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Contrasting community responses of root and soil dwelling fungi to extreme drought in a temperate grassland

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ABSTRACT

Fungal communities inhabiting plant roots and the soil diverge because they are shaped by differences in abiotic environment and plant filtering. Therefore, these two communities will also likely respond differently to climate change. However, such responses are poorly understood, especially for climate extremes with increasing frequency and intensity. Based on a long-term field experiment that simulated two types of extreme drought (chronic/intense) of once-in-20-year occurrence in the temperate grassland, we studied the response of soil and root fungal communities to extreme drought in association with plant communities. The species richness, community composition, and network stability of the root fungi were sensitive to extreme drought and showed legacy effects during recovery; notably, these responses were independent of extreme drought types. The sensitivity of the root community was mainly driven by rare symbiotic and saprotrophic fungal species, with abundant species remaining stable. In contrast, except for species relative abundances, soil fungal communities were resistant to drought. Structural equation modelling revealed that plant communities mediate drought effects on root fungal communities but not soil communities. Our findings highlight the climate sensitivity of root fungal communities and their response asymmetry to soil communities, with potentially profound consequences for ecosystem stability and functionality.

1. Introduction

Extreme climate events can have far-reaching ecological impacts on the structure and function of natural ecosystems (Reichstein et al., 2013; de Vries et al., 2018). Model projections of the frequency and magnitude of extreme climate events, such as extreme droughts, is increasing rapidly in future climate change scenarios (IPCC, 2021). Such extremes rare in the historical periods often have substantial ecological impacts (Xu et al., 2019; Bardgett and Caruso, 2020; Canarini et al., 2021). However, due to the unpredictability and variability of naturally occurring climate extremes, their ecological impacts have never been fully addressed, particularly for belowground communities (Bardgett and Caruso, 2020). Soil fungi are key soil microorganisms serving as decomposers, pathogens, and plant symbionts that hold enormous significance in maintaining ecosystem functionality (Powell and Rillig, 2018; Yang et al., 2018). With the foraging ability to access water and nutrients under stress, soil fungi have been considered to be highly resistant to climate perturbations (Barnard et al., 2013; Schimel, 2018). However, a significant proportion of soil fungal species inhabit the plant roots and are thus more strongly influenced by their plant hosts,

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including their physiological properties (e.g., photosynthesis (Karlowsky et al., 2018)), metabolism (e.g., root exudates (Williams and de Vries, 2020)), and plant community dynamics (e.g., diversity (Chen et al., 2017; Zhang et al., 2021)). Therefore, unlike the soil community, the root-associated fungal community is a dynamic assembly controlled by both the plant and the environment and thus could be highly sensitive to climate perturbations. Moreover, extreme drought may also have long-lasting legacy effects on root and soil fungal communities (i.e., fungal communities remain significantly changed after drought), as studies have shown that drought can have legacy effects on soil and plant communities, as well as their ecological functions (Anderegg et al., 2015; Kaisermann et al., 2017). However, studies examining how root and soil fungal communities respond to climate extremes are rare, particularly under natural conditions.

Plant roots harbor distinct fungal communities with unique resource utilization and environmental adaptation strategies (Hempel et al., 2007; Leroy et al., 2021). From the root surface to the cortical cells, there are various co-occurring fungal species, and each has the potential to modulate plant metabolism and immunity (e.g., mycorrhizal fungi (Smith and Read, 2010)). On the other hand, plants have evolved multiple strategies to control and regulate the interactions with their associated microbial species, including compartmentalization, defense, and reward mechanisms (Chomicki et al., 2020; Trivedi et al., 2020). For instance, with immune response and compartmentalized structures, such as intracellular compartments formed with arbuscular mycorrhizal (AM) fungi (Genre et al., 2020), plants can reward cooperative species (e.g., plant symbionts) and sanction or defend against other species (e.g., plant pathogens and saprotrophs) (Hacquard et al., 2016; Chomicki et al., 2020). With these strategies, plant roots filter out a significant number of soil fungal species and constrain a large number of species to low abundance (usually classified as rare species) (Hamonts et al., 2018; Pereira et al., 2019), thereby assembling their core communities. This kind of plant active control contributes to maintaining root community structure and thus potentially favors plant performance when faced with environmental changes. However, until now, responses of soil and root fungal communities to climate change regarding functional attributes and species abundance remain largely unexplored.

Plant community traits, including diversity (Prober et al., 2015; Xu et al., 2016; Chen et al., 2017), phylogenetic affiliation (Tedersoo et al., 2013), and productivity (Yang et al., 2017), have been shown to influence fungal community dynamics. Therefore, a key question is whether and how plant communities mediate the response of soil and root fungal communities to climate extremes. Plant diversity has long been considered to be one of the key factors driving the change of soil microbial communities. This also applies to root communities, as host identity has been identified as a key factor affecting the response of root fungal communities to grassland drought (Lagueux et al., 2020). Apart from plant diversity, plant productivity is closely related to plant photosynthesis aboveground, influencing carbon sequestration and production of root exudates belowground, with cascading effects on plant-fungal interactions. However, although plant productivity has been shown to be sensitive to climate extremes (Ciais et al., 2005; Hoover et al., 2014; Xu et al., 2019), its relationship with the response of soil and root fungal communities to climate extremes is still unclear.

Species interactions are believed to potentially influence the diversity and stability of microbial communities (Wardle, 2006; Ratzke et al., 2020). Within a microbial community, species can suppress other species through antagonistic strategies (competition) or support other species through cooperation and facilitation (Abrego et al., 2020; Hesse et al., 2021). Soil fungal networks are previously considered to be resistant to climate perturbations (de Vries et al., 2012, 2018; Zhou et al., 2021). However, unlike soil networks, studies on root fungal networks are very limited. As a host microbiome property, root fungal networks can be highly influenced by the plant (Foster et al., 2017). For example, theoretical studies suggest that the host tends to stabilize microbial communities by either limiting positive interactions, increasing microbial competition, or both (Coyte et al., 2015). This host strategy may help restrict species responses to small network modules, thereby avoiding propagation of the effect to the remaining network (Stouffer and Bascompte, 2011; Oliveira et al., 2014). Alternatively, plants may also tend to improve beneficial functions of microbial communities under stress by enhancing cooperation (i.e., positive interactions) between species (Hassani et al., 2018; Jiang et al., 2021). Therefore, climate change may potentially alter the root fungal interaction networks through the plant immune and defense system (e.g., through decreased photosynthesis, root exudates, and metabolites (Fuchslueger et al., 2014; Williams and de Vries, 2020)). However, we know little about how the interaction networks of root fungi respond to climate extremes and whether these responses are related to the response of the plant community.

Here, we studied the impacts of extreme drought on root and soil fungal communities based on a long-term grassland field experiment. In our previous study, we examined the response of soil AM fungi to extreme drought (Fu et al., 2021). In the present study, we focus on the overall response of the soil and root fungal community with extra emphasis on non-AM fungi. We propose three hypotheses in this study: (i) Root fungal communities would respond differently from the soil communities and show legacy effects after drought (recovery); (ii) The response of root fungal communities is closely associated with plant community responses, while the response of soil communities is largely explained by edaphic factors; (iii) Root fungal networks are less stable under extreme drought than soil fungal networks, and we expect this is mainly driven by the response of positive interactions between fungal species.

2. Methods and materials

2.1. Experimental design

We set up the field experiment (Extreme Drought in Grassland Experiment, EDGE) at Erguna Forest-Steppe Ecotone Research Station, Inner Mongolia, China (meadow steppe; MAT: -2.45 °C). The annual precipitation of the experimental site is 362 mm (1957–2017) (Fig. 1a). The dominant plant species are *Leymus chinensis*, *Carex duriuscula*, *Artemisia frigida*, *Stipa baicalensis*, *Pulsatilla turczaninovii*, *Cymbaria dahurica* and *Cleistogenes squarrosa*. Details of the experimental design have been described previously (Fu et al., 2021). In brief, based on historical climate data (1957–2017), we designed two types of extreme drought (chronic/intense) by manipulating the rainfall using light-transparent polyethene partial roofs (Fu et al., 2021). These two types of drought reduced roughly half of the mean annual precipitation and simulated extreme conditions of once-in-20-year events (Fig. 1a and b). We set up five treatments with two types of extreme drought (Intense/Chronic) using a randomized block design (Fig. 1a; Fig. S1): (i) control; (ii) intense drought (INT) − reducing 100% of the rainfall amount from June to July for 3 years (2015–2017); (iii) intense drought + recovery (INTR) − 2 consecutive years of intense drought (2015–2016) and drought ceased in the third year (2017); (iv) chronic drought (CHR) − reducing 66% of the rainfall amount from May to August for 3 years (2015–2017); (v) chronic drought + recovery (CHRR) − 2 consecutive years of chronic drought (2015–2016) and drought ceased in the third year (2017) (Fig. 1a). Totally we set up 30 experimental plots (6 blocks × 5 treatments) with 6 replicates for each treatment (Fig. S1).

We adopted the rainout shelter following Yahdjian and Sala's (2002) to passively reduce the rainfall during the growing season. To reduce the greenhouse effect, we used a steel-frame structure to raise the partial roofs 60 cm above the ground to allow free air exchange. Additionally, to hydrologically isolate the plot from the outside, we buried plastic films 1 m deep around each plot, and installed metal flashing 10 cm above the ground. In each 6 m × 6 m experimental plot, we chose the central 4 m × 4 m as the core experimental area and set the surrounding

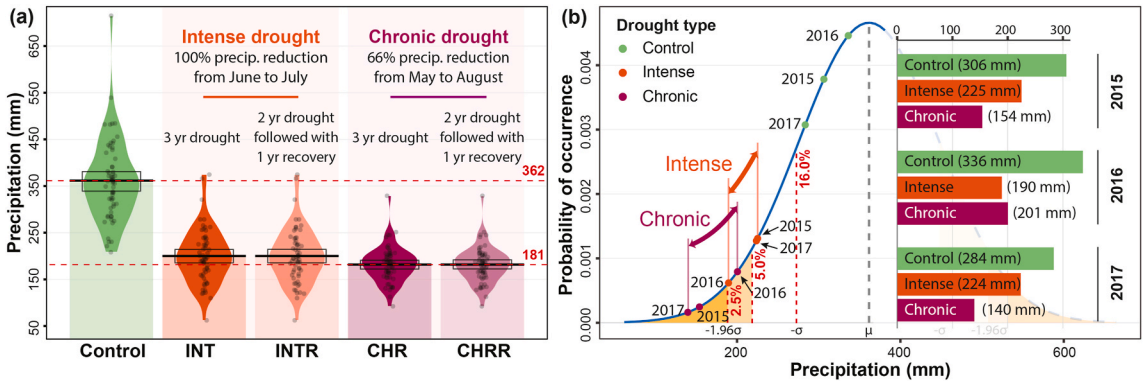

Fig. 1. Design and performance of the field experiment. (**a**) We designed two types of extreme drought based on historical climate data, namely intense and chronic drought, and each drought type reduced ~50% of rainfall amount compared with the control treatment. Within these two types of drought, we set up five treatments. Control; INT: 3 years of intense drought (2015–2017); INTR: 2 years of intense drought (2015–2016) followed by 1 year recovery (2017); CHR: 3 years of chronic drought (2015–2017); CHRR: 2 years of chronic drought (2015–2016) followed by 1 year recovery (2017). The black points represent the historical precipitation data from 1957 to 2017, the smoothed density curve showing the data distribution, the black line represents the mean annual precipitation, and the black box represents the 95% Bayesian highest density intervals (iterations = 1000). (**b**) The *in situ* performance of the experimental rainfall manipulations. The probability of occurrence of each drought type in 2015, 2016, and 2017 were mapped on the probability density curve calculated using historical precipitation data (1957–2017).

1 m area as a buffer zone (Fig. S1).

2.2. Sampling

Sampling was performed by the end of August 2017 and soil sampling was described previously (Fu et al., 2021). In brief, five topsoil cores (0–20 cm) were randomly taken from the middle and the four corners of the 4 m × 4 m core experimental area. Soil samples were homogenized by passing through 2 mm sieves, and the roots were manually picked up using tweezers, and 20 g bulk soil was stored at −80 °C for molecular analyses. To avoid the influence of subjectivity related to the sample source, the root samples were randomly numbered (i.e., 1-30) in processing. Plant litter and dead roots were identified by their color, physical appearance, and branching structures and then removed, and live roots were then thoroughly washed (ultrasonic cleaning was used) to remove the adherent soil (Fig. S2). We further stained the roots with trypan blue and found the root system was structurally intact (Fig. S2), indicating that the dead roots were effectively removed. All live roots were cut into 1 cm fragments and thoroughly mixed and stored at −80 °C for molecular analyses. Finally, 0.2 g roots and 0.5 g soil were used for DNA extraction.

2.3. Plant community survey and soil data collection

A plant community survey was carried out in August 2017 (Fu et al., 2021). The aboveground net primary productivity (ANPP) and diversity were recorded within 1 m^2 quadrat of each experimental plot. We tested the plant community survey efficiency using rarefaction curves and found that all the curves reached saturation (Fig. S3a), suggesting sufficient plant sampling to represent each plot. Root biomass was obtained using root auger (7 cm in diameter). The belowground net primary productivity (BNPP) was measured using the root ingrowth core method (described before (Ma et al., 2020)). In brief, before the experiment, soil cores (20 cm deep and 5 cm in diameter) were first drilled out and roots were removed; next, the original soil was re-filled with mesh bags; finally, BNPP was obtained by measuring the dry weight of the new roots in the mesh bags after the experiment. Soil physicochemical measurements, including soil total carbon and nitrogen content (elemental analyzer: Vario EL III, Elementar, Germany), pH value (digital pH meter: FE200, Mettler Toledo, Switzerland), available phosphorus (Olsen method (Olsen, 1954)), and moisture (gravimetric method: 105 °C oven-dried in a constant weight), have been described before (Fu et al., 2021).

2.4. DNA extraction, sequencing, and bioinformatics

Soil DNA was extracted from 0.5 g freeze-dried samples using FastDNA™ SPIN Kit for soil cooperated with FastPrep-24 5G homogenizer (MP Biomedicals, CA, USA) following the manufacturer's instructions. Frozen root samples (0.2 g per sample) were first grounded with liquid nitrogen, and then DNA was extracted using the same method as for soil samples. Fungal meta-barcoding was performed using a barcoded fITS7/ITS4 primer set (specifically designed for plant associated fungal communities) targeting the fungal ITS2 region (Ihrmark et al., 2012). All the PCR products (three replicates per sample) were checked using gel electrophoresis and then purified and pooled to construct the sequencing library using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, MA, USA). Finally, paired-end sequencing (2 × 250 bp) was conducted using the Illumina HiSeq 2500 platform.

The DADA2 algorithm was used for the bioinformatics analyses (ITS-specific version of the DADA2 workflow 1.8) (Callahan et al., 2016). The primers were first trimmed off the sequences using cutadapt (Martin, 2011). Low-quality reads (sequences contain "N" or the expected error being greater than 2) were filtered using *filterAndTrim* function; and then error rates were estimated using the *learnErrors* function and the denoised sequence table (ITS sequence variants) was constructed after sample inference and merging paired reads. Finally, after removing chimeras, taxonomic assignment was according to the UNITE database (general release of all eukaryotes) (Kõljalg et al., 2013) based on the naive Bayesian classifier method. Together, the DADA2 algorithm generated 4 321 655 denoised ITS reads (72 027 reads per root or soil sample on average) within 8 056 ASVs (amplicon sequence variants), of which 1973 ASVs were non-fungal (566 276 reads). The fungal ASVs table (6 083 ASVs) was rarefied to 17 000 reads per root or soil sample to ensure an equal sequencing depth. After rarefying, 5 664 ASVs were retained (4 880 and 2 233 ASVs for soil and root samples, respectively). The sequencing efficiency was tested using rarefaction curves, and all the root and soil samples reached saturation within 17 000 reads (Fig. S3b), suggesting sufficient sampling depth for all soil and root samples. All the subsequent data analyses were based on this rarefied fungal ASV table. Given that the amplification efficiency of general ITS primers for AM fungi was low (Lekberg et al., 2018), we did not obtain enough reads for downstream analysis. Therefore, in this study, we mainly focus on fungal groups other than AM fungi. The response of the soil AM fungal community was reported before (Fu et al., 2021), and the existence of AM fungi in roots was confirmed by microscopic

examination (Fig. S2b and also (Fu et al., 2021)). FUNGuild blast (version 1.1) (Nguyen et al., 2016) successfully annotated functional guild for 4 134 ASVs (73.0% of all the fungal ASVs), among them 1 738 ASVs were identified as saprotrophs (29.3%), 414 ASVs as pathotrophs (6.8%), and 615 ASVs identified as symbiotrophs (10.1%), respectively. Fungal guild responses in each treatment were compared using the response ratio (Hedges et al., 1999).

$$\text{Response ratio} = \ln\left(\frac{Response\ per\ sample}{\overline{Control\ mean}}\right)$$

where the response ratio of each functional guild was calculated using the ASVs richness response per sample under drought and the mean ASVs richness under control.

2.5. Statistical analyses

Fungal community data were analyzed using R statistics 3.6.0 (R Core Team, 2019), and the detailed analytical R scripts can be found in the supplementary materials. The fungal ASV richness and rarefaction curves were calculated using the vegan package (version 2.5.6) (Oksanen et al., 2007). Group comparisons were tested using the non-parametric Wilcoxon rank sum test. The pirateplot (Fig. 1a) was realized using yarrr package (version 0.1.5) (Phillips, 2017). We quantified the relative importance of plant and soil variables in predicting root and soil fungal richness responses using the random forest algorithm compiled in the randomForest (version 4.6.14) (Liaw and Wiener, 2002) and randomForestExplainer (version 0.10.0) packages (Paluszynska and Biecek, 2017). Regression analyses were performed using the *lm* function in R base installation, and the regression Akaike's information criterion (AICc) was calculated using the *AICc* function in the wiqid package (version 0.3.0) (Mike et al., 2020).

Fungal community composition shifts were assessed by unconstrained ordination analysis (PCoA) using the phyloseq package (version 1.30.0) (McMurdie and Holmes, 2013). To distinguish the effects of rare and abundant species on the response of the community composition, we used the Bray-Curtis and Sørensen (i.e., the binary version of the Bray-Curtis dissimilarity) dissimilarity matrix to characterize the species abundance and species presence/absence community composition shifts, respectively. We tested the significance of drought-induced fungal community composition shifts by permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations using the *adonis* function in the vegan package (Oksanen et al., 2007). Beta diversity partitioning was performed using Podani's method to decompose drought-induced fungal community shifts into species replacement and richness difference by setting control communities as references, respectively (Podani and Schmera, 2011). We first filtered ASVs that appeared in fewer than 5 communities in root or soil samples; and then we calculated pairwise fungal community dissimilarities between samples in drought treatments and control separately using the *beta.div.comp* function in adespatial package (version 0.3.14) (Sørensen-based species replacement and richness difference) (Borcard et al., 2018; Dray et al., 2020). Data visualization was realized using the ggplot2 package (version 3.2.1) (Wickham, 2016).

To quantify the response of fungal networks to extreme drought, we employed a metric named cohesion, which calculates the degree of complexity of a microbial community based on pairwise correlations and abundances of each taxon (Herren and McMahon, 2017):

$$\text{Positive cohesion} = \sum_{i=1}^{n} abundance_i \times positive\ connectedness_i$$

$$\text{Negative cohesion} = \sum_{i=1}^{m} abundance_i \times negative\ connectedness_i$$

Where n and m is the number of species with positive and negative connectedness values in a community. As suggested, we used the unrarefied taxon relative abundance table as input. The per.cutoff parameter was set to 0.3 to exclude taxa present in fewer than 9 samples (5–10 samples is suggested) to infer reliable correlations. Then pairwise Spearman correlation matrix between taxa was calculated using all root and soil samples, respectively. As recommended, we used all soil or root samples to infer species connectedness because our experiment was carried out at the local scale of the same fungal species pool (most OTUs are shared), and the algorithm needs sufficient change in relative abundance to distinguish the background or methodological noise in the data. Next, the 'taxon shuffle' null model was used to justify the compositional nature and the skewed distribution of the taxon relative abundance data. Finally, the average positive or negative corrected correlations were recorded as connectedness values. Theoretical and empirical studies show that the increase of negative associations and the decrease of positive associations predict stable microbial communities (Stouffer and Bascompte, 2011; Coyte et al., 2015; de Vries et al., 2018), so we use both positive and negative cohesion to quantify network stability:

$$\text{Network stability} = \frac{|Negtive\ cohesion|}{Positive\ cohesion}$$

where the greater the value, the more stable the microbial network. To sum up, instead of focusing on interactions of some specific species, we use community cohesion as a metric to justify the connectivity of species at the community level. In this way, we interpret the variation in cohesion as evidence for changes in community connectivity but not a direct proxy for species interactions.

The resistance and resilience of fungal richness to extreme drought were calculated using the metric developed by Orwin and Wardle (2004):

$$Resistance_{drought} = 1 - \frac{2|D_d|}{(C_0 + |D_d|)}$$

$$Resilience_{drought} = \frac{2|D_d|}{(|D_d| + |D_r|)} - 1$$

where D_d is the fungal richness difference between the control (C_0) and drought treatment (INT/CHR); D_r is the fungal richness difference between the control (C_0) and recovery treatment (INTR/CHRR). This index of resistance and resilience range from −1 to +1, and the greater the value, the stronger the resistance and resilience (+1 for maximal resistance and resilience), and lower (including negative) values indicate less resistance and slower recovery. For resistance, if $|D_d| \leq C_0$, the index will give values between 0 and 1; if $|D_d| > C_0$, the index will give negative values. For resilience, if $0 \leq |D_r| \leq |D_d|$, the index will give values between 0 and 1; if the $|D_r| > |D_d|$, the index will give a negative values.

Structural equation modeling (SEM) was performed using AMOS (version 24.0) to test hypothesized causal relationships for how extreme drought affect the root and soil fungal communities (Fig. 2). The *prior* model was initially constructed by the conceptual model outlined in Fig. 2, and then refined by the random forest and regression analysis. We used fungal species richness to represent root and soil fungal diversity responses, and precipitation deficits in 2017 (compared to mean annual precipitation) were used to quantify extreme drought. The SEM model was modified by stepwise removal of the least significant ($P > 0.05$) paths and then evaluated using model fit indices, including Chi-square (χ^2) tests ($P > 0.05$), root square error of approximation (RMSEA <0.06), Tucker-Lewis index (TLI ≥0.90), and comparative fit index (CFI ≥0.95) (Hu and Bentler, 1999; Fan et al., 2016).

3. Results

The taxonomic assignment showed that there were 7–8 fungal classes dominated the soil community in each treatment, among which

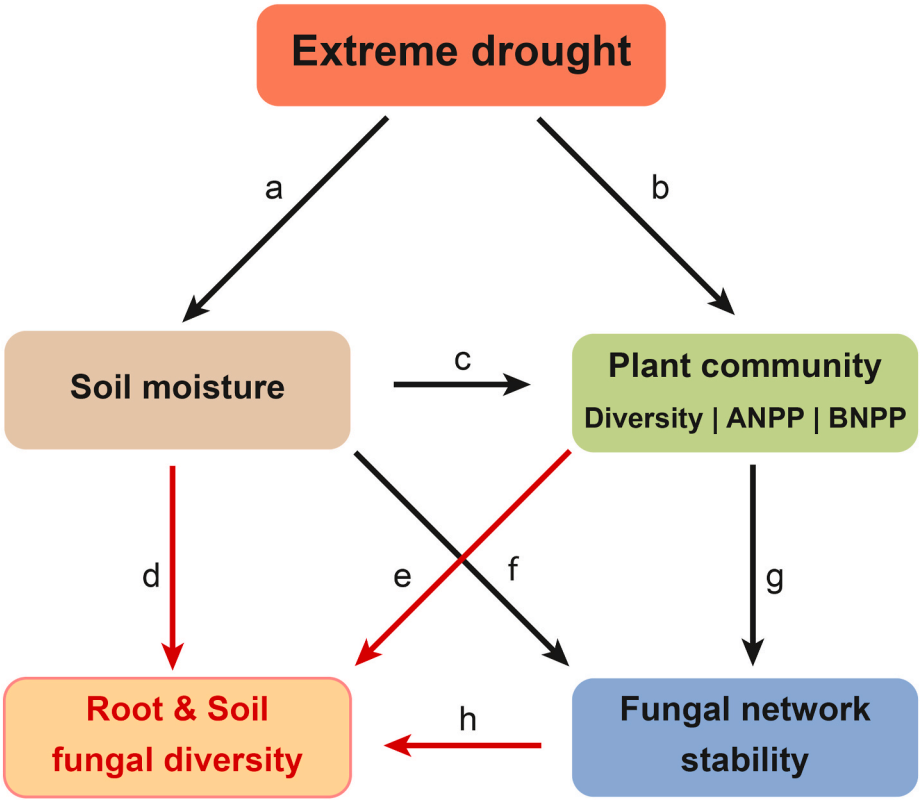

Fig. 2. Conceptual model for how extreme drought affects root and soil fungal diversity. (**a**) Reduced rainfall leads to decreased soil moisture. (**b, c**) Extreme drought could affect plant diversity and productivity via decreased soil moisture, increased heat stress, and atmospheric aridity (Reichstein et al., 2013; Xu et al., 2019; Zhou et al., 2019); and (**d**) Soil moisture can directly affect the growth, activity and mortality of soil fungi through water limitation (Schimel, 2018), which leads to the loss of species and changes of species interactions. We hypothesized that reduced soil moisture would affect root and soil fungal diversity and fungal network stability (**f**). Plants mainly use photosynthetic carbon (e.g., root exudates) and the immune and defense system to shape soil and root microbial communities to be more beneficial (Haichar et al., 2008; Philippot et al., 2013; Trivedi et al., 2020; Williams and de Vries, 2020). Therefore, we hypothesized that drought-induced productivity decline may impact the ability of plants to control soil and root fungal diversity (**e**) and network (**g**). Evidence is mounting that positive interaction among species help to form species-rich communities through enhanced resource utilization and niche amelioration (Gross, 2008; McIntire and Fajardo, 2014); thus we expect that drought-induced shifts in species interactions would have further impacts on root and soil fungal diversity (**h**).

Dothideomycetes, Eurotiomycetes, Geoglossomycetes, Leotiomycetes, Sordariomycetes, Agaricomycetes and Mortierellomycetes were particularly abundant (Fig. S4). The root community was mainly composed of 4–5 fungal classes in each treatment, with Dothideomycetes, Eurotiomycetes, Leotiomycetes, Sordariomycetes and Agaricomycetes were particularly abundant, with their relative abundances varying greatly as compared to that of the soil (Fig. S4). These abundant fungal classes mainly belonged to Ascomycota, Basidiomycota, and Mortierellomycota (Fig. S4). The ASVs richness of soil fungi was significantly higher than that of root fungi (679 versus 328 on average for control treatment) (Fig. 3a, b, c; Fig. S3b).

3.1. Fungal alpha diversity

The alpha diversity of root and soil fungi showed a divergent response pattern to drought. Compared with the control, the richness of root fungi was sensitive to drought treatments and increased significantly (~30%), while soil fungal richness remained stable with greater resistance to drought (Fig. 3a, b, c; Table 1). In the recovery phase, root fungal richness showed low resilience and remained high (Fig. 3b and c; Table 1). Notably, soil and root fungal richness did not differ significantly between the two drought types (Fig. 3b and c). The Shannon index (Fig. S5a) and inverse Simpson index (Fig. S5b) also showed similar response pattern. This divergent response pattern was also consistent at the fungal phylum level (Fig. S6a and b). For example, the richness of the most diverse fungal phylum Ascomycota increased significantly in roots under drought but remained stable in the soil. Although the overall soil fungal richness did not respond significantly, the richness of Glomeromycota and Chytridiomycota in soil responded significantly to drought (Fig. S6b) – the richness of Glomeromycota decreased significantly under continuous drought (INT/CHR), and the richness of Chytridiomycota increased significantly in all treatments.

For the response of the potential fungal functional guilds, the richness of root symbiotic (except for CHR) and saprotrophic fungi generally showed a significant positive response to drought, with pathogenic fungi in roots remained stable (except for CHRR) (Fig. 3d). However, in contrast to the richness responses, the relative abundance of fungal guilds within root communities remained stable across treatments (Fig. S7). For soil communities, the richness of symbiotic fungi was most responsive to drought (INT/CHR) but recovered promptly after drought ceased (INTR/CHRR); the richness of saprotrophic fungi were resistant to drought (INT/CHR) but responded positively after drought (INTR/CHRR); the richness of pathogenic fungi were generally resistant to extreme drought across treatments (Fig. 3d). In contrast to root communities, the relative abundance of soil fungal guilds was sensitive to drought. Specifically, compared to control, the relative abundance of symbiotic fungi decreased significantly under INT treatment ($P = 0.02$); the relative abundance of pathogenic fungi increased significantly in INTR ($P = 0.041$) and CHR ($P = 0.015$) treatments; for saprotrophic fungi, INT treatment only had a marginal effect ($P = 0.065$) (Fig. S7).

The rank-abundance curves illustrated that rare fungal species (relative abundance $\leq 0.1\%$ in each community) were more responsive to extreme drought in both root and soil communities (Fig. 3a). The regression analysis showed that the proportion of rare species in the fungal community was significantly correlated with the richness of root ($R^2 = 0.48$, $P < 0.001$; Fig. 3e) and soil fungi ($R^2 = 0.15$, $P = 0.04$; Fig. 3f).

Random forest analysis showed that plant productivity (both ANPP and BNPP) and soil moisture were the main potential drivers for root fungal richness responses, while soil moisture was the most powerful driver for soil fungal richness responses (Fig. S8a and b). The subsequent regression tests showed that both ANPP ($R^2 = 0.37$, $P = 0.002$; Fig. 4b) and BNPP ($R^2 = 0.16$, $P = 0.027$; Fig. 4c) were negatively correlated with root fungal richness, whereas soil moisture only showed marginal effects ($R^2 = 0.20$, $P = 0.053$ ns; Fig. 4a). Although the richness of soil fungi had no significant response to drought (Fig. 3c), its variation was significantly correlated to soil moisture ($R^2 = 0.28$, $P = 0.003$; Fig. 4e) and plant richness ($R^2 = 0.20$, $P = 0.012$; Fig. 4h).

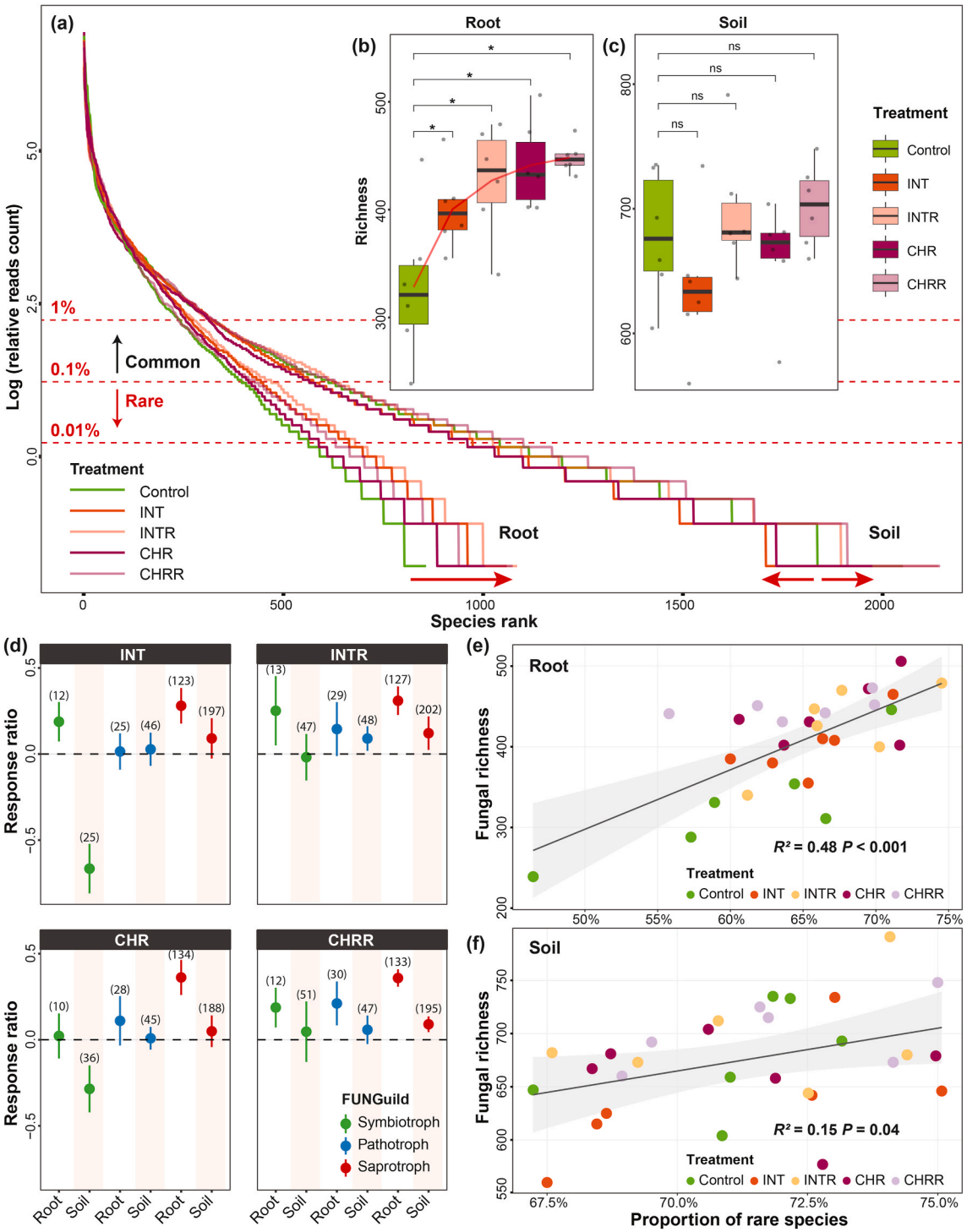

Fig. 3. Alpha diversity responses of root and soil fungi to extreme drought. (**a**) Species (ASVs) rank-abundance curve. Rare species were defined as species relative abundance under 0.1% in each community. (**b**) Root fungal richness response. (**c**) Soil fungal richness response. The black points jittered around the boxplot represent the raw data, significance was tested using Wilcoxon rank sum test (*$P < 0.05$). (**d**) The response ratio of the fungal guilds within each drought treatment. The response ratio was calculated using ASVs richness, the filled dots with whiskers represent the mean response ratio with 95% confidence intervals, and the mean ASVs richness in each treatment is given. The regression relationship of the proportion of rare species in each community with (**e**) root and (**f**) soil fungal richness. INT: intense drought; INTR: intense drought with recovery; CHR: chronic drought; CHRR: chronic drought with recovery.

3.2. Fungal beta diversity responses

Root and soil fungal community composition showed contrasting response patterns to extreme drought (Fig. 5a and b). Drought significantly affected the species presence/absence composition (PERMANOVA (Sørensen dissimilarity): $R^2 = 0.182$, $P < 0.001$) but not the species abundance composition (PERMANOVA (Bray-Curtis dissimilarity): $R^2 = 0.145$, $P = 0.23$) of the root community. In contrast, extreme drought had a significant effect on the species abundance composition (PERMANOVA: $R^2 = 0.174$, $P = 0.0021$) but not the species presence/absence composition (PERMANOVA: $R^2 = 0.148$, $P = 0.11$) of the soil community. Variance partitioning analyses showed that root

Table 1
The resistance and resilience of root and soil fungal richness to extreme drought.

Treatments	Average community resistance and resilience index values (±SE)		
	Soil fungal richness	Root fungal richness	*P* values (Soil vs. Root)
INT	0.84 ± 0.030	0.65 ± 0.060	0.041
INTR	−0.38 ± 0.23	0.058 ± 0.16	0.132
CHR	0.93 ± 0.039	0.49 ± 0.054	0.0050
CHRR	−0.13 ± 0.17	−0.028 ± 0.024	0.70

Note: Non-parametric Wilcoxon rank sum test was used for the analysis. The index values for INT and CHR treatments represent resistance (standardized by the undisturbed control); the index values for INTR and CHRR treatments represent resilience (standardized by the amount of change caused by the INT and CHR treatments, respectively) (Orwin and Wardle, 2004). SE: standard error.

fungal species presence/absence composition shifts were primarily explained by plant community variables (based on Sørensen dissimilarity; Fig. S10a), whereas soil fungal species abundance composition shifts were mainly explained by abiotic variables (based on Bray-Curtis dissimilarity; Fig. S10d). By setting control communities as references, beta diversity partitioning showed that species replacement played a major role in both root and soil fungal community shifts, but richness difference was more profound in root than in soil communities (Fig. 5c). In total, richness difference accounts for 23.3% of Sørensen dissimilarity in root communities, while only for 8.7% in soil communities (Fig. 5d).

3.3. Fungal species interaction responses

Extreme drought had no significant effect on soil fungal negative (Kruskal-Wallis test, $P = 0.98$) and positive (Kruskal-Wallis test, $P = 0.18$) community cohesion but significantly increased positive cohesion of the root fungal community (Fig. 6a and b). The root network stability, defined as negative cohesion/positive cohesion, significantly decreased under drought treatments (INT and CHR), and remained low after drought (CHRR) (Fig. 6c). In contrast, soil network stability increased significantly under drought treatments (INT and CHR) but returned to the control level in recovery treatments (INTR and CHRR) (Fig. 6c). Regression analyses showed that positive cohesion was positively correlated ($R^2 = 0.35$; $P < 0.001$), while network stability was negatively correlated ($R^2 = 0.34$; $P < 0.001$) with root fungal richness (Fig. 6d and e), but not with soil fungal richness (Fig. 6f and g).

3.4. Direct and indirect effects of extreme drought on root and soil fungal diversity

Based on the conceptual model outlined in Fig. 2 and above statistical analysis (Fig. 4; Fig. S8), we considered all the possible effects of extreme drought on fungal diversity (represented by species richness) and constructed the *prior* models for root and soil communities, respectively (Fig. 7; Fig. S9). In total, the structural equation model (SEM) explained 62% and 23% of the root and soil fungal richness responses, respectively (Fig. 7a and b). Extreme drought had direct negative effects on soil moisture (standardized estimates = −0.501, $P = 0.002$), ANPP (std. estimates = −0.642, $P < 0.001$), and plant richness (std. estimates = −0.717, $P < 0.001$), but a positive effect on BNPP (std. estimates = 0.963, $P < 0.001$) (Fig. 7a and b).

For root fungal communities, extreme drought had indirect negative effects on root fungal richness through ANPP (std. estimates = −0.555, $P < 0.001$) and BNPP (std. estimates = −0.450, $P < 0.001$) but a positive direct effect through soil moisture (std. estimates = 0.306, $P = 0.030$) (Fig. 7a). Through ANPP, extreme drought had an indirect positive effect (std. estimates = 0.460, $P = 0.005$) on root fungal network stability ($R^2 = 0.21$), and this species interaction had a negative effect on root fungal richness (std. estimates = −0.394, $P = 0.003$) (Fig. 7a). For soil fungal communities, extreme drought affected soil fungal richness ($R^2 = 0.23$) through soil moisture (std. estimates = −0.524, $P < 0.001$***), but not through plant richness (Fig. 7b).

4. Discussion

Based on a long-term field experiment on extreme drought in

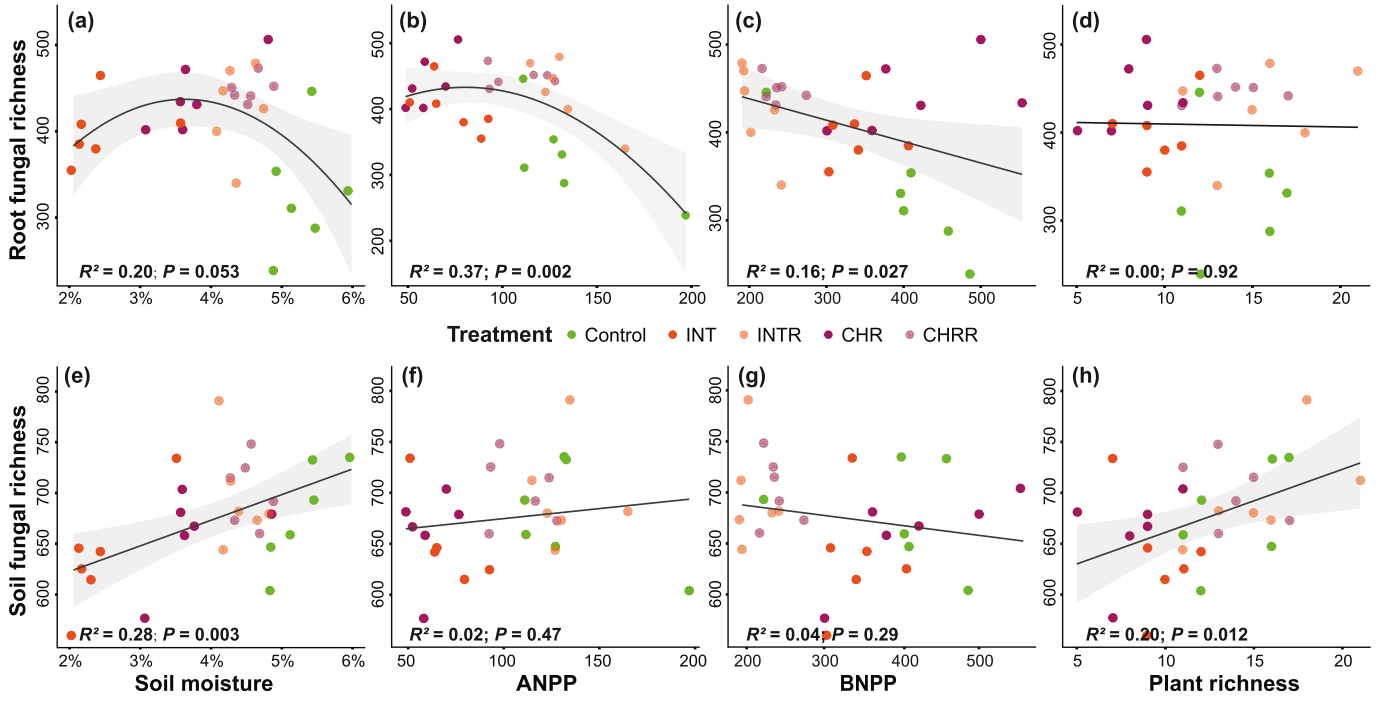

Fig. 4. Potential drivers of fungal alpha diversity response. (**a-d**) Response relationship of root fungal richness with (**a**) soil moisture, (**b**) ANPP, (**c**) BNPP, and (**d**) plant richness. (**e-i**) Response relationship of soil fungal richness with (**e**) soil moisture, (**f**) ANPP, (g) BNPP, and (h) plant richness. The fitting model (linear vs. curved) was selected based on AICc (Akaike's Information Criterion).

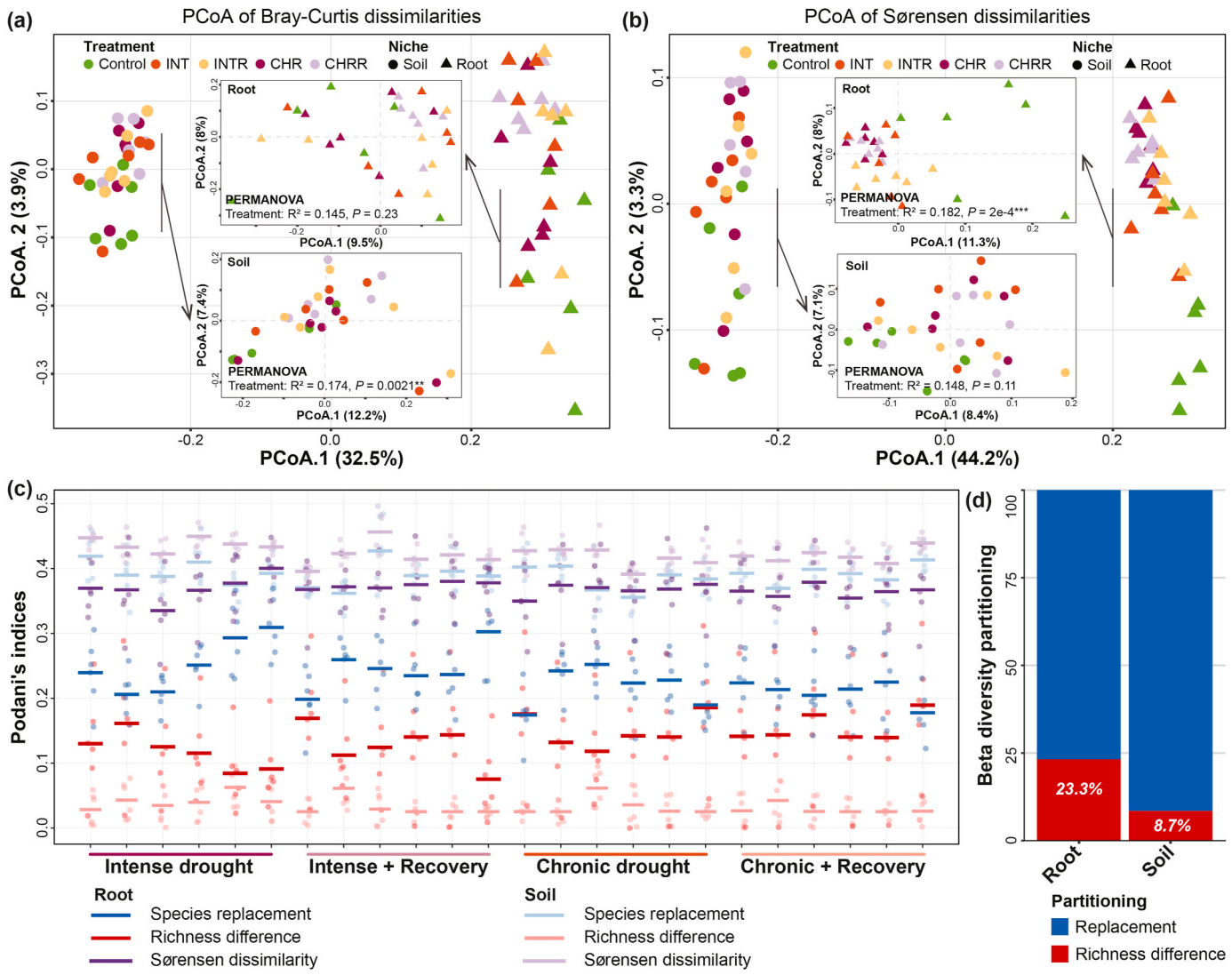

Fig. 5. Fungal beta diversity responses. (**a, b**) Principal coordinate analysis (PCoA, based on Bray-Curtis and Sørensen dissimilarity) of root and soil fungal communities. Significance was tested using PERMANOVA. (**c**) Fungal beta diversity partitioning of drought-affected communities with respect to control communities (Sørensen-based Podani's index of species replacement and richness difference). Each community in the drought treatment was compared with the 6 replicate communities in the control group separately, so each drought replicate consist of six data points, and every tile represents the mean of each data group. Deep (blue, red, and purple) and light colors (light blue, light red, and light purple) represent root and soil communities, respectively. (**d**) The contribution of species replacement and richness difference in total beta diversity of root and soil communities. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

temperate grassland, the present study provides new insights into the community response pattern of root and soil fungi and identified the main driving factors for the community response. In short, the study revealed four key findings: (i) the root fungal community was sensitive to extreme drought in terms of richness and species presence/absence composition. Furthermore, drought also showed legacy effects on the root fungal community after the drought ceased (i.e., recovery). Such drought sensitivity of the root fungal community was mainly driven by the positive responses of rare symbiotic and saprotrophic fungal species, with the abundant species in the community remaining stable. On the other hand, although the overall richness of soil fungi remained stable, the species abundance composition and functional groups (symbiotic and pathogenic fungi) responded significantly to drought. (ii) Statistical analysis showed that the response of root fungal richness was primarily correlated to plant productivity (i.e., ANPP and BNPP) but not soil moisture and plant richness, whereas soil fungal richness was correlated with soil moisture and plant richness. (iii) Extreme drought significantly increased positive cohesion of root fungal community and decreased root fungal network stability. Moreover, we found that root fungal positive cohesion and network stability were significantly correlated with root fungal richness, explaining more than 30% of root fungal richness variation. (iv) Based on the SEM analysis, we showed that the responses of fungal diversity in roots can be jointly explained by soil moisture, plant productivity (ANPP and BNPP), and fungal network stability, while the fungal diversity in soils can only be explained by soil moisture. Such divergent results imply that the plant community mediates drought responses of root fungal communities but not of soil communities.

The response divergence of the fungal community in roots and soils (Fig. 3; Fig. 5; Fig. 6) is likely related to the different niches for the two different fungal groups, with soil fungi more closely and directly interacting with the soil environment (Schimel, 2018), including soil organic carbon (Kyaschenko et al., 2017), while root fungi tend to be more strongly affected by plants and plant environmental response (Karlowsky et al., 2018). Sensitivity of root fungal richness, species presence/absence composition, and networks to extreme drought could be

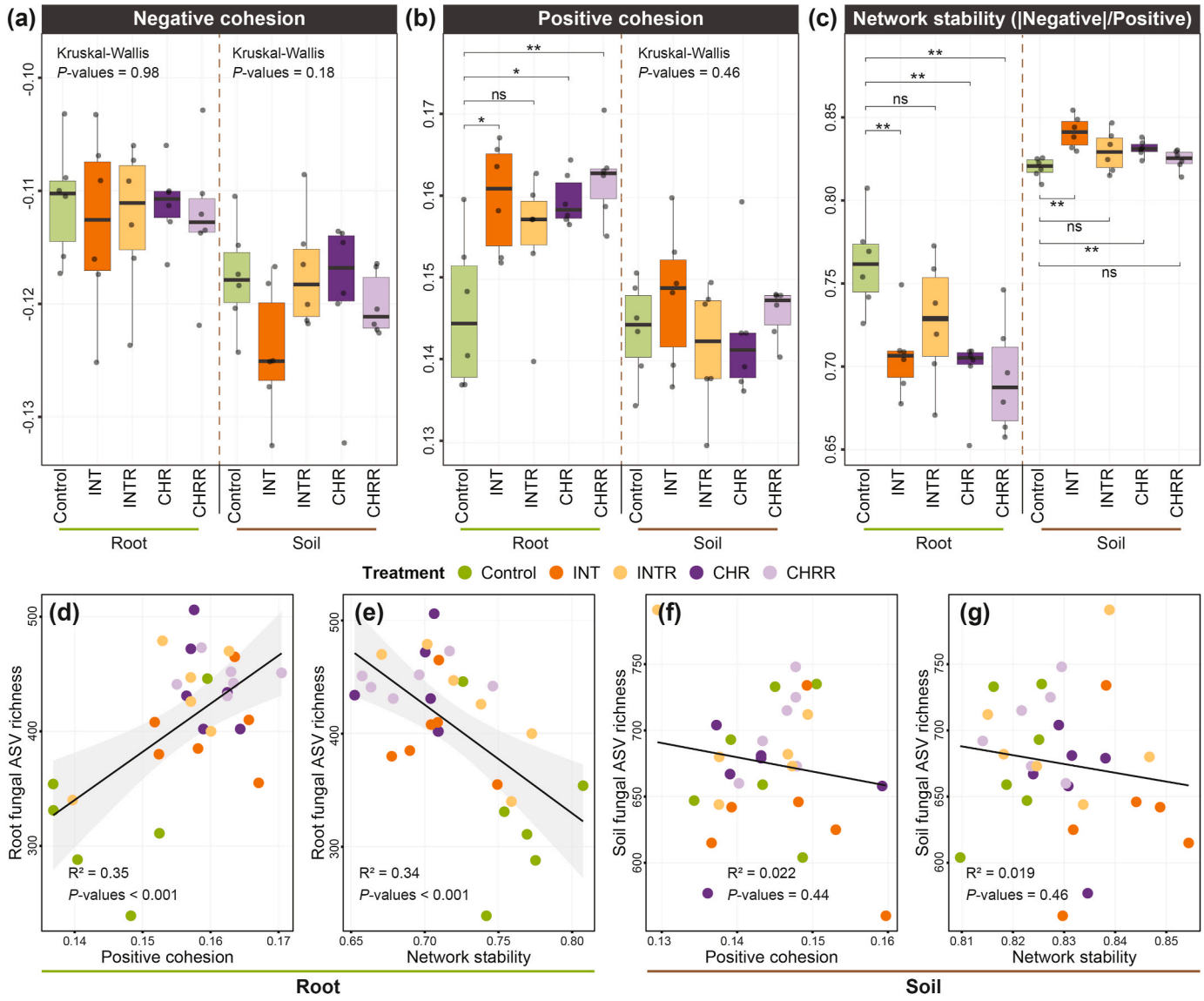

Fig. 6. Cohesion of fungal communities and its relationship with fungal species richness. (**a**) Negative and (**b**) positive community cohesion responses of root and soil fungi to extreme drought. (**c**) Responses of fungal network stability to extreme drought. The network stability was calculated by | negative cohesion|/positive cohesion. The relationship of (**d**) positive cohesion and (**e**) network stability with root fungal richness. The relationship of (**f**) positive cohesion and (**e**) network stability with soil fungal richness. The significance was tested using Wilcoxon rank sum test and indicated by asterisks (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$). INT: intense drought; INTR: intense drought with recovery; CHR: chronic drought; CHRR: chronic drought with recovery.

explained by the drought sensitivity of the plant community (Fig. 4b and c; Fig. 7a; Fig. S10a), particularly the plant productivity. In contrast, soil fungal communities were relatively stable in terms of richness (Fig. 3c; Table 1), species presence/absence composition (Fig. 5), and positive and negative community cohesion (Fig. 6a and b), which is in line with early studies suggesting that soil fungal communities were resistant to climate perturbations (de Vries et al., 2012, 2018; Manzoni et al., 2012; Zhou et al., 2021). Although the overall soil fungal communities were resistant, richness of the potential soil symbiotic fungi were sensitive to extreme drought (Fig. 3d). Such response is consistent with our previous study showing that AM fungal communities in the soil were sensitive to extreme drought (Fu et al., 2021). These results suggest that root-associated fungal groups are highly sensitive to extreme drought, both in the soil and in the roots. In this study, fungal richness did not show significant responses between drought types, suggesting drought type have limited effects on fungal community responses. However, this response pattern is different from our previous study on AM fungal communities, which showed that intense drought had a greater impact than chronic drought did (Fu et al., 2021). Notably, rare species in these two communities play a crucial role in richness responses to extreme drought (Fig. 3a), particularly for root communities (Fig. 3e and f). Theoretically, the rarity of species can be result from the process of community assembly (i.e., immigrant species often appear rare in a new community), biotic and abiotic interactions (Jousset et al., 2017). Moreover, as rare species often have a narrow ecological amplitude, they may be more sensitive to environmental perturbations (Gaston, 2008).

Compared to soil fungal communities, root communities are more affected by plants (Chomicki et al., 2020), and because of such restrictions, root fungal richness is substantially lower than that of soil fungi (Edwards et al., 2015) (also in this study Fig. 3b and c). Probably, the increase of root fungal richness among treatments could be caused by (1) the decreased plant immune and defense response; and/or (2) the enhanced plant recruitment; and/or (3) the improved fitness (or competitiveness) of some specific fungal groups. Because drought can impact plant physiological activity through water limitation, plants may

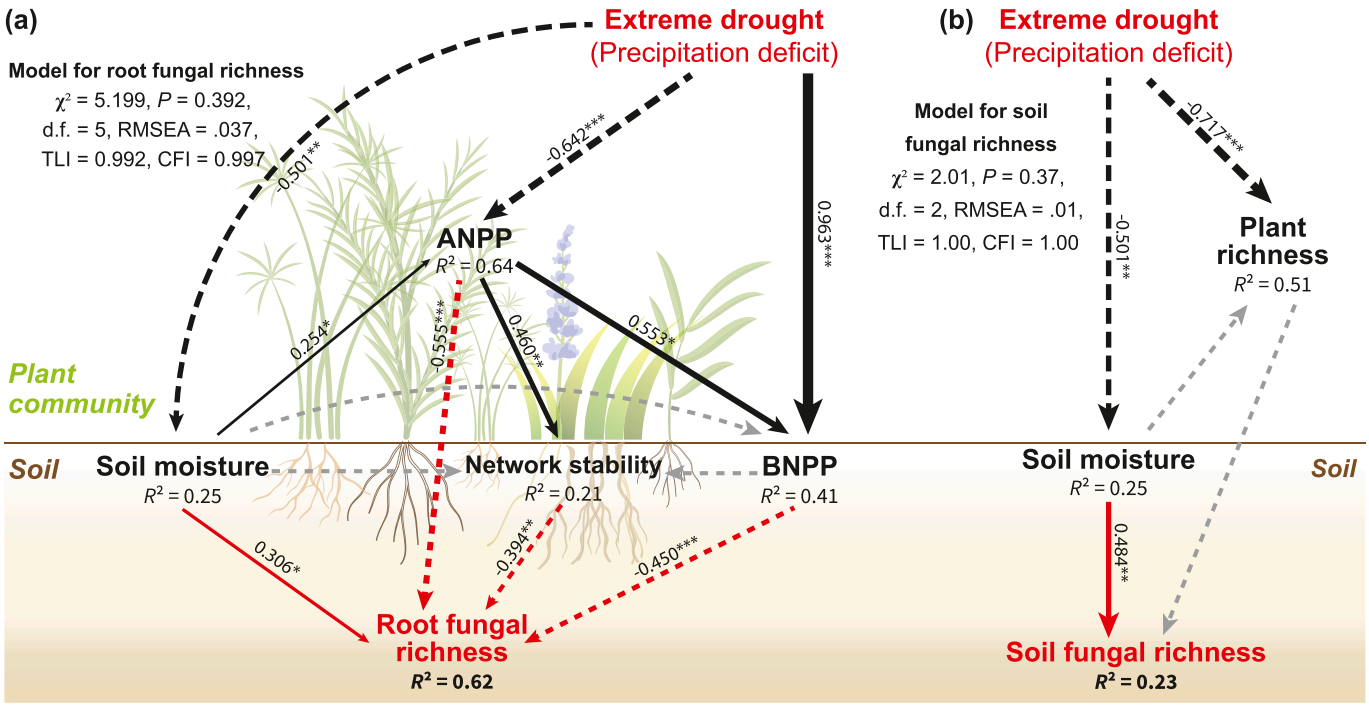

Fig. 7. Direct and indirect effects of extreme drought on root and soil fungal richness. Using a fitted SEM, we aim to identify the direct and indirect pathways through which soil moisture, plant community traits, and fungal network stability determine root (**a**) and soil (**b**) fungal richness exposed to grassland extreme drought. Continuous and dashed paths demonstrate positive and negative relationships, respectively. The gray dashed paths show the non-significant effects in the *priori* model. The width of the path was weighted by the standardized regression coefficients. Extreme drought was quantified using precipitation deficit of each treatment in 2017. The significance levels of each path were indicated by asterisks (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$). R^2 denotes the variance explained by the model. The fitness of the SEM model was indicated by chi-squared tests ($P > 0.05$), root mean square error of approximation index (RMSEA < 0.06), Tucker-Lewis Index (TLI ≥ 0.90), and comparative fit index (CFI ≥ 0.95).

face growth-defense tradeoffs, depending on stress conditions (Huot et al., 2014; Hou et al., 2021). In this regard, drought could decrease plant defense responses to soil fungi that may let more species invade plant roots in consequence. In addition, drought may alter plant carbon allocation strategies (both the amount and form) to belowground. For example, in a mountain grassland, drought reduced carbon allocation to bacteria but not fungi (Fuchslueger et al., 2014) and allocated more carbon to root storage (Hasibeder et al., 2015). This may also partly explain the positive responses of the potential saprotrophic fungi in roots under drought (Fig. 3d). Moreover, plants may actively recruit fungal species via the insurance effect (Jousset et al., 2017). For example, plants can promote the establishment of beneficial symbiotic fungi under drought by increased strigolactone biosynthesis (Ruiz-Lozano et al., 2016). This may explain the positive responses of the potential root symbiotic fungi under drought and particularly in recovery (Fig. 3d). The positive response of the potential saprotrophic fungi in recovery soils may suggest improved fitness under this condition and thus may contribute to the response of this group in roots. Because root growth was not affected by extreme drought in this study (Fig. S11) (also reported previously by meta-analysis (Liu et al., 2018)), and the relative abundance of the potential saprotrophic fungi remained stable in roots (Fig. S7), it is highly unlikely that the positive response of root fungal richness was caused by the decomposition of the roots. Taken together, these results indicate that functional affiliation may play crucial roles in the response of root fungal communities to climate change, yet their ecological relevance remains largely unknown.

In line with alpha diversity responses, the species presence/absence composition in roots also responded significantly to drought (Fig. 5b). However, the species abundance composition in roots remained unchanged (Fig. 5a), indicating that the composition of the abundant species remained stable in roots. The subsequent beta diversity partitioning analysis also supported the idea that the fungal community changes in roots were driven by species gain (richness difference) (Fig. 5c and d). In contrast, drought had a significant impact on the abundance composition of fungal species in soils but not on the species presence/absence composition (Fig. 5), suggesting soil abundant species were responsive to drought. Besides, the responses of root fungal communities to extreme drought showed legacy effects (richness, Fig. 3a, Table 1; community composition, Fig. 5a and b), which would influence plant-soil feedbacks, and thereby affect plant community dynamics and ecological restoration process (recovery phase) (Canarini et al., 2021). The community composition response of soil and root fungi is consistent with previous studies in North American grasslands (Ochoa-Hueso et al., 2018; Lagueux et al., 2020). However, in general, root fungal communities (alpha and beta) showed higher sensitivity to drought in this study, which is somehow contrary to the study in North America. Given fungal community responses are highly host and context dependent (Alzarhani et al., 2019; Lagueux et al., 2020), this contrast may be due to the divergent climate conditions (e.g., MAT, MAP, etc.) and plant sampling method (mixed vs. individual).

Theoretical studies suggest that the host tends to stabilize their microbial community by limiting positive feedbacks and weakening ecological interactions (Oliveira et al., 2014; Coyte et al., 2015); therefore, in reverse, the increased positive cohesion of root fungi (Fig. 6) may indicate that drought reduced the community stability. Empirical studies also supported the idea that environmental stress destabilized microbial networks with increased positive interactions (Hernandez et al., 2021). This is because positive interactions help establish positive feedback loops in the community, any response of individual species (or subgroups) will induce cascading effects on others reliant on the network, thereby may potentially reduce community stability under stress (Coyte et al., 2015). In contrast, soil networks showed strong resistance, echoing earlier work that the soil fungal networks were resistant to drought (de Vries et al., 2012, 2018; Zhou

et al., 2021). Besides, positive interactions between species can have the potential to improve species coexistence by improving their resources utilization and niche amelioration ability (Gross, 2008; McIntire and Fajardo, 2014), which may facilitate the migration of new species to the community. This mechanism may explain the positive correlations between positive cohesion and root fungal richness (Fig. 6d), indicating that positive interactions between species may contribute to increased root fungal richness under drought. Although network analysis is a powerful tool to understand the assembly process of the microbial community, it also faces enormous challenges in linking statistical inferences to reliable biotic interactions (Blanchet et al., 2020). Therefore, further work should make efforts to identify real fungal species interactions *in situ*.

Plant productivity has not been fully considered in soil and root microbial community studies. Unlike chronically acting climate change, climate extremes can evoke rapid responses of plant physiology and metabolism, resulting in rapid decrease of plant photosynthesis and productivity (Hoover et al., 2014; Xu et al., 2019), which may have profound impact on their associated fungal communities. Based on the SEM, we verified this concept that plant productivity (both ANPP and BNPP) mediated the effect of extreme drought on root fungal communities (Fig. 7a). Interestingly, the mediating effect of plant productivity can also be achieved by influencing fungal network stability in roots (Fig. 7a). Although the effect of species interactions on species coexistence has been widely recognized in plant and animal communities (McIntire and Fajardo, 2014; Calatayud et al., 2020), its role in the response of microbial communities to climate change has rarely been studied. In contrast to root communities, variations of soil fungal richness can only be explained by soil moisture but not plant richness (Fig. 7b), which indicates that plant and soil fungi responded independently from each other to extreme drought.

An ecologically stable and functional system should have either higher resistance, faster recovery, or both, in response to environmental perturbations. However, compared to the stable soil fungal communities (tested by both this study and previous ones (de Vries et al., 2012, 2018)), our results highlight that root fungal communities were sensitive to climate extremes. With the robust association between community responses of root fungi and the plant community, we can speculate that climate extremes have a major impact on the plant-fungal system, which could impose cascading effects on ecosystem processes and functions. In this regard, it will be crucial to evaluate how the functional traits of the root fungal community respond to climate change, and predict ecosystem responses upon inclusion of plant-soil feedback effects.

Author contributions

BC, MCR and JJ conceived the idea of this study. XH and QY designed the field experiment. WM, CX, HHW, QY and WL conducted the daily management of the field experiment and collected the plant data. WF, ZH and HW measured the soil physicochemical properties. WF performed the fungi community related experiments, analyzed all the data and created all the figures. WF, BC and MCR wrote the first draft, and all co-authors commented on the manuscript.

Data availability

The datasets generated and analyzed during the current study are available in the Figshare repository (https://doi.org/10.6084/m9.figshare.16908775.v2). The detailed analytical R scripts can be found in the supplementary materials.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All authors declare no conflict of interest concerning this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108670.

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