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Research

Nitrogen fertilisation disrupts the temporal dynamics of arbuscular mycorrhizal fungal hyphae but not spore density and community composition in a wheat field

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Summary

- Elucidating the temporal dynamics of arbuscular mycorrhizal (AM) fungi is critical for understanding their functions. Furthermore, research investigating the temporal dynamics of AM fungi in response to agricultural practices remains in its infancy.
- We investigated the effect of nitrogen fertilisation and watering reduction on the temporal dynamics of AM fungi, across the lifespan of wheat.
- Nitrogen fertilisation decreased AM fungal spore density (SD), extraradical hyphal density (ERHD), and intraradical colonisation rate (IRCR) in both watering conditions. Nitrogen fertilisation affected AM fungal community composition in soil but not in roots, regardless of watering conditions. The temporal analysis revealed that AM fungal ERHD and IRCR were higher under conventional watering and lower under reduced watering in March than in other growth stages at low ($\leq$ 70 kg N ha^{-1} yr^{-1}) but not at high ($\geq$ 140) nitrogen fertilisation levels. AM fungal SD was lower in June than in other growth stages and community composition varied with plant development at all nitrogen fertilisation levels, regardless of watering conditions.
- This study demonstrates that high nitrogen fertilisation levels disrupt the temporal dynamics of AM fungal hyphal growth but not sporulation and community composition.

Introduction

The Anthropocene, the current geological epoch marked by footprints of human activities, has resulted in major environmental disturbances that threaten the world's food supply (Thirkell *et al.*, 2017). Conventional, large-scale farming practices often rely heavily on the inputs of water and chemical fertiliser, whereas the role of microbiomes in promoting plant's absorption of soil water and nutrients is overlooked (Augé, 2001; Hartmann *et al.*, 2015; Ruiz-Lozano *et al.*, 2016; D'Hondt *et al.*, 2021). In consideration of the current economic and ecological burden of watering and chemical fertilisation, modern agriculture should harness microbiomes to maximise output and minimise input under future anticipated environmental disturbances (van der Heijden & Wagg, 2013; Bender *et al.*, 2016; de Vries *et al.*, 2020; D'Hondt *et al.*, 2021).

Within the plant microbiome, arbuscular mycorrhizal (AM) fungi represent a key group of root-associated mutualists, which form mutualistic relationships with upwards of 80% of terrestrial plant species (Smith & Read, 2008). In this association, AM fungi exchange soil-derived nutrients for host plant photosynthates (Smith & Read, 2008). In addition, AM fungi can also confer enhanced resistance to pathogens and abiotic stress, such as drought (Augé, 2001; Sikes *et al.*, 2010; Ruiz-Lozano *et al.*, 2016). Arbuscular mycorrhizal (AM) fungal vegetative structures are important to this symbiosis in which extraradical hyphae are responsible for accessing critical inorganic nutrients from the soil matrix and intraradical hyphae (e.g. arbuscules) form the interface for the exchange of these inorganic nutrients and plant photosynthates (Smith & Read, 2008). Arbuscular mycorrhizal fungi also form dormant asexual spores that are important propagules

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for fungal colonisation and dispersal that represent an important survival strategy under adverse environmental conditions (Chagnon *et al.*, 2013; Aguilar-Trigueros *et al.*, 2019). Therefore, the relative allocation of resources from the host plant to AM fungi reflects trade-offs in response to environmental perturbations, such as drought and shifts in nitrogen (N) availability (Chagnon *et al.*, 2013).

Previous studies have shown that drought and N fertilisation have positive, negative or neutral effects on AM fungal intraradical colonisation rate (IRCR), extraradical hyphal density (ERHD) and spore density (SD) (Supporting Information Table S1). For example, recent meta-analyses have indicated that N fertilisation significantly decreases AM fungal IRCR, but not ERHD and SD, suggesting that the directionality of these responses is context dependent and factors, such as soil N : phosphorus (P) ratio, pH, root biomass, and duration of fertilisation may play an important role in modifying these responses (Han *et al.*, 2020; Ma *et al.*, 2020). Therefore, important questions remain about how AM fungal strategies of growth investment might differ in response to both N deposition and drought. As mycorrhizal relationships function along a continuum from mutualism to parasitism based on environmental and species-specific contexts (Johnson *et al.*, 1997), the relative investment of AM fungi into vegetative growth and reproduction could act as an important lever in tipping these relationships towards more mutualistic or more parasitic interactions.

The effect of drought and N fertilisation on AM fungal community diversity and composition remains inconclusive (Table S1). For example, some studies have found that the response of AM fungal taxa in relative abundance to N fertilisation depends on environmental factors, such as soil P availability (Johnson *et al.*, 2003; Cotton, 2018; Treseder *et al.*, 2018; Lilleskov *et al.*, 2019). A recent meta-analysis indicates that N fertilisation significantly reduces the relative abundance of Glomeraceae and Claroideoglomeraceae, increases that of Archaeosporaceae and does not impact that of Gigasporaceae, Diversisporaceae and Paraglomeraceae (Han *et al.*, 2020). In addition, water availability may affect AM fungi directly or indirectly through changes in plant photosynthetic rate, root turnover and soil nutrients (Gao *et al.*, 2016; Chen *et al.*, 2017). While the separate effects of N fertilisation and watering reduction on AM fungi have received considerable attention in natural ecosystems (Table S1), to our knowledge, their combined effects on AM fungi have been poorly documented, especially from semiarid agroecosystems.

The temporal dynamics of microbes have been used to determine the factors that affect community structure and ecosystem processes (Bardgett *et al.*, 2005; Carini *et al.*, 2020; Broadbent *et al.*, 2021). AM fungi exhibit dynamic patterns over time due to the temporal development of host plants, shifts in habitat, seasonal climate fluctuations, time-dependent dispersal and interactions between AM fungal species (Werner & Kiers, 2015a; García de León *et al.*, 2016; Chaudhary *et al.*, 2020). While patterns of the temporal dynamics of AM fungal growth investment (IRCR, ERHD and SD) and community (richness and composition) have been extensively documented and compiled by Bahram *et al.* (2015) and Gao *et al.* (2019), important questions remain about how environmental perturbation might influence these temporal dynamics. At its most extreme, warming can lead to dysbiosis or the disruption of 'normal' symbiotic relationships (Greenspan *et al.*, 2020). Within the rhizosphere, recent studies have indicated that temporal dynamics of many bacterial associations with plant hosts are indeed disrupted by drought and warming (Naylor *et al.*, 2017; Xu *et al.*, 2018; Greenspan *et al.*, 2020; Broadbent *et al.*, 2021). However, to our knowledge, the temporal dynamics of AM fungi in response to agricultural practices, such as watering reduction and N fertilisation, remain largely unexplored.

Here, we investigated patterns of AM fungal growth and community dynamics throughout the growing period (four stages) in a wheat field subjected to treatments of watering (two levels) and N fertilisation (five levels) with three replicates (Fig. 1). Root and soil samples were collected to determine the impact of these important agricultural management practices on AM fungal IRCR, ERHD and SD. AM fungal communities in roots and soil were examined using Illumina MiSeq sequencing of 18S rDNA. We tested three hypotheses: H_1, that N fertilisation and watering reduction reduce AM fungal IRCR, ERHD and SD; H_2, that N fertilisation and watering reduction alter AM fungal community composition by decreasing the relative abundance of Glomeraceae and Claroideoglomeraceae and increasing that of Archaeosporaceae based on the meta-analysis of Han *et al.* (2020), and H_3, that N fertilisation and watering reduction disrupt the temporal dynamics of AM fungi.

Materials and Methods

Study site

This study was conducted at the Yucheng Comprehensive Experimental Station of the Chinese Academy of Sciences, Shandong Province in China (36°57′N, 116°36′E, 20 m above sea level). The site is in a typical temperate semiarid zone with a mean annual temperature of 13.3°C and annual precipitation of 559.8 mm according to long-term observations (1998–2015) (Zhao *et al.*, 2018). At the start of the experiment, the original soil had a pH of 8.3 and contained 0.5 g kg^{-1} of total N, 12.2 g kg^{-1} of organic matter, 12.9% sand, 65.1% silt and 22.0% clay, and is classified as a Calcaric Fluvisol according to the FAO-Uneson system (Zheng *et al.*, 2019).

Experimental design

The experiment was established in 2005 with a split-block design of watering reduction and N fertilisation. To keep soil field moisture capacity at 80%, one block (20 m × 40 m) received conventional watering according to the typical application of irrigation used by local farmers; the other block (20 m × 40 m) received reduced watering, to keep soil field moisture capacity at 60%. Each block contained 15 plots (each 5 m × 10 m), each of which included five N fertilisation levels consisting of 0 (N0), 70 (N1), 140 (N2), 210 (N3) and 280 (N4) kg N ha^{-1} yr^{-1}, in the form

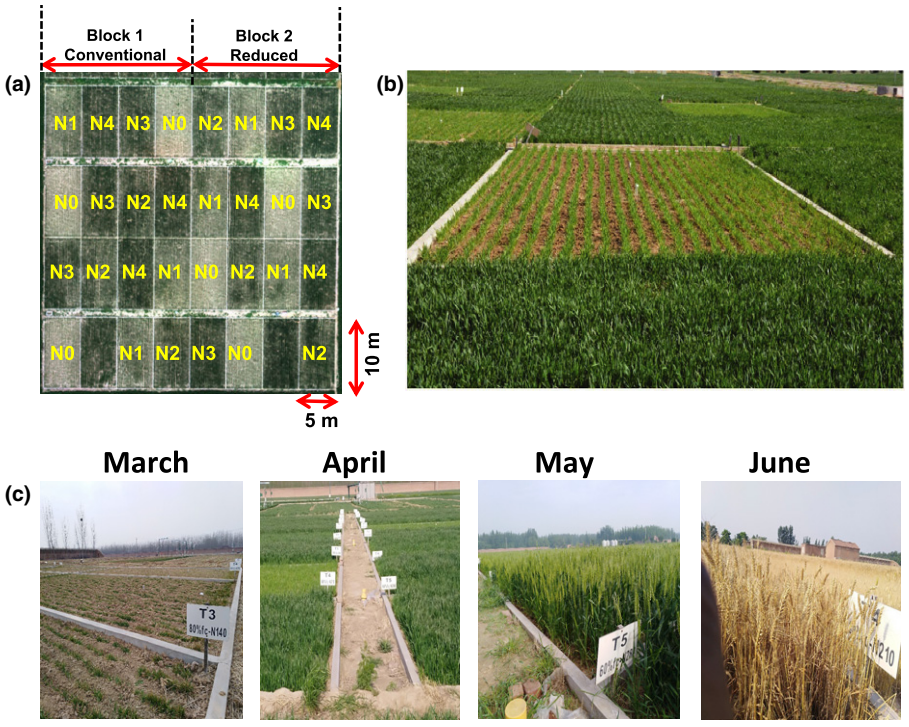

Fig. 1 Experimental design. (a) Aerial layout of the 30 plots (10 × 5 m^2 each) in a split-block design of two watering levels (conventional and reduced), each composed of five nitrogen (N) fertilisation levels (N0, N1, N2, N3, N4) with three plot replicates. N0, N1, N2, N3 and N4 indicate 0, 70, 140, 210 and 280 kg N ha^{-1} yr^{-1}, respectively. (b) To prevent movement of water and N between plots, plots are separated from each other by cement walls (20 cm thick and 60 cm deep in the soil). (c) Soil and root samples were collected at four growth stages along wheat development: 7 March (tillering stage), 9 April (jointing stage), 7 May (flowering stage) and 3 June (maturing stage) in 2018.

of urea. Each N fertilisation level had three replicates (plots), randomly distributed in the block (Fig. 1a). The highest N fertilisation level (N4) was set according to the typical application used by local farmers. Nitrogen was applied in two doses, first before wheat sowing on 1 October and then at the jointing stage on 20 March of the following year. Before sowing, to ensure plants were not limited by P or potassium (K), in total 80 kg ha^{-1} K_2O (as K_2SO_4) and 120 kg ha^{-1} P_2O_5 (as $Ca(H_2PO_4)_2$) were applied in each plot. To prevent movement of water and N between the individual plots, each plot was separated by a cement wall (20 cm thick) extending downward to a depth of 60 cm in the soil (Fig. 1b).

Soil and plant root sampling

In 2018, root and soil samples were collected on 7 March (tillering stage), 9 April (jointing stage), 7 May (flowering stage), and 3 June (harvesting stage) (Fig. 1c). Briefly, five soil cores (3.5 cm in diameter and 20 cm in depth) were randomly collected from the point immediately adjacent to plant roots in each plot and combined to generate one composite sample. The samples were immediately packed in an ice box and transported to the laboratory. Soil samples were sifted through a 1-mm mesh sieve, the recovered roots were washed with distilled water, and fine roots (< 2 mm in diameter) were cut into *c.* 1 cm fragments. Root samples and soil subsamples were stored at −80°C for DNA extraction and measurements of AM IRCR and ERHD (Tian *et al.*, 2013). Fresh soil subsamples were used for measurements of exchangeable NH_4^+–N and NO_3^-–N and moisture, and the remaining subsamples were air dried and stored at room temperature for measurements of AM fungal SD and other soil properties. In total, 240 samples (two watering conditions × five N fertilisation levels × four sampling growth stages × two sample types × three replicates) were collected.

Soil physicochemical and plant biomass analysis

Soil samples were dried at 105°C for 24 h to determine gravimetric moisture. Soil pH (soil to water = 2 : 5, w/v) was determined with a glass electrode (Thermo Orion T20, Columbia, MD, USA). Total soil carbon (C) and N were measured by direct combustion using a C/N Elemental Analyzer (Vario EL III; Elementar Analysensysteme GmbH, Hanau, Germany). Total soil P, calcium (Ca), magnesium (Mg), iron (Fe) and K were measured using an inductively coupled plasma-atomic emission spectrometer (ICP-AES) (iCAP 6300; Thermo Jarrell Ash Co., Waltham, MA, USA). Soil exchangeable NH_4^+–N and NO_3^-–N were extracted with a 2 M KCl solution (soil to water = 1 : 5, w/v) and determined using a continuous flow analyser (SAN^{++}; Skalar, Breda, the Netherlands). The information on soil properties is summarised in Table S2. The dry weights of aboveground plant biomass and grains in each plot at the harvesting stage were determined after oven drying at 60°C for 48 h.

AM fungal hyphal and spore measurement

Fungal hyphae were extracted from 4.0 g of frozen soil using the membrane filter method (Rillig *et al.*, 1999). Arbuscular mycorrhizal fungal hyphae were separated from non-AM fungal hyphae based on their morphology and staining colour (Miller *et al.*, 1995). The lengths of AM fungal hyphae were measured using a gridline intersect method by observing 135 fields of view for each

filter under a microscope (Nikon 80i, Osaka, Japan) and under ×200 magnification (Miller *et al.*, 1995). AM fungal spores were extracted from 20.0 g of air-dried soil using a wet sieving (bottom 38 μm mesh) and decanting method (Daniels & Skipper, 1982), and were counted under ×40 magnification (Nikon 80i). For AM fungal IRCR measurement, fine root segments (*c.* 1 cm long) were treated with 10% KOH solution at 92°C for 25 min, neutralised using 2% HCl solution and stained with lactic acid fuchsin dye at 92°C for 20 min (modified from McGonigle *et al.*, 1990). Fifty root segments were randomly selected from each sample and examined to quantify the IRCR using a magnified intersection method by observing 450 fields of view under ×200 magnification (McGonigle *et al.*, 1990).

Molecular analysis

Detailed description of DNA extraction, two-step PCR amplification and sequencing can be found in Maitra *et al.* (2019). Briefly, genomic DNA was extracted from 0.3 g frozen soil and roots using a PowerSoil® DNA Isolation Kit (MoBio Labs Inc., Carlsbad, CA, USA). The DNA concentration of each sample was measured using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). For Illumina MiSeq sequencing, amplicons of 18S rDNA were generated by a two-step PCR procedure respectively using GeoA-2 and AML2 primers (Schwarzott & Schüßler, 2001; Lee *et al.*, 2008) and NS31 and AMDGR primers (Simon *et al.*, 1992; Sato *et al.*, 2005). In the second round of PCR, the AMDGR primer was linked with 12-base barcode sequences for sample distinction (Table S3). The final PCR products were *c.* 300 base pairs (bp) in length and purified using an E.Z.N.A Gel Extraction Kit (Omega Bio-Tek, Norcross, GA, USA). The purified PCR products were pooled together with the same molar amount (100 ng) from each sample and adjusted to 10 ng μl^{-1}. Furthermore, a sequencing library was constructed by the addition of an Illumina sequencing adaptor (5′-GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG-3′) to the product using the Illumina TruSeq DNA PCR-Free LT Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The constructed library was submitted to an Illumina MiSeq PE250 platform for sequencing using the paired end (2 × 250 bp) option at the Environmental Genome Platform in the Chengdu Institute of Biology, Chinese Academy of Sciences, China.

Bioinformatics analysis

The raw sequences were filtered using Quantitative Insights into Microbial Ecology (QIIME) v.1.7.0 (Caporaso *et al.*, 2010). Quality control was conducted by excluding low-quality sequences containing either ambiguous bases, no valid primer sequence or barcode sequence, read length < 200 bp, or an average quality score < 20. The potential chimeras were subsequently checked using the CHIMERA.UCHIME command in MOTHUR v.1.31.2 (Schloss *et al.*, 2009) against the Maarj*AM* 18S rRNA gene database (Öpik *et al.*, 2010). The nonchimeric sequences were clustered into different operational taxonomic units (OTUs) at a 97% sequence similarity level based on the UPARSE pipeline using USEARCH v.8.0 after dereplication and singleton discarding (Edgar, 2013). Using a basic local alignment search tool (Altschul *et al.*, 1990), the representative sequence of each OTU, that is the most abundant, was selected and searched against the National Center for Biotechnology Information (NCBI) nucleotide database and Maarj*AM* 18S rRNA gene database. AM fungal OTUs were identified based on the closest BLAST hit annotated as 'Glomeromycotina' and *E* value < e^{-100}. The number of considered sequences per sample was normalised to the smallest sample size by using the SUB.SAMPLE command in MOTHUR to account for the influence of different sequence numbers on the analysis of AM fungal community.

Statistical analysis

All statistical analyses were performed in R v.3.5.1 (R Development Core Team, 2018). Any significant differences in soil total N, C, P, Mg, K, Fe, Ca, moisture, pH and exchangeable NH_4^+–N and NO_3^-–N among N fertilisation levels in conventional watering or reduced watering in each growth stage were compared using Tukey's honestly significant difference (HSD) test at $P < 0.05$. Rarefaction curves of the number of observed AM fungal OTUs in root and soil samples and sequences in each sample were calculated, using the SPECACCUM and RARECURVE commands respectively, in the VEGAN package (Oksanen *et al.*, 2017). Two-way analysis of variance (ANOVA) was used to examine the effect of N fertilisation, watering reduction and their interactions on the aboveground plant biomass and yield. Three-way ANOVA was used to examine the effect of N fertilisation, watering reduction, growth stage and their interactions on the SD, ERHD, IRCR, OTU richness and the relative abundance of abundant OTUs (relative abundance > 1%) and families of AM fungi, if these data satisfied the normality of distribution and homogeneity of variance before and after square root and logarithmic transformation. Then significant differences among N fertilisation treatments were compared using Tukey's HSD test at $P < 0.05$. Significant differences between watering conditions and among growth stages were then compared through pairwise comparison at $P < 0.05$. If these data did not satisfy the homogeneity of variance before and after square root and logarithmic transformation, a nonparametric Kruskal–Wallis test was carried out, then significant differences among N fertilisation levels, watering conditions and growth stages were compared by Conover's test using the 'post-hoc.kruskal.conover.test' function with Bonferroni correction in the PMCMR package (Pohlert, 2014).

The distance matrix for the AM fungal community (Hellinger transformation of the read counts of OTUs) was constructed by calculating dissimilarity using the Bray–Curtis method (Clarke *et al.*, 2006). To evaluate the effect of N fertilisation, watering reduction, growth stage and their interactions on AM fungal community composition, permutational multivariate analysis of variance (PerMANOVA) was carried out using the ADONIS command in the VEGAN package with 9999 permutations. In addition, the AM fungal community composition was subjected

to nonmetric multidimensional scaling (NMDS) using the METAMDS command in the VEGAN package. Using the 'envfit' function with 999 permutations, compartment (root and soil), five N fertilisation levels (N0, N1, N2, N3 and N4), four growth stages (March, April, May and June) and two watering conditions (conventional and reduced) were fitted as centroids, and soil pH, N, C, P, Mg, K, Fe, Ca, moisture, and exchangeable NH_4^+–N and NO_3^-–N were fitted as vectors onto the NMDS ordination plots to examine how AM fungal community composition was related to these variables.

Furthermore, biomarkers (from class to genus) of AM fungi with significantly different relative abundance between roots and soil and between no N fertilisation (N0) and different N fertilisation (N1, N2, N3, N4) levels were revealed using linear discriminant analysis (LDA) effect size (LEfSe) (Segata *et al.*, 2011), with a logarithmic LDA score of 2.0 as the threshold; this analysis was performed with default parameters on the GALAXY web-based interface (http://huttenhower.sph.harvard.edu/galaxy/).

Structural equation models (SEMs) were performed to examine the direct and indirect effects of N fertilisation, watering reduction, growth stage and soil variables on the AM fungal SD, ERHD, IRCR, OTU richness and community composition using the multilevel SEM (Shipley, 2009) implemented in the PIECEWISESEM package (Lefcheck, 2016), as described by Ji *et al.* (2019).

Results

Aboveground plant biomass and yield

The average aboveground plant biomass was 4.04 ± 1.49 (t ha^{-1}) and ranged from 1.11 ± 0.23 to 5.99 ± 0.31 and plant yield was 3.41 ± 1.35 (t ha^{-1}) and ranged from 0.89 ± 0.09 to 4.85 ± 0.26 (mean ± SD, $n = 3$). Two-way ANOVA showed that N fertilisation, but not watering reduction and their interactions, significantly influenced aboveground plant biomass and yield (Table 1). All N fertilisation levels significantly increased aboveground plant biomass and yield under both reduced and conventional watering conditions (Fig. 2a,b).

AM fungal SD, ERHD and IRCR

The average AM fungal SD was 8.96 ± 3.59 and ranged from 3.40 ± 0.25 to 18.63 ± 0.88 (spores g^{-1} dry soil), ERHD was 2.78 ± 0.12 and ranged from 1.59 ± 0.17 to 4.79 ± 0.20 (m g^{-1} dry soil), and IRCR was $76.43 \pm 1.39\%$ and ranged from $60.22 \pm 3.96\%$ to $97.42 \pm 1.06\%$ (mean ± SD, $n = 3$) (Fig. 3a–c). The three-way ANOVA revealed that AM fungal SD, ERHD, IRCR and the ERHD : IRCR ratio showed significant relationships with N fertilisation, watering reduction, growth stage and their interactions (Table 2).

Table 1 Two-way ANOVA showing the effect of nitrogen fertilisation, watering reduction and their interactions on aboveground plant biomass and yield.

		Aboveground plant biomass		Plant yield	
Source of variation	df	*F*	*P*	*F*	*P*
Nitrogen fertilisation (NF)	4	94.98	< 0.001	138.41	< 0.001
Watering reduction (WR)	1	0.09	0.756	0.33	0.572
NF × WR	4	1.19	0.342	0.54	0.707

Compared with the N0 treatment, AM fungal SD declined significantly at all N fertilisation levels in each growth stage, under both reduced and conventional watering conditions, except for a nonsignificance in April of N1 treatment under conventional watering condition (Fig. 3a). In addition, AM fungal SD was significantly higher in reduced watering compared with the conventional watering conditions in all growth stages for the N0 treatment and in March at the N1 treatment, but significantly lower in reduced watering conditions than in conventional watering conditions for the N1 treatment in April and May (Fig. 3a). The temporal analysis revealed that AM fungal SD decreased in June at all N fertilisation treatments under both watering conditions (Fig. 3a). This pattern suggests that the temporal dynamics of AM fungal SD were not influenced by N fertilisation and watering reduction.

Compared with the N0 treatment, under both the reduced and conventional watering conditions, AM fungal ERHD was significantly lower at all N fertilisation levels in each growth stage, except for March and June of the N1 treatment (Fig. 3b). AM fungal ERHD was significantly lower in reduced watering conditions than in conventional watering conditions in March for the N0, N1 and N2 treatments (Fig. 3b). The temporal analysis revealed that AM fungal ERHD was significantly higher in March than at other growth stages under conventional watering, but was lower in March than at other growth stages under reduced watering at the N0 and N1 fertilisation levels (≤ N1), but not for high N fertilisation levels (≥ N2) (Fig. 3b). This pattern suggests that the temporal dynamics of AM fungal ERHD were disrupted by high N fertilisation but not by watering reduction.

Under the reduced and conventional watering conditions, AM fungal IRCR was significantly lower for the N2, N3 and N4 treatments than for the N0 and N1 treatments in all growth stages (Fig. 3c). AM fungal IRCR was significantly higher in conventional watering than in reduced watering conditions in March for the N0 and N1 treatments (Fig. 3c). In addition, the ratio of AM fungal ERHD to IRCR was significantly decreased with N fertilisation (Fig. 3d). The temporal analysis revealed that AM fungal IRCR was significantly higher in March than in other growth stages under conventional watering, but was lower in March than in other growth stages under reduced watering at no and low N fertilisation levels (≤ N1), but not at high N fertilisation levels (≥ N2) (Fig. 3c). This pattern suggests that the temporal dynamics of AM fungal IRCR were disrupted by high N fertilisation but not by watering reduction.

The SEM revealed that the AM fungal SD, ERHD and IRCR were directly influenced by N fertilisation, growth stage and watering reduction (Fig. S1a). Furthermore, N fertilisation and watering reduction had indirect effects on the AM fungal SD,

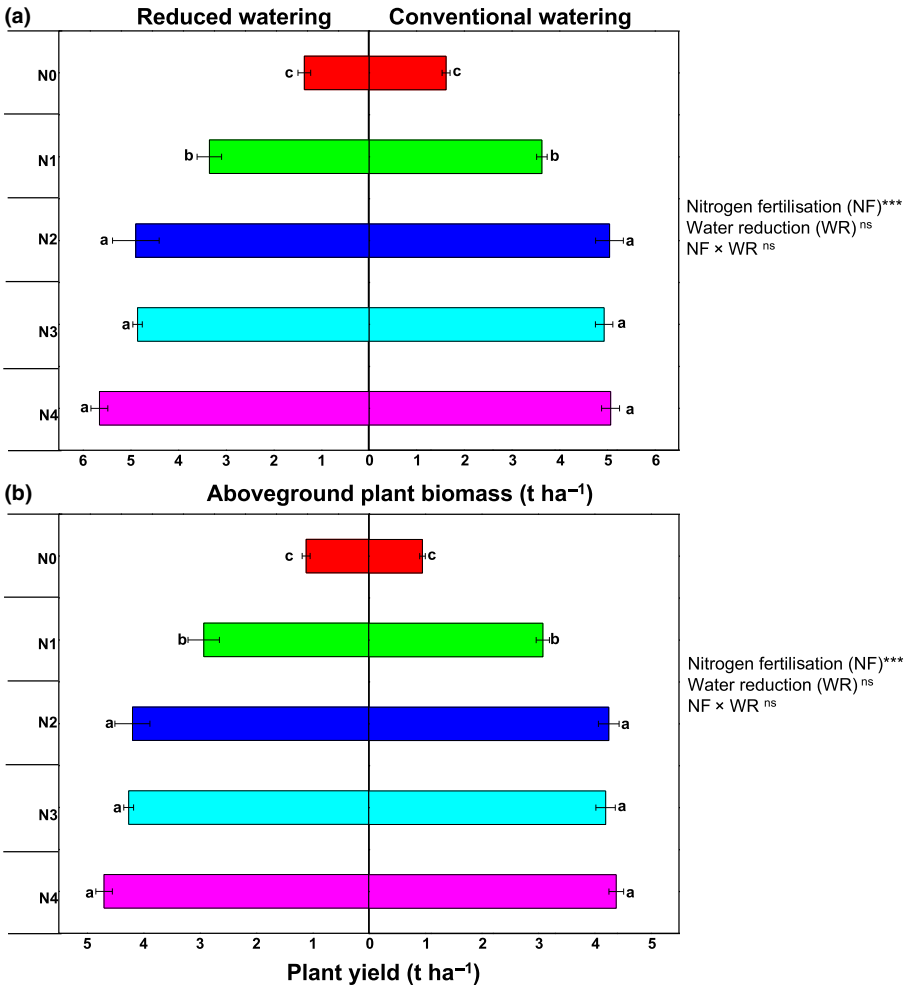

Fig. 2 Aboveground plant biomass (a) and yield (b) at different nitrogen (N) fertilisation levels and watering conditions at the harvesting stage. Two-way ANOVA showing the effect of N fertilisation, watering reduction and their interactions on aboveground plant biomass and yield. Data are means ± SD ($n = 3$). Bars without shared letters indicate significant difference at different N fertilisation levels in reduced and conventional watering conditions respectively according to Tukey's honestly significant difference (HSD) test. ns, $P > 0.05$; ***, $P < 0.001$. N0, N1, N2, N3 and N4 indicate 0, 70, 140, 210 and 280 kg N ha^{-1} yr^{-1}, respectively.

ERHD, and IRCR through soil moisture and/or total N (Fig. S1a).

Illumina MiSeq sequencing analysis and identification of AM fungi

In total, 12 588 267 high-quality 18S rDNA sequences were filtered from 12 895 818 raw sequences and clustered into 977 OTUs at a 97% sequence similarity level. Of these OTUs, 169 (10 293 271 sequences) were identified as AM fungal. As AM fungal sequences varied from 1428 to 113 847 across all 240 samples (Table S4), the sequence number was normalised to 1428 per sample, resulting in a normalised dataset containing 132 AM fungal OTUs (342 720 sequences) (Table S5). The 16 relatively abundant OTUs (relative abundance > 1%) accounted for 91.8% of the total AM fungal sequences, and the remaining 116 OTUs accounted for 8.2% (Fig. S2). Among these OTUs, four were exclusively recovered from roots, 41 were exclusively from soil and 87 were shared between roots and soil (Fig. S3; Table S6). Across all 132 AM fungal OTUs obtained, 78 belonged to the Glomeraceae (relative abundance 44.6%), 18 to the Diversisporaceae (32.8%), 19 to the Claroideoglomeraceae (10.95%), 14 to the Paraglomeraceae (10.86%), 1 to the Archaeosporaceae (0.72%), 1 to the Acaulosporaceae (0.06%), and 1 to the Gigasporaceae (0.01%) (Fig. S4). In addition, the rarefaction analyses revealed that the number of samples and sequences in each sample did not reach a plateau, suggesting that more samples and deeper sequencing would recover more OTUs in roots and soil (Fig. S5).

AM fungal OTU richness

The average observed AM fungal OTU richness was 29.03 ± 4.14 and ranged from 24 ± 4.00 to 36.33 ± 7.37 in roots, while in soil the average was 48.76 ± 6.44 and ranged from 40.67 ± 9.29 to 60.67 ± 5.69 (mean ± SD, $n = 3$) (Fig. 3e,f). Three-way ANOVA revealed that the AM fungal OTU richness in roots was significantly affected by N fertilisation, growth stage, the interaction between N fertilisation and growth stage, and the interaction between watering reduction and growth stage (Table 2). In soil, AM fungal OTU richness was significantly affected by N fertilisation, growth stage and the interaction between N fertilisation and watering reduction (Table 2). The temporal analysis revealed that AM fungal OTU richness in soil and roots differed significantly in some of the no fertilisation and N fertilisation levels under conventional or reduced watering

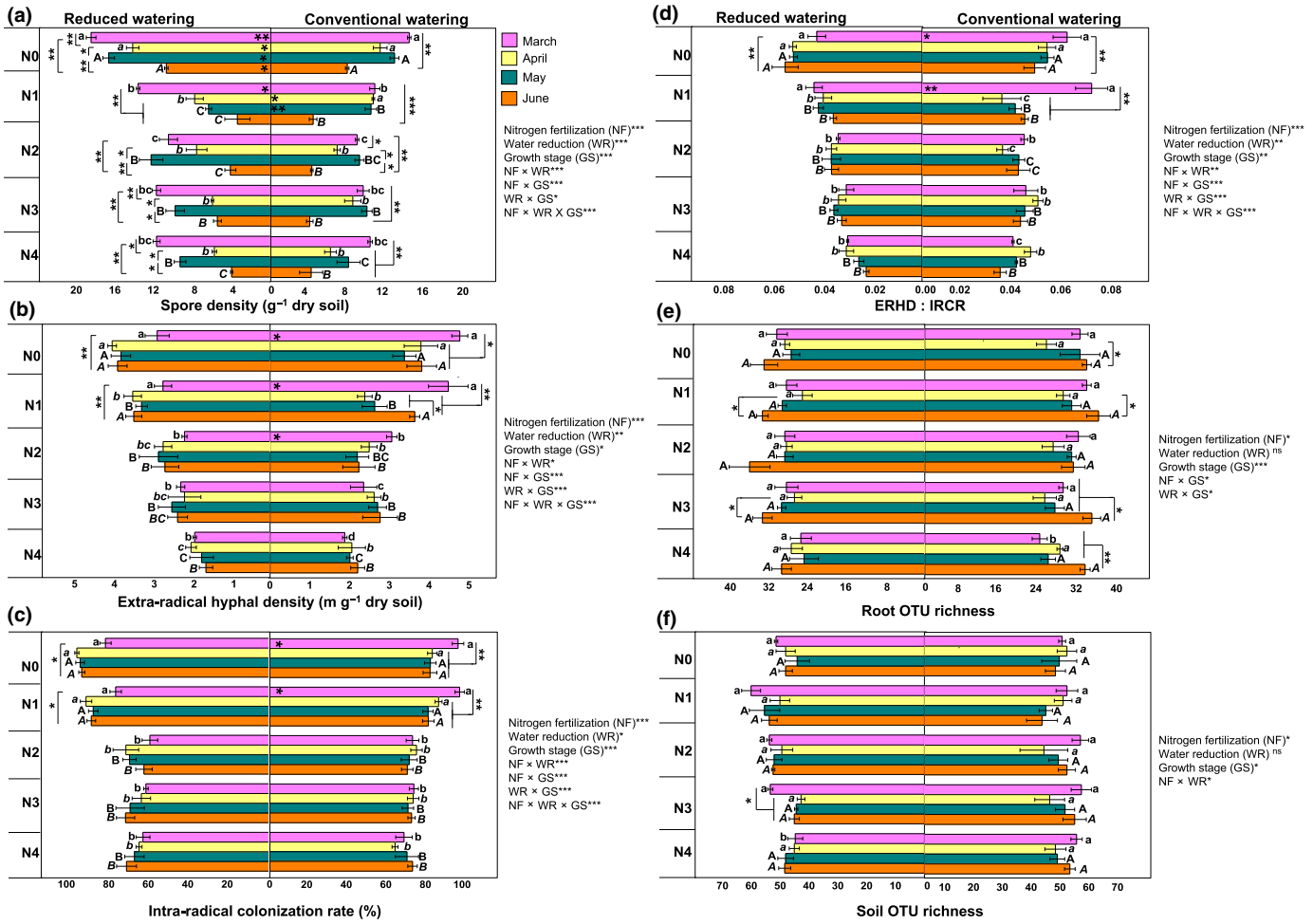

Fig. 3 Arbuscular mycorrhizal (AM) fungal (a) spore density, (b) extraradical hyphal density, (c) intraradical colonisation rate, (d) ratio of extraradical hyphal density to intraradical colonisation rate (ERHD : IRCR) and operational taxonomic unit (OTU) richness in (e) roots and (f) soil at different nitrogen (N) fertilisation levels, watering conditions and growth stages. Three-way ANOVA showing the effect of N fertilisation, watering reduction, growth stage and their interactions on AM fungal spore density, extraradical hyphal density, intraradical colonisation rate, ERHD : IRCR and OTU richness in roots and soil. Data are means ± SD ($n = 3$). Asterisks on the left horizontal columns indicate significantly higher values in reduced watering than in conventional watering, and asterisks on the right horizontal columns indicate significantly lower values in reduced watering than in conventional watering conditions. Bars with asterisks represent significant difference in different growth stages at the same N fertilisation level. Bars without shared uppercase, lowercase, italic uppercase and italic lowercase letters respectively indicate significant difference at different N fertilisation levels in March, April, May and June in reduced and conventional watering conditions according to Tukey's HSD test. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. N0, N1, N2, N3 and N4 indicate 0, 70, 140, 210 and 280 kg N ha^{-1} yr^{-1}, respectively.

Table 2 Three-way ANOVA showing the effect of nitrogen fertilisation, watering reduction, growth stage and their interactions on arbuscular mycorrhizal fungal spore density, extraradical hyphal (ERH) density, intraradical colonisation (IRC) rate, ratio of ERH density to IRC rate and operational taxonomic unit (OTU) richness in roots and soil.

		Spore density		ERH density		IRC rate		ERH density : IRC rate		Root OTU richness		Soil OTU richness	
Source of variation	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Nitrogen fertilisation (NF)	4	427.2	< 0.001	82.81	< 0.001	451.65	< 0.001	14.84	< 0.001	2.99	0.021	3.52	0.028
Watering reduction (WR)	1	152.91	< 0.001	10.95	0.002	4.61	0.01	6.45	0.01	0.43	0.522	1.21	0.072
Growth stage (GS)	3	767.41	< 0.001	2.41	0.04	61.92	< 0.001	3.25	0.03	17.28	< 0.001	2.4	0.01
NF × WR	4	61.75	< 0.001	2.58	0.03	7.74	< 0.001	3.31	0.02	1.61	0.181	2.78	0.02
NF × GS	12	17.64	< 0.001	3.49	< 0.001	41.69	< 0.001	5.67	< 0.001	3.04	0.042	0.93	0.525
WR × GS	3	38.12	0.02	9.27	< 0.001	8.02	< 0.001	11.16	< 0.001	2.21	0.031	0.16	0.922
NF × WR × GS	12	12.11	< 0.001	4.03	< 0.001	18.61	< 0.001	4.24	< 0.001	0.73	0.712	1.09	0.37

conditions (Fig. 3e,f). Therefore, we could not conclude if the temporal dynamics of AM fungal OTU richness were influenced by N fertilisation or watering reduction.

The SEM revealed that the AM fungal OTU richness in roots and soil was directly influenced by N fertilisation and growth stage, but not by watering reduction. In addition, N fertilisation and growth stage had indirect effects on AM fungal OTU richness in soil but not in roots through soil exchangeable NH_4^+–N (Fig. S1b).

AM fungal community composition

Three-way ANOVA and Kruskal–Wallis tests revealed that the relative abundance of eight abundant root OTUs and 12 abundant soil OTUs was significantly affected by growth stage and/or N fertilisation, but not by watering reduction (Figs 4a,b, S6, S7). The relative abundance of the Glomeraceae, Diversisporaceae, Claroideoglomeraceae, Paraglomeraceae and Archaeosporaceae was significantly affected by growth stage and/or N fertilisation in roots and soil (except for Paraglomeraceae in roots), but not by watering reduction (Figs 4c,d, S8a,b). For example, N fertilisation decreased the relative abundance of Claroideoglomeraceae and Paraglomeraceae, increased that of Diversisporaceae and Archaeosporaceae, and did not impact that of Glomeraceae in soil, but not in roots (Fig. 4c,d).

Regardless of N fertilisation treatments, LEfSe analysis showed that the relative abundances of the Paraglomeraceae, Claroideoglomeraceae, Acaulosporaceae and Archaeosporaceae were higher in soil, whereas Gigasporaceae, Diversisporaceae and Glomeraceae were more abundant in roots (Fig. 5a). In addition, the relative abundance of the *Paraglomus*, *Glomus*, *Claroideoglomus*, *Acaulospora* and *Archaeospora* was higher in soil, whereas *Rhizophagus*, *Scutellospora*, *Diversispora* and *Funneliformis* were more abundant in roots (Fig. 5a). The relative abundance of Archaeosporaceae (*Archaeospora*) and/or Diversisporaceae (*Diversispora*) was significantly higher at the N1, N2, N3 and/or N4 treatments than at the N0 treatment in soil but not in roots, while the reverse was observed for Paraglomeraceae (*Paraglomus*) and/or Claroideoglomeraceae (*Claroideoglomus*) (Fig. 5b–e).

PerMANOVA showed that the AM fungal community composition was significantly affected by compartment (root vs soil; $R^2 = 0.1999$, $P < 0.001$), growth stage ($R^2 = 0.098$, $P < 0.001$), N fertilisation ($R^2 = 0.046$, $P < 0.001$), watering reduction ($R^2 = 0.008$, $P = 0.020$), and the interaction between N fertilisation and watering reduction (soil only: $R^2 = 0.039$, $P = 0.012$) (Fig. 6a). The 'envfit' on the NMDS plot indicated that the AM fungal community composition in roots and soil significantly correlated with soil pH, NH_4^+–N, NO_3^-–N, moisture, N, P, K, N : P or C : N ratio (Fig. S9). Furthermore, AM fungal community composition in roots and soil was not significantly affected by watering reduction in each growth stage under different N fertilisation levels (Fig. 6b). By contrast, AM fungal community composition was significantly affected by N fertilisation in each growth stage in soil, but not in roots (except for June) (Fig. 6c).

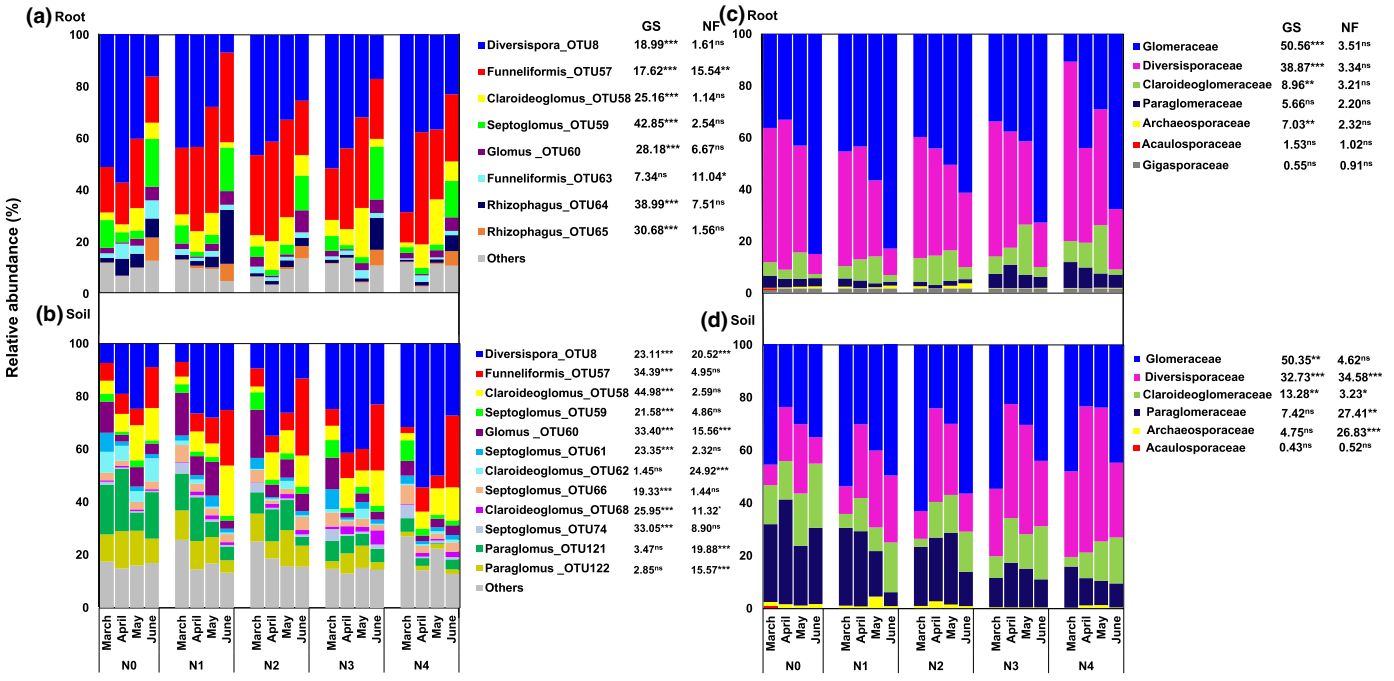

Fig. 4 Relative abundance of arbuscular mycorrhizal (AM) fungi in roots and soil at different nitrogen (N) fertilisation levels and growth stages. (a, b) The relative abundance of abundant (relative abundance > 1%) AM fungal operational taxonomic units (OTUs) in roots and soil. (c, d) The relative abundance of AM fungal families in roots and soil. Data are means ± SD ($n = 6$). Data for different watering treatments are merged because neither ANOVA nor Kruskal–Wallis tests showed any significant differences. (a, b) Others include the abundant AM fungal OTUs that were not significantly affected by N fertilisation (NF) and growth stage (GS) and the rare OTUs (relative abundance < 1%). ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. N0, N1, N2, N3 and N4 indicate 0, 70, 140, 210 and 280 kg N ha^{-1} yr^{-1}, respectively.

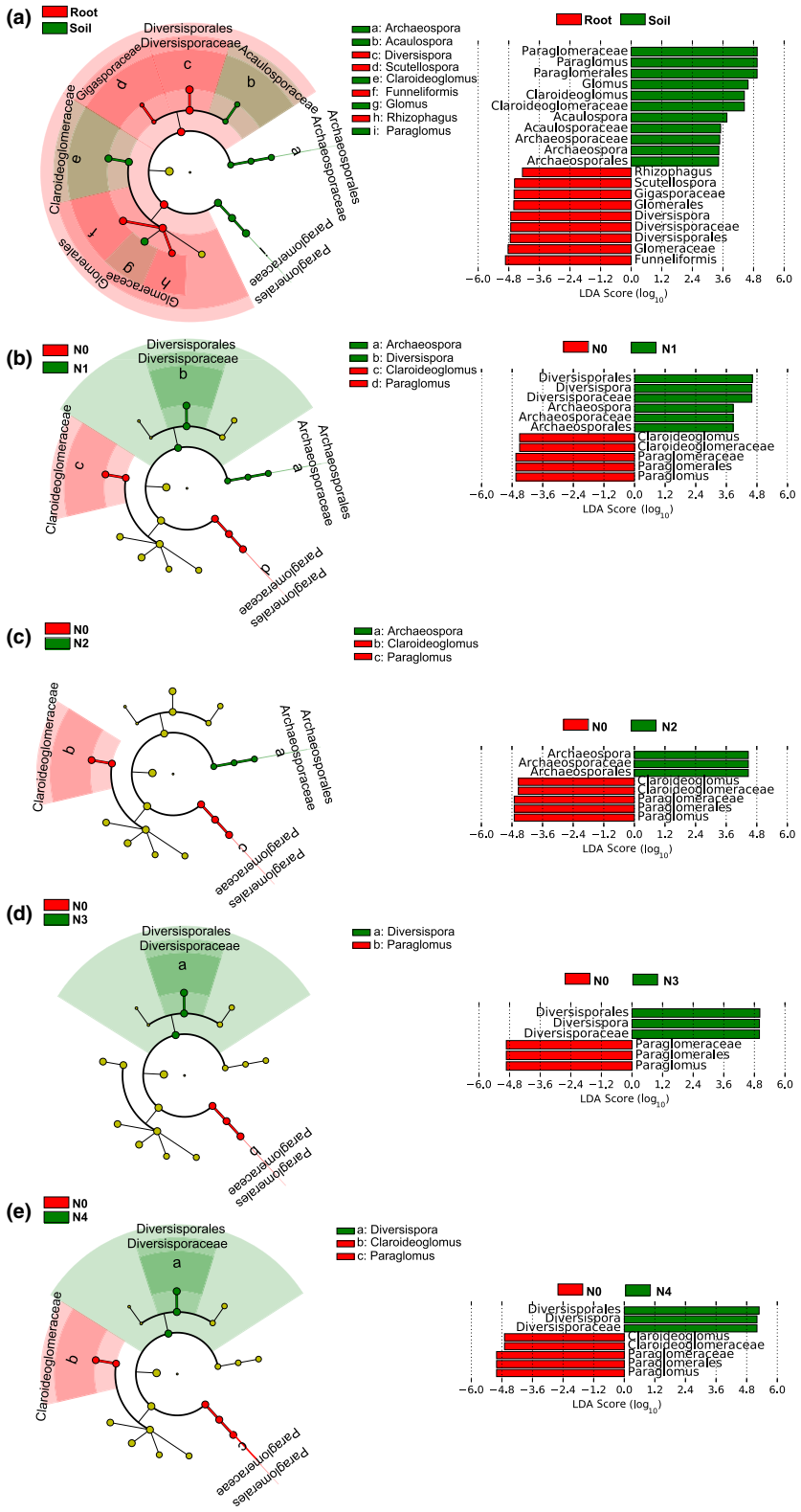

Fig. 5 Linear discriminant analysis (LDA) effect size analysis showing bias distribution of the relative abundance of arbuscular mycorrhizal (AM) fungal taxa. (a) The distribution of the relative abundance of AM fungal taxa in roots and soil. (b–e) The distribution of the relative abundance of soil AM fungal taxa between no nitrogen (N) fertilisation (N0) and different N fertilisation (N1, N2, N3, N4) levels. Significantly ($P < 0.05$) abundant AM fungal taxa are represented by red or green. Circles represent AM fungal phylogenetic levels from class to genus from the inside outwards. The AM fungal taxa with LDA values greater than 2.0 are displayed.

AM fungal community composition in roots and soil was significantly affected by growth stage at each N fertilisation level (Fig. 6d). Taken together, the temporal dynamics of AM fungal community composition in roots and soil were not influenced by N fertilisation and watering reduction.

The SEM revealed that the AM fungal community composition was directly influenced by N fertilisation and growth stage in soil and by growth stage in roots. Furthermore, N fertilisation and growth stage indirectly affected AM fungal community composition in soil but not in roots via soil exchangeable NH_4^+–N (Fig. S1c).

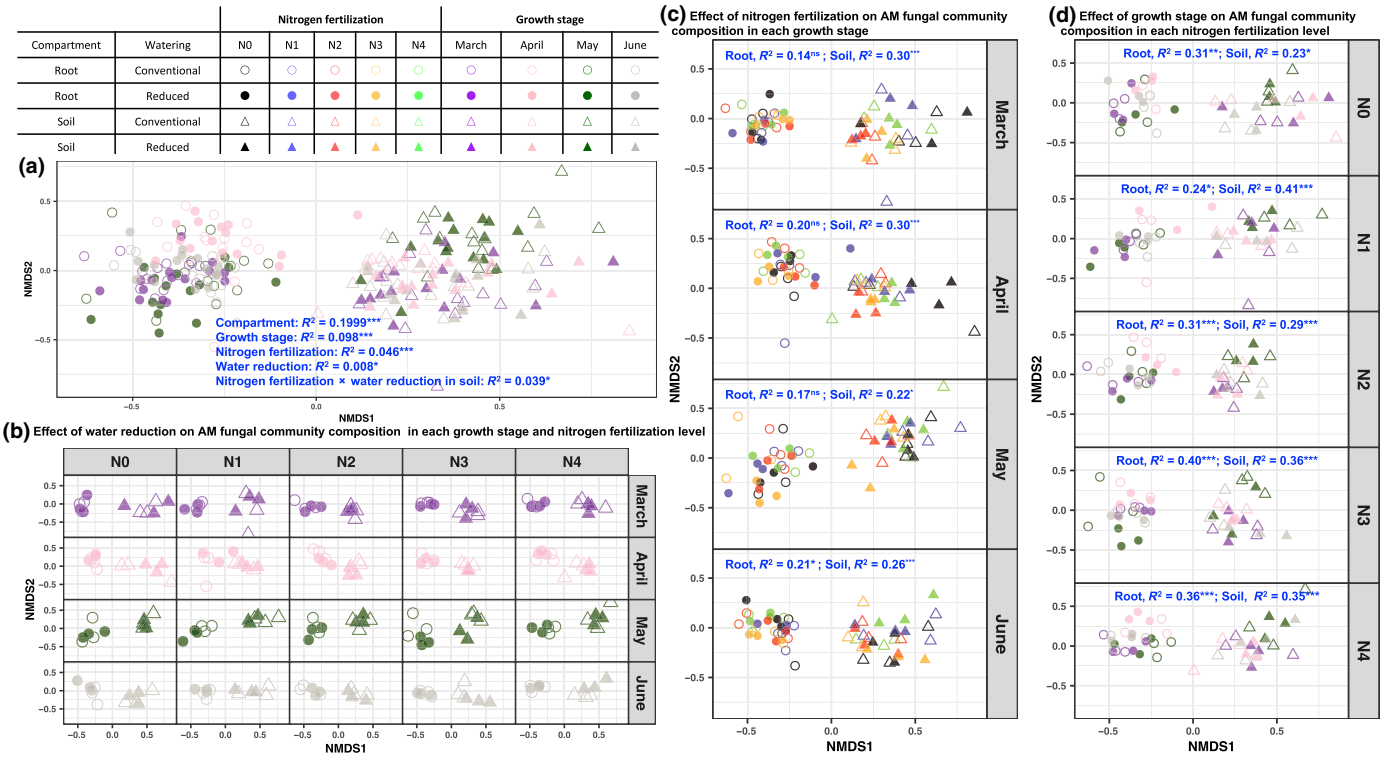

Fig. 6 Nonmetric multidimensional scaling (NMDS) of root and soil arbuscular mycorrhizal (AM) fungal community compositions at different nitrogen (N) fertilisation levels, watering conditions and plant growth stages. (a) Permutational analysis of variance (PerMANOVA) showing that AM fungal community composition was significantly affected by compartment (root vs soil), growth stage, N fertilisation, watering reduction and their interactions. (b) PerMANOVA showing that root and soil AM fungal community compositions at each growth stage and N fertilisation level were not significantly altered by watering reduction (all $P > 0.05$). (c) PerMANOVA showing that AM fungal community composition at each growth stage responded more strongly to N fertilisation in soil than in roots. (d) PerMANOVA showing that root and soil AM fungal community compositions at each N fertilisation level were significantly affected by growth stage. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. N0, N1, N2, N3 and N4 indicate 0, 70, 140, 210 and 280 kg N ha^{-1} yr^{-1}.

Discussion

Testing H_1: Nitrogen fertilisation and watering reduction reduce AM fungal IRCR, ERHD and SD

The H_1 is partially supported by our findings that N fertilisation reduced AM fungal ERHD, IRCR and SD. These results are consistent with previous studies (Table S1), as well as several extensive meta-analyses (Treseder, 2004; Zhou *et al.*, 2017; Zhang *et al.*, 2018; Han *et al.*, 2020; Ma *et al.*, 2020). The negative effect of N fertilisation on AM fungal biomass may be explained by the functional equilibrium model (Johnson, 2010), which describes how plants that experience less N limitation will invest less C in AM fungi as P is in luxury supply in our study site, thereby shifting the relationship between AM fungi and host towards a less mutualistic interaction (Fig. 7). In addition, we found that watering reduction reduced AM fungal ERHD and IRCR in early growth stage (March), but not in other growth stages at low N fertilisation levels (Fig. 3b,c). This may be because the precipitation was lower in March than in other growing stages (Fig. S10), which are likely to cause the suppression of photosynthetic activity and subsequent decline in nonstructural carbohydrates that might sustain AM fungal growth when soil water and N are less available in early plant development (Sapes *et al.*, 2021). Moreover, the adaptation of morphological root traits to watering reduction may alter the quality and quantity of root exudates, as demonstrated by Williams & de Vries (2020), and this may account for the observed decrease in the ratio of ERHD to IRCR in early plant development.

In addition, watering reduction increased AM fungal SD in all four growth stages at the N0 treatment and in March of the N1 treatment. However, for the N1 treatment, this initial increase in AM fungal SD in March was followed by a decrease with watering reduction in other growth stages (Fig. 3a). Our findings suggest how AM fungal sporulation has more complex cues associated with water availability and seasonality than was observed for N fertilisation, these are likely to be related to the complexities of C storage and investment by plants throughout the growing season when water becomes limiting. Whereas AM fungal SD is generally thought to decrease or be unaffected by watering reduction in previous studies (Table S1), our findings are consistent with recent findings from Tanzania–Serengeti where both historical water and N availability are low, and a significant increase of AM fungal SD was observed with increasing aridity (Stevens *et al.*, 2020). Therefore, the formation of a large number of spores might reflect the AM fungal strategy to survive

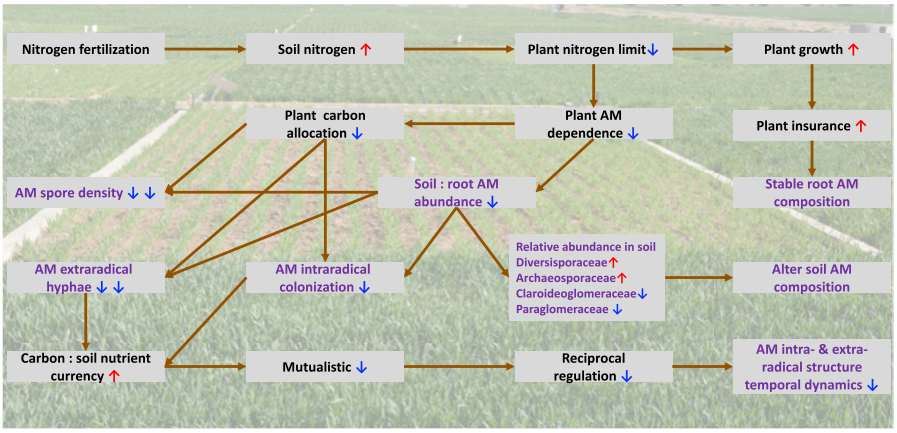

Fig. 7 A framework of the response of arbuscular mycorrhizal (AM) fungi to nitrogen (N) fertilisation. Our reports of AM fungal results are marked in purple text. Red up arrows (↑) represent an increase in response to N fertilisation, blue down arrows (↓) represent a decrease. Note the potential complex plant responses to N fertilisation, such as relieved plant N limitation, reduced plant dependence on AM fungi, reduced plant carbon allocation to AM fungi, stimulated plant growth and potential plant insurance. Subsequently, N fertilisation reduced the ratio of soil : root AM fungal biomass, extraradical hyphae, intraradical colonisation rate, spore density and the relative abundances of Claroideoglomeraceae and Paraglomeraceae, but increased that of Diversisporaceae and Archaeosporaceae in soil but not in roots. Nitrogen fertilisation may also reduce the availability of phosphorus and other soil resources, therefore increasing plant dependence on AM fungi, but this is not likely to be relevant in our case study in which phosphorus and other soil nutrients were added. Nitrogen fertilisation may also increase soil acidity, but it showed no significant influence on AM fungal community in our study with saline–alkaline soil. We cannot discount alternative causal pathways; it is also possible that N fertilisation may directly affect AM fungal communities, but these direct effects are not shown in the figure. The background photograph shows the overview of our study field. Photograph credit: Jing Li.

the contemporary harsh environment colimited by water and N availability. In addition, we acknowledge that the observed effect of watering reduction may be confounded with the potential natural gradient of soil conditions and water availability, while our experiment was carried out in a relatively homogenous agricultural field.

Furthermore, we detected significant interactive effects of N fertilisation, watering reduction and growth stage on AM fungal SD, ERHD and IRCR. For example, although N fertilisation decreased AM fungal SD, ERHD and IRCR, watering reduction had positive or negative effects on these AM fungal parameters at low, but not at high, N fertilisation levels in some growth stages. These findings suggest that the interactive effects of N fertilisation, watering reduction and growth stage on AM fungal SD, ERHD and IRCR are highly variable and context dependent, and are likely to be associated with host C allocation, fungal life-history trade-off and ecological stoichiometry (Johnson *et al.*, 2003; Kiers *et al.*, 2011; Hawkes & Keitt, 2015; Varela-Cervero *et al.*, 2016a,b; Chen *et al.*, 2017; Treseder *et al.*, 2018; Stevens *et al.*, 2020).

Testing H_2: Nitrogen fertilisation and watering reduction alter AM fungal community composition by decreasing the relative abundance of Glomeraceae and Claroideoglomeraceae and increasing that of Archaeosporaceae

The H_2 is partially supported by our finding that AM fungal community composition in soil was affected by N fertilisation but not by watering reduction. Indeed, we found that N fertilisation reduced the relative abundance of Claroideoglomeraceae and increased that of Archaeosporaceae, as the meta-analysis of Han *et al.* (2020). Although N fertilisation did not affect the relative abundance of Diversisporaceae and Paraglomeraceae (Han *et al.*, 2020), we found that the relative abundance of Diversisporaceae was increased and that of Paraglomeraceae was decreased by N fertilisation, as reported in previous studies (Borriello *et al.*, 2012; Liu *et al.*, 2012, 2015; Zhang *et al.*, 2020). In addition, Glomeraceae in relative abundance showed a neutral response to N fertilisation in our study, which was inconsistent with previous findings (Cotton, 2018; Treseder *et al.*, 2018; Lilleskov *et al.*, 2019; Han *et al.*, 2020). These different responses of AM fungal families to N fertilisation suggest that the ecological function, life-history strategy and species interaction may be different and context dependent, and are likely to be associated with host–AM fungi reciprocal regulation, ecological stoichiometry (N : P ratio), mycorrhizal dependence, plant C allocation and changes in soil environment (Figs 7, S1) (Johnson, 2010; Kiers *et al.*, 2011; Cotton, 2018; Treseder *et al.*, 2018; Lilleskov *et al.*, 2019; Han *et al.*, 2020).

Contrary to our second hypothesis, we found that AM fungal community composition in roots was generally not affected by N fertilisation and watering reduction. Our original prediction was that the benefit of AM associations for plants would decline when N became less limiting as a result of increased fertilisation. Instead, we observed that the host plant 'irrationally' maintained a relatively stable AM fungal community in roots. The relatively stable root AM fungal community may be attributed to the more stable root niche compared with the soil niche that is directly strongly affected by N fertilisation (Fig. S1c), resulting in the root AM fungal community mainly selected by the host rather than by N fertilisation (Werner & Kiers, 2015b). Furthermore, the AM fungi in roots are most likely to be alive, but soil could contain active, inactive or even dead AM fungi that cannot be

distinguished by DNA-based sequencing methods (Hempel *et al.*, 2007; Bainard *et al.*, 2014; Gao *et al.*, 2019). Therefore, it is likely that ecological and evolutionary factors shaping AM fungal community in soil are different from those shaping the root AM fungal community (Liu *et al.*, 2012). Similarly, N fertilisation was also found to correspond to shifts in the soil, but not root AM fungal community compositions in a lowland tropical forest (Sheldrake *et al.*, 2018), and soil properties did not correlate with AM fungal community composition in Tanzania–Serengeti (Stevens *et al.*, 2020). By contrast, N fertilisation caused shifts in both the root and soil AM fungal community compositions in environments typically presumed to be more nutrient rich, such as undeveloped grasslands (Liu *et al.*, 2012; Chen *et al.*, 2014) and a recently developed farmland (Zhang *et al.*, 2020).

In addition, the no effect of watering reduction on AM fungal community composition in soil and roots may be because soil field moisture capacity was maintained at 80% and 60% in conventional and reduced watering conditions in our particular study site. We postulate that these differences in watering reduction are likely to be inadequate to cause the expected shifts in AM fungal community composition. Moreover, this study was conducted in a semiarid site (559.8 mm mean annual precipitation), where historical contingency predicts that the AM fungal species existing at this site are likely to be already adapted to relatively low water availability (Compant *et al.*, 2010; Hawkes & Keitt, 2015).

Arbuscular mycorrhizal fungal families have been previously parsed into different functional groups based on spore morphology (Douds & Millner, 1999; Allen *et al.*, 2003; Oehl *et al.*, 2003). Alternatively, Weber *et al.* (2019) proposed to designate Glomeraceae, Claroideoglomeraceae and Paraglomeraceae as a rhizophilic guild that allocates more AM biomass to roots than soil, and to designate the Gigasporaceae and Diversisporaceae as an edaphophilic guild that allocates more AM biomass to soil than roots, based on the observations of a few studies (Hart & Reader, 2002; Varela-Cervero *et al.*, 2015, 2016a,b). However, we found that the relative abundances of Gigasporaceae, Diversisporaceae and Glomeraceae were higher in roots than in soil, whereas Paraglomeraceae and Claroideoglomeraceae were more abundant in soil than in roots (Fig. 5a). This divergence may be due to the differences in PCR primer usage, host plants and ecosystems in these studies (Table S7). Therefore, caution should be taken when classifying AM fungal families into 'edaphophilic' and 'rhizophilic' groups.

Testing H_3: Nitrogen fertilisation and watering reduction disrupt the temporal dynamics of AM fungi

The H_3 is partially supported by our findings that the temporal dynamics of AM fungal ERHD and IRCR were disrupted by high N fertilisation levels, but not by watering reduction throughout the host plant growth cycle. AM fungal intraradical and extraradical hyphal structures play a key role in exchanging the photosynthate and soil nutrients between the host and the fungus, which is reciprocally regulated in response to plant development and environmental fluctuations (Kiers *et al.*, 2011, 2016; Verbruggen *et al.*, 2012; Walder *et al.*, 2012; Walder & van der Heijden, 2015; Argüello *et al.*, 2016; van der Heijden & Walder, 2016). This disruption of the temporal dynamics by N fertilisation suggests that AM fungal extraradical and intraradical hyphae are less sensitive to plant development under N fertilisation. This decreased sensitivity can be attributed to the reduced dependence of the plant on the resources provided by AM fungi, thereby reducing plant C allocation to AM fungi, as well as reducing the soil versus root allocation of AM fungal biomass, which persists throughout the lifespan of the host plant. Therefore, the altered temporal viability of AM hyphal growth by N fertilisation may lead to potential dysbiosis that has an important ramification for plant nutrient acquisition from AM fungi in future environmental disturbances.

The H_3 is not supported by our findings that the temporal patterns of AM fungal SD and community composition were not impacted by N fertilisation and watering reduction. While AM fungal SD was reduced by N fertilisation, there may be strong evolutionary constraints on the reproductive phenology of AM fungi (Pau *et al.*, 2020). Additionally, the overall patterns of temporal dynamics of AM fungal community composition appeared resistant to N fertilisation and watering reduction. Traits that enable early colonisation of host plants, such as production of greater number of AM fungal spores may have important trade-offs with competitive ability (Aguilar-Trigueros *et al.*, 2019). Alternatively, host plants may selectively distribute rewards (in the form of greater allocation of photosynthate) to more beneficial partners that, over time, may result in changes in temporal dynamics in AM fungal communities (Kiers *et al.*, 2011). While patterns of succession have been observed for AM fungal communities in prior studies (Gao *et al.*, 2019), we found that neither N fertilisation nor watering reduction disrupted the temporal dynamics of the AM fungal community composition. These results imply that there are likely to be complex evolutionary constraints on AM fungal interactions within the rhizosphere that appear unaffected by these two important agricultural practices.

Conclusions

As common agricultural practice continues to impact agricultural sustainability, our research indicates how watering reduction and N fertilisation impact AM fungi that associate with wheat. Namely, AM fungal ERHD, IRCR and SD were significantly influenced by N fertilisation, watering reduction, growth stage and their interactions. In addition, AM fungal community composition had a stronger response to N fertilisation in soil than in roots throughout the growth cycle of plant irrespective of watering reduction. Interestingly, higher N fertilisation levels disrupted the temporal dynamics of AM fungal IRCR and ERHD, but not that of SD and community composition. The disruption of temporal dynamics of AM fungal hyphae implies a compromise of temporal functional trait trade-off of AM mutualism when the host plant is less dependent on AM fungi for soil-derived resources.

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Author contributions

L-DG, YZ, JL and BJB conceived and designed the study. BJB, JL, YZ and Y-LW, conducted fieldwork and BJB performed laboratory work. BJB and CG analysed and interpreted the data with vital contributions from L-DG, CEW, CAA, Y-LW, H-YG, X-CL and CW. BJB wrote the manuscript and L-DG, CG, CEW and CAA gave critical revisions. L-DG revised and approved the final manuscript. BJB and JL contributed equally to this work.

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Data availability

The representative sequence of each AM fungal OTU has been submitted to the European Molecular Biology Laboratory (EMBL) database (https://www.ebi.ac.uk/ena) (accession nos. LR792839–LR792970).

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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 SEM on the effects of nitrogen, water and time on AM fungal SD, ERHD, IRCR, richness and composition.

Fig. S2 Rank of the AM fungal OTUs by abundance.

Fig. S3 Venn diagram showing AM fungal OTUs shared and unique in roots and soil.

Fig. S4 Pie chart of the relative abundance of AM fungal families.

Fig. S5 Rarefaction curves with 95% confidence intervals for observed AM fungal OTUs.

Fig. S6 Temporal dynamics of AM fungal taxa in roots and soil.

Fig. S7 AM fungal OTUs in terms of compartment, nitrogen, water and time.

Fig. S8 AM fungal families in terms of compartment, nitrogen, water and time.

Fig. S9 NMDS of AM fungal community composition.

Fig. S10 Mean precipitation in March, April, May and June.

Table S1 A list of studies investigating the effect of nitrogen and water on AM fungal SD, ERHD, IRCR, richness and composition.

Table S2 Soil parameters in different nitrogen fertilisation treatments in reduced and conventional watering in March, April, May and June.

Table S3 Barcode sequences for each sample used in this study.

Table S4 AM fungal read counts per sample.

Table S5 Molecular identification of arbuscular mycorrhizal fungi.

Table S6 AM fungal OTUs shared and unique in root and soil.

Table S7 PCR primer usage, host plants and ecosystems in different studies.

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