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REGULAR ARTICLE

A foliar *Epichloë* endophyte and soil moisture modified belowground arbuscular mycorrhizal fungal biodiversity associated with *Achnatherum inebrians*

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Abstract
Background and aims Fungal symbionts, present in above and in belowground tissues, such as that of *Epichloë* endophytes and arbuscular mycorrhizal (AM) fungi, respectively, can modify the responses of host plants to environmental changes. Individual grass plants of the subfamily Pooideae can be host to both a foliar *Epichloë* endophytic fungus and root-associated AM fungi. Understanding the multiple interactions among above- and belowground symbionts and their host is an important step in understanding terrestrial ecosystems.
Methods A field experiment was conducted to study the effects of *E. gansusensis* endophyte and soil moisture on the belowground AM fungal biodiversity associated with *Achnatherum inebrians*, through amplicon sequencing technology. Soil properties were compared among stands using standard techniques.
Results Our results show that *E. gansusensis* increased root-associated AM fungal diversity under drought conditions, while decreasing diversity under the water addition treatment. Water addition and water stress treatments decreased the diversity and richness of the AM fungal community in rhizosphere soil compared to the normal treatment. The *E. gansusensis* altered the composition of the root-associated AM fungal community. Aboveground biomass was closely positively related to the abundance of *Funneliformis* in the root and the diversity of the rhizosphere soil AM fungal community was positively related to the soil total nitrogen and phosphorus.
Conclusion This study suggested that soil moisture regimes shifted the effects of *E. gansuensis* on the diversity of the root-associated AM fungal community from positive to negative; moreover, soil moisture and foliar *E. gansusensis* altered soil properties, thereby affecting belowground AM fungi.

Keywords Foliar *Epichloë* endophyte · Root-associated · Rhizosphere soil · AM fungi · Soil moisture · Biodiversity · *Achnatherum inebrians*

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Introduction

Arbuscular mycorrhizal (AM, phylum Glomeromycota) fungi participate in reciprocal symbiosis with roots of more than 80% of land plants, and these symbioses are the most common and widespread terrestrial plant symbioses (Smith and Read 2008). AM fungi contribute to improvements in the acquisition of mineral nutrients, such as phosphorus (P), nitrogen (N) and trace elements,

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through mycelial network structures in nutrient-deficient soil (Souza 2015; van der Heijden et al. 2015) and improve the tolerance of host plants to drought (Grümberg et al. 2015), heavy metals (Almeida-Rodríguez et al. 2015) and pathogens (Sikes et al. 2009; Malik et al. 2016) in return for 10%–20% of the photosynthetic carbon from host plants (Souza 2015; van der Heijden et al. 2015). The importance of soil AM fungal communities on key ecosystem processes is well studied, and AM fungal diversity is recognized to influence the plant productivity, plant-plant interactions, the structure and biodiversity of plant communities and the conservation and restoration of ecosystems (van der Heijden et al. 1998a b, 2015).

Host species and environmental conditions are dominant factors that have major effects on the composition and diversity of AM fungal communities in terrestrial ecosystems (Davison et al. 2015; Li et al. 2015; Souza 2015), and a successful symbiosis is very dependent on soil properties and the host plant (Souza 2015). The contribution of AM fungal communities is commonly affected by management practices in agricultural ecosystems (Lin et al. 2012; Li et al. 2015; Chen et al. 2016a) and resource availability in natural ecosystems (Liu et al. 2012, 2015). A common agricultural management practice affecting the AM fungal community composition is the application of fertilizers, such as those containing potassium (K), N and P, and these have positive, neutral or negative effects on the soil AM fungal community composition in agricultural ecosystems (Lin et al. 2012; Chen et al. 2016a; van Geel et al. 2016; Zhou et al. 2016). Water-deficit is another vital limiting abiotic factor for belowground AM fungal communities in agricultural and grassland ecosystems (Souza 2015). Studies found that the amount and distribution of rainfall can also affect root-associated and soil AM fungal communities in natural grasslands (Sun et al. 2013; Li et al. 2015).

Many studies have been conducted on the mutualistic associations between cool-season grasses of Pooideae and the seed-borne systemic fungal endophytes of the genus *Epichloë* (Schardl et al. 2005; Johnson et al. 2013; Becker et al. 2016; Soto-Barajas et al. 2016). The fungal endophytes are present in all tissues of host grasses other than the roots, and these vegetative tissues are symptomless (Christensen et al. 2008). Transmission of many *Epichloë* species is entirely vertical in nature, in the seeds of host plants (Schardl et al. 2005). Most studies on these symbiotic associations involved grasses of the genera *Lolium* and *Festuca*, as the presence of these endophytes enhances the fitness and productivity of host plants under abiotic and biotic stress in grassland and agricultural ecosystems (Johnson et al. 2013; Becker et al. 2016; Soto-Barajas et al. 2016). Drunken horse grass (*Achnatherum inebrians*, DHG), is a widespread perennial bunchgrass in grasslands of the semiarid regions of China (Shi 1997). In North-Western China, nearly every DHG plant was infected by either *E. gansuensis* (Li et al. 2004) or *E. inebrians* (Chen et al. 2015a). Studies have shown the presence of *E. gansuensis* improved the fitness of DHG under the presence of heavy metals (Zhang et al. 2010), low temperature (Chen et al. 2016b), drought (Xia et al. 2018), salt (Wang et al. 2018), fungal pathogenic attack (Xia et al. 2015, 2016) and insect pests stress (Zhang et al. 2012). The presence of alkaloids, including ergonovine and ergine, in *E. gansuensis*-infected DHG plants (Zhang et al. 2014) is associated with livestock toxicosis (Liang et al. 2017).

As mentioned above, host plants can receive protection from either the presence of an *Epichloë* endophye or from AM fungi, especially under drought stress (Hosseini et al. 2016; Mirshad and Puthur 2016; Xia et al. 2018). Grass species commonly form symbiotic associations with both a foliar *Epichloë* endophyte and belowground AM fungi, and the study of the interactions between above- and belowground microbes is important to understand community ecology (Bell-Dereske et al. 2017). Several studies have been conducted to investigate these effects of *Epichloë* endophytes on belowground AM fungi within a single grass species (Liu et al. 2011; Omacini et al. 2006; Rojas et al. 2016); most studies showed that the *Epichloë* endophyte reduced the root AM fungal colonization (Omacini et al. 2006; Antunes et al. 2008; Mack and Rudgers 2008; Liu et al. 2011; Slaughter et al. 2018), while some studies conversely indicated that *Epichloë* endophyte had a promoting (Larimer et al. 2012; Zhong et al. 2017) or neutral (Liu et al. 2011; Vandegrift et al. 2015; Slaughter and McCulley 2016) effect on root AM fungal colonization. These responses of belowground AM fungi to the presence of *Epichloë* endophytes may be affected by the composition of the root exudates (Novas et al. 2011; Guo et al. 2015) or the composition of the root volatile organic compounds (Rostas et al. 2015) of the endophyte-infected grasses.

Our previous research indicated that *E. gansuensis* significantly reduced the diversity of the DHG root-

associated fungal community (Zhong et al. 2018). Another study reported that watering primarily affected the variation of the root-associated and soil AM fungal communities (Li et al. 2015). However, little research has been conducted to investigate how the presence of *Epichloë* endophytes affects the diversity and composition of belowground AM fungal communities under different soil moisture treatments in agricultural or grassland ecosystems. A field experiment was conducted to explore the potential effects of soil moisture and *E. gansuensis* on the diversity and composition of the AM fungal community of plant roots and rhizosphere soil of DHG through amplicon sequencing technology, Therefore, we hypothesized that (1) the *E. gansuensis* endophyte and water stress/addition negatively affect the belowground AM fungal diversity and community composition associated with DHG, and (2) endophyte and soil moisture effects may occur through alteration of properties of rhizosphere soil.

Materials and methods

The origin of seeds

Twenty tillers of several individual *A. inebrians* plants were selected to determine the presence or absence of *E. gansuensis* by microscopic examination of leaf sheath pieces stained with aniline blue. Seeds were collected in 2011 from one plant with 100% endophyte-infected tillers. These seeds were divided into two parts; before planting, one part was treated with thiophanate-methyl fungicide to eliminate the ability of the endophyte to infect seedlings (Li et al. 2016) and the other part did not receive any treatment. Two hundred seedlings of each of the two parts were planted in an experimental field of College of Pasture Agriculture Science and Technology, Yuzhong campus of Lanzhou University (Yuzhong site) in 2012. The endophyte-infected status of all plants was checked by microscopic examination of leaf sheath pieces and seeds stained with aniline blue from 2012 to 2013, and these plants were individually labeled as endophyte-infected (EI) and endophyte-free (EF) plants. The seeds used for this study were obtained from the one EI and one EF *A. inebrians* plant in 2013.

Site description and experimental design

A total of nine field experimental plots (each plot: 4.8 m × 4.0 m) were established for this study in May 2014, located at the College of Pastoral Agriculture Science and Technology, Yuzhong campus (104°39′E, 35°89′N, attitude 1653 m) of Lanzhou University. Each plot was separated by a 1 m deep cement wall and each was evenly divided into two subplots along the center line of the long side, and inner walls with waterproof membrane were constructed between two subplots which were separated with a 1 m interval. The two subplots were established with either EI or EF DHGs seedlings, with a plant spacing of 40 cm. Before being planted, 50 seeds were selected randomly from the EI and EF seeds to confirm the endophyte infection status of the seeds used to establish these plots. The endophyte infection status of 50 EI and 50 EF selected seeds was determined, and the result showed that endophyte infections were 100% and 0 for the EI and EF seeds, respectively.

The three water treatments were performed from May to October in 2014–2016, with a split plot design that included three replicate plots. One treatment maintained the normal water content conditions (N), receiving only natural precipitation; 321 mm, 282 mm and 256 mm from 2014 to 2016, respectively. The second treatment was drought stress (D), with the plot being shielded when it rained. The third treatment was the water addition treatment (W), whose 45%–60% relative saturation soil moisture content was maintained through overhead sprinkle irrigation from automatic sprinklers.

Sampling

Soil and root samples were collected in October 2016 at the conclusion of the water treatments study. For each subplot, five soil and root samples were taken from five individual DHG plants with root crown width of approximately15 cm, using a 20 cm soil auger, and then these samples were mixed to form a composite sample. Root and soil samples were placed in an icebox and transported to the laboratory. In addition, the foliage was removed from each of the five sampled plants and the grass dry biomass was recorded when a constant weight had been reached in an 80 °C oven. In the laboratory, the root samples were shaken to remove the excess soil, and the residual soil remaining on the root was retained as rhizosphere soil. The root samples were gently washed

with tap water several times then rinsed with sterile water, and then were dried with sterilized filter papers. These root samples were stored at −80 °C prior to DNA extraction. Soil samples were passed through a 2.0 mm sieve and homogenized, and some were stored at 4 °C before soil chemical analysis, and the others were stored at −80 °C before DNA extraction.

Soil and biological properties

The soil pH was analyzed in soil/water mixtures at a ratio of 1:2.5. Plant available phosphorus (AP) was calculated by shaking 2.5 g air-dried soil for 30 min with 0.5 M sodium bicarbonate ($NaHCO_3$) solution at pH 8.5, and then extracts were colorimetrically analyzed using the molybdenum blue method (Robertson et al. 1999), Available potassium (AK) was extracted with ammonium acetate and analyzed using flame photometry (Helmke and Sparks 1996), and soil organic matter (SOC) was measured according to Nelson and Sommers (1982) using 0.25 mm sieved soil. Total nitrogen (TN), total P (TP), ammonium-N (AN) and nitrate-N (NN) in the soil were measured using a continuous flow analyzer (FIAstar5000Analyzer) (Zhao et al. 2014).

DNA extraction, nest PCR and sequencing

For root samples, 0.1 g fresh root of each sample was homogenized in liquid N, and a plant DNA kit (Tiangen, Beijing) was used to extract DNA. For soil samples, an E.Z.N. A.® Soil DNA Kit (OMEGA, Shanghai) was used to extract DNA from 0.5 g soil. DNA extraction samples were diluted 10 times with double-distilled water, and then stored at −80 °C prior to subsequent experiments. Glomeromycota sequences were amplified using the small subunit (SSU) rRNA gene primer. The first PCR reaction was performed with the universal fungal primers (AML1-F: 5'-ATC AAC TTT CGA TGG TAG GAT AGA-3′ and AML2-R: 5′-GAA CCC AAA CAC TTT GGT TTCC-3′) to amplify a fragment of approximately 800 bp from the 18S rRNA gene (van Geel et al. 2014). PCRs were carried out in a final volume of 20 μL with 10 ng genomic DNA and 0.8 μL of each primer (0.5 μM) using the Pfu PCR mastermix system (Tiangen Biotech) with the following cycling conditions: 95 °C for 120 s; 32 cycles (94 °C for 30 s; 55 °C for 30 s and 72 °C for 45 s) and 72 °C for 600 s. Successful products of the first amplification were diluted 1:100 and 2 μL of this dilution was used as a template in the second PCR with specific primer (AMV4.5NF: 5′-AAG CTC GTA GTT GAA TTT CG-3′ and AMDGR: 5'-CCC AAC TAT CCC TAT TAA TCAT-3′). PCRs were run with the same conditions as described above using the following cycling conditions: 95 °C for 120 s; 30 cycles (95 °C for 30 s; 55 °C for 30 s and 72 °C for 45 s); and 72 °C for 600 s. All PCRs were run on a GeneAmps PCR system 2700 (Applied Biosystems). PCR products were examined on a 1.5% (*w*/*v*) agarose gel with ethidium bromide staining. PCR products of each root and soil sample were purified with a PCR Purification Mini Kit (Aidlab Biotechnologies, Beijing, China) according to the manufacturer's instructions and then sent to Majorbio Pharm Technology (Shanghai, China) for amplicon sequencing.

Sequencing data

Those sequencing reads with ambiguous nucleotides, a quality score < 20, and lacking complete barcode and primer, were removed and excluded from further analysis. The remaining sequences were assigned to different operational taxonomic units (OTUs), with a 97% identity threshold using QIIME (http://qiime.org/scripts/assign_taxonomy.html). Sequences obtained from amplicon sequencing were subjected to the Silva (Release 128 http://www.arb-silva.de) to identity these OTUs. The resulting OTUs were compared using the National Center for Biotechnology Information (NCBI) website to obtained the similar OTUs from other studies to construct Neighbour-Joining (NJ) phylogenetic trees using MEGA 6.06 software. The DNA sequences used in this study have been deposited in Sequence Read Achieve (SRA) of NCBI database under accession numbers PRJNA574265.

Alpha and beta diversity analysis

Alpha diversity indexes were calculated using Mothur software (Schloss et al. 2009). Community richness was determined with Chao1 index through the following formula:

$$Chao1 = S_{obs} + \frac{F_1(F_1-1)}{2(F_2+1)}$$

where the S_{obs} which represents the number of observed OTUs, and F_1 and F_2 are the number of singletons and doubletons in each sample, respectively.

Community diversity was expressed as the Shannon index (H') and was calculated using the following formula:

$$H' = -\sum_{n=1}^{s} (P_i log_2 P_i)$$

where s is the number of OTUs and P_i is the proportion of the fungal community represented by the OTU*s*.

Principal coordinates analysis (PCoA) of DHG plant-root-associated and rhizosphere soil AM fungal communities based on OTUs level were performed using Bray-Curtis dissimilarities through the R-package Vegan (Oksanen et al. 2013). The statistically significant differences of root-associated and rhizosphere soil AM fungal communities under the different treatments were performed through permutational multivariate one-way analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM) based on the Bray-Curtis dissimilarities. Redundancy analysis (RDA) between grass biomass and root-associated AM fungal community composition and between soil properties and rhizosphere soil AM fungal community composition, was performed by CANOCO for Windows 4.5.

Statistical analyses

Differences in rhizosphere soil properties, root-associated and rhizosphere soil AM fungal community diversity and aboveground grass biomass under different endophyte and soil moisture levels were tested using a two-way analysis of variance (two-way ANOVA) in SPSS 22.0 (SPSS, Chicago, IL, USA). Significant differences in rhizosphere soil properties, root-associated and rhizosphere soil AM fungal community diversity and aboveground grass biomass between EI and EF DHG plants with the corresponding water treatment were examined by an independent-sample t-test, and significant differences in rhizosphere soil properties, root-associated and rhizosphere soil AM fungal community diversity, and aboveground grass biomass among the different soil moisture treatments were examined by one-way analysis of variance (one-way ANOVA). Tukey test was used to determine whether differences between the means were statistically significant. In all tests, a P value <0.05 was considered statistically significant.

Structural equation modeling

Structural equation modeling (SEM) was used to identify potentially causal linkages between explanatory variables and AM fungal diversity. Based on the linear regression results we estimated the strength of direct and indirect relationships among the considered variables. We checked the bivariate relationships between variables to ensure that linear models were appropriate. Several SEM models based on the known effects and potential relationships among the drivers of AM fungal diversity were constructed. The fit of each model using the χ^2-test was evaluated. SEM analyses were performed using AMOS 24.0 (Amos Development Co., Greene, Maine, USA). The remaining statistical analyses were conducted using SPSS 22.0 (SPSS, Inc., Chicago, Illinois, USA).

Results

Soil properties and grass biomass

Soil moisture and the status of foliar *Epichloë* endophyte had significant effects on DHG rhizosphere soil NN ($F_W = 48.621$, $P_W = 0.000$; $F_E = 8.385$, $P_E = 0.013$) and AN ($F_W = 23.397$, $P_W = 0.000$; $F_E = 132.816$, $P_E = 0.000$), and the interactions between the two factors were significant (Table 1). The soil AN of the N treatment was significantly ($P = 0.000$) higher than that of the D and W treatments (Fig. S1a), and soil NN significantly ($F_W = 48.621$, $P_W = 0.000$) increased with the increase of soil moisture (Fig. S1b). *Epichloë* endophyte significantly ($F = 4.047$, $P = 0.003$) decreased the soil AN under the D treatment, whereas, it significantly ($F = 0.119$, $P = 0.028$) increased the soil AN of the N treatment (Fig. S1a). Meanwhile, rhizosphere soil NN of EF DHG was significantly higher than that of EI DHG under the D ($F = 0.131$, $P = 0.008$), N ($F = 4.324$, $P = 0.001$) and W ($F = 0.472$, $P = 0.008$) treatments (Fig. S1b). Soil moisture ($F = 60.353$, $P = 0.000$) and *Epichloë* endophyte ($F = 26.741$, $P = 0.000$) had a significant effect on the rhizosphere soil AP; the soil AP significantly ($F = 19.746$, $P = 0.000$) decreased with the increase of soil moisture, and the rhizosphere soil AP of the EI DHG was significantly ($F = 5.349$, $P = 0.006$) higher than that of the EF DHG under the D treatment (Fig. S1c). Soil moisture had a significant ($F = 6.768$, $P = 0.008$) effect on the rhizosphere soil TN, and the soil

TN of the N treatment was significantly higher than that of the D and W treatment (Fig. S1d).

The soil moisture ($F = 6.695$, $P = 0.010$) and *Epichloë* endophyte ($F = 62.429$, $P = 0.000$) had a significant effect on the biomass of DHG; the DHG biomass of the W treatment was significantly higher than the DHG biomass of the D and N treatments, and the biomass of EI DHG was significantly higher than the biomass of EF DHG of the D ($F = 0.070$, $P = 0.029$), and N ($F = 3.780$, $P = 0.002$) and W ($F = 3.538$, $P = 0.011$) treatments (Table 1).

Diversity of AM fungal community

The interaction between soil moisture and *Epichloë* endophyte had significant ($F = 6.826$; $P = 0.010$) effects on the diversity of the DHG root-associated AM fungal community, as summarized by the Shannon index (Fig. 1a). The diversity of the EI DHG root-associated AM fungal community was significantly ($F = 0.083$, $P = 0.029$) higher than that of EF DHG under the D treatment, while the diversity of the EF DHG root-associated AM fungal community was significantly ($F = 3.593$, $P = 0.030$) higher than that of EI DHG under the W treatment (Fig. 1a).

Soil moisture had significant effects on the diversity ($F = 7.222$; $P = 0.009$) and richness ($F = 3.965$; $P = 0.048$) of the DHG rhizosphere soil AM fungal communities, as summarized by the Shannon and Chao1 indices, and the diversity of DHG rhizosphere soil AM fungal communities under the N treatment was significantly ($F = 6.533$, $P = 0.009$) higher than that under the D and W treatments (Fig. 1b). Furthermore, the richness of AM fungal communities in the DHG rhizosphere soil under the N treatment was significantly ($F = 0.550$, $P = 0.021$) higher than that under the W treatment. No significant difference was observed between the richness of the AM fungal communities in DHG rhizosphere soil between the D and N/W treatments (Fig. 1d). Finally, the diversity ($F = 20.640$, $P = 0.001$) and the richness ($F = 24.688$, $P = 0.044$) of the AM fungal community of DHG rhizosphere soil were significantly higher than those of the roots (Fig. 1).

Composition of AM fungal community

A total of 663,097 and 689,610 sequences were obtained from root and soil samples by using the AMV4.5/AMDGR primers, respectively (Table S1). All sequences were assigned to 86 OTUs with 97% similarity; 85 OTUs were detected in soil, and 74 OTUs were detected in the roots, and 73 OTUs were present in both the roots and the soil (Table S1 and Fig. S2). The 86 OTUs obtained were divided into 5 families, and 8 genera, and 4 OTUs were unclassified (Fig. S3).

The Glomeraceae (53 OTUs, 94.6% sequences) was the most abundant family in the DHG root-associated AM fungal community, and others included the Claroideoglomeraceae (11 OTUs, 4.6% sequences), Diversisporaceae, Paraglomeraceae and Archaeosporaceae (Fig. 2a, b, Table S1). The Glomeraceae (58 OTUs, 68.7% sequences) and Claroideoglomeraceae (13 OTUs, 29.8% sequences) were the dominant families in the DHG rhizosphere soil AM fungal community, and other OTUs were within the Paraglomeraceae, Diversisporaceae, and Archaeosporaceae (Fig. 2c, d, Table S1). The Glomeraceae was the most abundant family in all AM fungal communities in roots and rhizosphere soil under different soil moisture treatments and endophyte status (Fig. 2e, f). *Glomus* was the most abundant genus of AM fungal communities in all roots and most rhizosphere soil under different soil moisture treatments and endophyte status, whereas *Claroideoglomus* was the most abundant genus in the rhizosphere soil of EI DHGs under the W treatment (Table S1).

Rarefaction curves showed that the number of reads was sufficient to detect the majority of AM fungal sequence types from all soil and root samples, since the curves reached stable plateaus (Fig. S4ab). The PCoA and ANOSIM analysis results indicated that the composition of the root-associated AM fungal community was significantly ($P = 0.05$) different between the EI and EF DHGs (Fig. 3a and Table 2). However, no differences in the AM fungal community composition of DHG rhizosphere soil were observed under the different soil moisture treatments and endophyte status (Fig. 3b and Table 2).

Relationship among AM fungi, soil properties and grass biomass

Spearman correlation results indicated that the diversity of the AM fungal community of DHG rhizosphere soil was significantly ($P = 0.033$) and positively associated with rhizosphere soil TP (Table 3), while the richness was significantly ($P = 0.008$) positively correlated with the rhizosphere soil TN (Table 3). The first and second

Table 1 Rhizosphere soil properties and grass biomass associated with *Achnatherum inebrians* under different soil moisture (W) and endophyte (E) treatments, and results of two-way analysis of variance (two-way ANOVA) for the W and E on rhizosphere soil properties and biomass of *A. inebrians* (n = 3, D: drought, N: normal, W: water addition, EI: endophyte-infected and EF: endophyte-free)

Treatments	SOC (%)		TN (%)		TP (%)		NN (mg/Kg)		AN (mg/Kg)		AP (mg/Kg)		pH		AK (mg/Kg)		Biomass (g)
DEI	0.653 ± 0.023		0.059 ± 0.000		0.071 ± 0.002		3.521 ± 0.145		5.200 ± 0.188		8.062 ± 0.179		8.33 ± 0.06		21.32 ± 1.21		29.993 ± 5.3521
DEF	0.633 ± 0.037		0.060 ± 0.000		0.072 ± 0.000		4.575 ± 0.064		6.403 ± 0.156		6.827 ± 0.083		8.40 ± 0.09		17.03 ± 1.17		15.110 ± 5.5610
NEI	0.667 ± 0.006		0.061 ± 0.000		0.076 ± 0.003		5.473 ± 0.128		4.469 ± 0.350		6.793 ± 0.083		8.36 ± 0.07		16.69 ± 1.78		35.260 ± 4.3128
NEF	0.657 ± 0.034		0.060 ± 0.001		0.071 ± 0.001		4.930 ± 0.097		7.984 ± 0.083		6.033 ± 0.315		8.36 ± 0.07		18.02 ± 2.09		15.970 ± 1.6694
WEI	0.637 ± 0.063		0.057 ± 0.001		0.071 ± 0.001		4.226 ± 0.110		6.437 ± 0.308		5.613 ± 0.159		8.38 ± 0.08		21.01 ± 0.57		37.620 ± 0.255
WEF	0.679 ± 0.022		0.059 ± 0.000		0.068 ± 0.003		4.566 ± 0.153		8.217 ± 0.184		5.334 ± 0.151		8.41 ± 0.05		18.34 ± 1.46		25.183 ± 4.8350
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
W	0.154	0.859	8.480	**0.005**	2.097	0.166	48.621	**0.000**	23.397	**0.000**	60.353	**0.000**	0.154	0.859	1.396	0.285	6.965	**0.010**
E	0.022	0.885	2.203	0.164	2.652	0.129	8.385	**0.013**	132.816	**0.000**	26.741	**0.000**	0.392	0.543	2.470	0.142	62.429	**0.000**
W*E	0.446	0.651	2.297	0.143	0.909	0.429	22.233	**0.000**	13.662	**0.001**	3.553	0.061	0.166	0.849	1.964	0.183	1.040	0.383

Values are mean ± standard error. F is F-value, statistical value of F-test; *P* value is probability greater than the calculated value; significance $P \leq 5\%$, 1%, and 0.1% levels, respectively. Soil factors indicated include SOC (Soil Organic Carbon), TN (Total Nitrogen), TP (Total Phosphorus), AN (Ammonium Nitrogen), NN (Nitrate Nitrogen), AP (Available P), AK (Available potassium) and biomass (dry matter per plant). The number in blod indicated $P<0.05$.

axis of RDA explained 43.5% and 2.6% of the variance, respectively, and the grass biomass of DHGs was closely positively related to the abundance of *Funneliformis* in the DHG root-associated AM fungal community (Fig. 4a). The grass biomass was also positively related to the soil AP, AK and TP (Fig. 4a). Additionally, the first and second axis of RDA between the soil AM community and soil properties explained 35.6% and 21.2% of the variance, respectively (Fig. 4b), and the abundance of *Glomus* in the soil was positively related to the TP, AP and AK (Fig. 4b). Furthermore, the abundance of *Claroideoglomus*, *Funneliformis* and *Rhizophagus* in the soil was positively related to the TP, SOC and C:N ratio, and the abundance of the other three genera (*Diversispora*, *Paraglomus* and *Archaeospora*) was positively related to the soil TN, available N and pH (Fig. 4b).

The factors structuring AM fungal community diversity

The best Structural Equation Modeling (SEM) model ($\chi^2 = 15.281$, $Df = 15$, $P = 0.431$, $NFI = 0.872$, $RMSEA = 0.370$) explained 32.1% of the variations in the root-associated AM fungal community diversity and 79.3% variations in the rhizosphere soil AM fungal community diversity (Fig. 5). *E. gansuensis* increased the diversity of the root-associated and rhizosphere soil AM fungal community through increasing soil AP (Fig. 5). Soil moisture treatments decreased the diversity of the rhizosphere soil AM fungal community through decreasing soil AP and increasing soil pH (Fig. 5).

Discussion

Plant roots and soil are commonly associated with a highly complex AM fungal diversity in most terrestrial ecosystems (Lin et al. 2012; Chen et al. 2014; Davison et al. 2015; Li et al. 2015). AM fungal diversity in roots and plant rhizosphere soil have been traditionally detected and classified through morphological features (Bhadalung and Rungchuang 2005; Gai et al. 2012; Sun et al. 2013) and molecular sequencing technology (Davison et al. 2015). Now, sequencing technology is commonly used to study the AM fungal biodiversity of roots and soil in grassland (Chen et al. 2014; Li et al. 2014; Davison et al. 2015; Li et al. 2015) and agricultural (Lin et al. 2012; Chen et al. 2016a, b) ecosystems. The AM fungal community diversity and composition

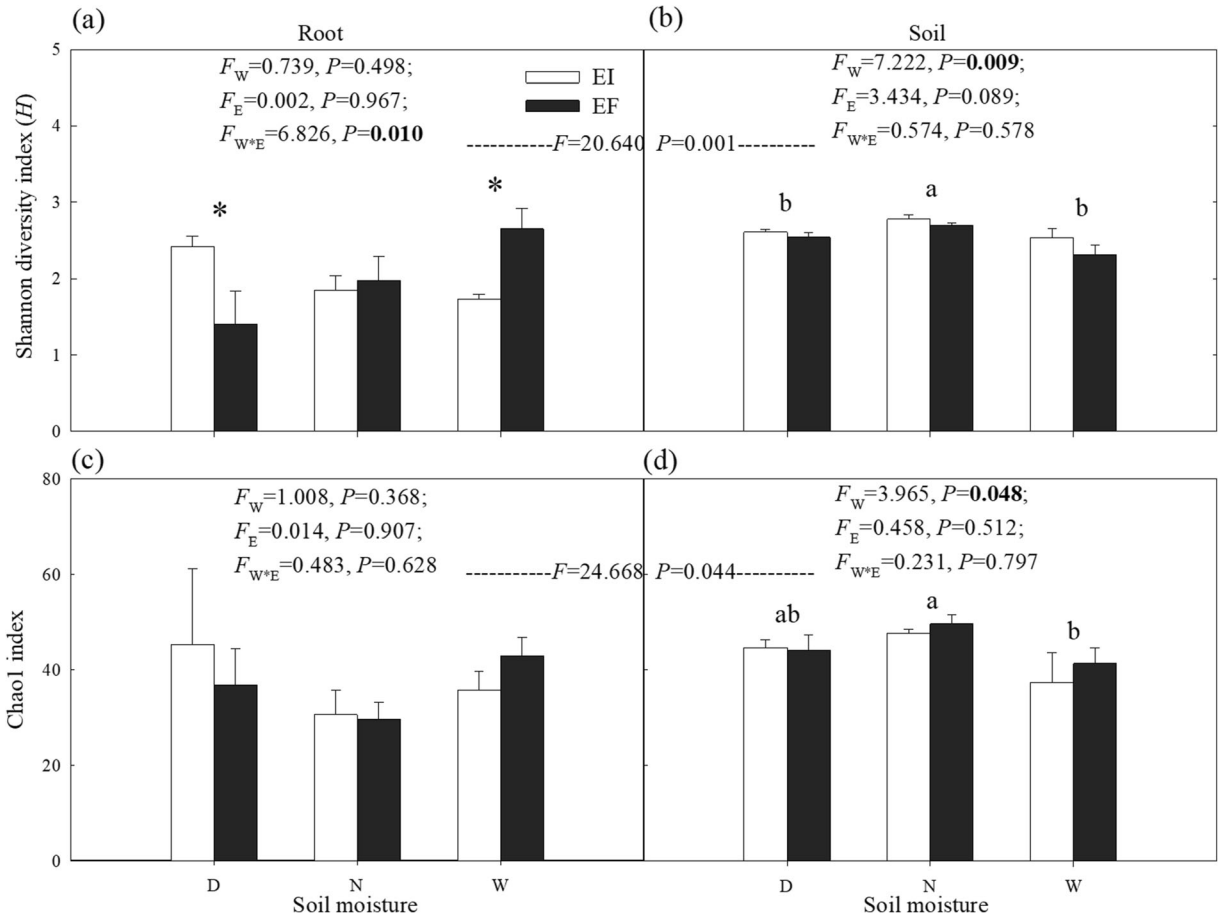

Fig. 1 Arbuscular mycorrhizal fungal community diversity in roots (**a** and **c**) and soil (**b** and **d**) under different soil moisture and endophyte treatments ($n = 3$, D: drought, N: normal, W: water addition, EI: endophyte-infected and EF: endophyte-free). Values are mean ± standard error (SE), with bars indicating SE. The asterisk (*) means significant difference at $P < 0.05$ (independent t-test) between and EI and EF plants at corresponding water content at 0.05 level. The a, b and c mean significant difference at $P < 0.05$ among corresponding water content at 0.05 level

were commonly and widely affected by host identity and environmental factors (Davison et al. 2015; Souza 2015). The present study used AM-fungal-specific primers to assess the effects of the presence of the systemic endophyte of the aboveground tissue of DHG, and the different soil moisture levels, on the AM fungal community diversity and composition in roots and rhizosphere soil through the application of amplicon sequencing technology.

Diversity of root-associated and rhizosphere soil AM fungal community

The most common use of sequencing techniques is the studying of microbial communities, especially AM fungal communities through different primer pairs (Erica et al. 2010; van Geel et al. 2014; Davison et al. 2015), and the AMV4.5NF-AMDGR primer pair commonly results in higher quality reads than other primer pairs, such as primer pairs NS31/AM1. (Erica et al. 2010; van Geel et al. 2014). This present study that used the AMV4.5NF-AMDGR primer pair indicated that the roots and the rhizosphere soil harboured a relatively high biodiversity in this DHG monoculture agroecosystem. The present study showed that the diversity and richness of the rhizosphere soil AM fungal community are significantly higher than those within the root-associated AM fungal community, as observed by other studies about grassland (Chen et al. 2014) and agricultural (Borriello et al. 2012) ecosystems. Glomeraceae (71.6% OTUs,

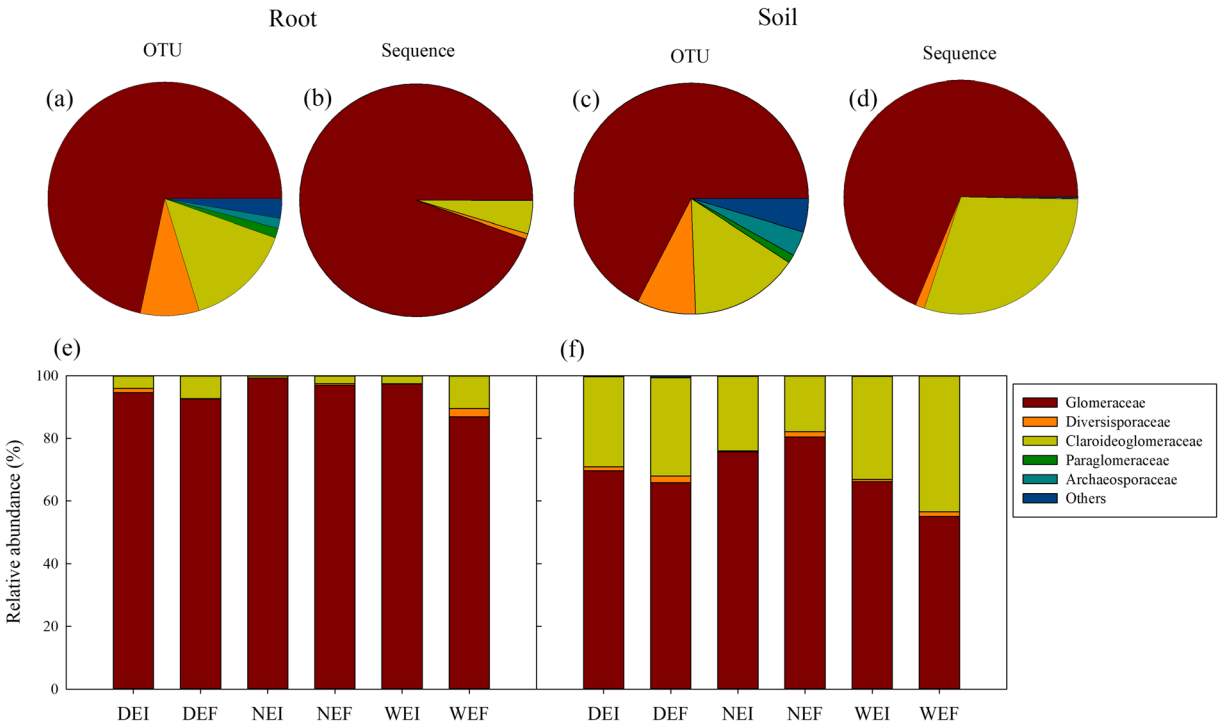

Fig. 2 The relative abundance of operational taxonomic units (OTUs) (**a**) and sequences (**b**) in all root samples, OTUs (**c**) and sequences (**d**) in all soil samples and different AM fungal families in roots (**e**) and rhizosphere soil (**f**) associated with *A. inebrians* under different soil moisture and endophyte treatments ($n = 3$, D: drought, N: normal, W: water addition, EI: endophyte-infected and EF: endophyte-free). Values are mean of three replicates

94.6% sequences in roots; 69.4% OTUs, 68.7% sequences in soil) was the most abundant family within all samples, and varied from 86.8% to 99.3% in the roots, and from 55.1% to 80.4% in the rhizosphere soil. These results indicate that the Glomeraceae is an active functional AM fungal group in all root and soil samples, which is in line with previous studies that Glomeraceae was the dominant AM fungal family in plant roots and soil in most terrestrial ecosystems (Lin et al. 2012; Li et al. 2014, 2015; van Geel et al. 2016). The present study also indicated that *Glomus* was the most abundant genus in soil AM fungal communities under all treatments and in root-associated AM fungal communities under most treatments; these results also agreed with the results of previous studies in which *Glomus* was the most abundant AM fungal genus associated with roots of *Elymus nutans* (Liu et al. 2012), *Heteropogon contortus* (Li et al. 2010) and soil in an agroecosystem (Lin et al. 2012). However, we also found *Rhizophagus* was the dominant genus in the DHG root-associated AM fungal community under the DEF, NEI and WEI treatments, similar to a study found that *Rhizophagus* was the most abundant genus in a root-associated AM fungal community associated with *Zea mays* in a high diversity cover crop system (Turrini et al. 2016). As expected, sequences belonging to the families Paraglomaceae and Archaeosporaceae were detected in the present study with the primer set AMV4.5NF/AMDGR. Meanwhile, the study by Erica et al. (2010) indicated that the primer set AMV4.5NF/AMDGR had advantage to amplify some AM fungal families such as Paraglomaceae over primers NS31/AM1.

Effects of soil moisture on biodiversity of belowground AM fungal community

Previous studies have indicated that the diversity and level of belowground microbial symbionts is affected by the availability of soil moisture, especially by a change in precipitation (Clark et al. 2009; Sun et al. 2013; Chen et al. 2014; Zhang et al. 2014) and by drought stress (Deepika and Kothamasi 2015; Naylor et al. 2017; Santos-Medellín et al. 2017; Xu et al. 2018). A study showed that mean

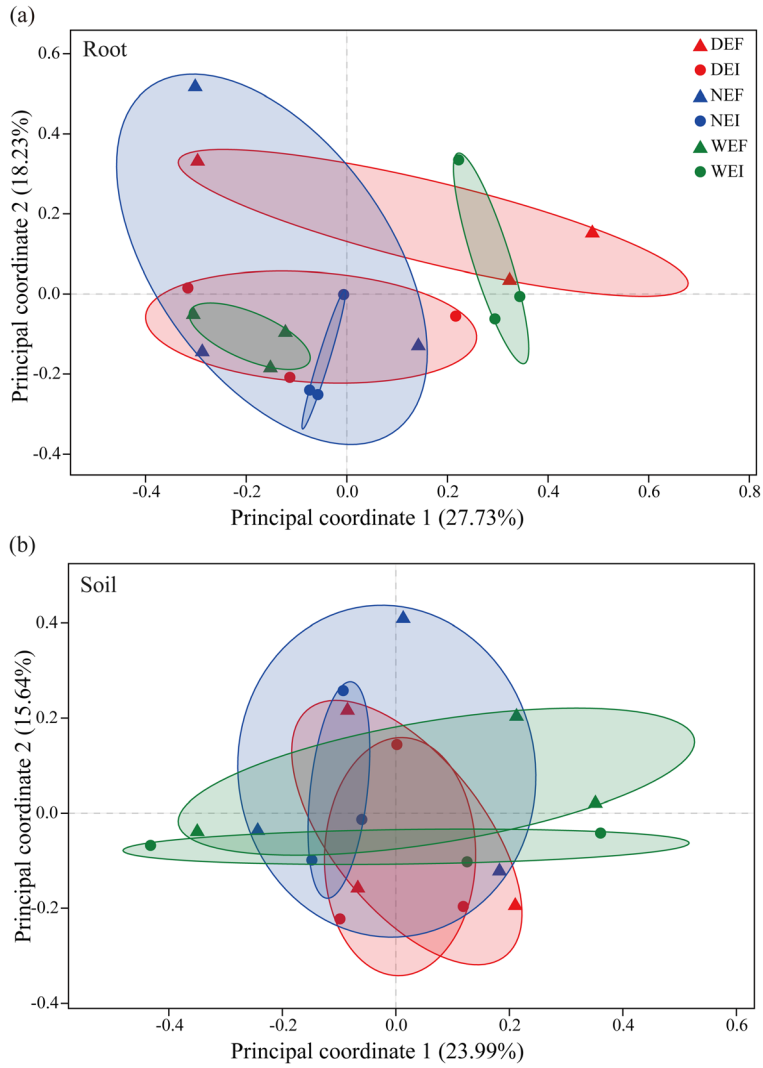

Fig. 3 Principal coordinates analysis (PCoA) of DHG plant root-associated (**a**) and soil (**b**) AM fungal communities at operational taxonomic units (OTUs) level based on the Bray-Curtis dissimilarities under different soil moisture and endophyte treatments ($n = 3$, D: drought, N: normal, W: water addition, EI: endophyte-infected and EF: endophyte-free)

Table 2 The statistical test of analysis of similarity (ANOSIM) and permutational multivariate one-way analysis of variance (PERMANOVA) to analyze differences in root-associated and rhizosphere soil AM fungal community compositions measured by amplicon sequencing under different soil moisture (W) and the status of foliar *Epichloë* endophyte (E) treatments

Type	Treatment	df	PERMANOVA		ANOSIM	
			F	*P*	*R*	*P*
Root	W	2	0.441	0.743	0.000	0.461
	E	1	2.571	0.105	0.333	**0.050**
	W*E	2	1.818	0.167		
Soil	W	2	0.936	0.497	−0.099	0.801
	E	1	0.722	0.566	−0.247	0.988
	W*E	2	0.313	0.953		

annual precipitation had strong positive effects on soil total fungal diversity (Tedersoo et al. 2014), and the increase of 30% precipitation increased the soil microbial biomass by 20% (Chen et al. 2015b), and increased precipitation reduced the spore germination of *Claroideoglomus etunicatum*, while it increased the spore germination of *Ambispora gerdemannii* (Sun et al. 2013). Additionally, drought altered the composition of the soil fungal community in a grassland ecosystem (Schmidt et al. 2017). Furthermore, drought also altered the composition of the root-associated fungal community of rice (*Oryza glaberrima*) (Santos-Medellín et al. 2017) and the AM fungal community of *Sorghum vulgare* (Deepika and Kothamasi 2015). In contrast, our study indicated that soil moisture had no effect on

Table 3 Spearman correlations of alpha diversity in root and rhizosphere soil AM fungal community to soil properties and grass biomass

Soil properties	Roots		Soil	
	Shannon	Chao1	Shannon	Chao1
AN	−0.287	−0.309	0.375	0.339
NN	0.195	0.026	−0.446	−0.080
TN	−0.145	−0.156	0.382	**0.604****
TP	−0.356	−0.437	**0.503***	0.364
AP	−0.107	−0.004	0.417	0.278
AK	−0.134	−0.211	0.006	−0.163
pH	0.228	0.410	−0.218	−0.343
SOC	0.215	−0.152	0.073	0.067
C/N	0.059	−0.101	−0.250	0.065
Available N	0.188	−0.018	−0.425	−0.140
N/P	−0.287	−0.309	0.375	0.339

Soil factors indicated include SOC (Soil Organic Carbon), TN (Total Nitrogen), TP (Total Phosphorus), AN (Ammonium Nitrogen), NN (Nitrate Nitrogen), AP (Available P), AK (Available potassium), C/N (Total Organic Carbon: Total Nitrogen) N/P (Available N: Available P), * indicated $P < 0.05$, ** indicated $P < 0.01$

the diversity and richness of the root-associated AM fungal community, in agreement with a previous study that both increased and reduced precipitation did not affect the diversity of the root-associated AM fungal community associated with *Ammophila breviligulata* (Bell-Dereske et al. 2017). The first hypothesis was supported by the present study that drought and water addition treatments reduced the diversity and richness of the rhizosphere soil AM fungal community in DHG.

Our results also supported the second hypothesis that soil moisture treatments resulted in the changes in the soil properties, and the variation in soil properties (e.g. soil TN, pH, NN and AP) brought changes to the diversity of the soil AM fungal community. A previous study showed that water treatments affected the soil microbe community by altering the availability of soil nutrients (Zhang et al. 2014). Studies also showed that the changes of soil availability of soil properties (Liu et al. 2015), especially soil moisture (Li et al. 2015) and soil pH (Dumbrell et al. 2011) could structure the diversity of the belowground AM fungal community in terrestrial ecosystems. Soil moisture has been thought to be a regulator of the soil AM fungal community assembly (Deepika and Kothamasi 2015) and spore sporulation (Sun et al. 2013; Souza 2015), and the favourable soil moisture for AM fungal spore germination was recorded as approximately 5–28% (Souza 2015).

Effects of foliar *Epichloë* endophyte on belowground AM fungi

The first hypothesis was supported by the present study that the *E. gansusensis* affected the diversity of the DHG root-associated AM fungal community from positive to negative with the increase of soil moisture and modified the composition of the DHG root-associated AM fungal community. Studies have found that foliar *Epichloë* endophytes affected the belowground microbial community (Rojas et al. 2016; Bell-Dereske et al. 2017; Zhong et al. 2017, 2018). Chu-chou et al. (1992) first found that the presence of *E. coenophiala* in tall fescue suppressed AM fungal colonization. Subsequently, most studies demonstrated, using pot experiments in greenhouses, that the presence of an *Epichloë* endophyte in host grasses could negatively (Mack and Rudgers 2008; Slaughter et al. 2018; Omacini et al. 2006; Liu et al. 2011; Zhou et al. 2016), neutrally (Antunes et al. 2008; Vandegrift et al. 2015; Slaughter and Mcculley 2016) and positively (Larimer et al. 2012; Arrieta et al. 2015; Zhong et al. 2017) affect mycorrhizal colonization. Moreover, the presence of an *Epichloë* endophyte in *A. sibiricum* decreased the spore density of *F. mosseae* in rhizosphere soil (Zhou et al. 2016), while the presence of *E. coenophiala* in tall fescue (*Festuca arundinacea*) increased the abundance of Glomeromycota in rhizosphere soil (Rojas et al. 2016). Our previous studies with *A. inebrians* plants host to *E. gansusensis* also indicated that the presence of the endophyte positively affected the mycorrhizal colonization (Zhong et al. 2017) and negatively affected the diversity of root-associated fungi (Zhong et al. 2018).

Our results support the second hypothesis that the foliar *Epichloë* endophyte affects the rhizosphere soil properties, such as AN, NN and AP, which affected the diversity of belowground AM fungi. Our second hypothesis was also supported by some previous studies which indicated that foliar *Epichloë* endophytes commonly result in change in rhizosphere soil properties (van Hecke et al. 2005; Guo et al. 2016), especially soil organic C (Iqbal et al. 2012), organic N (Buyer et al. 2011) and inorganic N (Franzluebbers and Hill 2005). Additionally, a study suggested that positive effects of an *Epichloë* endophyte on the phosphorus-solubilizing

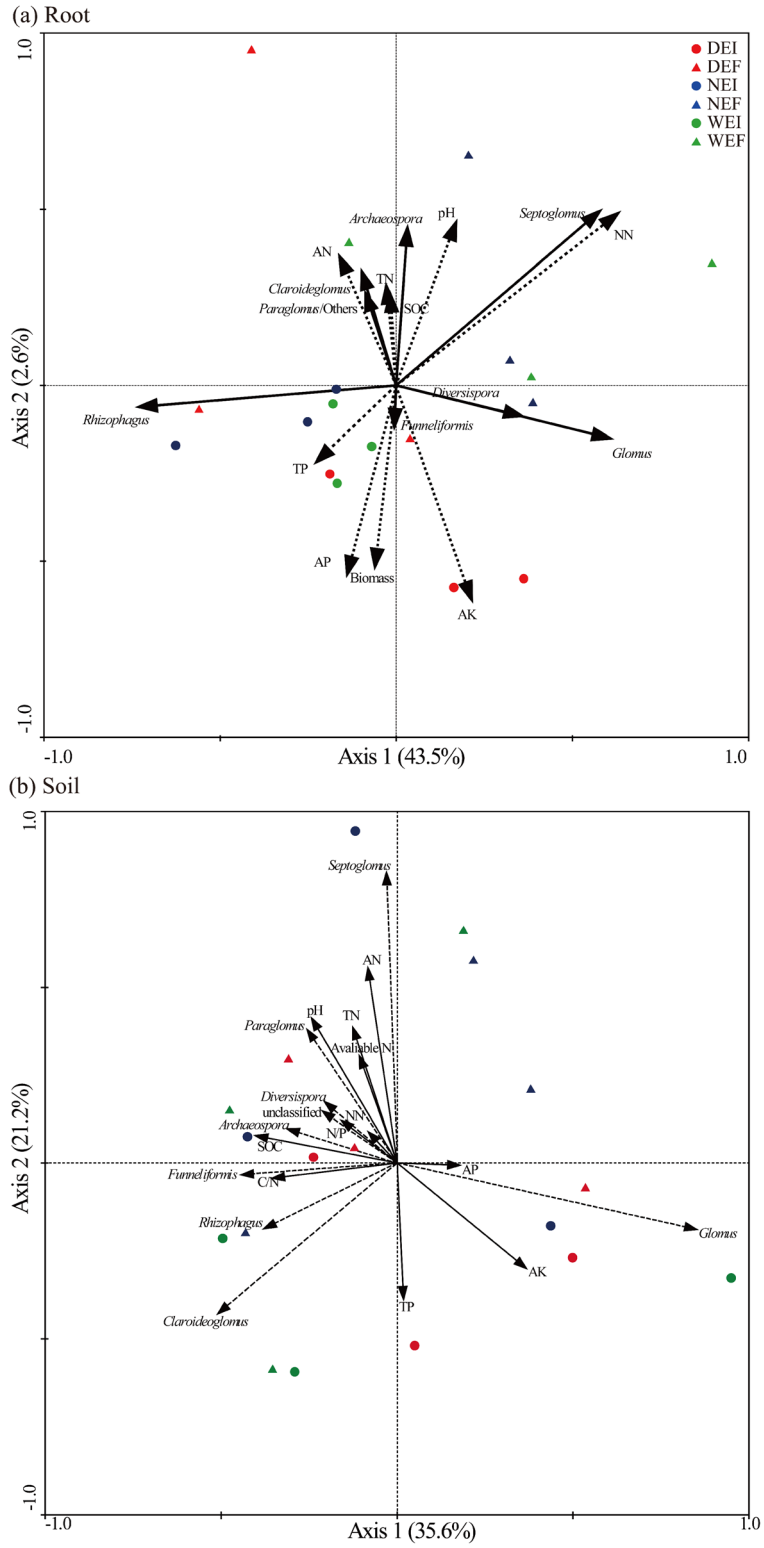

Fig. 4 Redundancy analysis of relative abundance of root-associated AM fungal genera, and grass biomass and soil properties (**a**), relative abundance of rhizosphere soil AM fungal genera and soil properties (**b**) under different soil moisture and endophyte treatments ($n = 3$, D: drought, N: normal, W: water addition, EI: endophyte-infected and EF: endophyte-free). The solid line indicates AM fungal genus, and dashed line indicates the soil properties and grass biomass. Soil factors indicated include TN (total N), TP (total P), AP (available P), SOC (soil organic carbon), AN (ammonium N), NN (nitrate N), AK (available potassium), biomass (dry matter per plant)

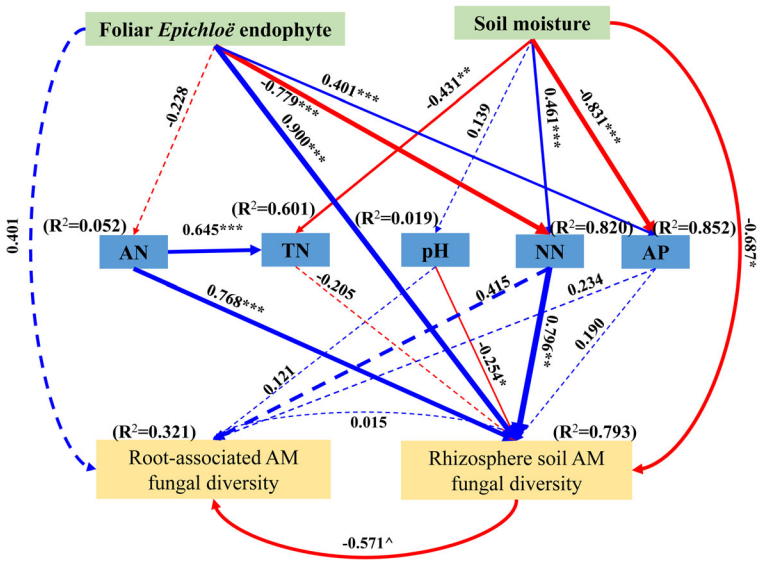

Fig. 5 The results of final structural equation modelling showing the causal relationships among foliar *E. gansuensis* endophyte, soil moisture, ammonium N (AN), soil total N (TN), soil pH, soil available P (AP), soil nitrate N (NN), root-associated and rhizosphere soil AM fungal community diversity. Arrows indicate significant relationships. The blue line represents the positive effect, red line represents the negative effect, and solid circular indicate significantly interactive effect. The width of arrows indicates the strength of the causal effect. R^2 values represent the proportion of variance explained for each variable. Model fit summary ($X^2 = 15.281$, $Df = 15$, $P = 0.431$, $NFI = 0.872$, $RMSEA = 0.370$) are shown. Numbers above arrows indicates path coefficients. $^P < 0.1$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

rhizosphere soil fungal diversity would generate an increase in the P available to plants (Arrieta et al. 2015). Studies have suggested that the presence of *Epichloë* endophytes could affect belowground AM fungi through a chemical function, with *Epichloë* exudates and root exudates of EI grass promoting development between plant roots and AM fungi (Novas et al. 2011; Vignale et al. 2018). Other studies revealed that the presence of an *Epichloë* endophyte in host grasses, even though hyphae are absent in roots, altered the composition of the root exudates (Guo et al. 2015) and root volatile organic compounds (Rostás et al. 2015), which may help asymbiotic mycelium growth at the root surface (Souza 2015). The aqueous extracts of the *E. coenophiala*-infected tall fescue foliage reduced the spore germination of *G. intraradices* by 10% compared to that of EF plants (Antunes et al. 2008), and that study also suggested that the inhibition may be caused by secondary metabolites including alkaloids produced by foliar *Epichloë* endophytes. Subsequent studies have detected the presence of endophyte-produced loline alkaloids in the roots of tall fescue (Patchett et al. 2008; Nagabhyru et al. 2013). Alkaloids including ergonovine and ergine, are present in aboveground DHG tissues (Zhang et al. 2014), but no studies were conducted to determine their presence in the roots and rhizosphere soil associated with DHG, so, we need further research to verify that foliar *E. gansusensis* affected the belowground AM fungi through alkaloids of the EI DHG grasses.

Relationships between the AM fungi and environmental factors

A successful symbiotic association between plant roots and AM fungi begins with AM fungal spore germination, which is affected by soil available resources, such as soil fertility and moisture (Souza 2015). Our second hypothesis was supported by this present study, which showed that water treatments and the presence of the *Epichloë* endophyte brought changes to the soil properties, and a close association was also observed between the belowground AM fungi and soil properties. A previous study indicated that the spore density of the AM fungi was significantly related to the soil pH and SOC (Gai et al. 2012), while the diversity and richness of the soil AM fungi were negatively related to the soil pH in this study; this suppression was explained by a study that had revealed that high pH reduced arbuscule and

vesicle formation of *G. intraradices* (van Aarle et al. 2002).

The present study showed that total available N and P available in the soil was significantly different among the different soil moisture treatments and the presence or absence of the *Epichloë* endophyte, and the diversity and richness of belowground AM fungi were closely related to the availability of soil N and P. Glomeromycota abundance in the soil fungal community was significantly positively correlated to soil NN (Chen et al. 2016a, b). Additionally, it has been shown that the addition of N and P fertilization promoted the relative abundance of *Glomus* and *Archaeospora* in soil; however, it suppressed the abundance of some major AM fungal genera (e.g., *Acaulospora*, *Scutellospora* and *Gigaspora*) in the soil (Lin et al. 2012); low P and higher available N could stimulate the AM fungal spores to grow (Hoeksema et al. 2011; Verbruggen et al. 2012; Pellegrino et al. 2015). Furthermore, available N and P were shown to have significant effects on the diversity and composition of the AM fungal community in association with two co-occurring perennial plants, *Pennisetum centrasiaticum* and *Kobresia* sp. (Li et al. 2014).

Many plant species depend on these mycorrhizal symbionts for growth and survival, and 80% of plant N and P is provided by mutualistic mycorrhizal fungi (van der Heijden et al. 2015). The present study found that the biomass of DHG was highly and positively related to the abundance of *Funneliformis* in a root-associated AM fungal community. One of three AM fungal OTUs in the DHG roots is likely to related to *F. mosseae*; this AM fungus significantly increased the shoot biomass (25-folds) and the photosynthetic efficiency (44%) of *Glycyrrhiza uralensis* (Chen et al. 2017). Nonetheless, belowground AM fungal communities can be partially attributed to stochastic processes in our study. This finding that AM fungal communities were codetermined by both stochastic (neutral-based) and deterministic (niche-based) processes in terrestrial ecosystems, is in agreement with previous studies (Lekberg et al. 2007; Dumbrell et al. 2011; Hazard et al. 2013).

Conclusions

This study revealed that soil moisture has stronger effects on the diversity and richness of the belowground AM fungal community than the foliar endophyte *E. gansuensis*. In addition, the study shows that the presence of *E. gansuensis* had different effects on the diversity of the DHG root-associated AM fungal community under different soil moisture treatments, and soil moisture can shift the effects of *E. gansuensis* on belowground AM fungal community diversity associated with DHG. Moreover, the water stress and water addition treatments significantly decreased the diversity and richness of the rhizosphere soil AM fungal community compared to the normal soil moisture treatment. Additionally, we also found that the biomass of DHG plants was highly positively related to their root-associated AM fungal abundance at the genus level, and the diversity and composition of the rhizosphere soil AM fungal community were also closely related to the rhizosphere soil properties. Further research is needed to explore the mechanism of the presence of *E. gansuensis* in DHG plants and soil moisture on the diversity and composition of the belowground AM fungal community through the composition and content of root exudates.

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