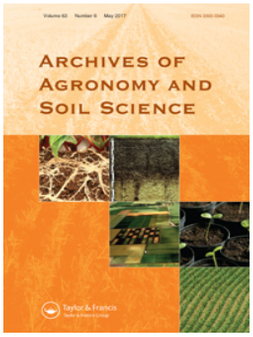

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Arbuscular mycorrhizal fungi diversity associated with two halophytes *Lycium barbarum* L. and *Elaeagnus angustifolia* L. in Ningxia, China

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ABSTRACT

The Arbuscular mycorrhizal fungi (AMF) community in saline soils of Ningxia, China, was rarely reported. Soils in the rhizosphere of two important food plants, *Lycium barbarum* L. (Goji) and *Elaeagnus angustifolia* L. (Oleaster), were sampled from Ningxia (Goji from Huinong, HNGQ; Goji from Yinchuan, YCGQ; Oleaster from Yinchuan, YCSZ) to investigate the AMF community. Thirty-three AMF species from 11 genera were identified in total. The dominant family and genera were Glomeraceae, *Acaulospora* and *Glomus*, respectively. *Septoglomus constrictum* was the most abundant species. The AMF community composition of Goji was different from that of Oleaster ($R = 0.26$, $p < 0.05$), while the AMF community from Huinong differed from Yinchuan ($R = 1.0$, $p = 0.01$). These findings suggest a high AMF diversity in Ningxia saline soils and the effect of host plant identity on AMF community composition. Furthermore, the AMF diversity index positively correlated with available potassium (AK), available phosphorus (AP), available nitrogen (AN) and organic matter (OM), but negatively correlated with electric conductivity (EC). This result demonstrated that a high level of salinity might reduce soil fertility and AMF diversity. The saline area with high diversity of the AMF community in Ningxia is promising for screening AMF isolates for utilization in crop production.

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KEYWORDS
AMF community; diversity; spore identification; salinity; edaphic factor

Introduction

Soil salinization is a critical environmental issue threatening food production (Zhu et al. 2014). Ningxia province, which is one of the most important food production bases in Northwest China, has a typical temperate semiarid climate (Wan et al. 2013). Long-term strong evaporation and excessive irrigation and fertilization lead to over-accumulation of salt in soils (Wang 2004). Arbuscular mycorrhizal fungi (AMF) have been demonstrated to efficiently alleviate the deleterious effect of salt stress on plants (Chandrasekaran et al. 2014; Kumar et al. 2015). Besides, an optimal fungal partner could stabilize the cooperation with the host plants (Kiers et al. 2011), and thus results in host preference (Martinez-Garcia & Pugnaire 2011). Recent works suggested that the autochthonous AMF manifest higher symbiotic efficiency in protecting host plants from salt stress (Garg & Pandey 2015). Therefore, exploring the native AMF community associated with plants in

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salinized soils may fortify the beneficial effects of AMF on plant growth, and thus to alleviate salt stress of agricultural productivity.

Lycium barbarum L. (Goji), which has important economic and medicinal value, is grown widely in Ningxia. Goji can be consumed as traditional Chinese medicine and dietary supplement to nourish the liver and the kidney, and brighten the eyes (Amagase & Farnsworth 2011). Goji berries and leaves are rich in polysaccharides, exerting a range of biological activities, including effects on aging, neuroprotection, antifatigue and antioxidant properties (Ma et al. 2009; Amagase & Farnsworth 2011). Moreover, rutin is the most abundant flavonoid in Goji berries and leaves, possessing the ability to scavenge free radicals and anticancer effect to protect the health of people (Dong et al. 2009). *Elaeagnus angustifolia* L. (Oleaster), another common tree species in Ningxia, is of great importance in maintaining the ecological balance in arid areas (Zhao et al. 2014). The fruits of Oleaster with biologically active compounds are capable of decreasing cholesterol and the atherogenic indices of humans (Nikniaz et al. 2016; Waili et al. 2016). However, the habitat of these food plants in Ningxia is seriously affected by salinization, thus reducing the yield and quality of food production. AMF might be a good agronomic practice to ameliorate the detrimental effect of salt stress on the food plants growth in Ningxia, as they have previously been confirmed as mycorrhizal (Riffle 1977; Zhang et al. 2010).

In this regard, it is important to study the AMF community associated with these important food plants in Ningxia and get to know the edaphic factors driving the AMF community in salinized soils. Furthermore, investigating the AMF community associated with Goji and Oleaster is essential for screening out the potential efficient AMF isolates, which in turn facilitate the production of these two important food plants. Therefore, the objectives of this study were: 1) to characterize the AMF community in the rhizosphere of Goji and Oleaster in the saline soil of Ningxia; 2) to clarify the edaphic factors driving the AMF community in salt-stressed soils; and 3) to explore the relationships among the AMF community, edaphic factors and plant identity.

Materials and methods

Sampling site description

This study was carried out in Ningxia province, which has a representative temperate continental climate. The annual average temperature is 5–9°C with great day-and-night temperature differences and long annual solar hours. The average annual precipitation is 300 mm and most of the precipitation irregularly occurs in summer.

Three sites were selected for sampling (Figure 1): a) HNGQ: soil samples collected from a Goji orchard farm in Huinong county (39°04′7″ N 106°35′44″E); b) YCGQ: soil samples collected from the Goji orchard of Goji research institute of Ningxia Academy of Agriculture and Forestry Sciences in Yinchuan (38°38′49″N 106°09′10″E); the orchards were arranged as monoculture with only Goji plants; and no companion plants existed in the well-managed Goji orchards; and c) YCSZ: Oleaster soil samples collected from the wild Oleaster forest in Yinchuan (38°37′38″′N 106°10′24″E). The weeds around and under Oleaster plants were removed before sample collection. The roots were carefully excavated and traced from the originating tree to ensure identity. The plant density for HNGQ, YCGQ and YCSZ was 0.99, 0.93 and 27.86 per square meter, respectively. The coverage of HNGQ, YCGQ and YCSZ was 17%, 16% and 43%, respectively. The soils of our sampling sites were a Fluvisol derived from river sediments.

Soil sampling

Three plots (10 m × 10 m) were randomly chosen in each site for samples collection. Soil samples were collected from the rhizosphere of five Goji plants in each plot of HNGQ and YCGQ, as well as five Oleaster plants in each plot of YCSZ, respectively. Five soil cores of 15–20 cm

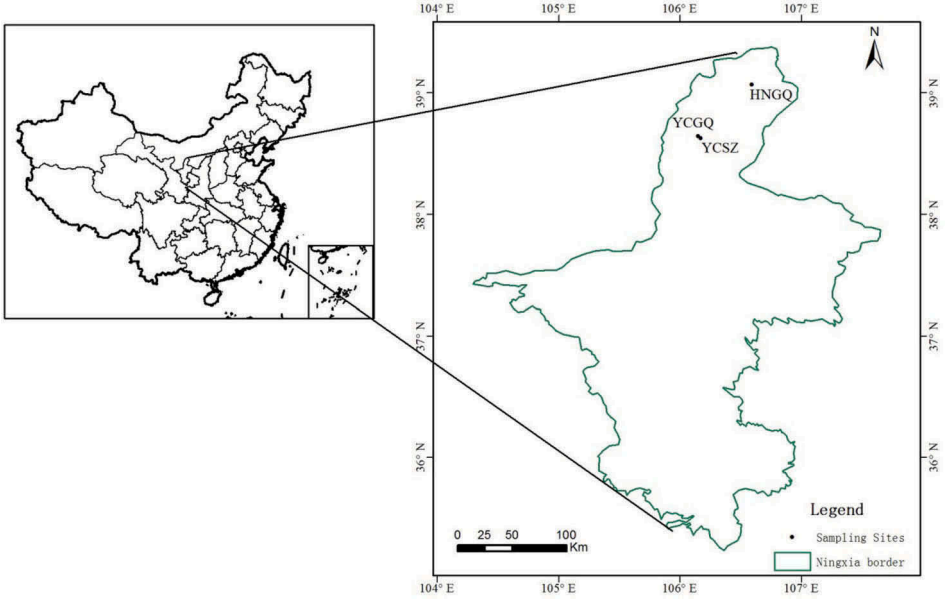

Figure 1. Sampling sites location for *Lycium barbarum* L. and *Elaeagnus angustifolia* L. in Ningxia, China. HNGQ – Goji soils from Huinong; YCGQ – Goji soils from Yinchuan; YCSZ – Oleaster soils from Yinchuan.

depth and 5 cm diameter were collected around each tree. The five soil cores from the same plot were mixed thoroughly to mask the differences usually existing around trees in open sites. The soils loosely and tightly bound to the root surfaces were removed by clean tweezers and brush, and defined as rhizosphere soils for spore extraction. The remaining extracted soils were used for chemical analyses. Fine roots were carefully separated using clean tweezers and collected from the soil cores.

Soil property analysis

The analyses of soil properties including total potassium (TK), total phosphorus (TP), total nitrogen (TN), available potassium (AK), available phosphorus (AP), available nitrogen (AN), organic matter (OM), cation exchange capacity (CEC), soil pH and electric conductivity (EC) were conducted. Part of the air-dried soil sample was ground to pass through a 2-mm sieve for chemical analysis. TK was determined using the sodium hydroxide alkali fusion-flame photometry method (Du et al. 2015). TP was analyzed by the sodium hydroxide alkali fusion-molybdenum antimony colorimetric resistance method (Hu et al. 2013). TN was determined by Kjeldahl digestions. AK was extracted by 1 M ammonium acetate and measured with flame photometry (Singh et al. 2012). AP was extracted and measured in a buffered alkaline solution with 0.5 M sodium bicarbonate. The extracts were quantified calorimetrically with a spectrophotometer (Hitachi, UV2300) at 660 nm (Olsen et al. 1982). AN was measured using a micro-diffusion method, in which NH_3 was released from the soil sample by NaOH and then absorbed by boric acid. The ammonium borate product was titrated with 0.01 M HCl (Conway 1957). OM concentration was determined by wet oxidation with potassium dichromate (Walkley & Black 1934). CEC was measured using the batch equilibrium method (Winistörfer 1995). Soil pH was determined using a pH meter (pHS-4C, Shanghai Leici Device Works, Shanghai, China) at a soil: water ratio of 1:2.5 (w/v). EC was determined using a digital conductivity meter (DDSJ-380A, Shanghai Leici Device Works, Shanghai, China) according to the instruction of the manufacturer.

AMF colonization estimation

The collected roots were washed with tap water gently, and dried. The fresh roots were cleared for 15 min in 10% KOH at 90°C, bleached in alkaline hydrogen peroxide for 20 min, acidified in 1% HCl and stained in trypan blue (Phillips & Hayman 1970). Colonization rate was measured using the gridline intercept method (Giovannetti & Mosse 1980).

AMF spore isolation, identification and density

To isolate AMF spores, 50 g rhizosphere soil from each plot was soaked in 1 L of water. The suspension was passed through 200- and 40-µm sieves to collect the spores. The spores from both sieves were vacuum-filtrated water washed into a filter paper in Buchner funnel. The spores were picked using a needle and counted under a stereoscopic microscope. For identification of AMF species, spores were mounted in polyvinyl alcohol-lactic acid-glycerine (PVLG) (Koske & Tessier 1983) to check the morphological features under a microscope equipped with a digital camera (Olympus U-TV0.63XC, Japan). The AMF species identification was performed based on the comparison of spore morphological features, the original diagnoses of AMF species and the reference culture description at http://invam.wvu.edu/the-fungi/classification. The Glomeromycotean classification of Redecker et al. (2013) and Schüßler and Walker (2010) at http://schuessler.userweb.mwn.de/amphylo/ was followed. Spore density for each plot was counted and expressed as spore number per gram dry soil.

Statistical analysis

AMF community composition was calculated using the following equation:

$$\text{Proportion of AMF spore}\,(\%) = \text{ni} \times 100/N \tag{1}$$

where ni is the number of AMF spore of a single species and N is the total number of AMF spores.

One-way analysis of similarity (ANOSIM) was performed on Bray–Curtis resemblance matrices (999 permutations) to determine the significance of differences between sites and plant species (PRIMER v5.2.8, Primer-E Ltd, UK).

Species richness was defined as the total number of species found in each plot. Shannon (H′) and Simpson (D) indices and Evenness were calculated using PAST 3.0 software (Hammer et al. 2001).

One-way ANOVA was used for analyzing the difference in soil factors, AMF colonization rate, spore density and diversity indices among the three sites on statistical analysis system (SAS), followed by Duncan's test (SAS version 8.01).

Pearson correlation between AMF diversity indices and edaphic factors were performed on SPSS (SPSS version 19.0).

Results

Soil properties

The soil properties from the three sampling sites are presented in Table 1. The soils from the three sites were uniformly alkaline, with the pH ranging from 8.16 to 8.56. EC of the three sampling sites increased in the following order: HNGQ<YCGQ<YCSZ. However, the decreasing order AK, AP, AN and OM was HNGQ>YCGQ>YCSZ. The relationship for TP and TN was HNGQ>YCGQ = YCSZ, while CEC showed the order as HNGQ = YCGQ>YCSZ. No differences for pH and TK were found for the three sampling sites. TP and TN were low, while OM was adequate when all the soil samples were taken into consideration. The soil fertility of the sampling sites decreased in order HNGQ>YCGQ>YCSZ.

Table 1. Soil properties of the sampling sites in Ningxia, China.

Sampling site	pH	AK (mg kg^{-1})	AP (mg kg^{-1})	AN (mg kg^{-1})	TK (g kg^{-1})	TP (g kg^{-1})	TN (g kg^{-1})	OM (g kg^{-1})	CEC (cmol(+) kg^{-1})	EC (dS m^{-1})
HNGQ	8.56 ± 0.25a	640 ± 19.70a	48.25 ± 3.81a	94.33 ± 6.19a	17.31 ± 3.81a	1.03 ± 0.13a	1.29 ± 0.19a	21.31 ± 3.11a	13.81 ± 1.64a	0.33 ± 0.06c
YCGQ	8.22 ± 0.18a	278 ± 29.94b	37.2 ± 2.81b	46.59 ± 4.83b	16.57 ± 4.92a	0.56 ± 0.11b	0.72 ± 0.10b	13.18 ± 1.91b	12.55 ± 1.75a	1.47 ± 0.11b
YCSZ	8.16 ± 0.10a	170.5 ± 22.97c	3.9 ± 0.41c	33.5 ± 5.94c	15.50 ± 2.11a	0.37 ± 0.06b	0.65 ± 0.13b	11.94 ± 0.75c	3.91 ± 0.94b	1.81 ± 0.09a

HNGQ – Goji soils from Huinong; YCGQ – Goji soils from Yinchuan; YCSZ – Oleaster soils from Yinchuan. Data with different letters in the same column means significantly different at $p = 0.05$. Data are means±SE (n = 3).

Table 2. AMF colonization and spore density in the surveyed fields of Ningxia, China.

	Colonization (%)	Spore density (spore number g^{-1} soil)
HNGQ	26 ± 3b	5.73 ± 0.87a
YCGQ	30 ± 3b	5.07 ± 0.90a
YCSZ	45 ± 5a	4.77 ± 0.27a

HNGQ – Goji soils from Huinong; YCGQ –Goji soils from Yinchuan; YCSZ –Oleaster soils from Yinchuan. Data with different letters in the same column means significantly different at $p = 0.05$. Data are means±SE (n = 3).

AMF colonization rate and spore density

All root samples surveyed in this study were colonized by AMF (Table 2). The highest AMF colonization occurred in Oleaster of YCSZ (45%), while the Goji plants from the other two sites had similar lower AMF colonization rates (HNGQ: 26%; YCGQ: 30%). The spore density (expressed per g dry soil) was moderate, ranging from 4.77 on YCSZ and YCGQ to 5.73 on HNGQ. No difference of spore density among the three sampling sites was observed.

AMF community composition

A total of 33 AMF species were identified in the soil samples from Ningxia province (Figure 2). They belonged to 10 genera and seven families (Acaulosporaceae, Ambisporaceae, Claroideoglomeraceae, Diversisporaceae, Glomeraceae, Pacisporaceae and Gigasporaceae). Most AMF spores were from the Glomeraceae family. Both genera of *Acaulospora* and *Glomus* took up a large proportion in the AMF community. Both *Septoglomus constrictum* and *Funneliformis coronatum* were found in the all sampling plots. In addition, *S. constrictum* was the most abundant species.

According to the ANOSIM for AMF, the communities were different between sites ($R = 1$, $p = 0.01$) and plant species ($R = 0.26$, $p < 0.05$) (Table 3). However, the plots from each site had similar AMF community composition.

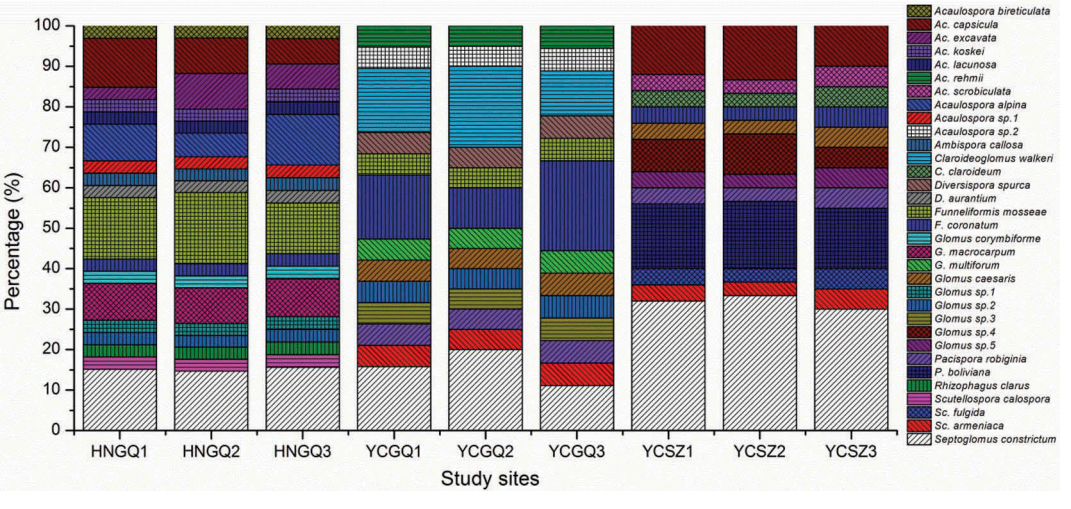

Figure 2. AMF community composition from the sampling sites in Ningxia, China. HNGQ – Goji soils from Huinong; YCGQ – Goji soils from Yinchuan; YCSZ – Oleaster soils from Yinchuan. (For interpretation of the references to color in this figure legend, the readers are referred to the web version of this article.)

Table 3. Analysis of similarity (ANOSIM) results for the comparison between samples from different sites and plant species.

Factor	Comparison	*R*	Sig. level
Site	Huinong–Yinchuan	1.0	0.01
Plant species	Goji–Oleaster	0.26	<0.05

Table 4. Diversity indices of AMF community in the sampling plots from the three sites in Ningxia, China.

	Species richness (E)	Shannon (H')	Simpson (D)	Evenness
HNGQ1	18	2.64	0.91	0.78
HNGQ2	18	2.64	0.91	0.78
HNGQ3	18	2.68	0.92	0.81
YCGQ1	13	2.42	0.90	0.87
YCGQ2	13	2.37	0.89	0.82
YCGQ3	13	2.43	0.90	0.87
YCSZ1	12	2.14	0.84	0.71
YCSZ2	12	2.07	0.82	0.66
YCSZ3	12	2.22	0.86	0.77
AVG				
HNGQ	18a	2.65a	0.91a	0.86a
YCGQ	13b	2.41b	0.89b	0.79a
YCSZ	12c	2.15c	0.84c	0.71b

HNGQ – Goji soils from Huinong; YCGQ – Goji soils from Yinchuan; YCSZ – Oleaster soils from Yinchuan. Data with different letters in the same AVG column means significantly different at $p = 0.05$. Data for AVG are means of three replicates.

AMF diversity

The species richness, and the Shannon (H') and Simpson (D) indices decreased in the following order: HNGQ>YCGQ>YCSZ (Table 4). However, HNGQ and YCGQ had similar Evenness, which was higher than that of YCSZ.

Relationship between soil factors and AMF diversity

Soil properties significantly correlated AMF colonization and community diversity indices, especially for AMF richness (Table 5). AMF species richness positively and significantly correlated with AK ($r = 0.98$, $p < 0.01$), AP ($r = 0.78$, $p = 0.01$), AN ($r = 0.96$, $p < 0.01$), TP ($r = 0.88$, $p < 0.01$), TN ($r = 0.81$, $p = 0.01$) and OM ($r = 0.80$, $p = 0.01$). However, EC negatively and significantly correlated with species richness ($r = -0.98$, $p < 0.01$), Shannon index ($r = -0.81$, $p = 0.01$) and Simpson index ($r = -0.91$, $p < 0.01$). The Shannon index positively and significantly correlated with AK ($r = 0.84$, $p = 0.01$), AP ($r = 0.97$, $p < 0.01$), AN ($r = 0.78$, $p = 0.01$), TP ($r = 0.72$, $p = 0.03$) and CEC ($r = 0.85$, $p < 0.01$). Simpson indices positively and significantly correlated with AK ($r = 0.94$, $p < 0.01$), AP ($r = 0.95$, $p < 0.01$), AN ($r = 0.89$, $p < 0.01$), TP ($r = 0.82$, $p = 0.01$), TN ($r = 0.74$, $p = 0.02$), OM ($r = 0.72$, $p = 0.03$) and CEC ($r = 0.82$, $p = 0.01$). Evenness positively and significantly correlated with AP ($r = 0.69$, $p = 0.04$). The AMF colonization rate positively and significantly correlated with EC ($r = 0.73$, $p = 0.03$), but negatively and significantly correlated with AP ($r = -0.80$, $p = 0.01$). Spore density positively and significantly correlated with pH ($r = 0.68$, $p = < 0.05$) and OM ($r = 0.76$, $p = 0.02$).

Discussion

Plants can establish symbiosis with AMF in natural salinized soils (Kumar et al. 2015). The extended hyphal networks enhance water and nutrients uptake (especially for P) of the host plants, subsequently alleviating the deleterious effect of salinity on growth (Porcel et al. 2012). On the other hand, a number of studies suggested the negative effects of P on AMF colonization (Yoshimura

Table 5. Pearson's correlation coefficient between AMF status and soil factors.

Item		Species richness	Shannon-H	Simpson-D	Evenness	AMF colonization	Spore density
EC	r	**−0.98**	**−0.81**	**−0.91**	−0.22	**0.73**	−0.22
	p	**<0.01**	**0.01**	**<0.01**	0.57	**0.03**	0.57
pH	r	0.55	0.44	0.52	0.15	−0.01	**0.68**
	p	0.12	0.24	0.15	0.71	0.98	**<0.05**
AK	r	**0.98**	**0.84**	**0.94**	0.29	−0.62	0.45
	p	**<0.01**	**0.01**	**<0.01**	0.46	0.08	0.23
AP	r	**0.78**	**0.97**	**0.95**	**0.69**	**-0.80**	0.47
	p	**0.01**	**<0.01**	**<0.01**	**0.04**	**0.01**	0.20
AN	r	**0.96**	**0.78**	**0.89**	0.22	−0.53	0.50
	p	**<0.01**	**0.01**	**<0.01**	0.58	0.14	0.17
TP	r	**0.88**	**0.72**	**0.82**	0.19	−0.42	0.32
	p	**<0.01**	**0.03**	**0.01**	0.62	0.26	0.40
TK	r	0.12	0.21	0.20	0.24	0.20	0.08
	p	0.75	0.58	0.60	0.53	0.61	0.85
TN	r	**0.81**	0.63	**0.74**	0.14	−0.21	0.17
	p	**0.01**	0.07	**0.02**	0.72	0.59	0.66
OM	r	**0.80**	0.60	**0.72**	0.13	−0.32	**0.76**
	p	**0.01**	0.09	**0.03**	0.74	0.40	**0.02**
CEC	r	0.65	**0.85**	**0.82**	0.66	−0.59	0.57
	p	0.06	**<0.01**	**0.01**	0.06	0.10	0.11

Data in bold mean the coefficient is significant.

et al. 2013), wherein plants are less dependent on AMF in P-rich conditions. In this study, the AMF colonization rate of Oleaster was higher than that of Goji. This result might be explained by the fact that YCSZ soil had lower AP and higher salinity, as the AMF colonization rate negatively correlated with AP ($r = -0.80$, $p = 0.01$) and positively correlated with EC ($r = 0.73$, $p = 0.03$). Another reason might be different plant species regulate AMF colonization via root traits, like dry matter content and root C:N (Legay et al. 2016). Furthermore, there was no difference in spore density among the three sampling sites. The fact of similar soil pH in the sampling sites might explain the identical spore density. In agreement with our study, spore density was found to be positively correlated with soil pH (Sivakumar 2013). Although OM positively correlated with spore density ($r = 0.76$, $p = 0.02$), the increasing trend of spore density was not significant along with OM gradients.

As a result of the vital role of AMF in nutrient cycling in saline ecosystem, the AMF community reinforced the relationship between plants and salinized soils (Smith & Read 2008). Previous researches revealed a high level of AMF diversity in salt-stress soils, such as 22 AMF species in European salt marshes (Wilde et al. 2009), and 19 and 30 AMF species salinized soils in the arid areas of Argentina (Becerra et al. 2014) and Spain (Estrada et al. 2013), respectively. Our results characterized 33 AMF species (including seven at the genus level) from the sampling sites, demonstrating a relatively high AMF diversity associated with Goji and Oleaster in Ningxia. In accordance with the saline soils in the dry areas of Argentina (Becerra et al. 2014) and Spain (Estrada et al. 2013), Glomeraceae was the most abundant family in the sampling sites. Given that *Glomus geosporum* was reported as a dominant species in salt marsh (Carvalho et al. 2004), *Septoglomus constrictum* (previously called *Glomus constrictum*) was the most abundant species in the sampling sites of Ningxia. Estrada et al. (2013) and Becerra et al. (2014) suggested the dominant position of *S. constrictum* in the salty soils of arid areas, which is in agreement with this study. These findings demonstrate that the dominant AMF species vary in different types of salinized soils. Furthermore, the high AMF diversity in Ningxia provides us great potential to select high-efficiency AMF and develop biofertilizers to facilitate plant growth and food production in salt stress (Liu et al. 2016).

The preference of host plants on AMF may lead to varied AMF communities (Hazard et al. 2013). Meanwhile, soil properties are also strong drivers of AMF community in dry areas (del Mar Alguacil et al. 2016). In the present study, the ANOSIM results showed that the AMF community between sites and plant species is different. Therefore, both plant species and soil factors had an impact on

the AMF community in the sampling sites of Ningxia. The influence of plant traits and abiotic factors on the root-colonizing AMF community in salt-stressed ecosystem has been reported before (Guo & Gong 2014). Our results extended the interaction of plant traits and soil factors driving the AMF community from within roots to salinized soils.

The higher diversity index of YCGQ (Goji) compared with YCSZ (Oleaster) from Yinchuan city was found in the present study. This may be attributed to the host difference, as indicated by Chen et al. (2012). Thus, host plant species differences should be taken into account when deciphering the factors affecting AMF diversity.

In addition to host plants, edaphic factors had a more pronounced effect on AMF diversity in this study. Salinity negatively and significantly correlated with AMF diversity in the sampling sites. This demonstrated the negative effect of salinity on AMF diversity in salty soils (Krishnamoorthy et al. 2014) as well as in roots of salt marshes (Guo & Gong 2014). In contrast to soil salinity, AK, AP, AN and TP positively and significantly correlated with AMF diversity. These soil factors represent soil fertility. The soils with relative higher fertility in nature enable better growth of plants, which may indirectly harbor higher AMF diversity. Moreover, evenness only positively and significantly correlated with AP, targeting the close relationship between soil phosphorus and AMF community (Johnson et al. 2013).

Conclusion

High AMF diversity (33 species) was identified with a relatively high spore density in the saline soils of Ningxia. Glomeraceae and *Septoglomus constrictum* were the most abundant family and species in the sampling sites. AMF community changes were attributed to host plants species as well as soil factors. Soil factors had significant effects on AMF diversity. Salinity negatively and significantly correlated with the AMF diversity index; however, AK, AP, AN and TP positively and significantly correlated with AMF diversity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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