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Effects of *Epichloë gansuensis* on root-associated fungal communities of *Achnatherum inebrians* under different growth conditions

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ABSTRACT

This study was conducted to investigate how *Epichloë gansuensis* endophyte and soil disturbance affect root-associated fungi (RAF) of *Achnatherum inebrians* (drunken horse grass, DHG), using Illumina sequencing techniques. The rhizosphere soil of wild endophyte-infected (W-EI) DHG had significantly ($P < 0.05$) higher available phosphorous and potassium, total organic matter, ammonium and nitrate nitrogen than cultivated soil. In addition, the rhizosphere soil of endophyte-infected DHG had significantly ($P < 0.05$) lower pH and nitrate nitrogen, and higher available phosphorous, than endophyte-free DHG under cultivated conditions. The sequencing provided a total of 54,413 sequences and these were assigned into 190 operational taxonomic units (OTUs) with 97% similarity. Ascomycota was the most dominant phylum in roots of three DHG populations. W-EI DHG had significantly ($P < 0.05$) higher RAF diversity than cultivated endophyte-infected (C-EI) DHG. The presence of endophyte significantly ($P < 0.05$) decreased RAF diversity under cultivation. The principal component analysis (PCA) and sample similarity analysis results indicated that both endophyte and soil disturbance could bring changes to RAF community composition. The RDA results demonstrated the RAF of W-EI DHG were positively correlated with soil properties, and the RAF of cultivated DHG roots were negatively correlated with soil properties. This study demonstrated that both endophyte and soil disturbance resulted in changes to the RAF communities.

1. Introduction

Plant tissues form a wide variety of symbiotic associations with microorganisms and the nature of the associations range from parasitism to mutualism (Baker et al., 1997; Van der Heijden et al., 2008; Philippot et al., 2013; Averill et al., 2014). An example of a mutualistic association with aboveground tissue is provided by systemic, seed-borne fungal endophytes belonging to the genus *Epichloë* (Leuchtmann et al., 2014). This type of fungal endophyte is found in many cool-season grasses of the subfamily Pooideae and hyphae are present in all tissues except roots (Christensen et al., 2008). Vegetative tissues of all associations are symptomless and transmission of many species is entirely vertical in nature, in the seed of host plants (Schardl et al., 2004; Christensen et al., 2008). However, with some *Epichloë* spp., horizontal transmission can occur arising from the production of stromata in which the sexual stage is produced and from which ejected ascospores can penetrate stigmata of flowers on neighbouring plants and colonize developing seeds (Chung and Schardl, 1997). The most studied associations are those involving grasses of the genera *Lolium* and *Festuca*, as they have enhanced persistence and productivity in forage livestock farming (Kuldau and Bacon, 2008; Becker et al., 2016; Soto-Barajas et al., 2016).

Roots are colonized, both internally and externally, by a wide range of root-associated fungi (RAF) (Keim et al., 2014; Wehner et al., 2014). The most studied RAF are those that form mutualistic mycorrhizal associations (Van der Heijden et al., 2015). The most abundant mycorrhizal fungi are those that form vesicles and arbuscules in roots and are connected to the soil via long hyphae (Smith and Read, 2008). They belonging to the phylum

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Glomeromycota, and are named arbuscular mycorrhizal fungi (AMF) (Smith and Read, 2008; Bonfante and Genre, 2010). Another type of mutualistic mycorrhizal association is that in which the fungi form a hyphal sheath over root tips and hyphae also surround the cortical cells; these ectomycorrhizal associations are common with tree species and provide a range of benefits (Van der Heijden et al., 2015; Martin et al., 2016). Most of the fungi producing ectomycorrhizal associations are Basidiomycota and Ascomycota (Van der Heijden et al., 2015).

Many other fungi form associations with roots with the nature of the associations including some that are probably mutualistic, some pathogenic, and some that are saprotrophic and breakdown dead root tissue, while the role of many is unknown (Hamilton and Bauerle, 2012; Wehner et al., 2014; Aguilar-Trigueros and Rillig, 2016). One group that is being intensively studied is referred to as the dark septate endophytes (DSE) (Bonfim et al., 2016; Santos et al., 2016). As with the known mycorrhizal fungi, they produce characteristic inter- and intra-cellular structures in root cortical tissues and some reports indicated that the associations between some DSE fungi and host plants might benefit both partners (Santos et al., 2016).

Drunken horse grass (*Achnatherum inebrians*, DHG), is a perennial bunchgrass in northwestern China, and is widely distributed in the arid and semi-arid grasslands in Gansu, Xinjiang and Inner Mongolia (Shi, 1997; Li et al., 2004). In these grasslands nearly every DHG plant will be host to either of two *Epichloë* spp., *Epichloë gansuensis* (formerly *Neotyphodium gansuense*) (Li et al., 2004; Leuchtmann et al., 2014) and *Epichloë inebrians* (Moon et al., 2007; Chen et al., 2015). Studies into the functional impacts of an *Epichloë* sp. in DHG have shown that its presence improves tolerance to some biotic and abiotic stresses including drought (Li et al., 2008), low temperature (Chen et al., 2016), some fungal pathogens (Xia et al., 2015, 2016), heavy metals (Zhang et al., 2010) and insect pests (Zhang et al., 2012). The presence of alkaloids, including ergonovine and ergine, in *E. gansuensis*-infected DHG plants, are associated with livestock toxicosis and thus they are little grazed (Zhang et al., 2014). Recent research has shown that these toxic plants provide a protected nursery enabling the reestablishment of the original plant flora in degenerated grasslands, the result of overgrazing (Yao et al., 2015). However, trials have indicated that endophyte-free DHG could be utilized as animal feed (Liang et al., 2017).

The traditional way that the colonization of roots by fungi was determined involves the isolation of fungi from surface-sterilized roots and also examination of clarified stained roots (Sun and Tang, 2012; Dalpé and Séguin, 2013). Although these procedures have provided much understanding there are limitations to the extent of information that can be obtained regarding the range of fungi present (Vierheilig et al., 2005; Sun and Tang, 2012). In recent years, sequencing techniques have enabled the identification of large numbers of fungal species in plants and soils in a wide range of environment conditions (Lin et al., 2012; Wehner et al., 2014; Kazeeroni and Al-sadi, 2016; Zhou et al., 2016a).

Grasses commonly form symbiotic relationships with many fungi including foliar endophytes and RAF such as AMF and DSE (Vandegrift et al., 2015; Slaughter and McCulley, 2016). *Epichloë* spp. endophytes are present in seeds prior to germination and their growth is synchronized with that of the host (vertical transmission) (Christensen et al., 2008), whereas RAF colonized roots horizontally (Smith and Read, 2008; Van der Heijden et al., 2015). Additionally, all of these fungi acquire carbon from host grasses, with the foliar-confined *Epichloë* endophytes being located within the tissues where photosynthesis occurs (Mack and Rudgers, 2008), and thus the presence of plant-nutrient dependent foliar endophytic fungi may inhibit the colonization of roots by some fungal species. Some studies have indicated that *Epichloë* endophytes can have different (suppression, promotion and no) effects on AMF, while having no effect on DSE in roots (Chu-Chou et al., 1992; Larimer et al., 2012; Vandegrift et al., 2015; Slaughter and McCulley, 2016; Zhou et al., 2016b). In addition, the RAF communities were commonly influenced by specific environmental factors (Blaalid et al., 2012, 2014; Fujimura and Egger, 2012; Yu et al., 2013). In the present study, the RAF communities of cultivated and wild DHG plants either with or without the foliar endophyte were examined. Based on previous findings two hypotheses were considered in this study. The first is that the occurrence of endophyte may influence or alter the RAF communities, and the second is that soil disturbance may also alter the RAF communities.

2. Materials and methods

2.1. Study site description and sample collection

The experimental study site, established in 2011, is located at the College of Pastoral Agriculture Science and Technology, Yuzhong campus (104°39′ E, 35°89′ N, attitude 1653 m) of Lanzhou University (Xia et al., 2015, 2016). A field area of DHG plants (32 endophyte-infected (EI) plots and 32 endophyte-free (EF) plots; each plot: 3.65 m × 1.30 m, 3 lines and 8 rows) were established using seeds collected from 100 EI and 100 EF DHG plants, respectively, that had been assessed by microscopic examination for the presence or absence of characteristic hyphae of *E. gansuensis* (Xia et al., 2015). To confirm that the seeds that were collected from both categories of plants were actually either EI or EF as expected, leaf sheaths of seedlings grown from each seed lot were stained with aniline blue and microscopically examined for the presence of the hyphae characteristic of the endophyte (Li et al., 2004, 2008). These plots were then established and were regularly watered and weeded. Four EI and EF experimental plots were randomly selected for this study. For each plot, five individual DHG plants were selected randomly, root and soil samples were collected using a 20-cm soil auger from close to each plant, roots samples were removed from each of the root and soil samples using tweezers following the soil being sieved, and these were combined as either root or soil samples. Four root and soil samples were also obtained from wild DHG plants at a nearby area of natural grassland (10 m × 100 m), with each root sample including lengths of five separate roots. The root and soil samples were placed in an icebox and transported to the laboratory. The root samples were gently washed with tap water several times, rinsed with sterile water, and then dried with sterilized filter paper. The root samples were stored at −80 °C prior to DNA extraction. Each of the four DHG plants in the natural grassland from which roots were obtained was later examined and this confirmed that all were infected with *E. gansuensis*.

2.2. Soil properties

The soil samples were air-dried, passed through a 2-mm sieve, and stored at 4 °C prior to being analyzed for pH (1:2.5, soil/water), available phosphorus (AP) (Olsen et al., 1954), available potassium (AK) (Helmke and Sparks, 1996), and soil organic matter (SOC) (Nelson and Sommers, 1982). Total nitrogen (TN), total P (TP), ammonium-N (AN) and nitrate-N (NN) in the soil were measured using a continuous flow analyzer (FIAstar 5000 Analyzer), and the plant-available N content was calculated as the sum of the AN and NN (Zhao et al., 2014).

2.3. DNA extraction and PCR

From each root sample (n = 12), 100 mg segments were

randomly chosen. After grinding with liquid nitrogen, total genomic DNA was extracted from root samples using a Plant DNA isolation kit (TIANGEN, Beijing). DNA quality and concentrations (purity ratio A_{260}/A_{280}) were measured using a Spectrophotometer (NanoDrop ND-1000), then the extracted DNA was diluted 10 times with double-distilled water. Agarose gel electrophoresis was used to detect the purity and concentration of DNA. Adequate amounts of samples were aliquoted into centrifuge tubes and the DNA samples were stored at −20 °C until use. Diluted genomic DNA was used as a template in conjunction with specific barcoded primers and high-fidelity enzymes (Phusion® High-Fidelity PCR Master Mix with GC Buffer; New England Biolabs) to guarantee high amplification efficiency and accuracy. The internal transcribed spacer (ITS1) genes of distinct regions were amplified using specific primers (ITS5-F: GGAAGTAAAAGTCGTAACAAGG and ITS2-R: GCTGCGTTCTTCATCGATGC) (White et al., 1990). The PCR amplification was performed using the following cycling conditions: 95 °C for 120s; 32 cycles (94 °C for 30s; 55 °C for 30s and 72 °C for 45s); and 72 °C for 10min. Amplicons were purified with the QIAGEN Gel Extraction Kit (QIAGEN, Germany) and then submitted to the Novogene Bioinformatics Technology Co. Ltd for the pyrosequencing. Finally, the library was sequenced on an Illumina HiSeq 2500 platform, generating 250 bp paired-ends reads.

2.4. Sequencing data

Sequences were analyzed through QIIME software (Version 1.7.0). A quality score <20, lacking complete barcode and primer were removed and excluded from further analysis. Chimeric sequences were removed using USEARCH software. The sequences were clustered into different OTUs with 97% similarity (OTU_{97}), and singleton OTUs (with only one read) were removed. OTU representative sequences were classified taxonomically through blasting against the UNITE fungal ITS database (https://unite.ut.ee/). After removing non-fungal OTUs, OTUs' abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed based on this output normalized data.

2.5. Alpha and beta diversity analysis

Diversity indexes were calculated using QIIME software (Version 1.7.0) and displayed with R software (Version 2.15.3). Richness index Chao1 (http://www.mothur.org/wik-i/Chao) and ACE (http://www.mothur.org/wiki/Ace) were determined using R software (Version 2.15.3). Shannon (http://www.mothur.org/wiki/Shannon) and Simpson (http://www.mothur.org/wiki/S-impson) diversity indices were determined using R software (Version 2.15.3). Principal component analysis (PCA) based on the fungal OTU levels, and redundancy analysis (RDA) between RAF phyla and soil properties, were performed by CANOCO for Windows 4.5. Significance of effects was tested using Monte Carlo permutation tests (499 permutations). The marginal effects, that is, the independent effect of each variable, were tested by auto-selection of each individual variable.

2.6. Statistical analyses

Data analyses were performed in SPSS 22.0 (SPSS, Chicago, IL, USA). The differences of fungal community composition and alpha diversity index among the three root-type samples, and soil properties among three different treatments, were tested by a one-way analysis of variance (ANOVA). Fisher's Least Significant Differences (LSD) test was used to determine whether differences between means were statistically significant. In all tests, P-value <0.05 was considered statistically significant.

3. Results

3.1. Soil properties

The soil TN, AP, TOC, AN and NN of W-EI treatment were significantly ($P < 0.05$) higher than those of C-EI/C-EF after the cultivated plots had been established for 6 yr. In terms of the cultivated EF and EI DHGs, AP in C-EI DHG rhizosphere soil was significantly ($P < 0.05$) higher than in the C-EF DHG, however soil pH and NN in C-EI DHG rhizosphere soil was significantly ($P < 0.05$) lower than in the C-EF DHG (Table S1).

3.2. Pyrosequencing information

A total of 54,413 sequences were obtained from 12 root samples in the present study (Table 1). These sequences were assigned to 190 OTUs. Taxonomic assignment of the OTUs identified at least four fungal phyla (Table 1). Ascomycota (139 OTUs; 41.1% sequences) and Basidiomycota (28 OTUs; 51.0% sequences) were the two dominant phyla, others were Chytridiomycota (4 OTUs; 0.1% sequences), Zygomycota (1 OTU; 6.0% sequences) and some unassigned OTUs (18 OTUs; 2.8% sequences) (Table 1).

The C-EI, C-EF and W-EI DHG RAF communities were primarily composed of Ascomycota and Basidiomycota (Table 1). Ascomycota was the most abundant phylum among C-EI, C-EF and W-EI RAF communities, Chytridiomycota (only the order Rhizophlyctidales) was only present in the cultivated DHG RAF communities. In terms

Table 1
Root-associated fungal phyla and their relative abundance of drunken horse grass under different treatments. Values are mean (n = 4). Different letters indicate significant differences at 0.05 level (**P < 0.01; *P < 0.05). Treatments: cultivated endophyte-infected (C-EI) drunken horse grass, cultivated endophyte-free (C-EF) drunken horse grass and wild endophyte-infected (W-EI) drunken horse grass in natural grassland.

Phylum/Order	Sequence	OTU	Relative abundance (%)			*F-value*	*P-value*
			C-EI	C-EF	W-EI		
Ascomycota	22363	139	53.13	62.61	41.11	0.681	0.531
Pleosporales	11775	40	38.73	21.30	17.26	1.067	0.384
Hypocreales	1847	13	3.48	3.72	7.32	1.129	0.365
Sordariales	1368	13	1.61b	5.89a	2.03b	5.027	**0.034**
Xylariales	1310	2	0.09	9.40	0.00	2.383	0.148
Chaetothyriales	968	9	2.39	3.58	1.26	0.580	0.580
Helotiales	229	3	0.42ab	0.77a	0.12b	4.485	**0.045**
Microascales	15	1	0.02	0.07	0.00	0.700	0.522
Capnodiales	62	1	0.07	0.00	0.09	0.765	0.493
Eurotiales	19	1	0.07	0.02	0.00	0.700	0.522
Pezizales	184	3	0.02	1.82	0.56	1.638	0.247
Saccharomycetales	117	6	0.12	0.00	0.65	3.897	0.060
Magnaporthales	32	2	0.00	0.37	0.14	0.860	0.455
Rhytismatales	7	1	0.00	0.02	0.00	1.000	0.405
Onygenales	4	1	0.00	0.00	0.00	–	–
Others	4426	43	8.14	21.70	11.37	–	–
Basidiomycota	27208	28	41.86	31.62	38.56	0.192	0.828
Agaricales	26883	17	41.58	29.61	37.72	0.268	0.771
Cantharellales	84	7	0.21	1.24	0.40	0.686	0.528
Corticiales	151	1	0.05	0.77	0.44	0.824	0.469
Cystobasidiales	3	1	0.02	0.00	0.00	1.000	0.405
Russulales	5	1	0.00	0.00	0.00	0.268	0.771
Zygomycota	3255	1	3.84	0.02	12.02	1.758	0.227
Mucorales	3255	1	3.84	0.02	12.02	1.758	0.227
Chytridiomycota	62	4	0.21	0.28	0.00	1.174	0.352
Rhizophlyctidales	62	4	0.21	0.28	0.00	1.174	0.352
Others	1525	18	0.96	5.47	8.30	–	–
Total	54413	190	100	100	100	–	–

of the relative abundance of RAF at order and genus levels (Table 1 and Fig. S1), there were significant ($P < 0.05$) differences among the roots of the three populations of DHG. The relative abundance of the order Sordariales in C-EF was significantly ($P < 0.05$) higher than C-EI and W-EI, and the relative abundance of the order Helotiales in C-EF was significantly ($P < 0.05$) higher than W-EI (Table 1).

3.3. Root associated fungal community diversity

The Shannon and Simpson indexes indicated *E. gansuensis* endophyte (C-EI and W-EI) significantly ($P < 0.05$) decreased the RAF diversity compared to C-EF, with the roots of the W-EI DHG plants showing significantly ($P < 0.05$) higher RAF diversity than C-EI (Fig. 1A, B). The Chao and ACE indexes indicated that there were no significant ($P > 0.05$) differences in RAF community richness among the three root types (Fig. 1C and D). The OTU_{97} numbers of cultivated (C-EI and C-EF) DHG roots was significantly ($P < 0.05$) higher than W-EI DHG roots (Fig. 1E). In addition, the presence of *E. gansuensis* endophyte in the foliage of cultivated DHG plants significantly ($P < 0.05$) increased the OTU numbers in the roots (Fig. 1E). OTU_{97} was significantly ($P < 0.05$) and negatively correlated with all soil properties except TP (Table 2). The Shannon and Simpson indexes were significantly ($P < 0.05$) and positively correlated with pH and N/P ratio, and the ACE index was negatively correlated with SOC (Table 2).

The PCA results showed that the contribution rate of the first principal component was 54.9% and the second was 23.3% (Fig. 2). The first principal component distinguished different RAF communities in C-EF and W-EI (Fig. 2). The second principal component distinguished different RAF communities in C-EI, C-EF and W-EI (Fig. 2).

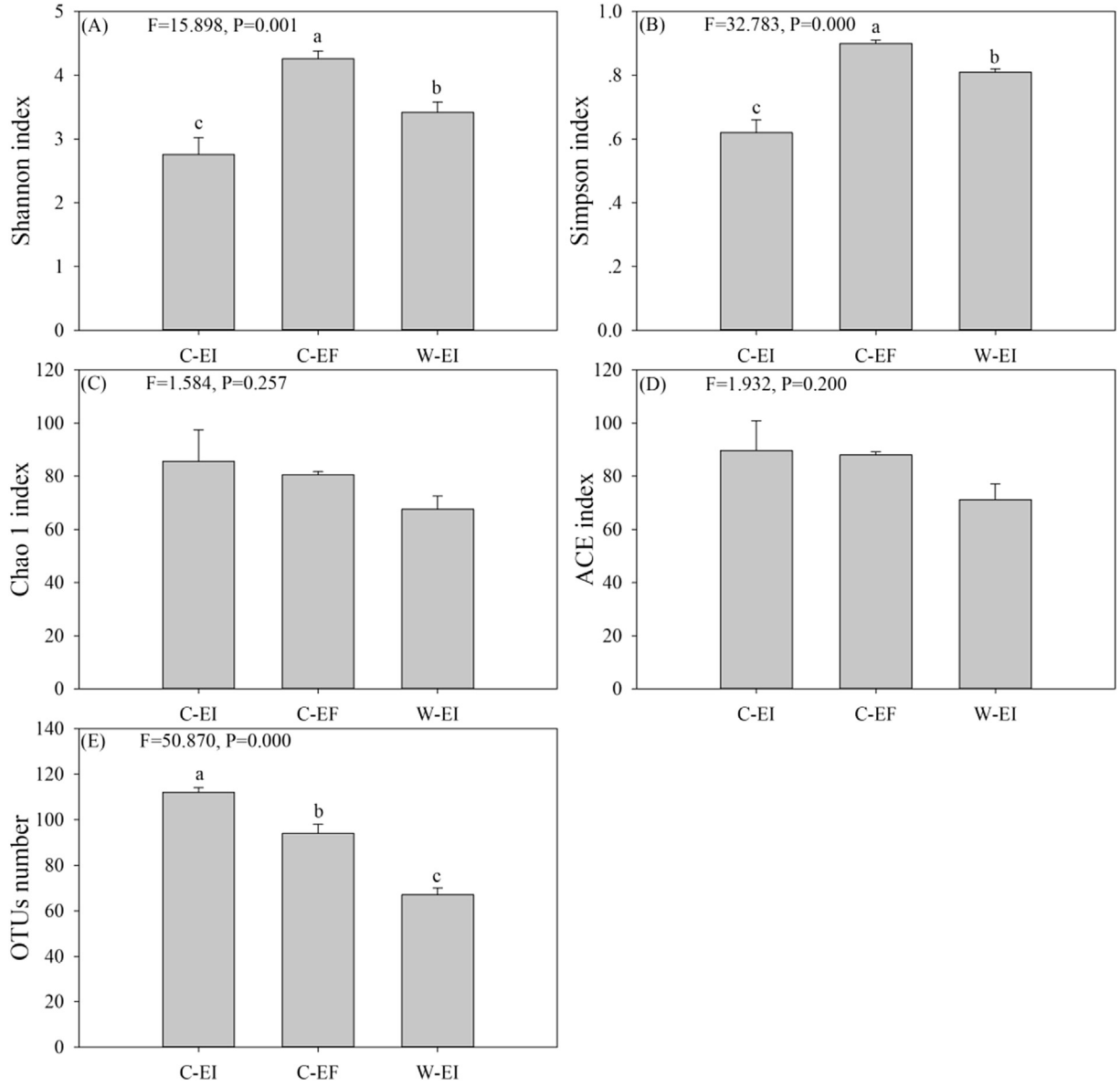

Fig. 1. Diversity indexes of root-associated fungal (RAF) communities of three drunken horse grass populations. Values are mean ± standard error (n = 4). Different letters indicate significant differences at 0.05 level. Treatments: cultivated endophyte-infected (C-EI) drunken horse grass, cultivated endophyte-free (C-EF) drunken horse grass and wild endophyte-infected (W-EI) drunken horse grass in natural grassland.

Table 2
Spearman correlations of soil properties and alpha diversity. Soil factors indicated include AP (Available P), TOC (Total Organic Carbon), AN (Ammonium Nitrogen), NN (Nitrate Nitrogen), AK (Available potassium), N/P (Available N: Available P). **P < 0.01; *P < 0.05.

Soil properties	Shannon	Simpson	Chao1	ACE	OTU_{97}
AP	−0.317	−0.073	−0.469	−0.538	**−0.759****
SOC	−0.033	0.219	−0.527	**−0.589***	**−0.899****
TN	0.017	0.237	−0.457	−0.504	**−0.918****
TP	0.327	0.463	−0.160	−0.128	−0.453
pH	**0.641***	**0.732****	−0.199	−0.159	**−0.684***
AN	0.092	0.352	−0.455	−0.499	**−0.907****
NN	0.059	0.311	−0.492	−0.529	**−0.905****
N/P	**0.741****	**0.887****	−0.301	−0.265	**−0.719****
AK	−0.080	0.153	−0.437	−0.503	**−0.851****

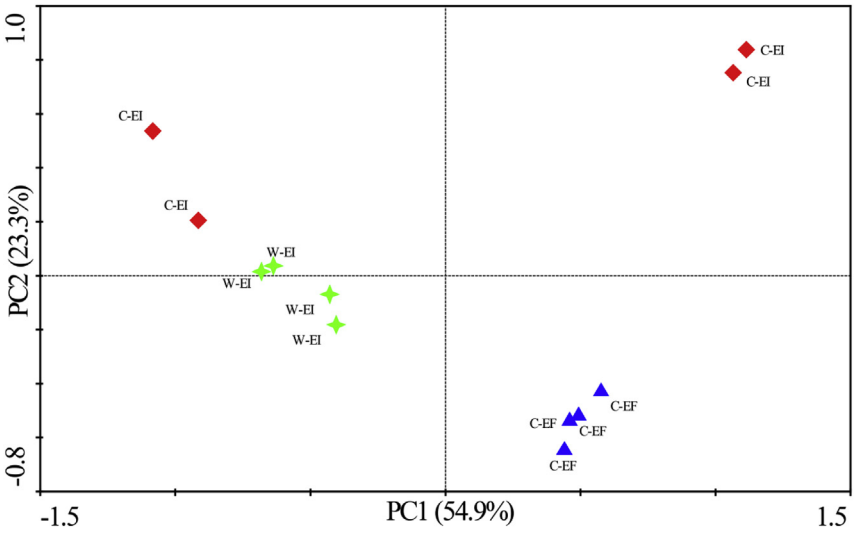

Fig. 2. Principal component analysis (PCA) of fungal diversity in three root samples. Scatter plot shows principal component 1 (PC1) versus principal component 2 (PC2). Percentages shown are percentages of variation explained by the components. Cultivated endophyte-infected (C-EI) drunken horse grass, cultivated endophyte-free (C-EF) drunken horse grass and wild endophyte-infected (W-EI) drunken horse grass in natural grassland.

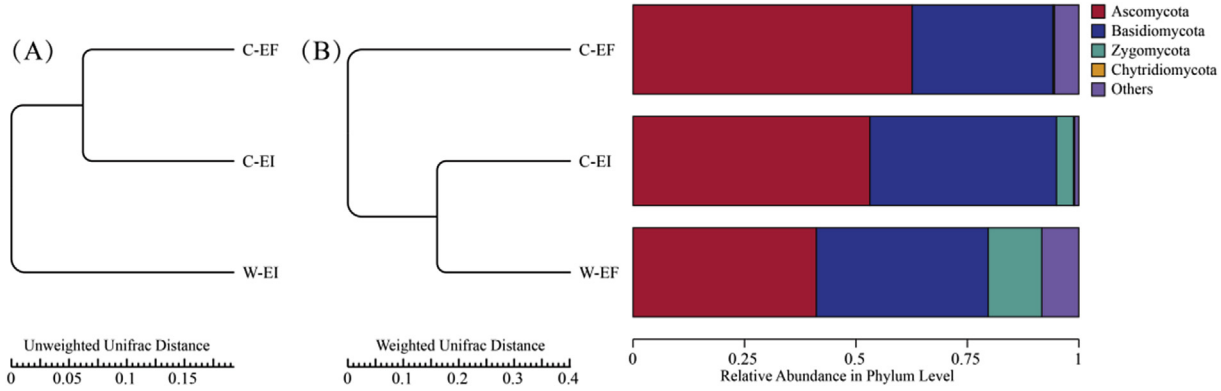

Fig. 3. UniFrac UPGMA cluster analysis of root-associated fungal communities of drunken horse grass under different endophyte and management practice treatments. (A) unweighted and (B) weighted pair group method with arithmetic mean. Figure was constructed based on Illumina sequencing data. Cultivated endophyte-infected (C-EI) drunken horse grass, cultivated endophyte-free (C-EF) drunken horse grass and wild endophyte-infected (W-EI) drunken horse grass in natural grassland.

Unweighted and weighted UniFrac analyses of fungal diversity clustered the different DHG root samples into two groups affected by the *E. gansuensis* endophyte or soil disturbance (Fig. 3). Unweighted UniFrac analysis showed that the three root RAF communities were divided into two groups according to the presence or absence of soil disturbance (Fig. 3A). Weighted UniFrac analyses divided three root RAF communities into two groups according to endophyte presence or absence (Fig. 3B).

3.4. Redundancy analysis

The first axis and second axis of RDA explained 77.8% and 8.8% of the variance, respectively (Fig. 4). According to RDA, soil properties was the most important factor to affect the RAF community

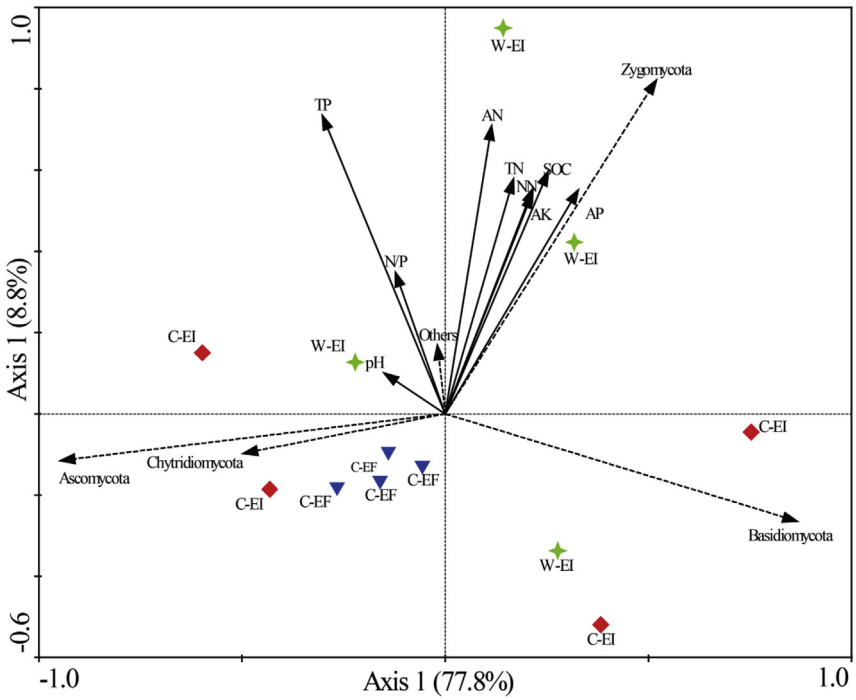

Fig. 4. Redundancy analysis (RDA) of relative abundance of root-associated fungal phyla and soil properties under different endophyte and management practice treatments. Figure was constructed based on Illumina sequencing data. Cultivated endophyte-infected (C-EI) drunken horse grass, cultivated endophyte-free (C-EF) drunken horse grass and wild endophyte-infected (W-EI) drunken horse grass in natural grassland. Soil factors indicated include AP (Available P), TOC (Total Organic Carbon), AN (Ammonium Nitrogen), NN (Nitrate Nitrogen), AK (Available potassium), N/P (Available N: Available P).

changes in wild conditions; the abundance of phyla in C-EF correlated with pH; while the fungal phyla in EI conditions were little correlated with any soil properties (Fig. 4). RDA also indicated that AP, AK, SOC, AN and NN had a positive effect on Zygomycota abundance and a negative effect on Ascomycota and Chytridiomycota. TP and N/P ratio had positive effects on Zygomycota and Ascomycota abundance and negative effects on Basidiomycota (Fig. 4).

4. Discussion

Sequencing techniques have been used previously to reveal soil and plant root microbial community composition (Wehner et al., 2014; Chu et al., 2016; Zhou et al., 2016a). In the present study, a total of 54,413 sequences were obtained, forming 190 OTUs, and roots of W-EI DHG plants had higher RAF diversity than roots of C-EI DHG plants. Furthermore, the presence of the foliage-confined endophyte, *E. gansuensis* decreased the RAF diversity under cultivation. Previous studies have shown that RAF community diversity and composition were affected by some factors, such as host species (Fujimura and Egger, 2012; Wehner et al., 2014; Aguilar-Trigueros and Rillig, 2016; Gao and Yang, 2016), parasitic pinewood nematode (Chu et al., 2016) and fertilization (Yu et al., 2013). Our results indicated that both foliar *Epichloë* endophyte and soil disturbance decreased RAF community diversity, and changed the RAF community composition of DHG. To our knowledge, this is the first study to quantify the effects of the aboveground *E. gansuensis* endophyte on RAF communities of DHG using sequencing techniques.

4.1. Root-associated fungal community composition

A plant root typically harbors a complex RAF community (Vandenkoornhuyse et al., 2002; Franken, 2012; Aguilar-Trigueros et al., 2014; Wehner et al., 2014; Aguilar-Trigueros and Rillig, 2016). In our study, the majority of OTUs (73.16%) belonged to the Ascomycota, a widespread and dominant phylum in different environmental conditions (Rodriguez et al., 2009; Lin et al., 2012; Kazeeroni and Al-sadi, 2016). The fungal order Pleosporales which was one of the most represented orders in RAF Ascomycota communities of semiarid areas (Porras-Alfaro et al., 2008; Márquez et al., 2012), comprised of 38.73%, 21.30% and 17.26% of C-EI, C-EF and W-EI DHG RAF communities, respectively. The most abundant OTU in Ascomycota in our study was identified as Phaeosphaeriaceae sp. Some fungal species of the genus *Phoma* belonging to the family Phaeosphaeriaceae are common plant pathogenic, saprotrophic or endophytic fungi (Lawrey et al., 2012). Similarly, Basidiomycota is a common and dominant phylum associated with grass (Vandenkoornhuyse et al., 2002; Porras-Alfaro et al., 2008; Márquez et al., 2012), shrub (Bjorbækmo et al., 2010), herb (Blaalid et al., 2012; Gao and Yang, 2016) and tree (Fujimura and Egger, 2012) roots. A high incidence of Basidiomycota was associated with roots of plants of the three DHG populations, and comprised 41.58%, 31.62% and 38.56% in C-EI, C-EF and W-EI DHG RAF communities, respectively. The overwhelming dominant Basidiomycota (98.88%) order was Agaricales in DHG RAF communities, which is in line with some studies of the grass *Bouteloua gracilis* and *Arrhenatherum elatius* (Vandenkoornhuyse et al., 2002; Porras-Alfaro et al., 2008; Márquez et al., 2012). However, the functional roles of Agaricales associated with grass roots are still poorly understood. The most abundant OTU (14.74%) belonging to Basidiomycota in DHG RAF communities was *Marasmiellus tricolor*. *M. tricolor* is associated with grass roots and rotting grass debris in open grassland (Henrici, 2016). Only 8.42% of our OTUs were of unknown fungal identity.

4.2. The effect of Epichloë sp. endophyte on root-associated fungal communities

Some previous studies have shown that soil fungal communities were affected by foliar *Epichloë* endophyte of tall fescue (*Festuca arundinacea*) (Rojas et al., 2016) and agricultural management (Lin et al., 2012; Siddiky et al., 2012; Zhou et al., 2016a). Our study indicated that *E. gansuensis* decreased DHG RAF community diversity and altered the DHG RAF community composition. An increasing amount of evidence is indicating that foliar *Epichloë* endophyte could suppress AMF colonization (Chu-Chou et al., 1992; Mack and Rudgers, 2008; Liu et al., 2011; Zhou et al., 2016b). However, Antunes et al. (2008) found *Epichloë bromicola* had no effect on *Glomus mosseae* and *Glomus etunicatum* in *Leymus chinensis*. Furthermore, Larimer et al. (2012) found that the presence of *Epichloë elymi* even promoted mycorrhizal colonization by *G. mosseae* in *Elymus hystrix* roots. In contrast to the results of AMF, the presence of an *Epichloë* endophyte had no significant effect on root DSE (Vandegrift et al., 2015; Slaughter and McCulley, 2016). In the present study, *E. gansuensis* suppressed the abundance of Sordariales and Helotiales in cultivated DHG RAF communities. Our results support our first hypothesis that foliar endophytes in DHG may alter RAF community composition. This may result from several causes: (I) *Epichloë* endophytes have temporal priority over root fungi because *Epichloë* endophytes are seed-borne (Schardl et al., 2004; Christensen et al., 2008), while root fungi continuously colonize grass roots from infectious progagules as they are forming and extending (Knapp et al., 2015; Van der Heijden et al., 2015). (II) *Epichloë* sp. endophytes could alter host grass nutritional requirements (Song et al., 2015; Soto-Barajas et al., 2016) and this may indirectly affect the RAF. (III) Infected plants could produce primary or secondary metabolites (e.g. alkaloids) or alter root exudates that could directly affect the root fungal symbiosis (Mack and Rudgers, 2008; Novas et al., 2011; Guo et al., 2015; Zhou et al., 2016b).

4.3. The relationships between soil properties and fungal community diversity and composition

Soil disturbance commonly resulted in a change of soil properties (Brito et al., 2012), and soil and root fungal communities are commonly and closely associated with environmental factors such as soil nutrient levels, climate and space distribution (Blaalid et al., 2012, 2014; Fujimura and Egger, 2012; Yu et al., 2013; Kazeeroni and Al-sadi, 2016). Our second hypothesis that soil disturbance could alter the DHG RAF communities was supported by the present study. Furthermore, resource availability could drive community assemblages of RAF of plants (Liu et al., 2015). Previous studies had demonstrated that chemical fertilization, space and soil types had a strong influence on soil fungal communities (Lin et al., 2012; Kazeeroni and Al-sadi, 2016; Zhou et al., 2016a). Long term balanced fertilization decreased soil AMF diversity (Lin et al., 2012; Zhou et al., 2016a); organic farm soil had higher richness than semi-oasis farm soil (Kazeeroni and Al-sadi, 2016). However, soil properties were also closely associated with RAF communities in terrestrial ecosystems (Blaalid et al., 2012, 2014; Fujimura and Egger, 2012; Yu et al., 2013). Organic fertilization altered RAF community composition of *Pisum sativum* (Yu et al., 2013). In addition, RAF communities of *Salix arctica* in different sites were strongly associated with soil properties such as AP, AN, NN and pH (Fujimura et al., 2008). These results are similar to the present study, as the RAF community diversity and richness of DHG are significantly associated with pH and SOC, and the number of OTUs in DHG RAF is also strongly linked to the soil properties in which DHG grows.

The present study demonstrated that *E. gansuensis* and soil disturbance brought changes to the RAF communities. In addition, there were strongly significant differences of rhizosphere soil AP and NN between C-EI and C-EF DHG. Further studies should mechanistically explore: (I) the effects of *E. gansuensis* endophyte on RAF communities under various available N and P levels using an accurate controlled experiment; and (II) the functions that secondary metabolites from EI and EF DHG having in sustaining RAF colonization.

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Supplementary data

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