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RESEARCH ARTICLE

Arbuscular mycorrhizal fungi attenuate negative impact of drought on soil functions

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Abstract

Although positive effects of arbuscular mycorrhizal (AM) fungi on plant performance under drought have been well documented, how AM fungi regulate soil functions and multifunctionality requires further investigation. In this study, we first performed a meta-analysis to test the potential role of AM fungi in maintaining soil functions under drought. Then, we conducted a greenhouse experiment, using a pair of hyphal ingrowth cores to spatially separate the growth of AM fungal hyphae and plant roots, to further investigate the effects of AM fungi on soil multifunctionality and its resistance against drought. Our meta-analysis showed that AM fungi promote multiple soil functions, including soil aggregation, microbial biomass and activities of soil enzymes related to nutrient cycling. The greenhouse experiment further demonstrated that AM fungi attenuate the negative impact of drought on these soil functions and thus multifunctionality, therefore, increasing their resistance against drought. Moreover, this buffering effect of AM fungi persists across different frequencies of water supply and plant species. These findings highlight the unique role of AM fungi in maintaining multiple soil functions by mitigating the negative impact of drought. Our study highlights the importance of AM fungi as a nature-based solution to sustaining multiple soil functions in a world where drought events are intensifying.

KEYWORDS
arbuscular mycorrhizal (AM) fungi, drought, global change, meta-analysis, multifunctionality, soil function

1 | INTRODUCTION

Ongoing climate change is predicted to intensify precipitation variability; one of the consequences is a progressive increase in the severity and duration of droughts (IPCC, 2021). As water is an essential resource for sustaining life, drought has been deemed a widespread crisis in terrestrial ecosystems (Schimel, 2018). Drought negatively affects plant productivity and the soil microbial community (Schimel, 2018), and, as a consequence, associated ecological processes driven by microbes, including soil aggregation, litter decomposition and soil nutrient cycling (Chen et al., 2023; Rillig et al., 2019). In addition, the frequency of precipitation could also exert a substantial impact on soil processes, even when the total amount of water remains unchanged (Bowles et al., 2018; Chen et al., 2023; Hoover et al., 2022). For example, intensified soil water variability (e.g. water supply with low frequency) promotes the breakdown of macro-aggregates compared to conditions with less variability in soil water status (achieved for example by watering

with high frequency), despite the total water supply remaining constant in both scenarios (Chen et al., 2023). Thus, there is a need to understand the mechanisms through which soil functions and processes react to alterations in both the amount and frequency of precipitation. Only with such information can we better understand and predict the shifts in soil functions in the context of future climate change.

Soil organisms play an indispensable role in maintaining soil functions and processes (Anthony et al., 2023; Delgado-Baquerizo et al., 2017). As an important functional group among soil organisms, arbuscular mycorrhizal (AM) fungi can form symbiotic associations with approximately 80% of terrestrial plant species, providing a wide array of benefits to their plant hosts in exchange for carbohydrates and lipids (Luginbuehl et al., 2017). Thus, much attention over the past decades has focused on the role of AM fungi in plant growth and its responses to drought (Abdalla et al., 2023; Augé, 2001; Cheng et al., 2021; Cosme, 2023; Püschel et al., 2020, 2021; Tang et al., 2023). For example, AM fungi have been shown to facilitate phosphorus uptake by plants even under severe drought stress (Püschel et al., 2021), and shape the evolution of plant adaptation to drought (Cosme, 2023). However, the impact of AM fungi usually extends beyond their influence on plants; in fact, they can affect soil functions and processes in several ways (Figure 1a). AM fungi have been estimated to utilize up to 20% of the plant net photosynthates (Jakobsen & Rosendahl, 1990), which are then released into the soil matrix via hyphal exudation (Drigo et al., 2010; Kaiser et al., 2015; Kakouridis et al., 2024). Exudates released by extensive hyphal networks in the soil can stimulate the activity of microbial communities around hyphal surfaces, and thus enzyme production (Cheng et al., 2012; Frey, 2019), which, in turn, could facilitate aspects of nutrient cycling in soils (Frey, 2019). Besides, AM fungi produce organic binding agents and can physically entangle and enmesh soil particles (Rillig & Mummey, 2006), thus contributing to the formation and stabilization of soil aggregates (Frey, 2019; Wu et al., 2023). Furthermore, AM fungi can improve the formation of soil aggregate and substrate hydraulic properties under drought (Ji et al., 2019; Püschel et al., 2021). Given that AM fungi can affect multiple soil functions simultaneously (that is, multifunctionality), their role in regulating the resistance of soil multifunctionality against drought requires further investigation. This information is essential to our understanding of the importance of this key symbiosis in maintaining multiple soil functions and processes in the currently unfolding scenario of increasing precipitation variability.

To address these knowledge gaps, we first conducted a meta-analysis to test the role of AM fungi in regulating soil functions in responses to water variability. On the one hand, we found that the studies included in the meta-analysis paid little attention to soil multifunctionality. On the other hand, almost all studies only explored the effect of the amount of water supply and little is known about the effect of watering frequency. To further investigate how AM fungi regulate soil multifunctionality in responses to both the amount and frequency of water supply, we then performed a greenhouse experiment, using a pair of hyphal ingrowth cores (that is, a paired design of static and rotated cores; Figure S1) to spatially separate the growth of AM hyphae and plant roots (Johnson et al., 2001). Meanwhile, we independently manipulated the amount (e.g. well-watered vs.

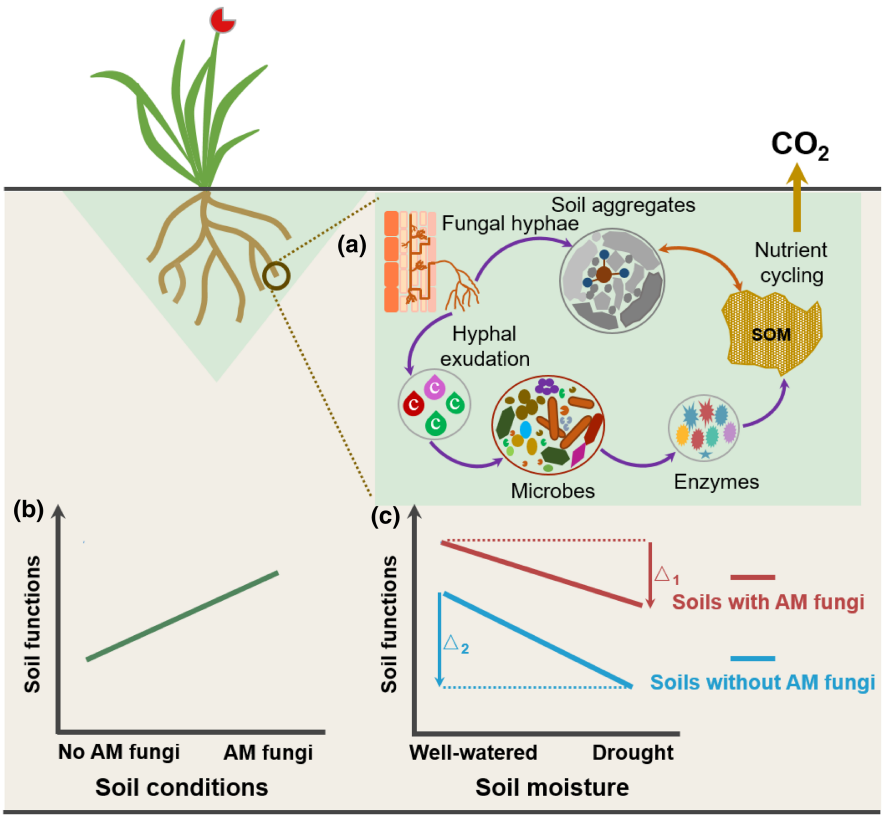

FIGURE 1 The potential pathways through which arbuscular mycorrhizal (AM) fungi influence multiple soil functions (a) and the hypotheses tested in the study (b and c). Releasing labile carbon in the form of exudation by extensive hyphal networks can influence the soil microbial community, and thus enzyme production. In addition, AM fungi produce organic binding agents and physically entangle and enmesh soil particles, both of which jointly contribute to the formation and stabilization of soil aggregate. These processes have the potential to regulate the dynamics of soil organic matters, and thus nutrient cycling in soils (a). Given the unique role of AM fungi, we hypothesize that AM fungi could facilitate multiple soil functions (b). Furthermore, we hypothesize that AM fungi could attenuate the negative impact of drought on multiple soil functions (i.e., $|\triangle_1| < |\triangle_2|$; c), and finally enhance the resistance of both against drought.

drought) and frequency (e.g. high vs. low) of water supply to simulate variability in water availability (Figure S2). Host plant functional group has been considered one of the important determinants of AM fungal functions (Hoeksema et al., 2010; Romero et al., 2023). Although many AM fungal species are capable of forming symbiosis with a wide range of host plants, there seems to be a substantial difference in the ecological consequences of specific plant-AM fungi combinations (Bever et al., 1996; Šmilauer et al., 2020). Therefore, three host plants (*Lolium perenne*, *Achillea millefolium* and *Trifolium repens*) from different plant functional groups (e.g. grass, forb and legume) were used as model species, which also significantly differ in functional traits (Table S1) (Lozano et al., 2020). In this study, we measured the following variables, including litter decomposition, soil DNA concentration, soil respiration, activities of four soil enzymes related to soil nutrient cycling, and soil aggregation. We calculated a soil multifunctionality index on the basis of these variables. Given the positive effects of AM fungi on soil aggregation and nutrient cycling (Ji et al., 2019; Li et al., 2023), we hypothesize that (1) AM fungi can promote multiple soil functions and thus multifunctionality (Figure 1b), regardless of watering amount and frequency; and that (2) AM fungi have the potential to attenuate the negative impact of drought on soil functions and multifunctionality (downward arrows in Figure 1c; that is, $|\triangle 1| < |\triangle 2|$), and, therefore, increase their resistance against drought.

2 | MATERIALS AND METHODS

2.1 | Meta-analysis

2.1.1 | Data collection

We conducted a literature search using the Web of Science (WoS, http://apps.webofknowledge.com/) and China Knowledge Resource Integrated Databases (CNKI, www.cnki.net/) on October 20, 2023, with the following keyword combinations for topic search: ("arbuscular mycorrhizal fung*" OR "AM fung*" OR "AMF" OR "AM symbios*") AND ("drought" OR "water deficit" OR "water stress" OR "water regime*") AND ("soil aggregat*" OR "decomposition" OR "soil enzym*" OR "respiration" OR "microbial activit*" OR "microbial biomass" OR "soil function*" OR "bacterial abundance" OR "fungal abundance" OR "microbial abundance"). In the WoS, databases used for our search included WoS Core Collection, Current Contents Connect, KCI-Korean Journal Database, MEDLINE and SciELO Citation Index. To avoid bias, the studies included in our dataset were required to meet the following criteria: (1) The study had to report at least one soil function of interest (see Table S2). (2) The study had to report the means of soil functions and sample sizes. (3) If a study used the static/rotated core, it must meet the following conditions to be included: (i) the only difference between the two ingrowth cores during the experiment was that one of them would be rotated; (ii) containing both well-watered (control) and drought treatment. (4) If a study used sterilized soil samples which were inoculated with AM fungal inoculum, it must meet the following criteria to be included: (i) the inoculated and non-inoculated groups must be identical except for the factor of AM fungal inoculation; (ii) both the inoculated and non-inoculated groups were required to have a control and drought treatment; (iii) to avoid any confounding effects, we excluded instances of AM fungal inoculation in combination with uncontrolled microorganisms (e.g. the non-AM fungal communities added only in the inoculated or non-inoculated groups); (iv) we excluded studies in which the non-mycorrhizal group was established using fungicides (e.g. benomyl) due to the potential non-target effects, as fungal inhibitors could eliminate other fungal groups, for example pathogens. The procedure for article selection followed the PRISMA guidelines (Figure S6). Finally, a total of 32 papers were compiled in our dataset. In each study, we extracted means, sample size, and either standard deviation (SD), standard error (SE), or 95% confidence interval (CI) if available. Unspecified error bars were treated as SE. When the results were graphically presented, the data were digitized using the software WebPlotDigitizer 4.6 (https://automeris.io/WebPlotDigitizer/).

2.1.2 | Calculation of effect size and variance

We used the natural log response ratio (lnRR), a metric widely used in meta-analysis (Hedges et al., 1999), to quantify the effects of AM fungi on soil functions:

$$\mathrm{lnRR} = \ln\left(X_T / X_C\right) = \ln X_T - \ln X_C, \quad (1)$$

where X_T and X_C are the mean values of soil functions in inoculated and non-inoculated groups, respectively. The variance (v) of each lnRR was calculated as follows:

$$v = \frac{SD_T^2}{N_T X_T^2} + \frac{SD_C^2}{N_C X_C^2}, \quad (2)$$

where SD_T and SD_C are the SD in the inoculated and non-inoculated groups, respectively; N_T and N_C are the sample size in inoculated and non-inoculated groups, respectively. If a study reported SE, then the corresponding SD was calculated as follows:

$$SD = SE \times \sqrt{n}, \quad (3)$$

where n is the sample size. If only 95% CI was reported in a study, the SD was calculated as:

$$SD = \left(CI_u - CI_l\right)\sqrt{n} / 2Z_{\alpha/2}, \quad (4)$$

where CI_u and CI_l indicate the upper and lower limits of the 95% CI, respectively. $Z_{\alpha/2}$ represents the Z score for a given level of significance (e.g. 1.96 at $\alpha = .05$). If in a study, microbial biomass was further separated into different groups, for example, bacterial biomass, fungal biomass and so on, then their sum value was considered as the microbial biomass. The corresponding SD values were calculated following the method of error propagation (Lorber, 1986):

$$SD_{sum} = \sqrt{SD_1^2 + SD_2^2 + \ldots + SD_n^2}, \quad (5)$$

where SD_{sum} is the new SD for the sum values, with SD_1, SD_2 and SD_n as the values of each component of microbial biomass. The same method was used to calculate phosphatase activity when a study simultaneously reported two or more activities of phosphatase, for example acid phosphatase, alkaline phosphatase, and neutral enzyme activity. In the studies that did not report SD, SE or 95% CI in our dataset, the *Bracken1992* approach was used to impute missing SD using the R package *metagear* (Lajeunesse, 2016). It should be noted that these methods have also been used to calculate the effect size of drought on soil functions.

2.2 | Experiment

2.2.1 | Experimental design

The experiment was carried out in a glasshouse at Freie Universität Berlin, Germany (Figure S3). A pair of hyphal ingrowth cores featuring openings covered with a pore size of 38 μm mesh (preventing penetration of plant roots but allowing hyphal passage) was inserted into each microcosm (Figure S1). One core remained stationary (AM hyphae access allowed), while the other was rotated to periodically sever the hyphae, creating a volume of soil free of AM fungal hyphal influence. To simulate water variability, we independently manipulated both the amount (well-watered vs. drought) and frequency (high vs. low) of water supply (Figure S2). In addition, three plants with widely differing functional traits (Table S1), *L. perenne* (grass), *A. millefolium* (forb) and *T. repens* (legume), were treated as host plants in this study. Each treatment is replicated eight times, resulting in a total of 96 microcosms (2 watering amount × 2 watering frequency × 3 plant species × 8 replicates). Microcosms were randomly distributed in the glasshouse and re-distributed every 2 weeks during the experiment (for more details on the experiment see sections below).

2.2.2 | Soils, hyphal ingrowth cores and litter bags

The soil used in the study was an Albic Luvisol collected from the top 20 cm of a local grassland experimental site of Freie Universität Berlin. Soil samples were passed through a 2 mm sieve and well homogenized, and then air-dried at room temperature. The properties of soil samples were as follows: 1.87% carbon, 0.12% nitrogen, 69 mg kg^{-1} phosphorus (calcium acetate-lactate), pH 7.1 (calcium chloride) (Rillig et al., 2010). Hyphal ingrowth cores were constructed using plastic tubes, 17.5 cm in length and 3 cm in diameter. Many openings with a size of 7 × 7 mm were cut into each tube (grid structure), the openings produced were equivalent to about 80% of the external surface area of the core. Nylon mesh with a pore size of 38 μm was attached to the wall and bottom of the core using silicone glue, which was chosen to prevent the penetration of plant roots but not AM hyphae. To determine the consequences of AM fungi on microbial decomposition, we made litter bags (3 cm × 3 cm) using 38 μm nylon mesh via an impulse sealer (Mercier Corporation). The litter bags were filled with 50 mg oven-dried and milled plant material (shoots of *Holcus lanatus*), and then sealed.

2.2.3 | Microcosm establishment and watering treatments

We established the microcosms using 96 pots (14.5 cm in diameter and 18 cm in height; Figure S3a). These pots were filled with two hyphal ingrowth cores and 1.9 kg soil to a final bulk density of 1.2 g cm^{-3}, simulating the bulk density at the field site (Figure S3b). Each core was filled with 220 g soil to the final bulk density, in which one litter bag was inserted at a depth of 8 cm (Figure S3b). Before transplanting, all microcosms were allowed to recover for 2 weeks at 70% of field water holding capacity (WHC) by weighing pots every 2 days. Seeds of the three host plants were surface sterilized with 70% alcohol for 2 min, germinated in a plastic box containing a mixture of air-dried soil and sterilized sand (50% w/w) at room temperature, and watered with sterilized water when needed. We ensured simultaneous germination of seeds from different plant species by altering the seeding time. Two seedlings of the same species were transplanted to positions on the diagonal in each pot (Figure S1). Starting from the completion of transplantation until the sampling phase, one ingrowth core remained stationary (static core), whereas the other one was rotated every 2 days by 2–3 mm horizontally (rotated core). In such static/rotated cores, hyphal connections are severed by rotating cores, thus eliminating hyphal access to the interior of the cores, while allowing such access in the static counterparts. The paired design of ingrowth cores provides us with an opportunity to study the impact of AM fungi on soil functions and processes, such as comparing soils without AM fungal activity (rotated core) to soils with AM fungal hyphae (static core), while keeping all other conditions identical. This method has been demonstrated to be highly effective and minimally disruptive in our preliminary experiment (Figure S4). The details of the preliminary experiment are provided in the Supplementary Methods. The microcosms were kept within a climate-controlled glasshouse with 22°C for 16 h during the day and 18°C for 8 h during the night. To ensure optimal lighting conditions, high-pressure sodium lamps were employed to supplement the light when the ambient light intensity dropped below 50 klx. All microcosms were maintained at 70% of WHC in the glasshouse for 8 weeks before the watering treatments began (Figure S3c).

In this study, we manipulated watering amount and frequency independently. To explore the effects of watering amount, these microcosms were maintained at 70% (well-watered) and 35% WHC (drought), respectively, by receiving different amounts of water (Figure S2). For each level of the watering amounts, the same amount of water was applied either in smaller amounts every day (high frequency hereafter) or in larger amounts every 3 days (low frequency hereafter) (Figure S2). The amount of water

replenished for each level of watering amount (high frequency) was determined by weighing pots every day; the recorded weights were used to calculate the amount of water supply for the low-frequency treatment (Figure S2). Therefore, both the high and low-frequency treatments for a given level of watering amount received the same amount of water overall. The watering treatments lasted 5 months before sampling. To correct the amount of water supply caused by changes in plant fresh biomass, we prepared an additional six microcosms for each species in each treatment. At the beginning and the third month of the watering treatment, we performed destructive sampling on three microcosms (3 replicates) for each species in each treatment, respectively. Plant fresh biomass (roots and shoots) was determined and used as a calibration parameter for water supply.

2.2.4 | Sampling and measurements

After 5 months of watering treatment, all of the ingrowth cores were harvested and immediately transported to the laboratory within ice boxes. Soil samples in each core were gently homogenized, and then divided into subsamples. Two grams of fresh sample was stored at −80°C for DNA extraction; 20 g was stored at 4°C for determination of soil CO_2 release; 5 g was stored at 4°C for measurements of soil enzyme activity. The remaining soil samples were air-dried for other analyses. In addition, we sampled plant roots, which were oven-dried at 40°C for determination of AM root colonization. Litter bags were stored at 4°C before further processing.

We measured the following soil functions and processes, including four enzyme activities related to soil nutrient cycling, litter decomposition, soil respiration, soil DNA concentration, and water-stable soil aggregates. The activities of phosphatase (organic phosphorus mineralization), β-D-celluliosidase (cellulose degradation), β-glucosidase (cellulose degradation), and N-acetyl-β-glucosaminidase (chitin degradation) were determined using a high throughput microplate assay (Jackson et al., 2013). The rate of litter decomposition was determined by calculating the proportional loss of litter weight during the experiment. We measured soil respiration using an infrared gas analyser (LiCOR 6400XT, Lincoln, NE, USA), which was used as an indicator of microbial activity (Yang et al., 2022). Soil DNA was extracted from 250 mg of fresh soil samples with DNeasy PowerSoil Pro Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions. DNA concentration was measured using a Qubit Fluorometer. The concentration of soil DNA has been widely employed as a powerful indicator for surface soil microbial biomass (Delgado-Baquerizo et al., 2016; Wagg et al., 2014), as it aligns closely with alternative methodologies indicative of microbial biomass (Gong et al., 2021; Taylor et al., 2002). Moreover, being a molecule rich in nitrogen and phosphorus, DNA could potentially function as a significant nutritional source for microbes (Pinchuk et al., 2008). Water-stable soil aggregates were measured by wet-sieving (Kemper & Rosenau, 1986), with some modifications (Yang et al., 2022). To verify the effectiveness of the hyphal ingrowth core system, we determined the length of AM fungal and non-AM fungal hyphae according to previous methods (Jakobsen et al., 1992; Mosse, 1959; Rillig et al., 1999). Percentage of plant roots colonized by AM fungi was determined to demonstrate the success of AM colonization following the magnified gridline intersect method (McGonigle et al., 1990). Full details of these measurements can be found in the Supplementary Methods.

2.2.5 | Assessing soil multifunctionality

In this study, we assessed soil multifunctionality using three approaches: (1) the averaging multifunctionality index, (2) the weighted multifunctionality index and (3) the principal component analysis (PCA)-based multifunctionality index. Before analyses, all individual soil functions were standardized between 0 and 1, as follows:

$$\text{Standardized soil function} = \frac{\text{soil function} - \min(\text{soil function})}{\max(\text{soil function}) - \min(\text{soil function})}, \quad (6)$$

where soil function is the raw value of each soil function. This transformation ensures that all individual soil functions are on the same comparable scale. We first calculated the averaging multifunctionality on the basis of the eight standardized soil functions. To down-weight highly correlated soil functions that we measured, we calculated the weighted multifunctionality index (Manning et al., 2018). To obtain this, we first performed a cluster analysis to generate a dendrogram tree using hierarchical agglomerative clustering. Then, the Elbow method was used to determine the optimal number of clusters, and the individual soil functions were weighted based on these clusters. Finally, the weighted multifunctionality index was derived by calculating the average of all weighted individual soil functions. The PCA-based multifunctionality index was calculated by summing the scores along oriented PCA axes weighted by their eigenvalues. It should be noted that the three multifunctionality indices are highly correlated with each other (Figure S5), underscoring that the selection of the multifunctionality index does not impact the integrity of our results. Therefore, we present the weighted soil multifunctionality in this study.

2.2.6 | Assessing resistance of soil functions against drought

To evaluate how AM fungi regulate the resistance of soil functions and multifunctionality against drought, we calculated the resistance index for static and rotated cores, respectively, as follows:

$$\text{Resistance index} = 1 - \frac{2\,|D - W|}{W + |D - W|}, \quad (7)$$

where D represents the soil functions under drought, and W is the mean value of soil functions under well-watered conditions. This index

offers the advantage of being both standardized by the control and constrained within a range of −1 (indicating minimal resistance) to +1 (reflecting maximal resistance), even in the presence of extreme values (Orwin & Wardle, 2004).

2.3 | Statistical analyses

2.3.1 | Meta-analysis

All statistical analyses were performed in R (R Development Core Team, 2019). We performed meta-analysis using the R package *metafor*. To test how AM fungi affect soil functions, we first calculated the weighted mean of lnRR ($\ln\overline{RR}$) for AM fungi and its 95% CI using a mixed-effects model (restricted maximum likelihood approach, REML), as follows:

$$\ln\overline{RR} = \frac{\sum_{i=1}^{m} w_i \ln RR_i}{\sum_{i=1}^{m} w_i}, \quad (8)$$

where m indicates the number of comparisons in the group, $\ln RR_i$ is the effect size of the ith study. The weighing of the ith study (w_i) was calculated as:

$$w_i = 1/v_i, \quad (9)$$

$$v_i = v + \tau^2, \quad (10)$$

where v_i is the variance of study (i), v indicates the within-study variance, and τ^2 represents the variance between studies. Because some studies provided more than one effect size in our dataset, we treated the observations nested within each study as a random factor in the mixed-effects model. The $\ln\overline{RR}$ was considered significant if the 95% CI did not overlap with zero. For ease of interpretation, the $\ln\overline{RR}$ was reverse-transformed and presented as a percentage change (%):

$$\text{Percentage change}\,(\%) = \left(e^{\ln RR} - 1\right) \times 100. \quad (11)$$

Second, to test whether AM fungi attenuate the negative effects of drought on soil functions, the approach of pooled estimation in the mixed-effects model was used to calculate $\ln\overline{RR}$ of drought and the corresponding 95% CIs for the moderator of AM fungi (e.g. with vs. without AM fungi). In these models, the overall heterogeneity of effect sizes can be divided into heterogeneity explained by moderators included in the model (Q_M) and residual heterogeneity (Q_E). We used the Q_M statistics to assess the significance of the moderator. Finally, to examine the potential impact of publication bias on the outcomes of this meta-analysis, we employed a funnel plot to assess the likelihood of publication bias, and the asymmetry of the funnel plot was examined through Egger's regression test. If asymmetry was identified, the Rosenberg fail-safe number was used to further test whether unpublished articles could potentially impact our conclusions. In addition, we used leave-one-out analysis to assess the robustness of our results. The quality of this meta-analysis was evaluated following the checklist of quality criteria proposed by (Koricheva & Gurevitch, 2014).

2.3.2 | Experiment

First, we applied a general linear model to test for effects of AM fungi, watering amount and frequency, plant species, and their interactions on soil functions and multifunctionality. Model residuals were checked to meet the assumptions of normal distribution and variance homogeneity. The data were log-transformed before analyses to meet the assumptions when needed. Second, we calculated the effect sizes of AM fungi to examine how AM fungi affect soil functions and multifunctionality using the Equation (1), in which X_T and X_C are the values of soil functions in static and rotated cores, respectively. We performed this calculation for each experimental unit (i.e. pot), given that both cores were included as pairs in each microcosm (i.e. paired design). A positive value of lnRR indicates a positive effect of AM fungi on soil functions and multifunctionality, while a negative value indicates the opposite. Third, to explore whether and how AM fungi regulate the responses of soil functions and multifunctionality to drought, we calculated the unpaired mean differences in soil functions and multifunctionality between drought and well-watered conditions for static and rotated cores, respectively, using the R package *dabestr* (Ho et al., 2019). This estimation is on the basis of a bootstrapping approach (5000 iterations), and directs attention to both the effect size and its accuracy. Furthermore, the insights provided by the effect size and confidence interval (CI) are reinforced by the depiction of the sampling error distribution plotted alongside. Fourth, we used a general linear model to test the effects of AM fungi, watering frequency, species and their interactions on the resistance of soil functions and multifunctionality against drought. Afterwards, the *t*-test was used to compare the difference in resistance index between static and rotated cores under a specific treatment. Given that rotated cores have the potential to cause changes in non-AM fungal hyphae and thereby affecting soil functions and multifunctionality, we treated hyphal length of non-AM fungi as a covariate in these linear models. Finally, to examine the extent to which rotated core-induced changes in non-AM fungal hyphae affect soil functions and multifunctionality, we used variation partitioning analysis to partition the effects of AM fungi and non-AM fungi (indicated by hyphal length) on soil functions and multifunctionality using the R package *vegan*.

3 | RESULTS

3.1 | Meta-analysis

We first performed a meta-analysis to examine how AM fungi affect multiple soil functions and the role of AM fungi in regulating the response of multiple soil functions to drought. On average, AM fungi promote soil functions (Figure S7). In terms of individual soil

functions, AM fungi had a positive effect on multiple soil functions, except for microbial abundance and activity and soil enzyme activity related to soil carbon cycling (Figure S7). Furthermore, we found that the positive effects of AM fungi on microbial biomass and activity, and soil aggregates were significantly higher under drought than under well-watered condition (Figure S8). We further calculated the effect size of drought on soil functions under AM fungi and without AM fungi conditions, respectively. Results showed that AM fungi significantly attenuate the negative impact of drought on microbial biomass and activity, soil aggregates and activities of soil enzymes related to P cycling (Figure 2).

In this meta-analysis, no evidence of publication bias for soil functions in response to AM fungi was observed (Figure S9, Egger's test: $p = .25$), which could be further demonstrated by the Rosenberg fail-safe numbers (Table S4). Although the funnel plots were asymmetrical for soil functions in response to drought (Egger's test: $p < .05$), the Rosenberg fail-safe numbers were much larger than $5k + 10$ (Table S4).

These findings suggest that the interpretation of our results is unlikely to be affected by publication bias. In addition, leave-one-out analysis showed that our findings are unlikely to be driven by a single influential study (Figure S10).

3.2 | Experiment

3.2.1 | Validation of experimental methods

Although the total water supply of high and low frequencies remained constant for a given level of watering amounts, we found

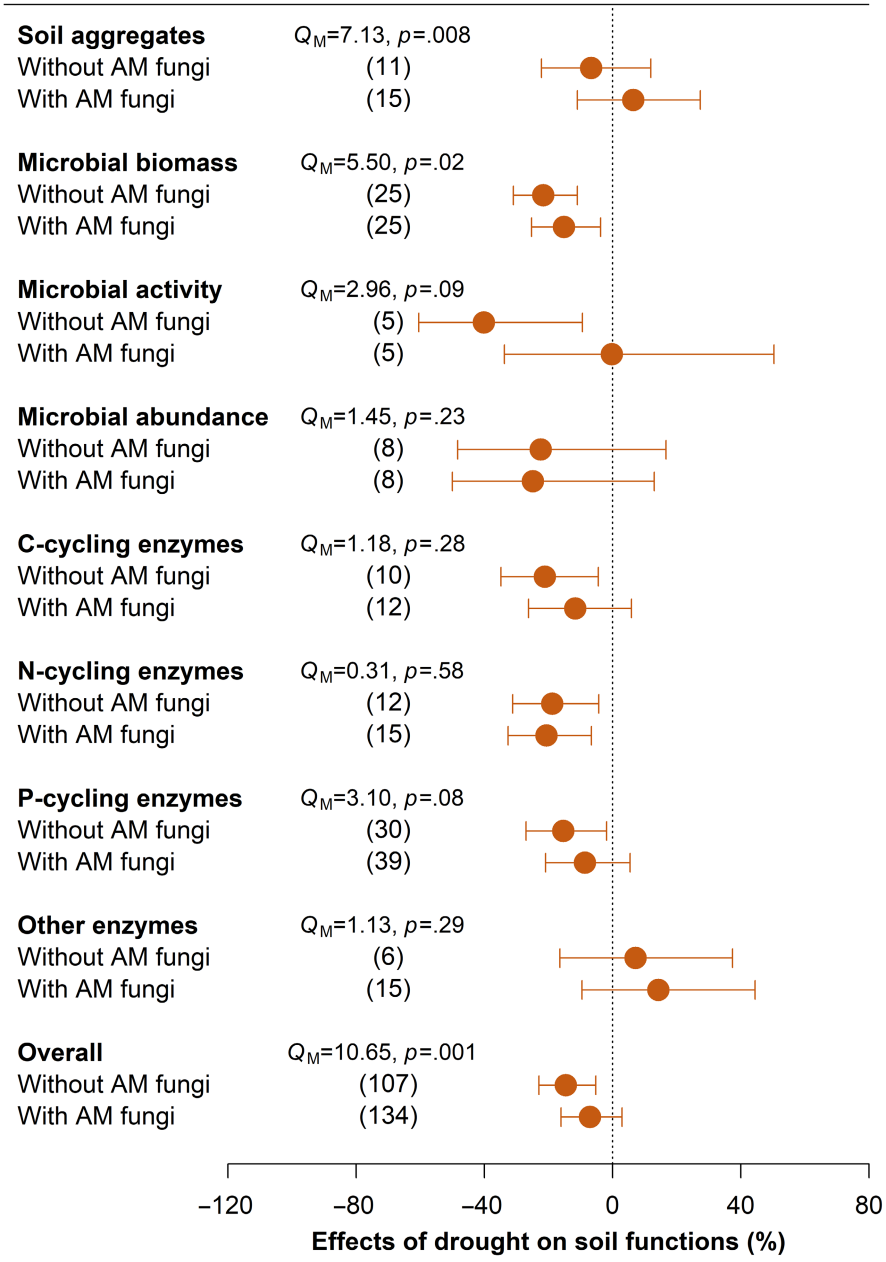

FIGURE 2 Meta-analysis of the effects of drought on soil functions in inoculated with arbuscular mycorrhizal (AM) fungi (with AM fungi) versus non-inoculated groups (without AM fungi). Circles and error bars represent the mean effect size and 95% confidence intervals (CIs), respectively. The numbers in brackets show the sample size. Overall refers to the pooling of all soil functions in the mixed-effects model. p-values derived from Q_M statistic indicate differences between the AM fungal treatments.

that low-frequency water supply significantly increased soil moisture variability (Figure S11). We found that only the identity of host species significantly affected AM fungal root colonization, with *T. repens* having the highest rate of colonization (Figure S12). Our results showed that rotated cores greatly reduced the length of AM fungal hyphae as expected (Figure S13). For non-AM hyphal length, a significant reduction was only observed in *L. perenne*, but not in *T. repens* and *A. millefolium* (Figure S13). The variance partitioning analysis suggested that changes in non-AM hyphae caused by core rotation have relatively little effect on soil functions and multifunctionality (0.01%–2.3%; Figure S14). Furthermore, the results from linear models showed that effects of AM fungi on soil functions and multifunctionality remain significant even when non-AM hyphal length is treated as a covariate (Table S5).

changed soil aggregates, soil DNA concentration, activity of β-D-celluliosidase and soil multifunctionality, we did not observe any significant interactions between AM fungi and watering frequency (Table S5). We found that all soil functions except for soil aggregates and litter decomposition were influenced by plant host identity (Table S5). However, a significant interaction between AM fungi and plant species was only observed for soil aggregates, litter decomposition and activity of N-acetyl-β-glucosaminidase (Table S5). Our results suggested that AM fungi had higher effects on litter decomposition and soil aggregates under *T. repens*, followed by the other two plant species, while the opposite pattern was observed for the activity of N-acetyl-β-glucosaminidase (Figure S18). In addition, we found that effects of drought on litter decomposition, activity of β-D-celluliosidase and soil multifunctionality were dependent on watering frequency (Table S5).

3.2.2 | Responses of soil functions and multifunctionality to AM fungi, watering amount and frequency, and plant species

Results of linear models showed that AM fungi significantly affected soil functions (except for litter decomposition) and multifunctionality (Table S5). On average, AM fungi had a positive effect on soil functions and multifunctionality (Figure S15). However, drought significantly decreased soil functions and multifunctionality (Table S5; Figure S16). We found that soil functions (except for litter decomposition and soil respiration) and multifunctionality were significantly affected by the interaction between AM fungi and watering amount (Table S5). Specifically, AM fungi had higher effects on soil functions and multifunctionality under drought than under well-watered conditions (Figure 3; Figure S17). Although watering frequency

3.2.3 | Effects of AM fungi on responses of soil functions and multifunctionality to drought

Our results showed that drought-induced negative impacts on soil multifunctionality were lower in the presence of AM fungal hyphae than in the absence of these fungi (Figure 4). Most individual soil functions followed similar patterns to those observed for soil multifunctionality (Figure S19). In addition, results of linear models showed that AM fungi significantly affected the resistance of all individual soil functions and multifunctionality against drought, except for litter decomposition and soil respiration (Table S6). Specifically, we found that soil functions and multifunctionality are more resistant to drought in soils with AM fungi compared to soils without AM fungi (Figure 5; Figure S20).

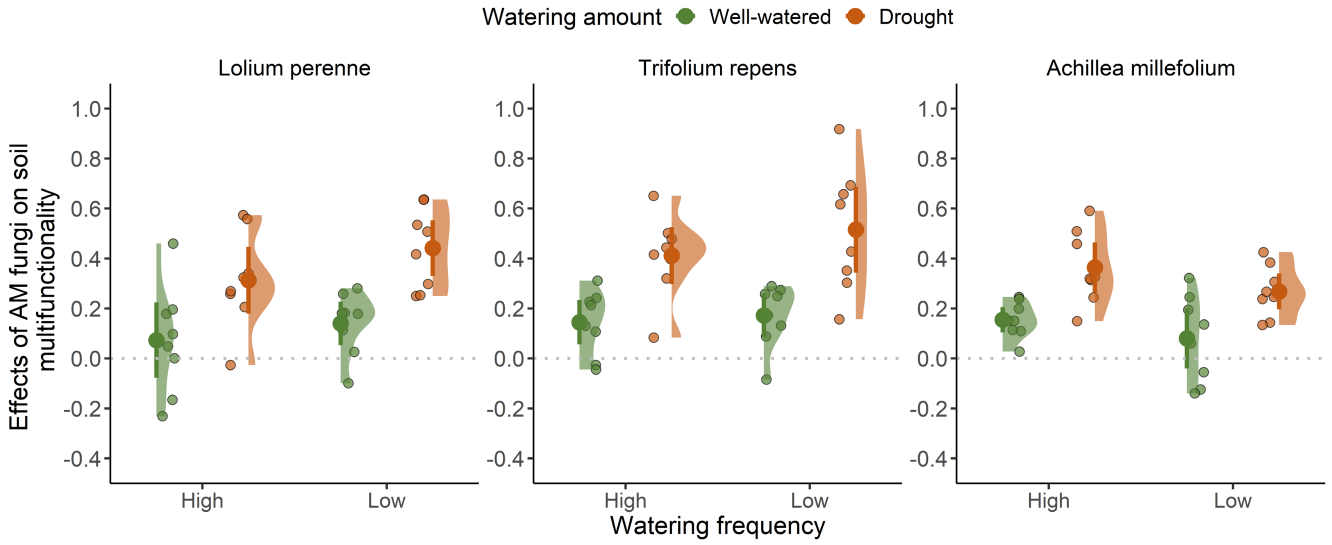

FIGURE 3 Effects of arbuscular mycorrhizal (AM) fungi on soil multifunctionality (LnRR) across watering amount (well-watered vs. drought; depicted as different colour symbols), watering frequency (high vs. low; *x*-axis labels) and plant species (different panels). Mean effect sizes and 95% confidence intervals (CIs) are presented ($n = 8$). A significant effect of AM fungi ($p < .05$) was considered if the 95% CIs do not overlap with zero (dotted line). Raw data are shown as swarm plots.

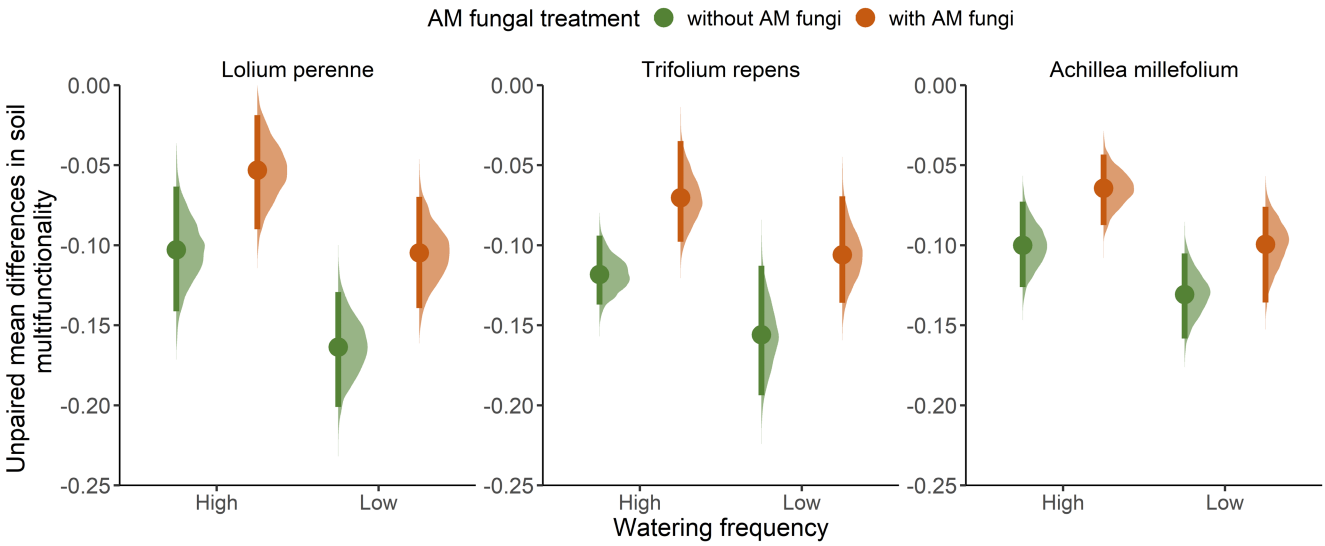

FIGURE 4 Unpaired mean differences (effect magnitude) in soil multifunctionality between drought and well-watered conditions (e.g. drought minus well-watered) across arbuscular mycorrhizal (AM) fungal treatments (without vs. with AM fungi; depicted as different colour symbols), watering frequency (high vs. low; *x*-axis labels) and plant species (different panels). The vertical lines around the mean represent 95% confidence intervals (CIs). Both the mean and 95% CIs are derived from bootstrapping approach (5000 iterations). The distribution of sampling errors is depicted as a curve.

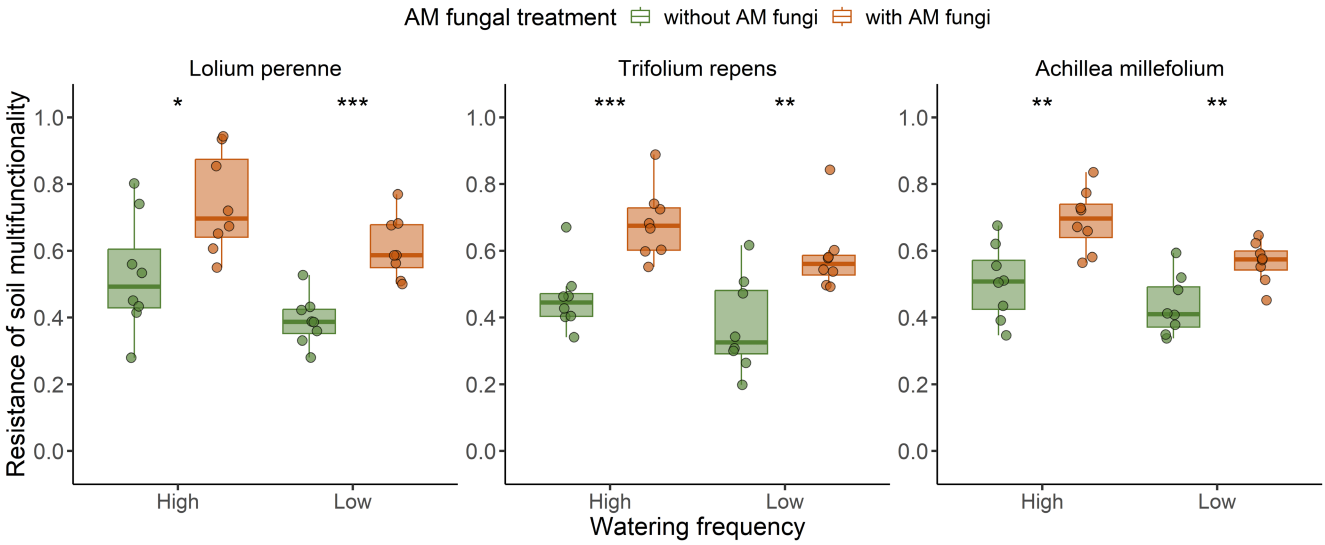

FIGURE 5 Resistance of soil multifunctionality against drought across arbuscular mycorrhizal (AM) fungal treatments (without vs. with AM fungi; depicted as different colour symbols), watering frequency (high vs. low; *x*-axis labels) and plant species (different panels). The horizontal lines inside each box represent the median ($n = 8$), and the ends of the boxes indicate the upper and lower quartiles. The whiskers are 1.5× the interquartile ranges. Raw data are shown as swarm plots. The asterisks show the significance between without and with AM fungal treatments (*t*-test: $^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$).

4 | DISCUSSION

In this study, we tested how AM fungi regulate soil functions and multifunctionality, and their responses to drought by using a pair of hyphal ingrowth cores to spatially separate the growth of AM fungal hyphae and plant roots. We found that core rotation greatly reduced the length of AM fungal hyphae in comparison with the static core, validating the experimental approach. Furthermore, there was no significant difference in other soil conditions, for example soil water content, soil pH and soil aggregates, between the rotated and static cores in unplanted microcosms. These results suggest that this partitioning method is highly effective and minimally disruptive in exploring the ecological consequences of AM fungi (Leifheit et al., 2014). Although physical rotation also reduced the non-AM fungal hyphae, but to a lesser extent, our analysis showed that rotation-induced changes in non-AM fungal hyphae have relatively little effect on

soil functions and multifunctionality. Therefore, this negligible side-effect resulting from rotation is unlikely to be an issue for the interpretation of our results.

4.1 | Positive effects of AM fungi on soil functions and multifunctionality

Consistent with the first hypothesis, our study including meta-analysis and greenhouse experiment provides strong evidence for a key role of AM fungi in maintaining soil functions and multifunctionality. On the one hand, AM fungi are commonly known for producing organic binding agents that facilitate the formation and stabilization of soil aggregates (Frey, 2019; Rillig et al., 2010; Rillig & Mummey, 2006; Wu et al., 2023). Furthermore, AM fungi physically entangle and enmesh mineral particles and organic matter through their mycelium, thus promoting aggregate formation (Rillig & Mummey, 2006). On the other hand, while AM fungi lack the capacity to function as saprotrophs (Tisserant et al., 2013), for example direct secretion of enzymes targeting soil nutrient cycling, they influence nutrient cycling through interactions with free-living saprotrophs around hyphal surfaces (Frey, 2019; Zhang et al., 2022). Up to 20% of the plant net photosynthates are generally allocated to the AM fungal partner (Drigo et al., 2010; Jakobsen & Rosendahl, 1990), part of which can be distributed throughout the soil matrix in the form of hyphal exudation (Drigo et al., 2010; Kaiser et al., 2015; Kakouridis et al., 2024). The hyphal exudates contain carbon-rich compounds that can promote microbial growth, and stimulate the activities of microbial extracellular enzymes related to soil nutrient cycling (Cheng et al., 2012; Kaiser et al., 2015; Zhang et al., 2022). Furthermore, releasing plant photosynthates by extensive hyphal networks is considered an efficient channel, through which hyphal exudation can be transported to microbes within soil microsites that are inaccessible by plant roots (Kaiser et al., 2015). Therefore, the hyphal pathway might offer more precise targeting of plant carbon to the saprotrophic microbial community around hyphal surfaces (Dickie et al., 2015; Kakouridis et al., 2024). In addition, fructose, a component of hyphal exudates, was shown to serve as a signal, triggering the expression of genes responsible for encoding phosphatase in bacterial cells (Zhang et al., 2018). These effects result in functional shifts and lead to changes in nutrient cycling, rendering the hyphosphere (the region under the influence of the AM fungal hyphae in the soil) a unique as well as an active functional zone within soils (Zhang et al., 2022).

Although no significant interaction between AM fungi and plant species on soil multifunctionality was observed, we found that the positive effects of AM fungi on soil aggregates and litter decomposition were the highest with *T. repens* (legume), followed by *A. millefolium* (non-leguminous forb) and *L. perenne* (grass). These host-dependent effects might be attributed to the following mechanisms. First, these host plants differ in the degree of dependency on AM fungi (Bergmann et al., 2020; Romero et al., 2023), which determines the influence of AM fungi on soil functions. The root system of *T. repens* belonging to legumes is characterized by thick, unbranched roots with few root hairs, which may impede roots in acquiring nutrients efficiently (Romero et al., 2023). In such cases, *T. repens* might rely more on the presence of AM fungi to acquire soil nutrients than grasses (*L. perenne*) with a well-developed, fibrous root system comprised of numerous fine roots specialized for efficient nutrient absorption (Brundrett, 2002). As such, this facilitative interaction between legume plant and AM fungi not only promotes plant growth (Romero et al., 2023), but likewise acts on soil functions. Second, plant hosts with diverse traits might harbour unique communities of AM fungi (López-García et al., 2017; Šmilauer et al., 2020), which have the potential to recruit distinct microbiomes (Emmett et al., 2021), and thus differentially affect soil functions (Nuccio et al., 2013; Wang et al., 2023; Zhang et al., 2022). For example, the AM fungal taxa from the *Claroideoglomeraceae* family were more frequently found in the roots of grass, whereas AM fungal taxa from the *Glomeraceae*, *Diversisporaceae*, and *Gigasporaceae* families were more commonly observed in the roots of forbs (Šmilauer et al., 2020). Furthermore, Firmicutes taxa showed a positive response to the AM fungus *Glomus hoi*, whereas Actinobacteria and Comamonadaceae taxa respond negatively to the identical fungal species (Nuccio et al., 2013). These diverse responses of microbial taxa to AM fungi yield varying effects on soil functions, such as litter decomposition and nitrogen cycling (Nuccio et al., 2013). Finally, plant functional group may contribute to different nutrient composition of root exudates and root biomass, and thus the colonized AM fungal communities, but this potential mechanism warrants further study.

4.2 | AM fungi attenuate the negative impact of drought on soil functions and multifunctionality

We further explored the role of AM fungi in regulating responses of soil functions and multifunctionality to drought. In line with our second hypothesis, both meta-analysis and greenhouse experiment show that AM fungi attenuate the negative impact of drought on soil functions and multifunctionality, and therefore, increase their resistance against drought. In general, drought-induced changes in soil hydrological pressure cause soil to shrink and swell, which may trigger the breakdown of soil aggregates (Chen et al., 2023; Denef et al., 2001). Similar to the way plant roots function, AM fungal hyphae contribute to the formation and stabilization of soil aggregates as discussed above (Rillig & Mummey, 2006; Wilson et al., 2009), and even increase soil water repellency (Rillig et al., 2010). Moreover, the favourable effects of AM fungi on soil aggregates persist under drought condition (Ji et al., 2019). Given the unique role of AM fungi in aggregate formation and stabilization, the soil aggregates without presence of AM fungi are more sensitive to drought, which has been demonstrated by both our greenhouse study and meta-analysis. To sum up, our study suggests that AM fungi attenuate the negative impact of drought on soil aggregates and thus increase their resistance against drought.

Drought was shown to negatively affect soil microbial communities, for example, decreasing microbial growth and activity (Manzoni et al., 2012), and then associated ecological processes, for example enzyme production, litter decomposition and soil CO_2

release (Lozano et al., 2021; Rillig et al., 2019). First, the increased soil osmotic potential caused by drought likely exerts pressure on soil microbes directly (e.g. growth and activity) (Schimel, 2018). Besides, drought can impact soil functions by changing the availability of substrates to microbes through processes such as dissolution, diffusion and transport (Schimel, 2018). Because most substrates utilized by microbes as energy sources are soluble in water (Tecon & Or, 2017), drought-induced reduction in substrate availability thus limits microbial growth and activity and then may impact microbially driven functions. Nevertheless, this unfavourable situation could potentially be averted due to the presence of AM fungi, as they offer readily available carbon substrates for soil microbes through the secretion of hyphal exudates (Kaiser et al., 2015; Kakouridis et al., 2024). Furthermore, recent studies indicate that AM fungal hyphae can serve as a "highway" for bacteria, facilitating their efficient movement towards nutrient patches, which enhances the utilization of these isolated substrates (Jiang et al., 2021; Zhang et al., 2022). Collectively, these processes mediated by AM fungi could contribute to maintaining soil connectivity, sustaining microbial activity, and thus facilitating nutrient cycling under drought, which, in turn, helps prevent bacterial dormancy and mortality, even amidst a significant decrease in soil moisture levels (Hestrin et al., 2022). By bolstering microbial functioning in soils exposed to drought, AM fungi could potentially mitigate the destabilizing impact of drought on soil functions, and enhance their resistance against drought.

This study offers important insights into the role of AM fungi in resistance of soil multifunctionality against drought, but has some potential limitations that point to future research directions. First, although this study showed positive effects of AM fungi on drought resistance of multiple soil functions (Ji et al., 2019; Püschel et al., 2021), this might be specific to the studied system. In addition, a bias of publication and referencing is likely to have influenced the dissemination of unfavourable mycorrhizal outcomes (Lehto & Zwiazek, 2011). Therefore, more studies carried out using a wider range of ecosystem types and soil conditions are needed to validate this point. Second, the well homogenized soil samples used in this study were not sterilized, and thus the hyphal growth of saprotrophic fungi is likely somewhat disrupted by the core rotation. Although our results demonstrated that core rotation has relatively little effect on hyphal growth of non-AM fungi and the effects of AM fungi on soil functions and multifunctionality remain significant even when accounting the influence of non-AM fungi, this still interferes to some extent with the ability to attribute the observed positive effects to AM fungi exclusively. Thus, experimental designs focusing on the impact of filamentous non-AM fungal species are needed in future studies to address this issue.

5 | CONCLUSION

In this study, we showed that AM fungi maintain multiple soil functions, including soil aggregation, soil respiration, microbial biomass and associated processes of soil nutrient cycling, and thus multifunctionality. More importantly, we found that AM fungi could attenuate the negative impact of drought on soil functions and multifunctionality, and thus increase the resistance of both against drought. Furthermore, this buffering effect of AM fungi persists across different frequencies of water supply. These findings highlight the important role of AM fungi in enhancing soil functions and multifunctionality by mitigating the negative impact of drought. Our study provides important insights into the role of AM fungi in maintaining soil functions and multifunctionality, highlighting a huge potential of AM fungi as a nature-based solution to sustaining multiple soil functions in a world where drought events are intensifying. At the same time, our results highlight the need for renewed efforts to conserve AM fungi in different ecosystem types, including agricultural land and grasslands.

AUTHOR CONTRIBUTIONS

Bo Tang: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Jing Man:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **Anika Lehmann:** Formal analysis; writing – review and editing. **Matthias C. Rillig:** Conceptualization; funding acquisition; methodology; project administration; supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.26123989.

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SUPPORTING INFORMATION

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

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