associated species against pathogens than ECM fungi for ECM-associated species. These together may explain why AMRA was not selected by the best AM model. This may also indicate that pathogen richness in the roots of AM-associated species is more associated with root traits, thereby leading to a strong relationship between pathogen richness and root traits. By contrast, because ECM fungi well protect roots from pathogen infection (Marx 1972), a significant relationship between root traits and pathogen richness was not detected in ECM-associated species. These results are in line with the PSF experiments that the strength of negative PSF was related to root traits of AM-associated species (Xi et al. 2021), and that the strength of positive PSF of ECM-associated species depended on ECM colonisation (Segnitz et al. 2020). Indeed, previous studies on negative PSF usually focus on species-specific soil pathogens, but this study suggests that the diversity of pathogens should be incorporated into PSF estimation for understanding their contribution to maintaining tree species diversity in forests.

In contrast to our third hypothesis, the first three orders were better than the first two orders in explaining pathogen richness (Table 1). Specifically, $SRL_{1–3}$ rather than $SRL_{1–2}$ was retained in the best selected model explaining pathogen richness in the roots of AM-associated species. Our results suggest that third-order roots may also need to be considered when studying root-pathogen interactions.

In summary, this study provided evidence of the defence-acquisition tradeoff in AM-associated species, suggesting that the 'do-it-yourself' root strategy is associated with elevated pathogen pressure. This finding could explain the relationship between root traits and the strength of negative PSF of AM-associated species. For ECM-associated species, EMRA rather than root morphological traits showed a negative relationship with pathogen richness, which highlights the strong protection of ECM fungi against soil pathogens. These findings not only extend the framework of RES but also improve the understanding of the difference in the strength of PSF between AM- and ECM-associated species, and thus their roles in maintaining tree species diversity in forests.

Speculation and perspective

It is known that root traits are involved in negative PSF (Cortois et al. 2016, Semchenko et al. 2018, Xi et al. 2021), but the underlying mechanisms remain unclear, which warrants further study in the future. Specifically, how pathogen community in roots is correlated to root traits may be of particular interest. Addressing this question will contribute to understanding why the strength of negative PSF depends on pathogen richness (varies with root traits; Semchenko et al. 2018), even though host-specific pathogens have been well acknowledged to play key roles in inducing negative PSF. Moreover, the detected relationships between root traits and negative PSF may also provide a promising future direction to fully understand the persistence of a large number of rare species in nature (36.5% of terrestrial plants on Earth; Enquist et al. 2019). Namely, it is worth investigating whether rare plant species own unique fine traits, thereby testing the rare-species advantage hypothesis (providing a stabilizing mechanism for species coexistence) and better conserve rare species in the future.

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Author contributions

Dong Dai: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Software (lead); Visualization (lead); Writing – original draft (lead). **Jiarong Yang**: Data curation (equal); Investigation (equal); Writing – review and editing (equal). **Yougui Wu**: Project administration (equal); Resources (lead); Writing – review and editing (equal). **Wenhua Zhang**: Data curation (equal); Investigation (equal); Methodology (equal). **Yajing Liu**: Data curation (equal); Investigation (equal). **Xian Wu**: Writing – review and editing (equal). **Hua Xing**: Investigation (equal); Validation (lead); Writing – review and editing (equal). **Yu Liu**: Funding acquisition (lead); Project administration (lead); Software (equal); Supervision (lead); Validation (lead); Writing – review and editing (lead).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.mkkwh713m> (Dai et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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