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Research

Community response of arbuscular mycorrhizal fungi to extreme drought in a cold-temperate grassland

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Summary

- Climate extremes pose enormous threats to natural ecosystems. Arbuscular mycorrhizal (AM) fungi are key plant symbionts that can affect plant community dynamics and ecosystem stability. However, knowledge about how AM fungal communities respond to climate extremes in natural ecosystems remains elusive.
- Based on a grassland extreme drought experiment in Inner Mongolia, we investigated the response of AM fungal communities to extreme drought in association with plant communities. The experiment simulated two types of extreme drought (chronic/intense) of once-in-20-year occurrence.
- AM fungal richness and community composition exhibited high sensitivity to extreme drought and were more sensitive to intense drought than chronic drought. This community sensitivity (i.e. decline in richness and shifts in community composition) of AM fungi can be jointly explained by soil moisture, plant richness, and aboveground productivity. Notably, the robustness of the plant–AM fungal community co-response increased with drought intensity.
- Our results indicate that AM fungal communities are sensitive to climate extremes, and we propose that the plant community mediates AM fungal community responses. Given the ubiquitous nature of AM associations, their climate sensitivity may have profound consequences on plant communities and ecosystem stability under climate change.

Introduction

Climate change is reordering and shifting the ecological communities as organisms respond to the changing environments (Bellard *et al.*, 2012). Species, populations, and ecological communities do not, however, respond promptly to gradual climate changes. Rather, extreme climate events, which are historically rare at a given regional scale, are more relevant to the impacts of climate change on ecosystems (Easterling *et al.*, 2000). Such extreme climate events are predicted to be a primary manifestation of future climate change and have been demonstrated to have profound impacts on natural ecosystems (IPCC, 2014; Xu *et al.*, 2019). However, due to the unpredictability and high variability of extreme climate events, knowledge about their effects on soil communities remains limited, particularly under natural conditions. Arbuscular mycorrhizal (AM) fungi are key soil microbes that can form intimate association with most terrestrial plants called AM symbiosis, and hold enormous significance in maintaining ecosystem stability (Smith & Read, 2010; Powell & Rillig, 2018; Yang *et al.*, 2018). Previous studies indicated that soil fungal communities were resistant to climate perturbations (de Vries *et al.*, 2018). However, AM fungi are obligate plant symbionts, so their response to environmental changes may be mediated by plant communities. To date, how AM fungal communities respond to climate extremes and the potential links with plant communities remain largely unexplored.

Within the AM association, the fungi trade nutrients for carbon with their plant partners (Smith & Read, 2010; Kiers *et al.*, 2011; Wang *et al.*, 2017), such that both partners can functionally help each other to resist a wide range of biotic and abiotic stresses (Smith *et al.*, 2009; Delavaux *et al.*, 2017; Wu *et al.*, 2019). For instance, AM associations could alleviate plant

drought stress by altering hormonal profiles (Ruiz-Lozano *et al.*, 2016), increasing water transport via upregulating AM fungal aquaporin genes (Li *et al.*, 2013), and stabilizing plant stoichiometric homeostasis via enhancing plant nutrient uptake (Bowles *et al.*, 2018). Plants, in turn, would transfer a proportional amount of photosynthetic carbon to AM fungi (Kiers *et al.*, 2011). However, climate extremes have immediate impacts on plant carbon assimilation and stimulate plants to allocate more carbon to belowground parts (Hasibeder *et al.*, 2015; Liu *et al.*, 2018), which could decrease plant aboveground productivity (Hoover *et al.*, 2014; Xu *et al.*, 2019). Subsequently, such an adaptive strategy of plants reduces partitioning of recently assimilated carbon to AM fungi (Fuchslueger *et al.*, 2014; Karlowsky *et al.*, 2018), which may have cascading effects on the responses of AM fungal communities to environmental change. However, solid evidences to support such a hypothesis is still scarce. This lack of knowledge hampers our ability to understand and predict the community dynamics of AM fungi and their relevance to ecosystem resistance and resilience under climate change.

The community dynamics of AM fungi depend on dispersal limitation, abiotic filtering, and biotic interactions (Zobel & Öpik, 2014; Vályi *et al.*, 2016; Zhang *et al.*, 2021). Translating these driving forces into specific processes, several hypotheses have been formulated to aid our understanding of AM fungal community assembly mechanisms. However, these ideas focus on different driving forces separately, making them less powerful under climate change. For instance, the passenger and driver hypotheses (Hart *et al.*, 2001), which assume that plants can drive AM fungal community dynamics or *vice versa*, maybe impractical for community assembly shaped by strong environmental filtering (Davison *et al.*, 2015; Van Geel *et al.*, 2018). Likewise, the habitat hypothesis also has its drawbacks for not considering complex cross-kingdom interactions and feedbacks (van der Heijden *et al.*, 1998; Rillig *et al.*, 2014; Neuenkamp *et al.*, 2018). In fact, climate change can affect the AM fungal community directly by changing the abiotic environment or indirectly via changes in the plant community. Therefore, a joint conceptual framework – that incorporates AM fungal community responses driven by abiotic forces while recognizing their mutualistic nature with plants that make their community responses unique – is needed to describe the community response patterns of AM fungi to climate change.

Efforts to investigate AM fungal community responses to climate extremes face the immense challenges of the unpredictability, rarity, and high variability of naturally occurring climate extremes. To overcome this difficulty, we designed an *in situ* extreme drought experiment in a cold-temperate grassland with two types of rainfall manipulations to simulate chronic and intense drought. For the chronic drought, we reduced the growing season rainfall by 66% from May to August; and for the intense drought, we suspended 100% rainfall from June to July (Fig. 1a). Both types of drought reduced roughly 50% of mean annual precipitation and simulated a once-in-20-year event, which meets the criteria of climate extremes (Smith, 2011; Slette *et al.*, 2020) (Fig. 1b,c). Besides, we also set recovery treatments after drought to study the resilience of AM fungal communities. Specifically, we intended to address how AM fungal communities respond to grassland extreme drought, and whether the community response of AM fungi was associated with the plant community. Because AM fungi are obligate plant symbionts, we hypothesize that, unlike other fungal clades, their community resistance and resilience would be related to plant community responses (i.e. plant productivity and richness) and plant adaptive strategy. Further, we assume that patterns of climate change (i.e. chronic and intense drought) can affect the extent to which AM fungi respond to climate extremes and influence their association with plant communities. Given that AM fungal communities show phylogenetic relatedness in response to environmental changes (Liu *et al.*, 2015; Chen *et al.*, 2017), we expect extreme drought also affect the phylogenetic community structure of AM fungi. Finally, we predict that environmental factors, especially soil moisture and plant community variables, jointly influence community responses of soil AM fungi.

Materials and Methods

Study site and experimental design

The experiment was set up at the Erguna Forest-Steppe Ecotone Research Station (50°10′N, 119°22′E), northeast China. The climate of the research area belongs to the cold-temperate continental monsoon climate, with a mean annual precipitation of 362 mm and a mean annual temperature of −2.45°C. The soil type at this site is classified to chernozem (US soil taxonomy classification) and rich in carbon and nitrogen. The dominant plant species are *Carex duriuscula*, *Leymus chinensis*, *Artemisia frigida*, *Pulsatilla turczaninovii*, *Stipa baicalensis*, *Cymbaria dahurica* and *Cleistogenes squarrosa* (Supporting Information Table S1). The experimental grassland was used for hay harvesting before 2013, and since then it has been fenced to prevent both human and livestock interference.

We arranged five treatments with two types of extreme drought (Fig. 1a): (1) Control; (2) CHR: 3 yr (2015–2017) of chronic drought; (3) CHRR: 2 yr (2015–2016) of chronic drought followed by 1 yr (2017) recovery; (4) INT: 3 yr (2015–2017) of intense drought; (5) INTR: 2 yr (2015–2016) of intense drought followed by 1 yr (2017) recovery. For the chronic drought (CHR/CHRR), we imposed a 66% precipitation reduction from May to August throughout the growing season, whereas for the intense drought (INT/INTR), we reduced 100% precipitation from June to July (Fig. 1a,b). Both types of drought reduced about 50% of annual precipitation with low occurrence incidence (most of them < 5%) based on historical climate data (Fig. 1b,c). To eliminate the potential heterogeneity of the experimental area, the experiment was laid out in a randomized block design with six blocks, and five treatments were randomly assigned to each block. Therefore, we set up 30 plots altogether (six blocks × five treatments) with six replicates for each treatment. We tested the treatment and block effects using AM fungal richness and found that block had no effect on AM fungal community responses (two-way ANOVA: treatment effect: $P < 0.001$, df = 4; block effect: $P = 0.47$, df = 5).

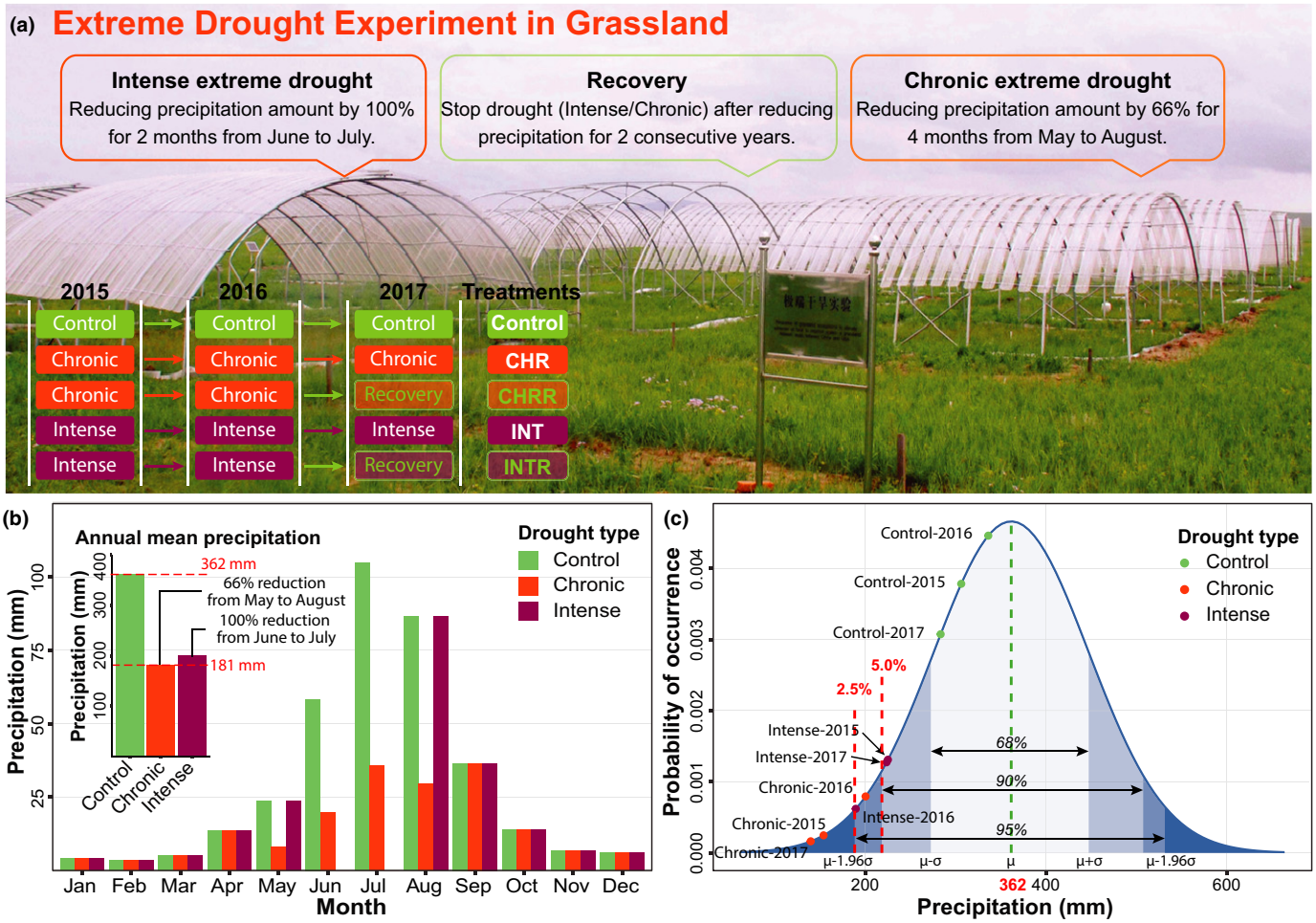

Fig. 1 Experimental design of the grassland extreme drought experiment. (a, b) Experimental design with brief introductions. During the growing season of 2015–2017, the chronic and intense droughts were implemented by reducing the ambient rainfall using light-transparent partial roofs. The inset in (b) shows both drought types result in similar annual mean precipitation based on historical precipitation data. (c) The performance of the experimental setup. The rainfall manipulation resulted in statistical extreme drought conditions in 2015, 2016 and 2017 by mapping on the estimated probability density curve based on historical precipitation data (1957–2017).

We followed the rainout shelter design developed by Yahdjian & Sala (2002) with some modifications to minimize the microclimate effect caused by the experimental facilities. We used high light-transparent polyethylene partial roofs (Beijing Plastics Research Institute, Beijing, China) to reduce the rainfall amount passively (Fig. 1a). The metal scaffold supported the partial roofs aboveground to minimize glasshouse effects by permitting free air flow (Fig. S1a). Before setting up the experiment, we assessed the microclimate effect: the partial roof permitted over 90% light transmission without air and soil temperature changes (automatically gathered with sensors). To prevent water flow into the plot, we hydrologically isolated the plot by trenching 1 m deep around the plot and lined the trench with plastic films and metal sheets (Fig. S1b). In the 6 m × 6 m plot area, we selected the central 4 m × 4 m area for further research, with the surrounding 1 m zone serving as a buffer zone (Fig. S1c). This experiment is part of the global Drought-Net research network in China (https://drought-net.colostate.edu/).

Plant and soil analyses

We collected soil samples by the end of August 2017 after the chronic drought. In each plot, five soil cores (0–20 cm depth; 3.8 cm in diameter) were randomly taken from the middle and four corners of the core experimental area and pooled to generate one composite soil sample for the plot. Therefore, totally 30 soil samples were collected. Soil samples were kept in sterile plastic bags and stored with ice bags in a portable cooler box before transfer to the laboratory for further processing. The soil auger was cleaned using tap water between plots and dried using wipes. Then, we homogenized the soil samples by passing through a 2 mm-sieve and collected the roots (stored at −20°C) for AM fungal colonization measurement. Sieves and tweezers were carefully sterilized by using 75% alcohol and cleaned with water to avoid cross-contamination between samples. Finally, we divided the soil samples into three subsamples and stored them at room temperature (air dried for the measurement of soil physicochemical properties),

4°C (for the measurement of soil biotic properties), and −80°C (for soil DNA extraction), respectively.

Fresh roots were first cleaned using tap water and cut into *c.* 1 cm fragments. We cleared the roots using 10% potassium hydroxide (KOH, 25 min, 90°C), rinsed in 2% hydrochloric acid (HCl, 5 min), then stained in 0.05% trypan blue (30 min, 90°C), and finally destained using lactic acid–glycerol solution. Thirty root fragments per plot were randomly selected for microscopic inspection according to Trouvelot's method at magnification ×200 (Trouvelot *et al.*, 1986). The mycorrhizal colonization intensity (M%) and the abundance of arbuscules were calculated using MYCOCALC software (https://www2.dijon.inrae.fr/mychintec/Mycocalc-prg/download).

The air-dried soil samples were ground using a ball mill (MM400; Retsch, Düsseldorf, Germany) and passed through 0.15 mm-sieve for further analysis. The elemental analyzer (Vario EL III; Elementar, Langenselbold, Germany) was used for total soil carbon and nitrogen measurement. Soil pH was measured using a soil to water ratio of 1 : 2.5 with a digital pH meter (FE200; Mettler Toledo, Greifensee, Switzerland). Soil available phosphorus was measured following the Olsen method (Olsen, 1954). Soil moisture was measured monthly using the gravimetric method (oven-dried (105°C) to a constant weight), and the mean soil moisture content of each plot (from May to August) in 2017 was used for subsequent data analysis.

In the same week we did soil sampling, the aboveground part of each plant species was collected (41 plant species in total; Table S1) by clipping each plant individual at the ground level in a 1 × 1 m^2 area. We collected the root biomass (0–20 cm) using a root auger (7 cm in diameter). Three soil cores were randomly collected within the 1 × 1 m^2 area after plant community investigation, and the roots were cleaned using tap water. The belowground net primary productivity (BNPP, 0–20 cm) was measured using the root ingrowth core method described earlier (Ma *et al.*, 2020). All the roots were oven-dried (80°C) to a constant weight in the laboratory and weighed to calculate the belowground plant biomass of 1 × 1 m^2 area by area conversion.

DNA extraction and amplicon sequencing

Soil samples stored at −80°C were first freeze-dried (FreeZone 4.5; Labconco, Kansas City, MO, USA), and then soil DNA was extracted from 500 mg freeze-dried soil samples using FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) preceded by Fastprep-24 5G sample homogenization (MP Biomedicals) following the manufacturer's instructions. We used general fungal internal transcribed spacer (ITS) primers, since they have been shown to have comparable effectiveness with AM fungi-specific small subunit rRNA primers in detecting AM fungal community responses to environmental changes (Kohout *et al.*, 2014; Berruti *et al.*, 2017; Lekberg *et al.*, 2018). More importantly, these primers enable direct comparison of AM fungi with other phyla across the fungal kingdom at the community level. The extracted DNA was amplified using a barcoded primer set (fITS7/ITS4) targeting the fungal ITS2 region (Ihrmark *et al.*, 2012). This ITS2 primer set previously has been used in AM fungal community studies (e.g. Gomes *et al.*, 2017; Deveautour *et al.*, 2020, etc.); it amplifies a sequence length similar across AM fungal families (MaarjAM database (Öpik *et al.*, 2010; Lekberg *et al.*, 2018)), thus avoiding sequencing bias that favors shorter reads in the Illumina platforms (Castaño *et al.*, 2020). For the ITS PCR amplification, each of the 25 µl PCR reaction system contained 12.5 µl 2 × Premix Taq (Takara Biotechnology, Dalian, China) and 2 µl diluted DNA template. The PCR amplification was performed with an initial denaturation at 94°C for 5 min, 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s followed by 10 min extension at 72°C, and the PCR products were checked using agarose gel electrophoresis. Three PCR replicates were performed for each sample and then pooled together to generate a composite PCR product. After PCR purification and pooling, sequencing libraries were constructed using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. Paired-end (2 × 250 bp) sequencing was performed using the Illumina HiSeq 2500 sequencing platform.

Bioinformatics and statistical analyses

DADA2 (v.1.12.1) was used for the bioinformatics analyses (Callahan *et al.*, 2016). We used the ITS-specific version of the DADA2 workflow (1.8) to infer denoised ITS sequence variants (or amplicon sequence variants, ASVs (Callahan *et al.*, 2017)). Briefly, primers were firstly removed using *cutadapt* (Martin, 2011); secondly, low-quality reads were filtered by *filterAndTrim* function; and then error rates were estimated for sample ASVs inference; next, chimeras were removed after merging paired reads; finally, taxonomy was assigned using the UNITE database (general release of all eukaryotes (2 February 2019)) (Kõljalg *et al.*, 2013). Overall, the DADA2 algorithm generated an ASV table of 30 samples × 6894 ASVs (2169 792 reads), of which 1669 ASVs were nonfungal (188 202 reads). Among all the fungal ASVs (1 981 590 reads), 489 ASVs (15 814 reads) were identified as AM fungi. We used two approaches to analyze soil AM fungal community responses. For the first approach (dataset1: rare species approach), we rarefied the total fungal reads to 52 000 per sample to ensure an equal sampling depth. This approach treats AM fungi as a subset of the fungal data (widely used in rare species studies), which eliminates the effects of different fungal reads and retained abundance variation of AM fungi among the fungal communities. To eliminate the effect of reads difference in following diversity analysis, we rarefied the AM fungal reads to 216 per sample in the second approach (dataset2: traditional approach). We tested the sequencing efficiency using rarefaction curves (Fig. S2). The fungal rarefaction curve reached saturation around 15 000 reads per sample (Fig. S2a), suggesting 52 000 reads per sample was sufficient to cover almost all the fungal species. In line with this result, rarefaction curves of AM fungi in both dataset1 and dataset2 reached its plateau in almost all samples (Fig. S2b,c). Although reads in sample INT1, INT2, INT4 and INT8 are less than 216 in dataset2, their rarefaction curves all reached saturation within the existing reads, suggesting

sufficient sequencing depth within these samples (Fig. S2c). Besides, the within treatment variance of AM fungal relative abundance is significantly lower than that of between treatments (Fig. S3b), suggesting limited randomness of sequencing variation within treatments. Taken together, by deep ITS sequencing, we obtained sufficient reads to uncover the completeness of the AM fungal diversity in this experiment.

We analyzed the diversity of both datasets using the VEGAN R package (v.2.5.6, Oksanen *et al.*, 2007). The two datasets showed consistent results in both α and β diversity responses (α diversity: Fig. 2b vs Fig. S4; β diversity: Fig. S5a,b vs Fig. S5c,d), and also the response relationship with plant community (diversity-diversity (Fig. 2e vs Fig. S6a): $R^2_{dataset1} = 0.22$ vs $R^2_{dataset2} = 0.25$; diversity-productivity (Fig. 2f vs Fig. S6b): $R^2_{dataset1} = 0.26$ vs $R^2_{dataset2} = 0.27$). As we used general fungal ITS primers, it is more reasonable to analyze community data using dataset1 taking relative abundance variation into account, and also compare AM fungal responses with other fungal groups. Therefore, we use dataset1 for the subsequent community response analysis (results of dataset2 could be found in Supporting Information). Because AM fungal richness, Shannon, and inverse Simpson index showed a similar response pattern (Fig. S4), we took richness for downstream analyses.

General data processing and statistical analyses were performed in R (v.3.6.0) (R Core Team, 2019). The main R packages used in the data analyses are listed in Table S2, and detailed steps are

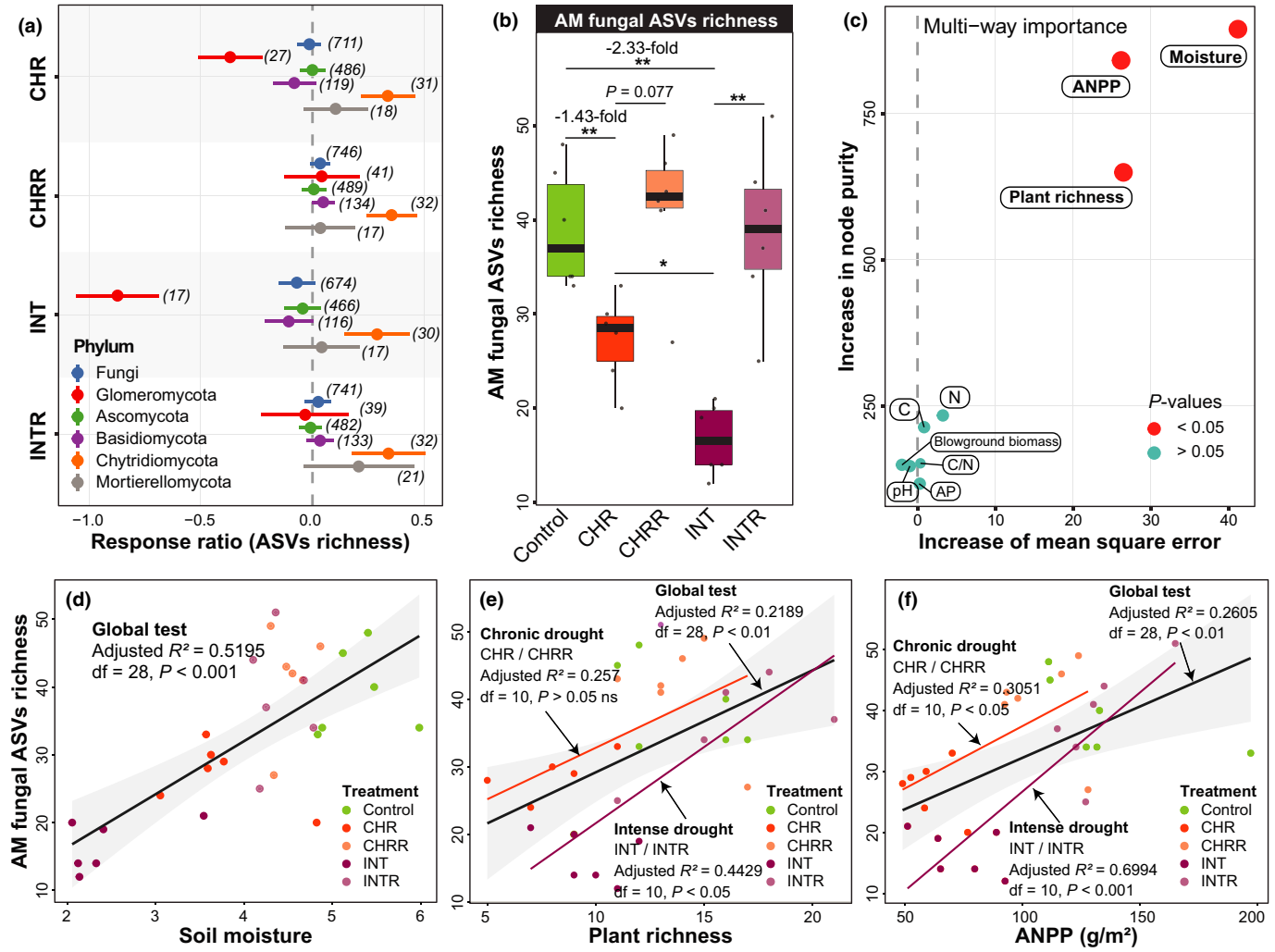

Fig. 2 Arbuscular mycorrhizal (AM) fungal alpha diversity responses to extreme drought and the potential drivers. (a) Standardized responses of the main fungal phyla using ASVs (amplicon sequence variants) richness. The filled dots and whiskers represent the mean response ratio and 95% confidence intervals. The mean fungal richness was annotated beside each whiskered dot. (b) The response of AM fungal ASVs richness. The black points jittered around the boxplot represents the raw data; the horizontal lines marked treatments in comparison, and significance of difference was tested by Wilcoxon signed-rank test (*, $0.01 \leq P < 0.05$; **, $P < 0.01$). (c) Random forest analysis showing the relative contribution of biotic and abiotic factors in predicting AM fungal ASVs richness response to extreme drought (the more important the index, the greater the values). Abiotic variables: soil moisture (2017), soil carbon (C), nitrogen (N) content, C/N, pH, and available phosphorus (AP) content; biotic variables: aboveground net primary productivity (ANPP), belowground biomass and plant richness. (d) The relationship of soil moisture and the AM fungal richness response. (e) The response relationship of AM fungal ASVs richness and plant richness. (f) The response relationship of AM fungal ASVs richness and ANPP. All the linear regressions fitted using the OLS model and the gray shading area around represents the 95% confidence intervals; ns, nonsignificant. CHR, chronic drought; CHRR, chronic drought followed by recovery; INT, intense drought; INTR, intense drought followed by recovery.

described later and also in the code description (GitHub repository: https://github.com/dreamerfuwei/Community-responses-of-plants-and-AM-fungi.git).

Random forest algorithm (randomForest package (v.4.6.14); Liaw & Wiener, 2002) was used for quantifying the relative contribution of biotic and abiotic factors in predicting the responses of AM fungal richness to extreme drought, the *P*-values were calculated using a one-sided binomial test as compiled in the randomForestExplainer R package (v.0.10.0) (Paluszynska *et al.*, 2019). We used response ratio $\log_e RR = \log_e$(Response per sample/Control mean) (Hedges *et al.*, 1999) to normalize the responses of AM fungal richness, plant richness, and aboveground net primary productivity (ANPP) so that they can be compared directly. To analyze the community composition shifts of AM fungi, we employed unconstrained principal coordinates analysis (PCoA, based on Bray–Curtis dissimilarity), and the significance was tested using permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) (realized by *adonis* and *anosim* function in vegan R package (Oksanen *et al.*, 2007), respectively). Then, we used distance-based redundancy analysis (db-RDA, based on Bray–Curtis dissimilarity) to detect any community composition shifts of AM fungi constrained by abiotic and plant variables. This approach allows using Bray–Curtis dissimilarity as input, which calculates community composition shifts and corresponds to PCoA (Legendre & Anderson, 1999). The significance of db-RDA was tested using random permutations ($n = 9999$ for all analyses). Given that AM fungal species could have multiple ITS ASVs (Thiéry *et al.*, 2016; Bruns *et al.*, 2018) and the ASV inference may further amplify this intraspecific variation (Callahan *et al.*, 2016), we performed the ordination analysis at both ASV-level and genus-level (Fig. S5). To construct the phylogenetic tree, we first performed a sequence alignment (*AlignSeqs* function) of all the AM fungal ASVs using the decipher R package (v.2.18.1) (Wright, 2016) and constructed a neighbor-joining (NJ) tree in the phangorn R package (v.2.7.0) (Schliep *et al.*, 2017); next, we fitted a maximum likelihood tree (GTR model) using the NJ tree as a starting point (Callahan *et al.*, 2016). The net relatedness index (NRI or -SES_{MPD} (standardized effect size of mean pairwise distance)) and nearest taxon index (NTI or -SES_{MNTD} (standardized effect size of mean nearest taxon distance)) were calculated using the *ses.mpd* and *ses.mntd* functions in the picante R package (v.2.7.0) (Webb *et al.*, 2002; Kembel *et al.*, 2010). We chose the *independentswap* null model (999 randomizations) in *ses.mpd* and *ses.mntd* functions because it is suitable for communities with variations in diversity and richness (Horn *et al.*, 2014; Egan *et al.*, 2017). The phylogenetic community structure of AM fungi was realized in the phyloseq R package (v.1.30.0) (McMurdie & Holmes, 2013) using weighted UniFrac distance (Lozupone & Knight, 2005).

The plant–AM fungi co-occurrence network was constructed using Spearman correlation according to the method described by Ramirez *et al.*, (2018) with some modifications. Given that network analysis is strongly influenced by the number of effective species (Inverse Simpson) and the sparsity of the species table (Weiss *et al.*, 2016), we first filtered out all the species that appear less than three times among all samples. This approach lowered the sparsity of the combined species table with a reasonable number of effective species (12.8). Then we calculated Spearman correlation coefficients (ρ) and *P*-values using *corr.test* function in the psych R package (v.2.1.6) (William, 2017), and the *P*-values were adjusted using the Benjamini–Hochberg method. Next, *P*-values < 0.01 were filtered. Finally, the correlation network was visualized using igraph package in R (v.1.2.6) (Csardi & Nepusz, 2006).

Amos software (v.24.0) was used to construct the structural equation model (SEM). The prior model was constructed based on the published literature and our hypotheses. In brief, plant richness was reported to have positive relationships with AM fungal richness (Hiiesalu *et al.*, 2014); we hypothesized that plant diversity could mediate AM fungal community responses to extreme drought. Soil moisture can directly influence soil AM fungal species through water limitation. Drought can induce plant adaptive strategy to allocate more carbon to belowground (Hasibeder *et al.*, 2015). This strategy could reduce plant photosynthesis aboveground, which has negative impacts on the carbon supply to AM fungi. Data from all treatments were used to generate the model, the ANPP to BNPP ratio was used to quantify the adaption strategy of plants, and the db-RDA1 was used to quantify community composition shifts of AM fungi. The model was modified by stepwise removal of the least nonsignificant ($P > 0.05$) 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 & Bentler, 1999; Fan *et al.*, 2016).

Results

Arbuscular mycorrhizal fungal community responses to extreme drought

Based on the denoised AM fungal ASV inference, we successfully obtained 489 AM fungal ASVs belonging to seven families, including Acaulosporaceae (one ASV), Ambisporaceae (one ASV), Archaeosporaceae (11 ASVs), Claroideoglomeraceae (44 ASVs), Diversisporaceae (25 ASVs), Glomeraceae (372 ASVs), and Paraglomeraceae (10 ASVs); and 19 genera, including *Ambispora* (one ASV), *Archaeospora* (seven ASVs), *Claroideoglomus* (41 ASVs), *Diversispora* (14 ASVs), *Dominikia* (104 ASVs), *Funneliformis* (five ASVs), *Glomus* (64 ASVs), *Kamienskia* (four ASVs), *Paraglomus* (10 ASVs), *Rhizophagus* (55 ASVs), *Septoglomus* (35 ASVs), and eight unclassified genera (149 ASVs) (Fig. S3a). AM fungal communities responded differently from other fungal clades, with AM fungal richness exhibiting low resistance to extreme drought but rapid recovery (high resilience) after drought, whereas other fungal clades were generally resistant (Fig. 2a). AM fungal richness decreased significantly after 3-yr of continuous drought (CHR/INT), however, recovered to the control level 1 yr after the cessation of drought (CHRR/INTR). Generally, the impact of intense drought was stronger than that of chronic drought (Fig. 2b). The relative abundance, Shannon and

inverse Simpson index of AM fungi showed a similar response pattern, with significant declines under both drought modes (CHR/INT) (Figs S3b, S4b,c). AM fungal community composition also varied significantly after 3-yr of continuous drought (CHR/INT), but the community composition recovered to control status after 1 yr of recovery (CHRR/INTR) (ASVs level: Adonis test, $R^2 = 0.17$, $P < 0.001$, ANOSIM test, $R^2 = 0.21$, $P < 0.01$, Fig. S5a; Genus level: Adonis test, $R^2 = 0.36$, $P < 0.001$, ANOSIM test, $R^2 = 0.30$, $P < 0.001$, Fig. S5b). The phylogenetic structure of AM fungal communities was significantly affected by extreme drought (Fig. 3). The null model analyses showed that, under intense drought (INT), NRI (Fig. 3a) and NTI (Fig. 3b) were significantly lower than expected by chance (i.e. values of NRI and NTI significantly negative). In addition, extreme drought significantly affected the phylogenetic beta diversity of AM fungal communities (Adonis test: $R^2 = 0.22$, $P < 0.01$; ANOSIM test: $R^2 = 0.15$, $P < 0.01$; Fig. 3c).

AM fungal ASVs richness, plant richness, and ANPP responses were normalized using the response ratio for direct comparisons, and the results showed a steadily consistent synergistic response pattern under the two types of extreme drought (Fig. 4a). The response of AM fungi to intense drought (INT) was significantly greater than that to chronic drought (CHR) ($\Delta RR = 0.504$, $P < 0.01$), whereas AM fungal richness recovered to control level in both recovery treatments (CHRR/INTR) (Fig. 4a). By contrast, there was no significant difference in ANPP and plant richness response between the two drought modes (CHR/INT; Fig. 4a). The AM fungal colonization intensity was significantly higher under CHR treatment than control (Fig. 4b), whereas other treatments did not have a significant difference compared to control. Further regression analysis showed a significant positive relationship between AM fungal colonization intensity and AM fungal response ratio under continuous drought (CHR/INT) (adjusted $R^2 = 0.46$, $P < 0.01$; Fig. 4c).

Potential drivers of arbuscular mycorrhizal fungal alpha diversity

Random forest analysis showed that the abrupt responses of AM fungal richness were best predicted by soil moisture, ANPP, and plant richness (Fig. 2c). Further correlation analyses revealed that responses of AM fungal richness were significantly and positively correlated with soil moisture (adjusted $R^2 = 0.52$, $P < 0.001$; Fig. 2d), plant richness (adjusted $R^2 = 0.22$, $P < 0.01$; Fig. 2e) and ANPP (adjusted $R^2 = 0.26$, $P < 0.01$; Fig. 2f). Moreover, the correlation coefficients between AM fungal richness and plant community responses differed between drought modes. The adjusted R^2 of the correlation between plant richness and AM fungal richness increased from 0.257 ($P > 0.05$, ns) under chronic drought to 0.443 ($P < 0.05$) under intense drought (Fig. 2e); and the adjusted R^2 of the ANPP and AM fungal richness boosted from 0.305 ($P < 0.05$) under chronic drought to 0.699 under intense drought ($P < 0.001$) (Fig. 2f).

Potential drivers of arbuscular mycorrhizal fungal beta diversity response

By using constrained ordination analysis (db-RDA, adjusted $R^2 = 0.29$, $P < 0.001$), we showed that soil moisture, ANPP, and plant richness were among the best predictors for AM fungal community shifts, whereas AM fungal colonization and soil physicochemical properties did not (Fig. 5a). The subsequent variance partitioning analysis showed that the abiotic variables were the main driving force for AM fungal community shifts, however, with a great portion (> 40%) of effect through the plant community (Fig. 5b). Further regression analysis showed that ANPP (adjusted $R^2 = 0.38$, $P < 0.001$; Fig. 5c) and plant richness (adjusted $R^2 = 0.23$, $P < 0.01$; Fig. 5d) were significantly correlated with the first axis of db-RDA, which explained 16.8% ($P < 0.001$) variance of AM fungal community composition shifts. Notably, correlation coefficient of ANPP ($R^2_{chronic} = 0.15$, $P > 0.05$ to $R^2_{chronic} = 0.73$, $P < 0.001$; Fig. 5c) and plant

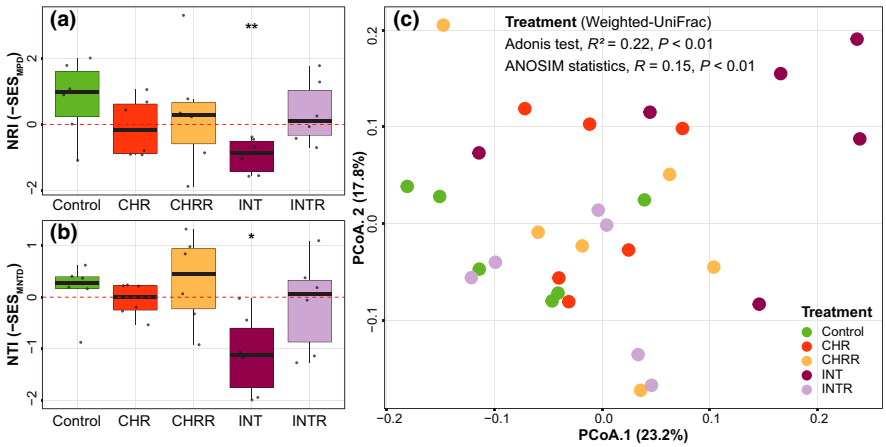

Fig. 3 Phylogenetic community responses of arbuscular mycorrhizal (AM) fungi. (a) The net relatedness index (NRI) and (b) nearest taxon index (NTI) of phylogenetic structure within AM fungal communities of each treatment. The black points jittered around the boxplot represents the raw data; asterisks indicate index values significantly nonzero (*t*-test; *, $0.01 \leq P < 0.05$; **, $P < 0.01$). (c) Unconstrained ordination (principal coordinates analysis (PCoA)) showing weighted UniFrac distance between treatments. CHR, chronic drought; CHRR, chronic drought followed by recovery; INT, intense drought; INTR, intense drought followed by recovery.

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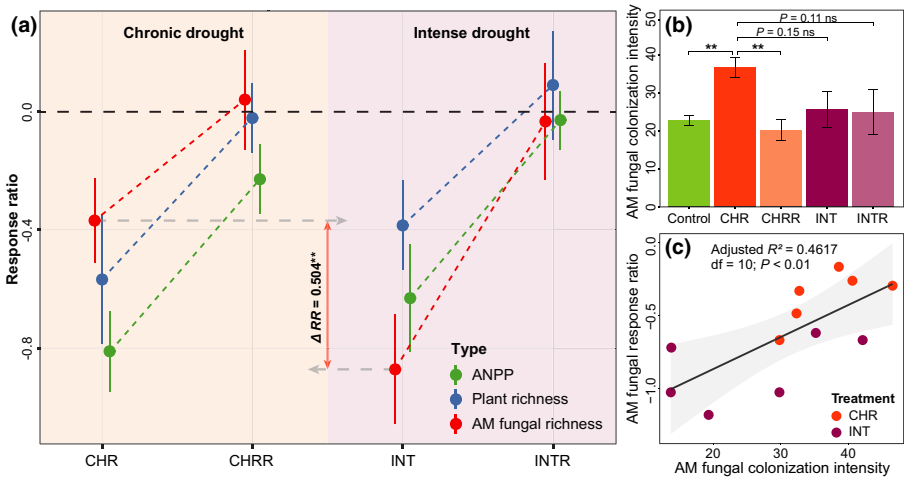

Fig. 4 Standardized arbuscular mycorrhizal (AM) fungal diversity and plant community responses. (a) The response ratio of AM fungal richness, plant richness, and aboveground net primary productivity (ANPP). The filled dots and whiskers represent the mean response ratio and 95% confidence intervals. AM fungal richness, plant richness, and ANPP response ratios (relative to control) were calculated using AM fungal ASVs (amplicon sequence variants) richness (red), plant richness (blue), and ANPP (green), respectively. Δ*RR*, average variation of response ratio between CHR and INT treatment. (b) AM fungal colonization intensity. The horizontal lines marked treatments in comparison, and significance of difference was tested using the Wilcoxon signed-rank test (**, $P < 0.01$), ns, nonsignificant; error bars represent ± SE calculated per treatment. (c) The response relationship between AM fungal colonization intensity and AM fungal response ratio (calculated using AM fungal ASVs richness). The linear regressions were fitted by using the OLS model and the gray shading area around represents the 95% confidence intervals. CHR, chronic drought; CHRR, chronic drought followed by recovery; INT, intense drought; INTR, intense drought followed by recovery.

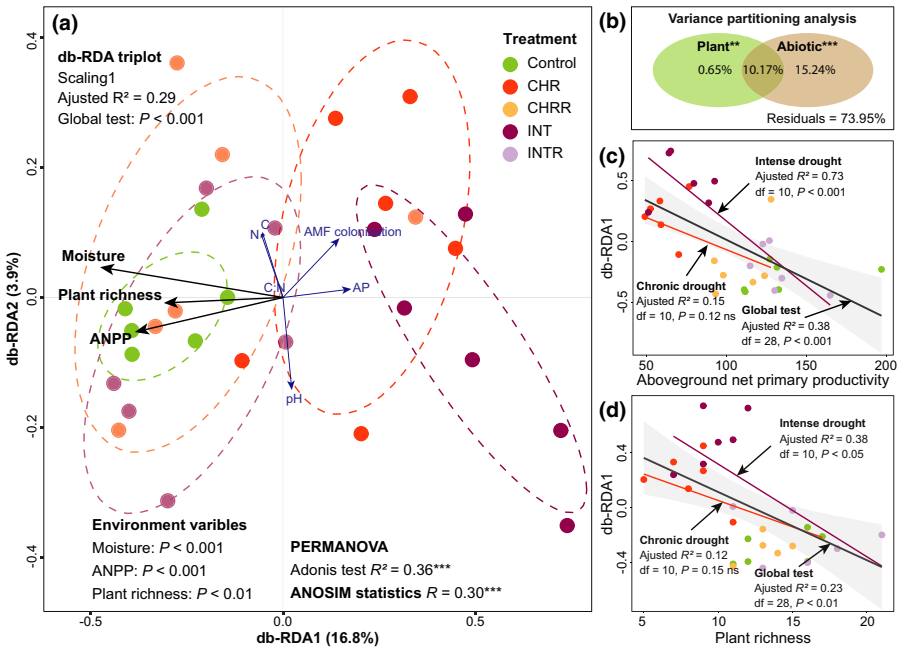

Fig. 5 Arbuscular mycorrhizal (AM) fungal community response and its potential drivers. (a) Constrained ordination analysis (distance-based redundancy analysis (db-RDA) at genus level) revealed the main factors responsible for the AM fungal community compositional response (Permutation test ($n = 9999$); *, $0.01 \leq P < 0.05$; **, $0.001 \leq P < 0.01$; ***, $P < 0.001$). (b) Variance partitioning analysis of plant and abiotic variables in predicting AM fungal community composition shifts (Permutation test ($n = 9999$); *, $0.01 \leq P < 0.05$; **, $0.001 \leq P < 0.01$; ***, $P < 0.001$). (c) Regression analysis of aboveground net primary productivity (ANPP) and the constrained AM fungal community variation (db-RDA1). (d) Regression analysis of plant richness and the constrained AM fungal community variation (db-RDA1). The linear regressions were fitted by using the OLS model and the gray shading area around represents the 95% confidence intervals. CHR, chronic drought; CHRR, chronic drought followed by recovery; INT, intense drought; INTR, intense drought followed by recovery.

richness ($R^2_{chronic}$ = 0.12, $P > 0.05$ to $R^2_{chronic}$ = 0.38, $P < 0.05$; Fig. 5d) with db-RDA1 markedly increased from chronic drought to intense drought.

Plant–arbuscular mycorrhizal fungi co-occurrence network

The integrated correlation network included 179 predicted non-randomly distributed biotic edges among 41 plant species (productivity) and 489 AM fungal ASVs (Fig. 6a; Table S3). The network showed a strong co-response of plants and AM fungal species (Fig. 6a). Specifically, most of the inter-kingdom links were limited to AM fungal genera *Dominikia*, *Glomus*, *Rhizophagus*, *Claroideoglomus*, *Diversispora*, and an unclassified genus of Glomeraceae, and most of these fungal genera belonged to *Glomerales* (except for *Diversispora*) (Fig. 6b). For the plant community, *Artemisia dracunculus, Calamagrostis angustifolia*, *Cleistogenes squarrosa*, *Potentilla acaulis*, and *Potentilla verticillaris* had the largest number of inter-kingdom links with AM fungi (Table S4). The co-occurrence network also contains intra-kingdom links within plant species or AM fungal ASVs. For example, AM fungal ASVs within genera *Dominikia*, *Glomus*, *Septoglomus*, and *Diversispora*, and plant species of *Cleistogenes squarrosa*, *Pulsatilla turczaninovii* had the largest number of intra-kingdom links in the network. Notably, ASVs within the AM fungal genera *Septoglomus*, *Paraglomus* only showed intra-kingdom links (Fig. 6b).

Plant community mediates soil arbuscular mycorrhizal fungal community responses to extreme drought

As shown in Fig. 7(a) ANPP to BNPP ratio (used to quantify plant adaptive strategy) was significantly decreased under continuous drought (CHR/INT) compared to control; by contrast, ANPP to BNPP ratio was significantly increased compared to control under recovery (CHRR/INTR). Further regression analysis showed a significant positive relationship between plant adaptive strategy and AM fungal richness (adjusted R^2 = 0.36, $P < 0.001$; Fig. 7b). The SEM was performed to test the direct and indirect effects of extreme drought on soil AM fungal community responses (Fig. 7c). In total, the model explained 64% and 88% of the AM fungal richness and community composition (db-RDA1) responses, respectively. In the model, precipitation had direct effects on AM fungal community composition (standard estimates = −0.21, $P < 0.05$) and richness (standard estimates = 0.58, $P < 0.001$) via changes in soil moisture, while also had indirect effects on AM fungal richness through plant adaptive strategy (standard estimates = 0.38, $P < 0.01$).

Discussion

Despite the fundamental role of the symbiotic association in driving plant and AM fungal community dynamics (Tedersoo *et al.*, 2020), it remains unclear how AM fungal communities respond to and recover from climate extremes in natural ecosystems and whether this response is associated with plant communities. In this study based on a grassland extreme drought experiment, we found that: (1) AM fungal richness and community composition showed low resistance but high resilience to extreme drought; (2) responses of AM fungal richness and community composition can be jointly explained by soil moisture, plant richness, and aboveground net plant productivity; (3) regression analysis showed that the robustness of the plant–AM fungal synergistic community response increased with drought intensity; (4) further network analysis showed that species of Glomerales dominated the AM fungal community response with plant species, indicating its key role in the plant–AM fungi interactions under drought. Our findings suggest that soil AM fungal communities were sensitive to climate extremes, and this sensitivity was associated with plant community dynamics. Based on the experimental results we proposed that plant drought adaptive strategy mediated AM fungal community responses to extreme drought.

The AM symbiosis dates back 450 million years ago (Ma) (Brundrett & Tedersoo, 2018), and likely helped plants to adapt to the early harsh environments of terrestrial ecosystems (Wang *et al.*, 2020). Within the mutualism, plant carbon is the only energy source for the fungus, which is usually constrained by

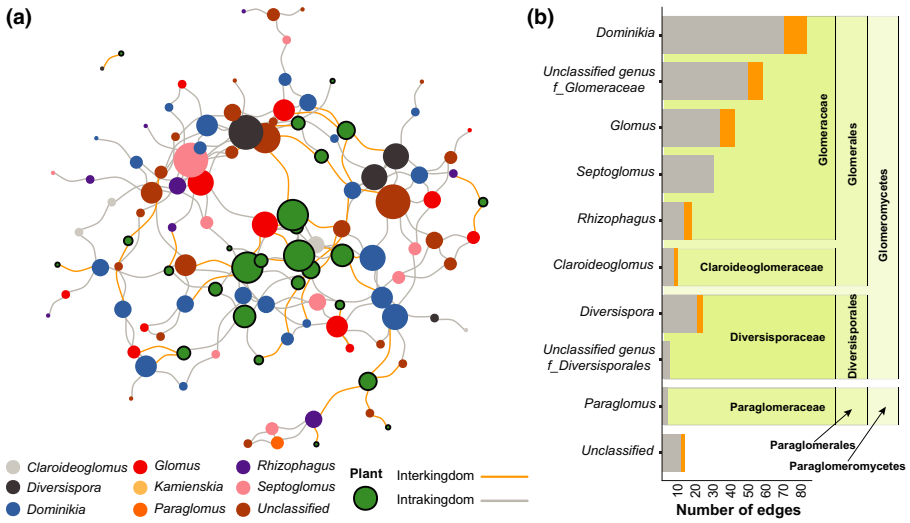

Fig. 6 Network analyses for exploring plant–arbuscular mycorrhizal (AM) fungi cross-kingdom correlation. (a) Plant–AM fungal cross-kingdom co-occurrence network. Nodes represent AM fungal ASVs (amplicon sequence variants) and plant species productivity; edges represent significant Spearman correlations ($P < 0.01$). Node size were weighted by the number of its edges. Gold lines represent inter-kingdom correlations; gray lines represent intra-kingdom correlations. (b) Edge distribution across AM fungal phylogenetic taxa.

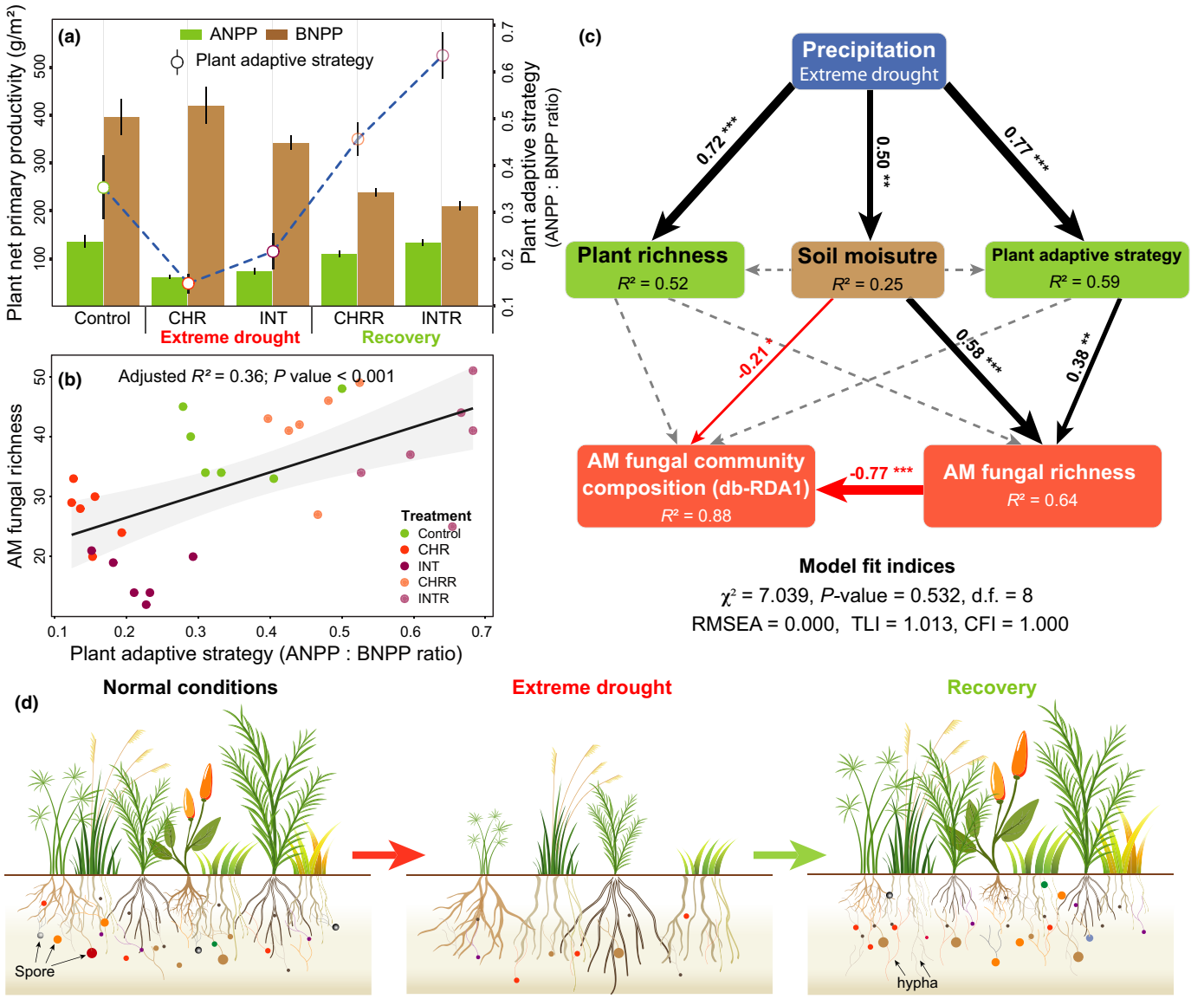

Fig. 7 A hypothesis on how plant community mediates soil arbuscular mycorrhizal (AM) fungal community responses to extreme drought. (a) Plant aboveground net primary productivity (ANPP), belowground net primary productivity (BNPP) and plant adaptive strategy responses to extreme drought. The plant drought adaptive strategy was quantified using ANPP : BNPP ratio. The hollow dots and whiskers represent the mean response ratio and 95% confidence intervals; error bars represent ± SE calculated per treatment. (b) The response relationship of plant adaptive strategy and AM fungal ASVs (amplicon sequence variants) richness. The linear regressions were fitted by using the OLS model and the gray shading area around represents the 95% confidence intervals. (c) Using a fitted structural equation model (SEM) to identify the direct and indirect pathways through which extreme drought affects the soil AM fungal community. Significant pathways are marked by: *, $0.01 \leq P < 0.05$; **, $0.001 \leq P < 0.01$; ***, $P < 0.001$. (d) Schematic diagram on the synergistic community response of plant and AM fungi. In brief, extreme drought decreased plant photosynthesis but allocated relatively more carbon to plant roots. This plant adaptive strategy reduced plant carbon supply to AM fungi, which had negative impacts on AM fungal communities. In the recovery process, plants allocated more carbon to the aboveground, increasing plant photosynthesis, which in turn supported AM fungal community recovery and development. CHR, chronic drought; CHRR, chronic drought followed by recovery; INT, intense drought; INTR, intense drought followed by recovery.

plant photosynthesis linked to plant aboveground biomass. Thus, finely-tuned interplay evolved between plants and AM fungi, mainly based on the carbon-for-nutrient exchange (Kiers *et al.*, 2011; Wang *et al.*, 2017). Such an exchange could explain our findings that AM fungal community responses can be explained by aboveground net plant productivity (Figs 2f, 5). Subsequently, the symbiotic association would further influence community responses of both partners within a sufficient temporal scale (Chomicki *et al.*, 2019). In our case, a positive correlation between plant and AM fungal richness was observed after 3 yr of extreme drought (Fig. 2e). The symbiotic mutualism has already been addressed in laboratory studies and may explain the diversity–diversity relationships between plants and AM fungi. For example, plants preferentially allocate more photosynthate to

the more beneficial AM fungal partners (Bever *et al.*, 2009); in turn, AM fungi would trade more phosphorus for carbon from plants when alternative AM fungi competitors are present (Argüello *et al.*, 2016). This preferential-interplay may lead to phylogenetic clustering of AM fungal communities, which is frequently reported in undisturbed natural ecosystems (Horn *et al.*, 2014; Liu *et al.*, 2015; Egan *et al.*, 2017; also in this study where five of six control plots had NRI and NTI higher than zero; Fig. 3a,b). However, the AM fungal communities became phylogenetically over-dispersed upon intense drought (NRI and NTI significantly lower than zero; Fig. 3a,b) (Webb *et al.*, 2002), which may indicate decreased preferential-interplay by both partners. This response pattern is in line with some early studies reporting the phylogeny of the AM fungal communities could be over-dispersed upon elevated abiotic stress levels such as upon fertilizer inputs (Liu *et al.*, 2015). However, how the carbon-nutrient exchange regulates the AM fungal community response to climate changes remains largely unknown.

It is universally acknowledged that environmental factors play a crucial role in shaping AM fungal communities (Vályi *et al.*, 2016; Deveautour *et al.*, 2020). In line with these previous studies, our results also showed that soil moisture has a significant effect on AM fungal diversity (Figs 2d, 5). Interestingly, Glomeromycota (or Glomeromycotina) is the only fungal phylum that showed significant negative responses to drought (Fig. 2a,b). This unique response was tightly associated with plant community responses (Figs 2e,f, 4, 5), suggesting that the symbiotic association with plants is the reason why the response of AM fungal community differs from that of other fungal clades. Given the positive interactions between AM fungi and plant community responses (Fig. 2e, f), we argue that plant and AM fungal communities may respond cooperatively to grassland extreme drought. This finding differs from previous empirical and theoretical studies highlighting that AM fungi drive plant community dynamics or *vice versa* (Hart *et al.*, 2001). Such inconsistency could be caused by the experimental setup and the spatial scale of the research (Vályi *et al.*, 2016; Hempel, 2018). Previously, plant–AM fungi interaction studies usually control one partner to investigate its effect on the other, which neglected dynamic community changes of both partners, and which may thus not be applicable to predict climate-driven community dynamics in natural ecosystems. Likewise, large-scale investigations may suffer from biogeographical constraints (Veresoglou *et al.*, 2019), environmental heterogeneity (Horn *et al.*, 2017), and diversified community successional stages (Gao *et al.*, 2019), which could mask plant–AM fungal community interactions. This synergistic response may explain that the robustness of this community association increases with drought intensity (Figs 2e,f, 5c, d), perhaps indicating intensified mutual interdependence between plants and AM fungi.

The sensitivity of individual species to drought may also influence community responses. Specifically, drought-induced growth, reproduction, and mortality responses may differ among plants and AM fungal species, and also between drought treatments, thereby inducing local response inconsistencies. This may also explain the variation of the association robustness from chronic to intense drought (Figs 2e,f, 5c,d). For plant and AM fungal species, stress tolerance could be increased by the symbiotic association (Smith *et al.*, 2009). Specifically, if we take mycorrhizal colonization as an indicator of plant–AM fungi interdependence, then both partners may benefit more from the intensified mycorrhizal colonization under drought. This may partially explain our results that, compared with INT treatment, AM fungi have a lower drought sensitivity under CHR treatment (Fig. 4a), where mycorrhizal colonization was significantly higher (Fig. 4b,c).

The co-occurrence network analysis can help to identify potential interactions between plants and AM fungi and may indicate whether they respond to environmental changes in the same way (Barberán *et al.*, 2012; de Vries *et al.*, 2018). By doing so, we detected a strong cross-kingdom network between plant species (productivity) and the AM fungal ASVs in response to extreme drought (Fig. 6). This result reinforces our point that plant communities are involved in AM fungal community responses. For plant–AM fungal interactions, the links between plants and AM fungi probably mean AM fungal community responses to extreme drought can be affected by plants and *vice versa*. Given that AM fungal functions show phylogenetic conservatism within AM fungal clades (Maherali & Klironomos, 2007; Yang *et al.*, 2017), it is highly likely that different AM fungal clades interact differently with plants (Öpik *et al.*, 2009). This concept might explain why the integrated network showed a nonrandom distribution pattern, with most of the inter-kingdom links involving Glomerales (Fig. 6b). Previous synthetic studies showed that species from Glomerales (e.g. *Glomus deserticola*, *Claroideoglomus etunicatum*) had the largest effects on plant drought resistance (Augé *et al.*, 2015). By contrast, AM fungal ASVs in *Septoglomus*, *Paraglomus*, and an unclassified genus of Diversisporales only showed intra-kingdom links (Fig. 6b). This absence of abundance-productivity relationships of these AM fungal genera with plants may suggest that they interact differently with plants or lack of competitiveness with other AM fungal genera. For example, introduction of nonnative *Rhizophagus irregularis* could outcompete native AM fungal communities composed of *Diversispora*, *Septoglomus* and *Paraglomus* species (Symanczik *et al.*, 2015). Nevertheless, very few studies have yet compared the interactiveness of different AM fungal clades with plants; it is thus too early to formulate the interaction patterns through the plant–AM fungal co-occurrence network.

The adaptive strategy of plants to environmental stresses has not been fully considered in most AM fungal community studies. Climate change not only affects the composition of plant communities (Hoover *et al.*, 2014), but also influences plant carbon allocation (Hasibeder *et al.*, 2015; Liu *et al.*, 2018). The plant adaptive strategy to drought stress could reduce carbon supply to belowground AM fungal communities due to decreased plant photosynthesis aboveground (Fuchslueger *et al.*, 2014; Karlowsky *et al.*, 2018). Possibly, the carbon starvation would reduce AM fungal richness through (1) enhanced interspecific competition; and/or (2) plant preferential carbon allocation to the more beneficial AM fungal species (Bever *et al.*, 2009; Kiers *et al.*, 2011); and/or (3) carbon demand (or sensitivity) differences between species. However, further investigations should be carried out to test these potential mechanisms particularly under

field conditions. Additionally, the SEM also supports that precipitation can have a considerable positive effect on AM fungal richness through plant adaptive strategy (Fig. 7c). When plant adaptive strategy was replaced by ANPP, the model showed no significant effect of ANPP on AM fungal richness and community composition. This may suggest that although higher ANPP ensures higher aboveground photosynthesis, how plants affect AM fungi depends on the carbon allocation strategy of plants under environmental stress. Therefore, the positive correlation between ANPP and AM fungal diversity under extreme drought is likely a manifestation of this plant adaptive response. Taken together, the present study suggests that plant adaptive strategy may play a key role in AM fungal community responses to extreme drought (Fig. 7d).

Climate change is imposing enormous threats to natural ecosystems and has caused substantial alterations of biodiversity patterns across ecosystems (Bellard *et al.*, 2012). Integrating and disentangling the interplay between aboveground plants and belowground microbes remains an essential step to understand the ecological impacts of climate change. AM fungi, an ancient group of fungi forming mutualistic symbioses with the majority of land vascular plants (Brundrett & Tedersoo, 2018), hold enormous significance for an integrated ecosystem. Our findings clearly uncovered the sensitivity of AM fungal communities and their response associations with plant communities under extreme drought. With the predicted increasing frequency and severity of climate extremes worldwide, such community sensitivity of AM fungi is expected to have substantial impacts on its ecological functions and further influence plant community dynamics aboveground.

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Author contributions

BC, WF, MCR and JJ conceived the idea of this study. XH, HHW 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, HW and AZ measured the soil physicochemical properties. WF performed AM fungi related experiments, analyzed all the data and created all the figures. WF, BC, HHW and MCR wrote the first draft of the manuscript, and all co-authors contributed substantially to the revisions.

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Data availability

Raw sequencing data and metadata supporting the results have been deposited in Figshare (https://doi.org/10.6084/m9.figshare.14495811.v2).

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Supporting Information

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

Fig. S1 Experimental design and setup.

Fig. S2 Rarefaction curve of fungal reads in each sample.

Fig. S3 AM fungal community composition.

Fig. S4 AM fungal alpha diversity calculated using dataset2.

Fig. S5 Principal coordinates analysis (PCoA, Bray–Curtis) of AM fungal community response to extreme drought.

Fig. S6 Regression analysis of AM fungal ASVs richness with plant richness and ANPP based on dataset2.

Table S1 All the plant species observed in the experimental plots.

Table S2 List of R packages and functions for data processing and statistical analyses.

Table S3 Properties of plant–AM fungi network.

Table S4 The number of inter-kingdom edges of plant species.

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