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RESEARCH ARTICLE

Non-random tree species loss shifts soil fungal communities

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Abstract

1. Ongoing global changes speed up forest diversity loss, typically in a non-random manner, with potential consequences for soil fungal communities. However, it remains elusive how non-random tree species loss, based on distribution ranges or specific traits, impacts soil fungal communities.
2. Here we employed a large-scale field experiment where random and non-random tree species losses were experimentally manipulated, to estimate soil fungal responses using high-throughput sequencing. Two non-random scenarios were studied: non-random loss of either rarer species (measured by regional frequency) or more acquisitive species (measured by specific leaf area [SLA]).
3. We found that soil fungal communities were more profoundly affected by the non-random rather than the random tree species loss. The preferential loss of higher-SLA species resulted in significant decreases in soil fungal diversity, shifts in community composition and simplifications in the fungal co-occurrence network. Moreover, both the diversity and relative abundance of saprotrophic fungi decreased, whereas those of ectomycorrhizal fungi increased with SLA-oriented tree species loss. With the biased loss of rarer species, plant pathogenic fungi showed decreases in diversity and shifts in community composition. Compared with the random scenario, the significant effects of non-random tree species loss are closely related to the covaried trait dominance of plant communities, notably to the changes concerning community life-history traits and mycorrhizal associations.
4. *Synthesis*: Our study provided novel evidence that the non-random, trait-oriented tree species loss exerted far-reaching effects on soil fungal community structures compared with the random species loss, highlighting the potential of trait-based predictions for understanding the dynamics of soil fungal communities. Modified

ecosystem functioning under disturbances causing non-random tree species loss in the real world can, therefore, be expected owing to the trait-based plant–soil fungal interactions.

KEYWORDS
BEF-China, co-occurrence network, functional trait, fungal functional guild, mycorrhizal association, non-random tree species loss, soil fungal community

1 | INTRODUCTION

Accelerating biodiversity loss (Betts et al., 2017; Pimm et al., 2014; Purvis et al., 2000) triggers the rapid growth of biodiversity experiments over the past decade (Cardinale et al., 2012; Midgley, 2012). Forest biodiversity experiments are typically conducted using synthetic communities based on a random assembly strategy assuming equal extinction risks among species (Purvis & Hector, 2000). However, local species extinctions (extirpations) in the real world are typically non-random and accompanied by predictable changes in community structures (Duffy, 2009; Zavaleta et al., 2009). Mounting theoretical and empirical evidence indicates that rare species with narrow distribution ranges suffer from higher extinction risks when being faced with habitat fragmentation (Payne & Finnegan, 2007; Staude et al., 2019; Vincent et al., 2020). In addition, traits can serve as potential indicators for assessing the likelihood of local extirpation, given their effectiveness in predicting species responses to environmental changes (Esquivel-Muelbert et al., 2020; Kröber et al., 2012). For example, species featuring acquisitive life-history traits, generally characterized by stress intolerance (Grime, 2002), are expected to be more vulnerable to stress-based disturbances, such as droughts (Greenwood et al., 2017). Therefore, non-random species loss scenarios should be considered to fully understand the implications and extrapolations of species loss for biodiversity conservation and management (Bannar-Martin et al., 2018; Bracken et al., 2008; Bruelheide et al., 2014; De Laender et al., 2016; Duffy, 2009; Lepš, 2004; Peh & Lewis, 2012; Schmid & Hector, 2004; Wardle, 2016).

Tree species loss may strongly affect soil fungal communities (Gan et al., 2022; Hiiesalu et al., 2017; Peay et al., 2013) and subsequently influence ecosystem functioning (Ma et al., 2023; Ruijven et al., 2020). A loss in plant species diversity can influence the quantity, quality and heterogeneity of plant-derived resources (De Dyn & Van der Putten, 2005; Hooper et al., 2000; Waldrop et al., 2006). Plant species identity and community composition, both of which are intrinsically related to plant traits, can also modify soil fungal communities (Chen et al., 2019; Pei et al., 2016; Prada-Salcedo et al., 2021; Wardle et al., 2004). As suggested by *the mass ratio hypothesis* (Grime, 1998; Smith et al., 2020), dominant traits in plant communities may exert primary effects on determining above- and below-ground interactions (Richard, 2017). However, the random extinction scenario, which assumes equal extirpation probabilities for all species, might underestimate, or even cancel out the diversity effects derived from the dominant traits (Zavaleta & Hulvey, 2006). Conversely, under the trait-oriented non-random species loss, a diversity gradient may be observed that coincides with a directional shift in community trait composition, potentially revealing the trait-driven processes (Solan et al., 2012; Zavaleta & Hulvey, 2006). In a few cases where non-random species loss has been tested experimentally, the simulated non-random diversity loss exerted either stronger or weaker effects on productivity than random diversity loss (Chen et al., 2020; Smith et al., 2020; Wolf et al., 2021). However, the impacts of non-random tree species loss on soil fungal communities remain largely unexplored, which is necessitated given the potential trait-based linkages between plant–soil fungal interactions.

Soil fungi can be functionally classified into different guilds with distinct lifestyles and nutrient acquisition strategies (Tedersoo et al., 2014; Zanne et al., 2020), which are expected to respond differently to random versus trait-oriented non-random tree species loss. Given the intimately mutualistic-symbiotic relationships, ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) fungi may depend more on host tree species identity and their mycorrhizal status than on tree species diversity per se (Gao et al., 2013; Heklau et al., 2021; Nguyen, Williams, et al., 2016; Tedersoo et al., 2010). Saprotrophic fungi, pivotal in decomposition, are strongly shaped by the quantity and quality of soil and litter substrates, which may in turn depend on both plant traits and diversity (Nguyen, Williams, et al., 2016; Prada-Salcedo et al., 2021; Setälä & McLean, 2004). Pathogenic fungi can either be diluted or amplified under specific diversity loss depending on shifts in community competence traits, with more competent communities potentially enhancing pathogen maintenance and transmission (Halliday et al., 2020; Johnson et al., 2015; Keesing & Ostfeld, 2021; Rohr et al., 2020). Apart from the separate responses of these guilds, the integrated co-occurrence networks further provide valuable insights into the complexity and interaction potential among fungal guilds or taxa under biotic and abiotic environmental changes (Bakker et al., 2014; Barberan et al., 2012; Gan et al., 2022; Guseva et al., 2022). Profiling responses of soil fungi from individual guilds to integrated networks would greatly enhance our comprehensive understanding of how soil fungal communities respond to the random versus non-random tree species loss.

Except for plant communities, soil fungal communities are also shaped by a broad range of edaphic, topographic and spatial variables, such as soil pH, moisture and nutrient contents (Gan et al., 2022; Schappe et al., 2020; Weissbecker et al., 2018). These abiotic factors, partly independent of plant communities, introduce a high degree of context dependency and dispersal limitation on soil fungal assembly, which may even overshadow the effects of plant communities (Weissbecker et al., 2018). Accordingly, variations in environmental conditions across sampling plots should be accounted for when evaluating the effects of random versus non-random tree species losses (Table S1).

In this study, we aimed to assess how soil fungal communities respond to tree species richness under different tree extinction scenarios, as simulated by a long-term field experiment with tree community plantations ranging from mixtures of eight, four and two species and monocultures. The experiment was carried out in a subtropical forest biodiversity and ecosystem functioning experiment in China (BEF-China, Bruelheide et al., 2014). Specifically, two non-random extinction scenarios were designed when setting up the experiment as compared to the random scenario, assuming that species with higher specific leaf area (SLA) indicative of a faster life-history strategy (the SLA-oriented scenario), and locally rarer species with narrow distribution potential (the rarity-oriented scenario) would experience higher extinction risks, respectively (Bruelheide et al., 2014). We profiled soil fungal communities based on ITS2 amplicon sequencing and hypothesized that (1) the diversity, community composition and co-occurrence structure of soil fungi would be more strongly affected by the non-random than the random tree species loss. In particular, these distinct responses are expected to be driven by shifts in plant community traits under the non-random scenarios. Given the distinct life strategies, we expected varying sensitivities across fungal functional guilds under different non-random scenarios. Specifically, (2) under the SLA-oriented scenario, the preferential loss of high-SLA species would potentially shift the plant community from fast-growing to slow-growing. As a result, decreases in diversity and relative abundance for both saprotrophic and pathogenic fungi were expected, attributable to the reduced resource quality and enhanced defensive features of plant-derived substrates (Prada-Salcedo et al., 2021). Conversely, the diversity and relative abundance of EcM fungi might increase due to the typical alignment between EcM-tree dominance and slow-growing traits (Averill et al., 2019). As indicated by the pathogen adaptation theory that locally common or widespread hosts generally exhibit greater competence and tolerance for pathogen transmission and maintenance than rare species (Lively & Dybdahl, 2000), we predicted that (3) the diversity and relative abundance of pathogenic fungi would increase with the preferential loss of locally rare species.

2 | MATERIALS AND METHODS

2.1 | Study site and extinction design in BEF-China

The study was conducted in the Biodiversity-Ecosystem Functioning Experiment China Platform (BEF-China) at Xingangshan, Jiangxi Province, southeastern China (29.08°–29.11° N, 117.90°–117.93° E, Figure 1a). This region belongs to a typical subtropical climate zone, with a mean annual temperature of 16.7°C and a mean annual precipitation of 1821 mm (Yang et al., 2013), where subtropical mixed evergreen broadleaved forests are distributed.

The BEF-China experiment consists of two main experimental sites (i.e. Site A and B) with 566 plots (25.8 × 25.8 m^2 per plot) in total, which were established in 2009 and 2010, respectively (Bruelheide et al., 2014). In each plot, 400 tree individuals were planted in a grid of 20 rows and 20 columns, with a grid size of 1.29 × 1.29 m. Tree species richness was experimentally varied ranging from monocultures to mixtures of two, four, eight and 16 species under one random and two types of non-random extinction scenarios (Bruelheide et al., 2014). At each site, 24 native broadleaved tree species were used as the species meta-pool to establish different species compositions (Figure S1a). A 'broken-stick' design was applied to conduct the random scenario. In detail, three partly overlapping starting compositions of 16 species each were randomly and sequentially partitioned into two non-overlapping halves, resulting in the eight-, four- and two-species mixtures and monocultures (Figure 1b).

Considering that extinction incidents occurring in natural ecosystems are typically biased and more prone to species with certain characteristics, two non-random extinction scenarios, featuring specific orders in tree diversity loss sequences, were deliberately designed according to the following two considerations: (i) that a locally rarer species would be more vulnerable to lose, for example, as a result of habitat fragmentation, and (ii) that a species with a higher SLA would be more susceptible to stress-based environmental disturbances, such as drought, because the trade-offs in the leaf economic spectrum render them less resistant (Figure 1b, Bruelheide et al., 2014). Accordingly, the 20 most common species or the 20 species with the lowest SLA from the meta-pool of 24 species were chosen as the starting compositions for the rarity-oriented or the SLA-oriented scenario, respectively (Figure S1a). The rarer species or species with higher SLA were then sequentially eliminated from the starting 20-species composition to yield decreasing richness levels from 16- to eight-, four- and two-species mixtures (Figure 1b). Three different species compositions were established at each richness level that shared comparable degrees of commonness or SLA. The monoculture plots for the non-random scenarios were available from the random scenario series. More details about the design of the BEF-China experiment are addressed in Bruelheide et al. (2014).

2.2 | Soil sampling

To investigate the distribution of soil fungal communities and the associated soil conditions, soil sampling was conducted at site A of BEF-China across four tree species richness levels (one-, two-, four- and eight-species richness) under the random, rarity-oriented and SLA-oriented extinction scenarios. A total of 59 plots were chosen in this study (23 plots for monoculture + 4 plots × 3 extinction scenarios × 3 tree richness levels for mixtures = 59 plots, Figure 1a). For species mixtures of eight, four and two species, we randomly

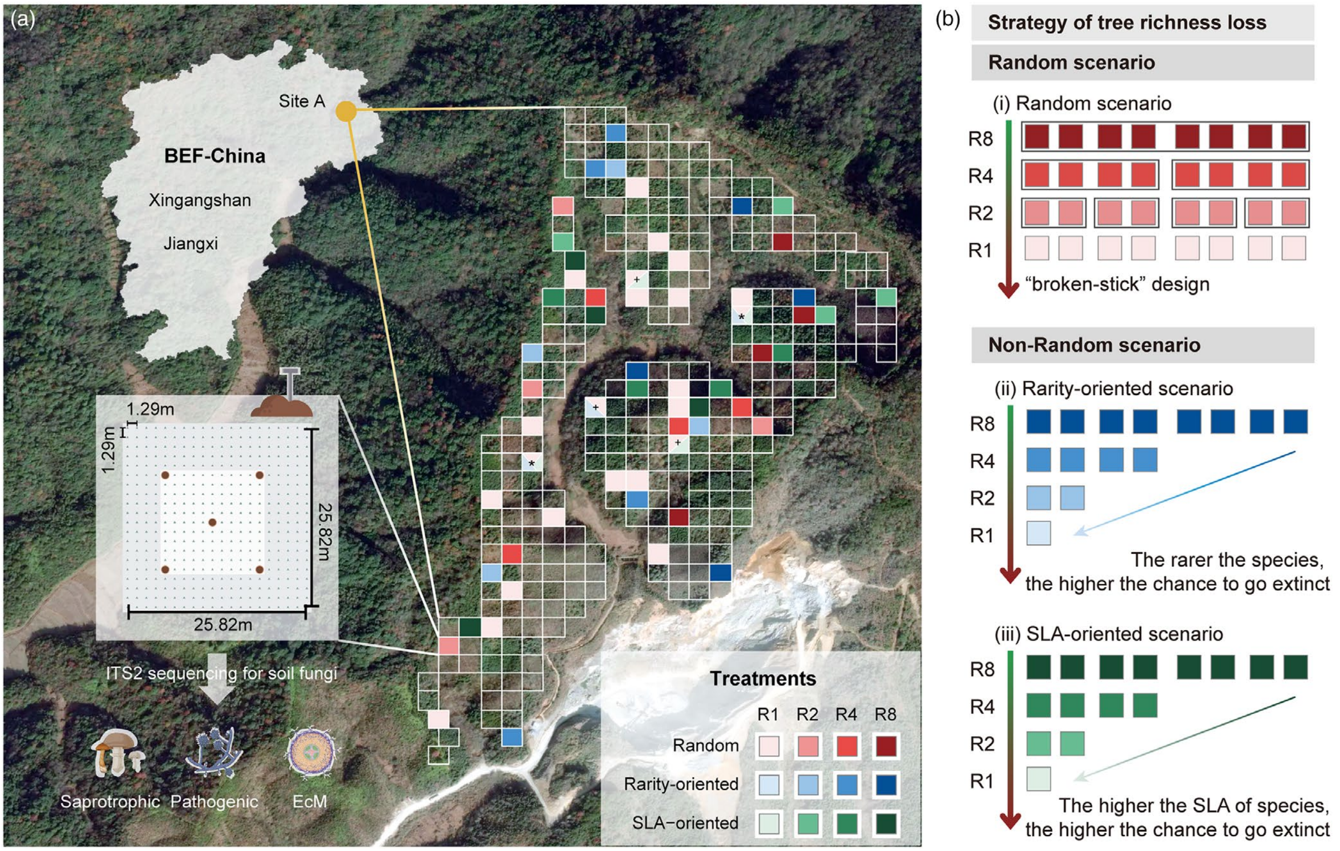

FIGURE 1 Overview of the sampling design. (a) Map of Jiangxi province with the location of BEF-China Site A, the within-site soil sampling design, and the within-plot sampling method is shown. (b) Diagram depicts the design of tree species loss under the (i) random versus two non-random [i.e. (ii) rarity-oriented and (iii) SLA-oriented] scenarios (modified from Bruelheide et al., 2014). Note that some monoculture plots were designated to be included for multiple scenarios in the statistical analyses, with the label "+" or "*" indicating the specific monoculture plots shared by two or three scenarios, respectively. More details are available in Section 2. SLA, specific leaf area.

selected four plots from all available plots as replicates. For monocultures, 23 plots encompassing all species encountered in the mixture plots were selected. Particularly, monoculture plots were not completely or evenly shared among the three scenarios in subsequent analyses. For each non-random scenario, only the plots of the component species from the two-species mixtures were used for the monoculture treatment, representing the species with relatively extreme trait values (i.e. the most locally common species or species with the smallest SLA). Therefore, the monoculture plots of *Schima superba*, *Cyclobalanopsis glauca*, *Lithocarpus glaber* and the plots of *Castanopsis sclerophylla*, *Lithocarpus glaber*, *Cyclobalanopsis glauca*, *Cyclobalanopsis myrsinifolia* were chosen for the rarity- and the SLA-oriented scenario, respectively (Figure S1b). For the random scenario, monoculture plots of all component species included in the mixtures were sampled, which also encompassed all the monoculture plots used for the non-random scenarios (Figure S1b). Monoculture plots with overlapping species identities were thus shared across different scenarios, with their measurements included multiple times in the analyses, resulting in a higher number of analysed units (66) than experimental sampling plots (59). The attributes of tree species encountered in this study are shown in Table S2 and Figure S1b.

In October 2017, soil samples were collected from all selected plots. Soil cores (3.5 cm in diameter and 5 cm in depth) were sampled at five points per plot (one point at the centre and four points equidistant from the centre towards corners of the plot, Figure 1a). These five subsamples were then homogenized into one composite soil sample. After being sieved using a 2 mm mesh to remove large stones, roots and other residues, each soil sample was divided into three subsamples and properly stored. One sub-sample was air-dried at room temperature for physicochemical measurements. For inorganic nitrogen measurement and DNA extraction, the other two subsamples were stored at −20°C and −80°C, respectively.

2.3 | Tree biomass, community composition and functional traits

During the period from September to October 2017, we measured tree height and ground diameter (5 cm above-ground) as growth parameters of all surviving tree individuals within the central 6 × 6 planting positions in the plots of the monocultures and two-species mixtures, and within the central 12 × 12 positions in the plots of four- and eight-species mixtures. Individual tree biomass (kg) was calculated using the allometric equation corrected with size-dependent conversion factors (Equation 1) employed in the BEF-China experiment (Huang et al., 2018), where GD indicates the ground diameter (m) and H indicates tree height (m). We summed the biomass of individual trees investigated in each plot and then scaled up to calculate community-level above-ground biomass (AGB, kg ha^{-1}).

$$\text{Biomass factor} = 269.13\,\text{kg}\,\text{m}^{-3} - 141.96\,\text{kg}\,\text{m}^{-6} \times \left(H \times \pi \left(\frac{\text{GD}}{2} \right)^{2} \right) \tag{1}$$

Tree community composition of each focal plot, including the component species and their abundance, both in designed and as observed in 2017, was recorded. The designed tree community compositions were further indexed by the scores of the first two axes extracted from a principal component analysis (PCA) (TreeComp0_PC1 and TreeComp0_PC2, Figure S2a) and then applied to the subsequent analyses to characterize the effects of designed tree community composition. Tree community compositions observed in 2017 were used for the following calculations of community traits.

To capture interspecific trait variations and minimize context heterogeneities, nine leaf functional traits of each tree species were collected from the high-diversity plots with 16- or 24-species mixtures in 2011 and 2012 (Bongers et al., 2021; Eichenberg et al., 2015; Kröber et al., 2014). The selected traits were related to resource acquisition ability (i.e. SLA, leaf dry matter content [LDMC], leaf nitrogen content [LNC], leaf carbon-to-nitrogen ratio [C:N], leaf phosphorus content [LPC] and leaf magnesium content [LMg], leaf physical defence [i.e. leaf toughness] and chemical defence [i.e. leaf phenol and tannin concentrations]). Further details about the trait measurements are described by Kröber et al. (2014) and Eichenberg et al. (2015). To synthesize the collected functional traits, we performed a PCA and extracted the scores of the first two axes to present two typical trait syndromes. Community functional dominances were then evaluated using the community-weighted mean (CWM) values of these trait PCs by weighting the survived abundance of component species based on the community compositions observed in 2017 (CWM_Trait_PC1, CWM_Trait_PC2). We also calculated the CWM of SLA (CWM_SLA), as it is the key trait in guiding the SLA-oriented scenario. Finally, to determine the mycorrhizal association type as a trait, we classified each tree species into corresponding mycorrhizal types (Table S2, Bongers et al., 2021). Given that only AM-tree and EcM-tree species were included in this study, we then assessed mycorrhizal dominance by calculating the percentage of survived EcM-tree individuals per plot in 2017 (EcM_perc).

2.4 | Topographical, spatial and soil parameters

Based on the GPS measurements at the beginning of the BEF-China platform in 2009, the topographic features of each plot were obtained using a 5 m digital elevation model, including altitude, slope and aspect. Sine and cosine transformations were used to decomposing aspect into eastness and northness, respectively, and then corrected by slope (Equation 2, Roberts, 1986). To depict the spatial structure of sampling locations, the GPS coordinates of sampling locations were decomposed into the distance-based Moran's eigenvector map vectors (dbMEMs) using *adespatial* package (Dray et al., 2022). The first 14 vectors, corresponding to significant positive Moran's *I* indices and hinting at positive spatial autocorrelation (Figure S3), were used as the spatial variables in the subsequent analyses.

$$\begin{cases} \text{Northness}_{\text{cor}} = \tan(\text{slope}) \times \cos(\text{aspect}) \\ \text{Eastness}_{\text{cor}} = \tan(\text{slope}) \times \sin(\text{aspect}) \end{cases} \tag{2}$$

For soil sampled in 2017, several soil parameters were determined in the laboratory. Gravimetric soil water content was measured by oven-drying fresh soil samples at 105°C for 48 h. Soil pH was measured with a pH electrode (PB-10; Sartorius, Goettingen, Germany) with a 1:2.5 soil–water ratio. The inorganic nitrogen (i.e. NH_4^+ and NO_3^-) was extracted from fresh soil samples by 1 M KCl and then quantified colorimetrically using a flow injection analyser (Autoanalyzer 3 SEAL; Bran and Luebbe, Norderstedt, Germany). Air-dried soil samples were ground to measure soil organic carbon and total nitrogen, which were determined using a $K_2Cr_2O_7$ oxidation method and a Kjeldahl method, respectively. An inductively coupled plasma optical emission spectrometer (ICP-OES; iCAP 6300; Thermo Fisher Scientific, Waltham, MA, USA) was used to measure total soil phosphorus content and soil potassium content, with air-dried soils digested using a tri-acid-melting method (HF-HNO_3-$HClO_4$). To depict a multivariate equivalent of soil conditions, all measured soil parameters were subjected to a PCA. The scores of the first two PC axes were extracted and used in the subsequent analyses (Soil_PC1 and Soil_PC2, Figure S2b; Table S3a).

2.5 | Soil genomic DNA extraction, fungal PCR amplification and sequencing

Soil microbial genomic DNA was extracted from 0.25 g fresh soil using the PowerSoil™ DNA isolation kit (Qiagen DNeasy PowerSoil, Germany) according to the standard procedure of the manufacturer's instructions. We amplified the fungal ribosomal internal transcribed spacer 2 (ITS2) region using the primers of ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990), with a 20 μL polymerase chain reaction (PCR) mixture consisting of 4 μL of five-fold FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 0.2 μL of BSA and 10 ng of template DNA. The PCR amplification was performed on an ABI GeneAmp®9700 thermocycler with the following settings: 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s and finally 72°C for 10 min. Then, the PCR products were isolated using 2% agarose gels and purified using the AxyPrep DNA Gel Extraction

Kit (Axygen Biosciences, Union City, CA, USA). The amplicons were sequenced following the paired-end amplicon sequencing protocol via the Illumina Miseq platform at the Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

2.6 | Bioinformatics analyses

In total, 3,824,589 raw pair-ended sequences were recovered after preliminary quality control, including demultiplexing and removing adapter, primer and poor-quality sequences. The software package Fungal ITS Extractor (Bengtsson-Palme et al., 2013) was used to extract the ITS2 regions of the remaining sequences. The extracted sequences were then imported into the Quantitative Insights into Microbial Ecology 2 (QIIME 2) version 2019.10 (Bolyen et al., 2019) to perform the following bioinformatics analyses. The sequences were filtered to exclude replicates and singleton sequences and then clustered into operational taxonomic units (OTUs) at a threshold of 97% similarity. The potential chimeras were checked and removed by using both of the programmes with or without a UNITE reference database (Abarenkov et al., 2020). To avoid sequencing artefacts, OTU sequences with frequencies in fewer than five samples were discarded. The representative sequence (i.e. the most abundant sequence for each OTU) of each remaining OTU was taxonomically assigned based on the UNITE database version 02.02.2019 (Abarenkov et al., 2020) using the naïve Bayesian classifier (Bokulich et al., 2018). After removing non-fungal OTUs, the final OTU table was rarefied to the same sequencing depth to avoid biased diversity estimation, with a threshold of the smallest sequencing size (No. reads of 12,435, Figure S4), using the *vegan* R package (Oksanen et al., 2022).

All OTUs identified as fungi were further classified into putative functional guilds based on the FunGuild and FungalTraits databases (Nguyen, Song, et al., 2016; Põlme et al., 2021) and several published datasets (Gao et al., 2014; Tedersoo et al., 2014). The functional guild of AM fungi was not analysed separately in this study due to insufficient sequencing reads (0.2238% of all reads, 10 samples failed to detect AM sequences, Figure S4e). Thus, we focused on the community structures of saprotrophic, putative plant pathogenic and EcM fungi in the following analyses. The detailed characteristics of fungal sequencing data in our study were shown in the Notes S1.

2.7 | Statistical analysis

All the data handling aforementioned and statistical analyses were performed using R (version 3.6.3, R Core Team, 2022), and all figures were generated using *ggplot2* (Wickham, 2016) and *patchwork* (Pedersen, 2022) packages.

To fully characterize soil fungal communities, we quantified the diversity and community composition of total fungi and main functional guilds (i.e. saprotrophic, plant pathogenic and EcM fungi). Fungal diversities were assessed by observed OTU richness, Shannon index and relative abundance (only for each functional guild). Fungal community compositions were characterized by pairwise Bray–Curtis dissimilarities based on Hellinger-transformed abundance matrices. To further characterize fungal co-occurring structure, we constructed the co-occurrence networks of total fungi under each scenario based on significant cross-OTUs Spearman's correlations (i.e. $\rho > 0.6$ and $p < 0.05$) and then extracted topological features about network size (number of nodes and links), connectivity (network average degree, natural connectivity and the average shortest path length) and modularity (modularity score, Method S1). Additionally, to estimate the potential of co-existing fungi to exploit similar resources under different diversity scenarios, we calculated the Levins' niche overlap coefficient between all pairs of OTUs (Method S1, Levins, 1968).

To determine how tree species richness under different extinction scenarios affected tree community traits (i.e. CWM_Trait_PC1, CWM_Trait_PC2, CWM_SLA and EcM_perc), overall soil fungal community characteristics (i.e. the diversity estimates of total fungi and their main functional guilds, and fungal co-occurrence network topological features), nested linear models were fitted (i.e. Log_2-transformed tree species richness nested in tree extinction scenario). With the nested model, we firstly evaluated the main effect of tree extinction scenario and the interaction effect between tree extinction scenario and tree species richness. Secondly, the specific effects of tree species richness on fungal community characteristics under the random, rarity- and SLA-oriented scenarios were separately extracted using the same nested model. To improve the normal distributions of model residuals, CWM_SLA, the Shannon diversity of total fungi and saprotrophic fungi, the relative abundance of saprotrophic and pathogenic fungi and the average path length of the fungal co-occurrence network were $\log_e$-transformed. Moreover, to account for the combined effects of plant, soil, topographic and spatial variables, as well as to estimate the importance of the candidate variables in determining the prediction accuracies for the diversity estimates of total fungi and their main functional guilds, and the topological features of fungal co-occurrence networks, Random Forest (RF) models were implemented using *randomForest* (Liaw & Wiener, 2002) and *rfPermute* (Archer, 2022) packages (Method S2).

To assess the overall impacts of tree species richness on community compositions of total fungi and main functional guilds under different tree extinction scenarios, we performed nested (i.e. Log_2-transformed tree species richness nested within tree extinction scenario) permutational multivariate analyses of variance (PERMANOVA) with 9999 permutations. To further calculate and compare the impacts of tree species richness under each scenario, we re-analysed the same nested model on a second type of coding, by splitting the variable of tree species richness into three dummy variables. Each dummy variable represents tree species richness under the random, rarity- and SLA-oriented scenario, respectively, with values of the samples absent from the specific scenario set to zero. Fungal community compositions were further visualized based on the principal coordinate analyses (PCoA). Spearman's partial Mantel tests were conducted to evaluate the relationships between fungal community composition and all candidate variables concerning plant, soil and topographic features, by controlling spatial distances (i.e. the significant positive vectors of dbMEMs) with 9999 permutations using the *linkET* package (Huang, 2021). All the candidate and controlled variables were *z*-score standardized and then transformed into Euclidean distances in the partial Mantel tests. Moreover, variation partitioning analyses were employed to assess and disentangle the relative contributions of the candidate variable groups, including tree community attributes, soil conditions as well as topographic and spatial features, to the variations in fungal community compositions. All the analyses of PERMANOVA, PCoA and variation partitioning were conducted using the *vegan* package (Oksanen et al., 2022).

3 | RESULTS

3.1 | Plant community trait composition

The PCA of the nine leaf functional traits revealed that the first two axes accounted for 38.67% and 24.72% of the total variations (Figure 2a). The first PC axis primarily reflected life-history traits, where higher scores indicated faster-growing features with higher SLA, N, P and Mg content, while lower scores indicated slower growth and greater physical defences with lower SLA and higher LDMC and toughness (Figure 2a; Table S3b). The second PC axis was represented by plant chemical defences, with higher scores correlating with higher contents of phenol and tannin, indicating stronger chemical-defenced (Figure 2a; Table S3b). Notably, mycorrhizal associations of tree species were closely aligned with the axis of leaf life-history traits, with AM-trees generally exhibiting faster-growing features and EcM-trees showing slower-growing features (Figure 2a).

Tree CWM of both PC1 and PC2 based on the nine leaf traits decreased with declined tree species richness under the rarity- and SLA-oriented scenarios (Figure 2b,c). Meanwhile, tree species loss under both non-random scenarios led to increased dominance of EcM-tree species (Figure 2d) and decreased CWM of SLA (Figure 2e). In contrast, under the random scenario, the CWMs of plant functional traits and mycorrhizal proportions remained unchanged with varying tree species richness (Figure 2b–e).

3.2 | Fungal alpha-diversity and relative abundance

To characterize the overall impacts of treatments on the fungal diversity estimates, we analysed the variations in the observed OTU richness, Shannon diversity index and relative abundance across total fungi and the main functional guilds. The results across three indices were similar, with tree extinction scenario, and the interaction effects between tree extinction scenario and tree species richness being significant or marginally significant, except for the relative abundance of plant pathogenic fungi (Table S4). Under the non-random scenarios, richness and Shannon diversity of total fungi decreased with SLA-oriented tree species loss, while only the total fungal richness declined with the preferential loss of rare species. In

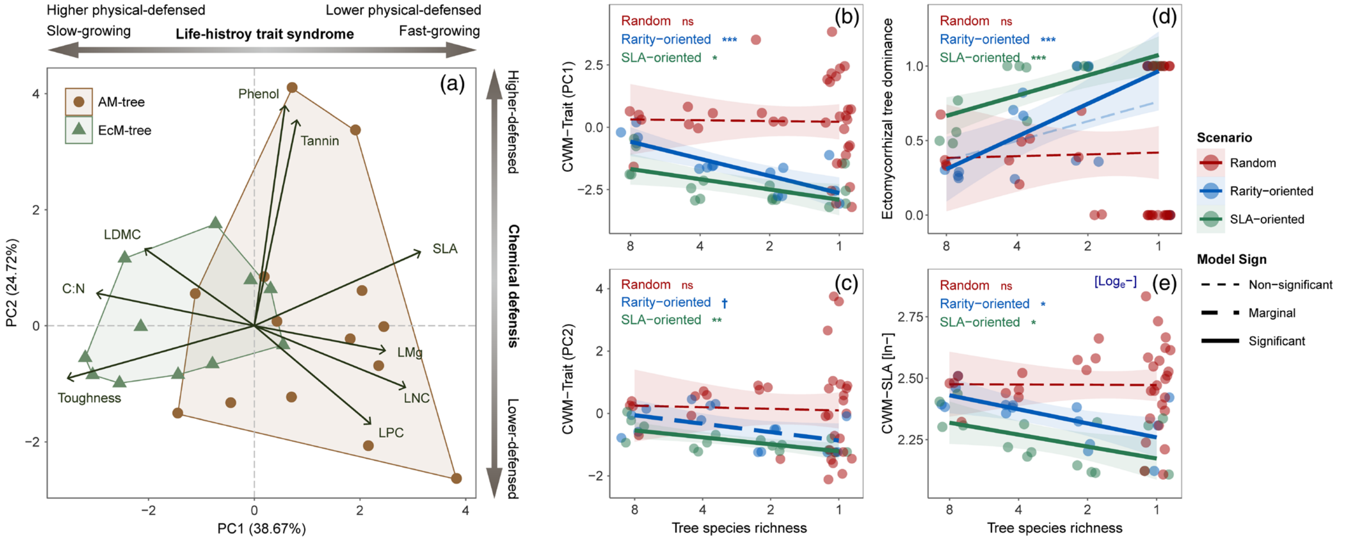

FIGURE 2 Responses of tree community traits to tree species loss under different scenarios. (a) PCA on the nine leaf functional traits are shown. The responses of (b) CWM of the first PC axis, (c) CWM of the second PC axis, (d) EcM-tree dominance and (e) CWM of SLA to tree species loss under different extinction scenarios are shown. Lines represent fitted linear regressions with tree species richness $\log_2$-transformed, where bold solid lines denote significant relationships ($p < 0.05$), bold dashed lines indicate marginal significance ($0.05 \le p < 0.1$) while regular dashed lines indicate non-significant relationships ($p \ge 0.1$). Note that there is one outlier point of EcM-tree dominance in the monocultures under the rarity-oriented scenario (d). The dark blue regression line was fitted after removing the outlier point, while the light blue line was regressed with all samples. Asterisks indicate the statistical significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; $^{\dagger}p < 0.1$ and ns, $p \ge 0.1$). CWM, community-weighted mean; EcM, ectomycorrhizal; PCA, principal component analysis; SLA, specific leaf area.

contrast, no significant changes in richness and Shannon diversity of total fungi were observed under the random scenario (Figure 3a,b; Table S4). Under the SLA-oriented scenario, decreasing tree species richness resulted in significant declines in the richness, Shannon diversity and relative abundance of saprotrophic fungi, non-significant variations in those of plant pathogenic fungi, and significant increases in those of EcM fungi (Figure 3c–k; Table S4). With declining tree species richness under the rarity-oriented scenario, the richness and Shannon diversity of plant pathogenic fungi significantly decreased, the richness, Shannon diversity and relative abundance of EcM fungi increased, while saprotrophic fungi showed marginal declining trends in the richness and relative abundance (Figure 3c–k; Table S4). Under the random scenario, there were no significant changes in richness, Shannon diversity or relative abundance for any fungal functional guilds (Figure 3c–k; Table S4).

Random Forest analyses showed that vegetation attributes, soil conditions, topographic and spatial variables collectively explained more than 36% (62.36 ± 16.76%) of the variations in richness, Shannon diversity and relative abundance of total, saprotrophic and EcM fungi, while relatively lower portions of the variations were interpreted for plant pathogenic fungi (Figure S5a; Table S5). Across different groups of the candidate variables, plant attributes generally showed greater importance in determining model prediction accuracy than soil, topographic and spatial variables (Figure S5c). Particularly, tree community functional traits (i.e. the CWMs of SLA and the first two PCs of leaf traits) and community mycorrhizal dominance (i.e. EcM-tree percentage) exerted consistently strong influences in modifying soil fungal richness, Shannon diversity and relative abundance (Figure S5b). An exception was observed for the Shannon diversity of plant pathogenic fungi, which exhibited stronger correlations with sampling spatial features (i.e. the dbMEM vectors), followed by tree community functional traits (Figure S5b).

3.3 | Fungal community composition

The effects of tree species richness on the community compositions of total fungi and main functional guilds, pointing to directional patterns yielded by linear analysis, were dependent upon extinction

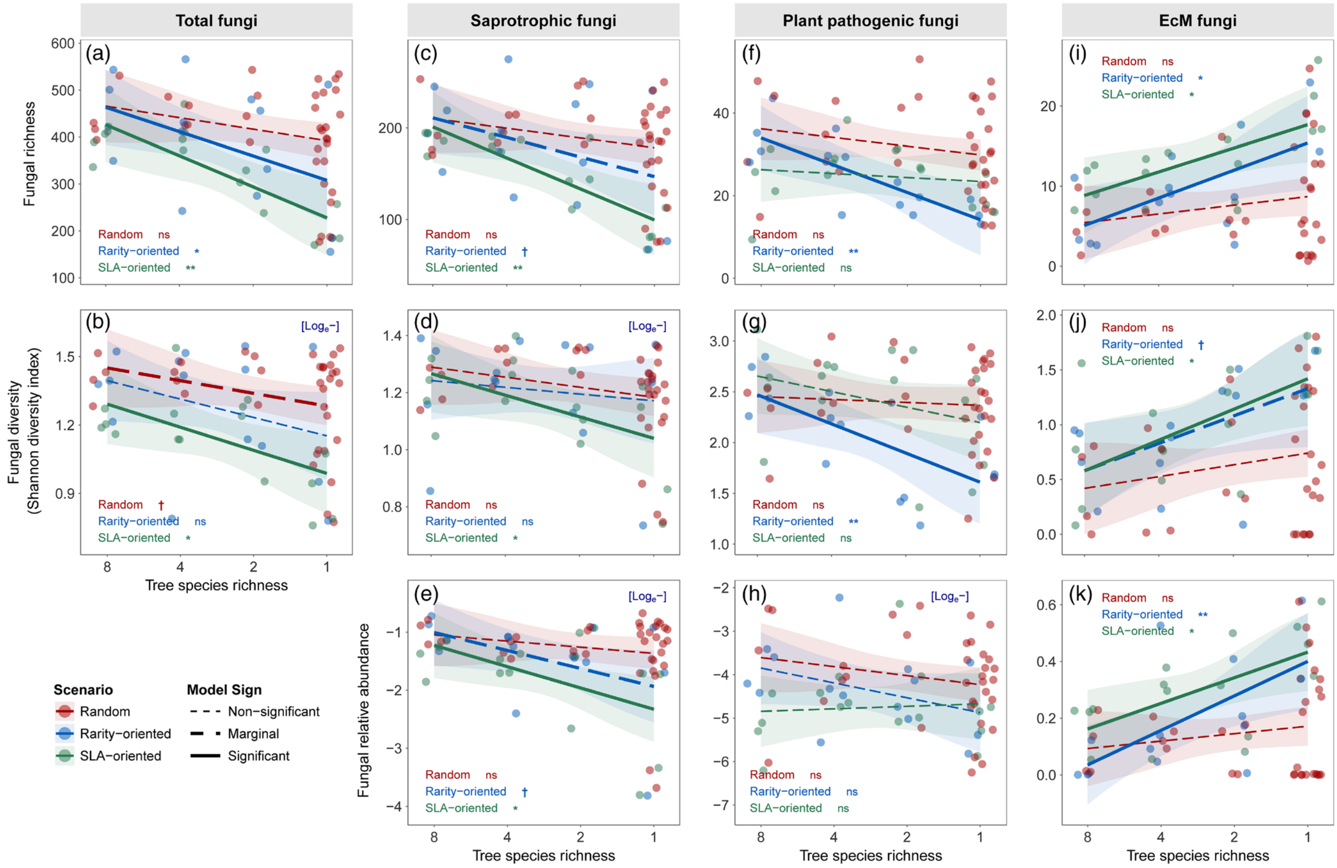

FIGURE 3 Responses of soil fungal richness, Shannon diversity and relative abundance to tree species loss under different scenarios. As tree species richness declined under different scenarios, the responses in observed OTUs richness, Shannon diversity index and relative abundance for (a, b) total fungi, (c–e) saprotrophic fungi, (f–h) putative plant pathogenic fungi and (i–k) EcM fungi are shown. Lines represent fitted linear regressions between tree species richness ($\log_2$-transformed) nested in each extinction scenario and fungal diversity or relative abundance, where bold solid lines denote significant relationships ($p < 0.05$), bold dashed lines indicate marginal significance ($0.05 \le p < 0.1$), and regular dashed lines indicate non-significant relationships ($p \ge 0.1$). Asterisks indicate the statistical significance (**$p < 0.01$; *$p < 0.05$; $^{\dagger}p < 0.1$ and ns, $p \ge 0.1$). EcM, ectomycorrhizal; OTU, operational taxonomic unit.

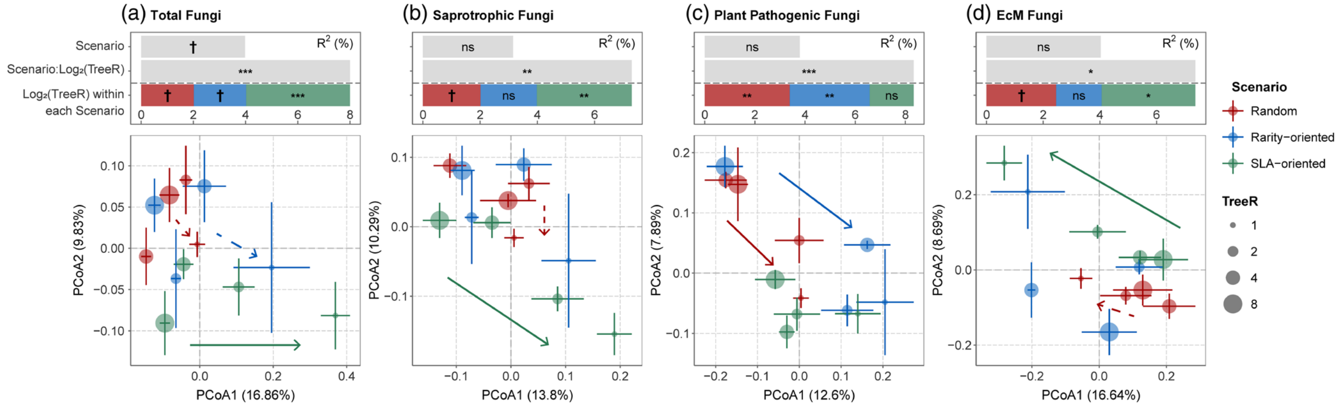

FIGURE 4 The distributions of soil fungal community composition with tree species loss under different extinction scenarios. The statistics of nested permutational multivariate analysis of variance (PERMANOVA), and principal coordinate analysis (PCoA) ordinations for (a) total, (b) saprotrophic, (c) putative plant pathogenic and (d) EcM fungal community compositions are shown. Fungal community composition was characterized by the Hellinger-transformed Bray–Curtis pairwise dissimilarity. The top bar plots illustrate the variances explained by tree species richness ($\log_2$-transformed), the interaction effects between tree species richness and extinction scenario, as well as the effects of tree species richness under the random, rarity- and SLA-oriented scenario separately, as revealed by the nested PERMANOVA tests. The arrows in the bottom ordinations indicate the directional shifting trends of fungal community composition in response to tree species loss, where the solid arrows represent significant trends ($p < 0.05$) and the dashed arrows indicate marginally significant trends ($0.05 \le p < 0.1$). Asterisks indicate the statistical significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; $^{\dagger}p < 0.1$ and ns, $p \ge 0.1$). EcM, ectomycorrhizal; SLA, specific leaf area.

scenarios, as indicated by the significant interaction effects between scenario and tree species richness in PERMANOVA analyses (Table S6). Under the random scenario, shifts in fungal community compositions with decreasing tree species richness were evident for plant pathogenic fungi and, to a limited extent, for total fungi, saprotrophic and EcM fungi (Figure 4; Table S6). Visually on the PCoA ordinations, plant pathogenic fungal communities from tree monocultures, two-species mixtures and higher-species mixtures (i.e. four- and eight-species mixture) exhibited distinct clustering, with samples from two-species mixtures positioned intermediately under the random scenario (Figure 4c). Additionally, community compositions of total, saprotrophic and EcM fungi were less distinctly separated, primarily distinguishing monocultures from mixed-species mixtures (Figure 4a,b,d). Under the rarity-oriented scenario, community compositions of plant pathogenic fungi shifted significantly with decreasing tree species richness (Table S6), with samples from different tree species richness clustering separately in the PCoA ordinations, and those from four-species mixtures tending to cluster in the middle between eight-species mixtures and the other groups (Figure 4c). Marginally significant shifts in community compositions with rare tree species loss were observed for total fungi, while no significant trends were revealed for saprotrophic and EcM fungi (Table S6; Figure 4a,b,d). SLA-oriented tree species richness loss significantly shifted community compositions of total, saprotrophic and EcM fungi (Table S6), with samples from different tree species richness tending to form separate clusters and distributing directly along the gradient of tree species richness in the PCoA ordinations (Figure 4a,b,d). The shifts in community compositions were not evident for plant pathogenic fungi under the SLA-oriented scenario (Table S6; Figure 4c).

Variation partitioning revealed that, compared with soil, topographic and spatial variables, tree community features explained a greater proportion of the variations in the community compositions (i.e. the Bray–Curtis dissimilarities) of total, saprotrophic and plant pathogenic fungi (Figure S6a–c). Notably, more variation in the EcM fungal community was captured by spatial patterns, followed by tree community characteristics (Figure S6d). As deciphered by the partial Mantel test controlling for the spatial patterns of sampled plots, EcM-tree dominance and the CWM of leaf life-history traits (PC1) significantly correlated with community compositions of total, saprotrophic, plant pathogenic and EcM fungi (Figure S7). In addition, tree community composition, above-ground biomass and soil parameters were correlated with community compositions of total, saprotrophic and plant pathogenic fungi (Figure S7). The CWM of SLA and leaf chemical defence traits (PC2) was more associated with total, saprotrophic and EcM fungal community composition (Figure S7).

3.4 | Fungal co-occurrence network structure

Based on the significant cross-OTUs Spearman's correlations ($\rho \ge 0.6$, $p < 0.05$), we constructed fungal co-occurrence networks under the random, rarity- and SLA-oriented scenarios separately, which consisted of 723, 260 and 206 nodes (OTUs), respectively, and 2273, 367 and 435 links, respectively, all with a high proportion of positive links (0.965, 0.973 and 0.975, respectively). The degree patterns of all the networks followed power-law distributions (Figure S8), exhibiting scale-free network features, with the distribution of network links being independent of network size.

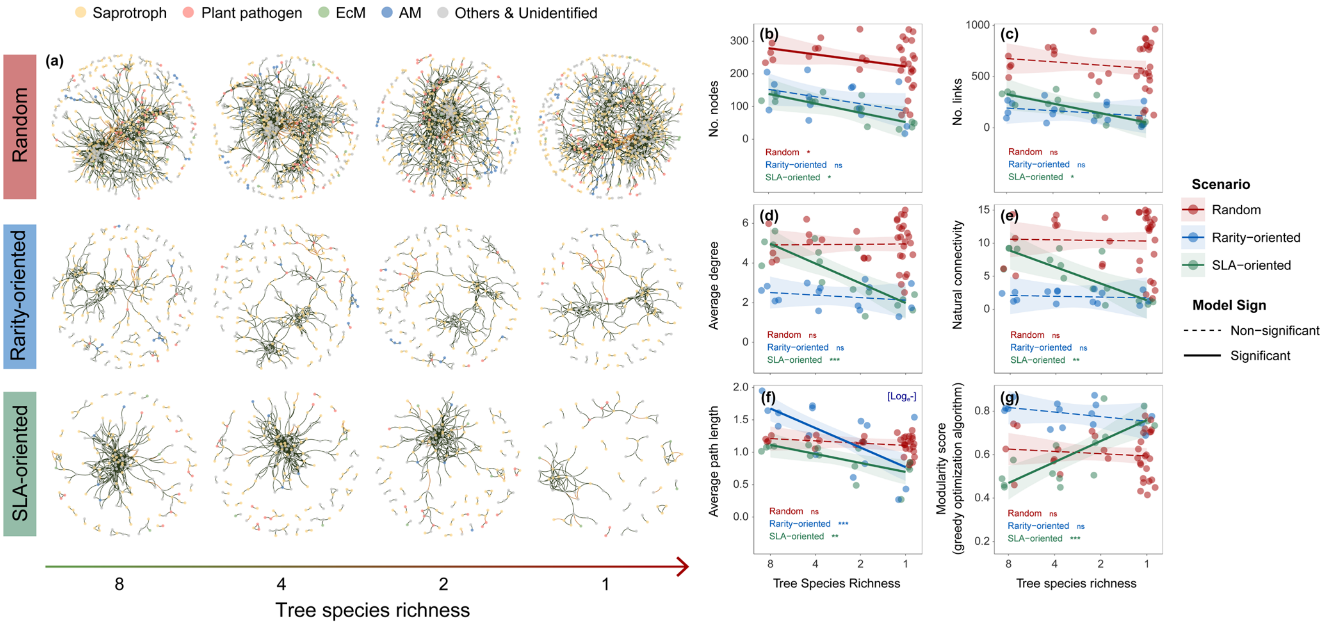

FIGURE 5 Responses of soil fungal co-occurrence network features to tree species loss under different extinction scenarios. (a) Visualization of fungal co-occurrence networks along with tree species loss under different tree extinction scenarios is shown. The nodes within the networks represent individual OTUs, where different colours indicate different functional guilds. The edges represent significant Spearman correlations ($\rho > 0.6$, $p < 0.05$). Topological and architecture features of the co-occurrence networks are further extracted and shown, including (b) the nodes number, (c) links number, (d) average degree, (e) natural connectivity, (f) average path length and (g) modularity score index based on the greedy clustering algorithm. The lines represent fitted linear regressions of the considered network features by tree species richness ($\log_2$-transformed) nested in each scenario, where the bold solid lines denote significant relationships ($p < 0.05$), and the regular dashed lines indicate non-significant relationships ($p \geq 0.05$). Asterisks indicate the statistical significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ and ns $p \geq 0.05$). OTU, operational taxonomic unit.

The fungal co-occurrence networks showed varied responses to decreasing tree species richness under the random, rarity- and SLA-oriented scenarios [Scenario:Log_2(TreeR), $p < 0.05$, Figure 5]. Tree species loss under the SLA-oriented scenario largely reduced the number of network nodes and links, average degree, natural connectivity and average path length, collectively implying a decrease in network complexity (i.e. decreasing network size and connectivity, Figure 5a–f). Additionally, we also found that SLA-oriented tree species richness loss led to a significant increase in fungal network modularity (Figure 5g) and a decrease in average community niche overlap (Figure S9). As tree species richness decreased under the random and rarity-oriented scenarios, the node number and average path length of fungal networks decreased, respectively, while the other network topological features did not show significant changes (Figure 5).

As revealed by RF analyses, more than 33% (43.75 ± 8.68%) of the variations in fungal network topological features, including node number, link number, average degree, natural connectivity and average path length, were explained by the candidate environmental factors (Figure S5d; Table S5). On average, plant community features exerted more important roles than soil, topographic and spatial variables in determining these network topological features (Figure S5f). Plant community compositions, functional traits and mycorrhizal associations emerged as the most influential factors (Figure S5e). Tree community compositions, soil parameters, aspect and plot spatial patterns collectively, but to a limited extent, accounted for only 17.5% of the variations in fungal network modularity (Figure S5d,e; Table S5).

4 | DISCUSSION

Plant species are usually lost non-randomly, driven by the interaction between species trait and environmental change at the local scale (Freville et al., 2007; Zavaleta et al., 2009). Therefore, it is imperative to incorporate different trait-oriented species loss strategies into BEF studies for a better understanding the consequences of biodiversity loss in the real world (Lepš, 2004; Wolf et al., 2021; Zavaleta & Hulvey, 2006). Based on a manipulated subtropical tree diversity experiment, we found that the simulated non-random tree species loss, namely by the preferential loss of locally rarer or higher-SLA species, exerted different and more far-reaching effects on soil fungal communities than random tree diversity loss, supporting our hypothesis (H1). Specifically, the diversity and community composition of soil fungi and the main fungal functional guilds, and the co-occurrence network features of total fungi shifted, especially under the SLA-oriented scenario (Figures 3–5).

The issue of random versus non-random species loss has been acknowledged both in theoretical considerations (Garcia-Valdes et al., 2018; Larsen et al., 2005) and field experiments

(Chen et al., 2020; Schuldt et al., 2015; Suding et al., 2005; Wolf et al., 2021), but mostly focused on the responses of above-ground subsystems, such as productivity and invasion resistance. Our study on forest stands and soil fungi provides novel insights into below-ground biodiversity that non-random and trait-oriented tree species loss may have profound impacts on soil fungal communities, for example as a result of climate changes or stress-based disturbances. Soil fungi, comprising a large portion of soil biodiversity (Bardgett & Van der Putten, 2014; Tedersoo et al., 2014), play a critical role in maintaining multiple ecosystem processes and functions, such as organic matter decomposition, nutrient cycling, plant community production and stability (Delgado-Baquerizo et al., 2016; Liu et al., 2022; Wagg et al., 2021). Accordingly, substantial changes in ecosystem processes delivered by soil fungal communities could be expected under the non-random tree species loss, suggesting a potential explanation for the distinct effects of non-random diversity loss on above-ground ecosystem functioning previously observed in the same biodiversity experimental platform (Chen et al., 2020).

By comprehensively evaluating the potential drivers of soil fungal communities, our results supported the joint roles of spatial, abiotic (soil properties and topographic features) and biotic (tree community attributes) in shaping soil fungal community structures (Gan et al., 2022; Singavarapu et al., 2021; Weissbecker et al., 2018). Notably, plant community features served the most important drivers in our study (Figures S5–S7). Compared with the random scenario, shifts in tree community trait compositions under the non-random scenarios emerged as crucial determinants of the observed changes in soil fungal communities (Figure 2; Figures S5–S7). These findings support *the mass ratio* hypothesis where the dominant traits within communities underpin the non-random diversity effects (Diaz et al., 2007; Grime, 1998). Moreover, recent trait-based BEF experiments, deliberately manipulating assemblages to decouple the effects of trait composition and diversity gradient, further reinforce the predominant roles of plant traits on soil biota and the relevant functions (Pichon et al., 2020; Steinauer et al., 2017).

4.1 | Functional guild-dependent responses in fungal diversity, relative abundance and community composition to non-random tree species loss

We found distinct responses across different fungal functional guilds to different tree species loss scenarios, highlighting the guild-specific responses of soil fungi to environmental changes (Nguyen, Williams, et al., 2016; Põlme et al., 2021; Schappe et al., 2020). For saprotrophic and pathogenic fungi, we predicted that the responses would primarily occur under the SLA-oriented scenario (H2). What we observed, however, showed that the responses under both non-random scenarios were similar, characterized by decreases in diversity and relative abundance for saprotrophic fungi and increases in those for EcM fungi (Figure 3). These changes in saprotrophic and EcM fungal communities were largely correlated with the overall shifts in plant community trait compositions (Figures S5–S7), supporting that soil fungal diversity and community composition may be predicted by plant functional traits (Pei et al., 2016; Prada-Salcedo et al., 2021). The most important traits in our study included EcM-tree dominance, community-level SLA and community-level trait syndrome for life-history strategies. Notably, shifts in these crucial trait compositions were similar under both non-random tree diversity gradients (Figure 2), probably explaining the comparable fungal responses.

In detail, as tree species richness decreased under the non-random scenarios, the covaried trait compositions were characterized by slowed life-history strategies and increased dominance of EcM-tree species (Figure 2). On the one hand, the increases in diversity and relative abundance of EcM fungi are primarily driven by the increasing dominance of EcM-tree species, capturing host preference and density dependence for EcM fungi (Gao et al., 2013; Tedersoo et al., 2010; Weissbecker et al., 2018). On the other hand, both slow-growing and EcM-associated tree species ultimately contribute to accumulating recalcitrant litter and slow decomposition rates (Cornwell et al., 2008; Garnier et al., 2004; Wardle et al., 2002). The decreases in litter quality and nutrient cycling rate limit resource availability for free-living saprotrophic fungi, thereby reducing their diversity and relative abundance (Prada-Salcedo et al., 2021). Moreover, the reversed pattern, with saprotrophic fungi being more enriched in tree species-rich-and-faster communities and EcM fungi in tree species-poor-and-slower communities, may be reinforced by the Gadgil effects (Gadgil & Gadgil, 1971, 1974). In other words, the dominant EcM fungi potentially suppress the activity of saprotrophic fungi by outcompeting them for plant-derived organic compounds and soil available resources (Bödeker et al., 2016; Högberg et al., 2003), which may be strengthened under ecosystems with slower nutrient cycling and poorer soil nutrient availability.

For soil-borne pathogenic fungi, their transmission and maintenance potential can be predicted by host species' competence, which is generally greater for faster-growing or broadly distributed species (Johnson et al., 2013, 2015; Lacroix et al., 2014). Accordingly, we expected that the prior loss of higher-SLA species may correspond to a decrease in plant community competence, thus reducing pathogenic diversity and abundance, whereas a reversed pattern was expected for the prior loss of rare species (H3). However, not supporting our initial hypothesis, neither non-random tree species losses affected the relative abundance of soil pathogenic fungi (Figure 3h), which might be explained by the neutralized changes in overall plant community competence. Here we could evaluate plant community competence by the decoupled physical and chemical defence traits (Figure 2a). As tree species loss under the non-random scenarios, we found that tree community physical defence traits increased while the chemical defence traits decreased (Figure 2b,c). Such contrasting shifts collectively neutralized overall changes in community competence, which may explain the lack of responses in pathogenic abundance at the community level. Similar neutral community-level responses of pathogen infestation to tree species richness have also been reported (Rutten et al., 2021). However, significant dilution effects emerged in their further species-level analyses (Rutten et al., 2021). This underscores the importance to investigate the species-specific pathogenic responses, either dilution (Mommer et al., 2018) or amplification, which may be obscured when examined at the community level (Cappelli et al., 2022; Rutten et al., 2021), offering deeper insights into pathogenic dynamics under tree diversity loss (Johnson et al., 2015).

In comparison, we found significant decreases in pathogen diversity and shifts in community composition in response to the preferential loss of rare species (Figures 3f,g and 4c). This finding is in line with the *diversity begets diversity* hypothesis, which suggests that a diverse plant community fosters a wide range of host habitats, thereby supporting a higher diversity of pathogens, with specialist pathogens benefiting the most (Johnson et al., 2015; Rottstock et al., 2014). The asymmetric host–parasite interactions, where local abundant hosts tend to harbour a higher proportion of specialist enemies (Vázquez et al., 2005), likely strengthen the positive relationships between tree species richness and pathogenic fungal diversity under the rarity-oriented scenario rather than under other scenarios. To further clarify this distinction, deeper investigations at the individual tree species level to differentiate generalist versus specialist pathogens are recommended.

4.2 | Fundamental changes in co-occurrence network features shaped by tree species richness loss under the SLA-oriented scenario

The observed fungal co-occurring networks in our study predominantly showed positive correlations, suggesting potential for mutualistic interactions, shared environmental requirements or responses to environmental fluctuations in tandem (D'Amen et al., 2018; Guseva et al., 2022). As tree species richness decreased, decreasing network size and connectivity, and increasing modularity were encountered only under the SLA-oriented scenario (Figure 5), which were primarily determined by plant community features and spatial patterns (Figure S5e,f). These results suggest the primary effects of plant-derived resource filtering and dispersal limitation (Barberan et al., 2012; Blanchet et al., 2020) in driving fungal co-occurrence networks under the non-random tree species loss.

With tree species loss under the SLA-oriented scenario, the decreases in network size and connectivity could be coupled (Figure 5a–f), indicating a simplified fungal community characterized by decreased diversity and interaction intensity. This is in line with the patterns revealed by the microcosm-based experiments manipulating soil microbial diversity (Shen et al., 2022; Wagg et al., 2019). Moreover, each of the delimited sub-clusters within co-occurring networks could represent a distinct fungal group that occupies a specialized habitat (e.g. a suitable host species or a specific soil patch) or that exhibits similar ecological behaviours (Barberan et al., 2012; Freilich et al., 2010; Godoy et al., 2018). Accordingly, the increasing trend of network modularity (Figure 5g) can correspond to the decreasing average niche overlap (Figure S9c) within fungal communities in response to the SLA-oriented tree species loss. These results jointly suggest a substantial reduction of redundancy in fungal communities and potentially hint at a decrease in community stability and a heightened susceptibility to environmental disturbances (May, 1972; Yuan et al., 2021).

4.3 | Experiment limitation and the way forward

In this study, we assessed the non-random tree species loss under environmental changes by assuming two plausible disassembly orders based on plant distribution ranges or traits. With such directional non-random extinction designs, caution is warranted when understanding our findings: (1) To address potential confounding effects between plant diversity and community trait composition due to the unbalanced non-random species loss strategies, the random scenario was incorporated as a null model for comparisons against any non-random scenario (Bruelheide et al., 2014); (2) Our non-random scenarios exemplified only two specific non-random species loss scenarios, which cannot fully represent the species loss events driven by complex interactions between species fitness and environmental changes in the real world. For example, while the higher-SLA species were assumed to be more vulnerable to stress-based disturbances in our design, they were more likely to thrive under subsidy-based disturbances, such as N deposition (Suding et al., 2005). Future non-random designs should broaden the range of proxy traits, covering both above- and below-ground attributes, and apply the varied disassembly rules tailored to specific disturbances. Additionally, we recommend trait-based BEF experiments that deliberately manipulate species assemblages with orthogonal CWM trait values and diversity levels (Ebeling et al., 2014), to more effectively decouple the impacts of species richness and trait composition within a single experiment (Diaz et al., 2007).

5 | CONCLUSIONS

We conclude that the assumed non-random tree species loss in our study, characterized by the prior loss of tree species with higher specific leaf area or locally rarer species, had much stronger effects on fungal community structures than random tree species loss. These effects were primarily mediated by the changes in plant community trait composition, emphasizing the potential of trait-based frameworks in understanding soil fungal dynamics. The responses of soil fungal communities may have profound implications for ecosystem processes or functioning mediated by plant–soil feedbacks, particularly in response to biodiversity loss under the directional, non-random environmental pressures, such as climate change or stress-based disturbances.

AUTHOR CONTRIBUTIONS

Sirong Zhang, Naili Zhang and Keping Ma conceived and designed this research; Sirong Zhang conducted the experiment and analysed

the data; Xiaojuan Liu and Helge Bruelheide provided important data of site census; Sirong Zhang led the writing of the manuscript; Naili Zhang, Wim H. Van der Putten, G. F. (Ciska) Veen, Helge Bruelheide, Xiaojuan Liu and Keping Ma contributed critically to the improvement and revision of the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70029.

DATA AVAILABILITY STATEMENT

The data of plant and soil properties acquired in the experiment are all deposited in Figshare at https://doi.org/10.6084/m9.figshare.28539767 (Zhang et al., 2025). The soil sequencing data on fungal communities have been submitted in the NCBI Sequence Read Archive (SRA) under Project ID PRJNA1027142.

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SUPPORTING INFORMATION

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

Figure S1. Tree species encountered within different scenarios at (a) Site A of BEF-China experiment and (b) in our study.

Figure S2. Principal component analyses on (a) the designed plant community composition and (b) sampled soil properties.

Figure S3. The positive vectors of the distance-based Moran's eigenvector map vectors (dbMEMs) based on the GPS coordinates of sampling locations.

Figure S4. Rarefaction curves of sequencing samples.

Figure S5. Random Forest heatmaps indicating the relationships and importance of candidate variables in determining the prediction accuracies of fungal community attributes.

Figure S6. Variation partitioning analyses showing the variations in soil fungal community compositions explained by groups of vegetation, soil, topographic and spatial variables.

Figure S7. Correlations between soil fungal community compositions and candidate environmental factors.

Figure S8. Degree distributions of fungal co-occurrence networks.

Figure S9. Habitat niche overlap of fungal community.

Figure S10. Comparisons of co-occurrence network features between the observed and the simulated random networks.

Figure S11. Overview of sequencing assignment.

Figure S12. Relative abundance of OTUs across tree species richness under different scenarios.

Table S1. Ecological mechanisms underlying the effects of the candidate variables on soil fungal communities.

Table S2. Attributes of tree species included in this study.

Table S3. Detailed information of the principal component analyses (PCA) on (a) soil parameters and (b) tree leaf functional traits.

Table S4. Detailed statistics about the nested linear models of fungal diversity measures.

Table S5. Filtered parameters of the final best-fit Random Forest models for soil fungal community characteristics.

Table S6. Statistics of the nested PERMANOVA analyses.

Table S7. Overview of sequencing assignment.

Method S1. Inference of fungal co-occurrence network.

Method S2. Inference of Random Forest models.

Notes S1. Overview of the fungal sequencing data.

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