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Full Length Research Paper

Community diversity of bacteria and arbuscular mycorrhizal fungi in different ecological regions of Loess Plateau in Shaanxi Province of China

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The research of rhizosphere microorganism community structure in degraded areas has been a focus recently. The aim of this study is to analyze the community diversity of bacteria and arbuscular mycorrhizal fungi (AMF) in the rhizosphere of *Amorpha fruticosa* L., *Hippophae rhamnoides* L. and *Robinia pseudoacacia* L. in three different ecological regions of Loess Plateau in Shaanxi Province of China by terminal restriction fragment length polymorphisms (T-RFLP). Results obtained that the AMF and bacterial diversity differed greatly between regions and native plants species. Species richness and the Shannon diversity index of bacteria and AMF in rhizosphere of *R. pseudoacacia* were higher than that of *H. rhamnoides* and *A. fruticosa* in the three regions. The results of principal component analysis and redundancy analysis (RDA) indicated that host plants had no strict specificity with both AMF and bacterial community diversity while environmental condition did great influence on AMF community diversity and organic matter content, and pH were primary influencing factor. The community diversity of AMF was significant correlative to that of bacteria ($p<0.01$). These results suggest that the environmental conditions exhibit greater influence on the community diversity of AMF than the host plants. And organic matter and pH are more indicative of the change of community diversity of bacteria and AMF.

Key words: Loess Plateau, arbuscular mycorrhizal fungi, rhizosphere bacteria, community diversity, terminal restriction fragment length polymorphisms (T-RFLP).

INTRODUCTION

The inland Loess Plateau of north-central China has special topographical and geological features. Soil erosion in this region is severe, partly due to the climatic conditions, industrial pollution and destruction of vegetation. Therefore, the research of improving ecological environment on the Loess Plateau has become a hot recently (Shen, 2005). Arbuscular mycorrhizal fungi (AMF) play an important role in vegetation restoration because of symbiosis with plant root; they can facilitate mineral absorption by the host plant, stabilize and improve soil structure, affect the population structure and preserve species diversity (Bothe et al., 2010). Leigh et al. (2009) indicate that uptake from organic N could be important in AM symbiosis for both plant and fungal partners and that some AM fungi may acquire inorganic N from organic sources. Rillig and Mummey (2006)

reviewed the contribution of AMF to soil structure at various hierarchical levels. Stevens et al. (2010) found alter AMF prevalence in wetlands could significantly alter plant community structure by directly affecting seedling growth and development. In recent years, AMF have been identified as important species associated with native trees on the Loess Plateau (Tang et al., 2006; Zhang and Tang, 2006). In addition, traditional microbio-logical methods targeting morphological or physiological characteristics, many culture-independent methods have been used. For example, using the BIOLOG method, Zhang et al. (2008) examined microbial community diver-sity of mycorrhizospheres in five tree species, *Caragana microphylla* L., *Hippophae rhamnoides* L., *Sophora viciifolia*, *Pinus tabulaeformis* Carr. and *Robinia pseudoacacia*. The results demon-strated that the different tree species differ significantly in both mycorrhizal infection rates and microbial functional diversity. Zhang et al. (2010) revealed that the AMF and bacterial Shannon diversity index in the rhizosphere of *H. rhamnoides* was higher than that of *Caragana korshinkii* and AMF communities had a significant positive correlation with the bacterial communities in Zhifanggou watershed on the Loess Plateau.

Amorpha fruticosa L. is heavily resistant to cold stress, drought, salinity and many other adverse conditions. Generally, *A*. *fruticosa* forms nodules and mycorrhiza, and improves its nitrogen fixation capacity by hosting AMF (Song et al., 2009). By comparison, *H. rhamnoides* grows fast and possesses strong root germination, as well as rich rhizobium (Zhang, 2005). *R. pseudoacacia* is a pioneering concomitance tree species for eco-environment restoration on the Loess Plateau which can improve soil cohesion (Du et al., 2008). These tree species can not only improve the local ecological environment, but also may help conserve water and soil.

Given the importance of plant survival for eco-restoration on the Loess Plateau, it appears important to analyze the microbial diversity in the rhizosphere of *A*. *fruticosa*, *H*. *rhamnoides* and *R*. *pseudoacacia* to reveal symbiotic interactions between rhizosphere microorganisms and the host plants. No study has been reported on the microbial diversity in the rhizosphere of these tree species. In this study, terminal restriction fragment length polymorphisms (T-RFLP) was applied to the study of bacterial and AMF communities in the rhizosphere of *A*. *fruticosa*, *H*. *rhamnoides* and *R*. *pseudoacacia* in different ecological regions of Loess Plateau, in an attempt to reveal the local ecological status of AMF and the relationship between AMF and bacterial communities. Results obtained will provide a theoretical basis for the application of eco-environment restoration.

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Abbreviations: AMF, Arbuscular mycorrhizal fungi; **T-RFLP,** terminal restriction fragment length polymorphisms; **PCR,** polymerase chain reaction; **T-RF,** terminal restriction fragment; **OTU,** operational taxonomic unit; **PCA,** principal component analysis; **RDA,** redundancy analysis; **OM,** organic matter content; **TN,** total nitrogen; **AP,** available phosphorus; **AK,** available potassium.

MATERIALS AND METHODS

Site and sampling

The research sites lie in the Loess Plateau in northwest of China. The plant nursery of Northwest A and F University in Yangling (108°04 ′, 34°16 ′) is located in Central Shaanxi Plain at altitude of 468 m, with average annual rainfall of 635 mm, and characterized by Manured loessial soil (a well developed agricultural soil). *P*. *tabulaeformis, Quercus variabilis* Blume and *A*. *fruticosa* are dominant vegetation species. This area has a good ecological environment and optimum climate. The Zhifanggou watershed (109°19 ′, 36°51 ′) is located in Loess Hilly and Gully region of Western Shaanxi province at altitude of 1125 m, with average annual rainfall of 484 mm and loessal soil. The dominant vegetation comprised of *P*. *tabulaeformis*, *A*. *fruticosa*, *C*. *korshinkii* and *R*. *pseudoacacia*. This site is the focal point for ecological reconstruction on the Loess Plateau. The Liudaogou watershed (110°21 ′, 38°47 ′) is located in a wind-water erosion belt in the Loess Plateau of Western Shaanxi province at an altitude of 1255 m, with average annual rainfall of 441 mm and aeolian sandy soil. The dominant floral species are *C*. *korshinkii*, *H*. *rhamnoides*, *A. fruticosa* and *Salix matsudana* Koidz. This site has been subject to severe soil erosion and pollution due to mining, with most natural vegetations destroyed.

Five sampling plots (20×20 m ^{2}) were randomly chosen in each plant in May 2008. Rhizosphere soil (Clegg and Gobran, 1997) was collected from 5-25 cm depths using a four points sampling method. Soil samples (of about 1 kg) from the same plot were mixed thoroughly as test samples. Each treatment has three repeats. The soil samples then placed in plastic bags for transportation to the laboratory, where they were stored at -20°C before an alysis. Soil parameters of sampling plots are given in Table 1.

DNA extraction and purification

Total DNA was extracted according to method described by Zhou et al. (1996) Total genomic DNA was then purified using a gel-DNA-recovery-kit (DP1702, Bioteke, Beijing).

Polymerase chain reaction (PCR) amplification

The forward primers of bacterial and AMF were labeled with 6-FAM fluorescence. The variable V3 region of the bacterial 16S rDNA was amplified using 8-27F (5′(6-FAM)-AGAGTTTGATCCTGGCTCAG-3′) (Dunbar et al., 2001; Yuan et al., 2007) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Sawamura et al., 2010). The variable V3-V4 region of the AMF 18S rDNA was amplified using AM1 (5′(6-FAM)-GTTTCCCGTAAAGGCGCCGAA-3′) (Helgason et al., 1998) and NS31 (5′-TTGGAGGGCAAGTCTGGTGCC-3′) (Simon et al., 1992). Reaction mixtures (20 µl) included 10 pmol primers, 2 µL 10×buffer, 1.2 µL 25 mmol/L $MgCl_2$, 1.6 µL dNTP mix, 2 µl purified template DNA and 0.2 µL Taq DNA polymerase (5 U/µl, TaKaRa, Japan). Thermal cycling of bacteria included an

Table 1. Soil parameters of sampling plots.

Sample ID	Sampling plot	Host planT	Organic matter (g/kg)	Total nitrogen (g/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	pH
LA	Liudaogou watershed	*A. fruticosa*	8.11±0.04	0.05±0.04	4.44±0.06	89.68±0.03	7.95±0.01
LH		*H. rhamnoides*	8.56±0.02	0.04±0.07	4.91±0.05	50.61±0.04	8.01±0.03
LR		*R. pseudoacacia*	10.64±0.02	0.11±0.05	2.58±0.05	196.97±0.05	7.68±0.04
ZA	Zhifanggou watershed	*A. fruticosa*	16.59±0.03	0.32±0.05	5.19±0.02	118.68±0.05	7.94±0.01
ZH		*H. rhamnoides*	17.77±0.05	0.24±0.02	3.05±0.01	55.48±0.07	7.99±0.02
ZR		*R. pseudoacacia*.	19.29±0.04	0.22±0.02	2.02±0.03	157.97±0.03	7.89±0.02
YA	Yangling nursery	*A. fruticosa*	21.54±0.02	0.72±0.03	14.70±0.04	216.15±0.03	7.78±0.02
YH		*H. rhamnoides*	21.23±0.05	0.59±0.06	8.64±0.04	235.27±0.04	7.82±0.04
YR		*R. pseudoacacia*.	24.78±0.03	0.32±0.04	9.85±0.05	162.29±0.01	8.00±0.03

LA, *A. fruticosa* sample collected in Liudaogou watershed; **LH,** *H. rhamnoides* sample collected in Liudaogou watershed; **LR,** *R. pseudoacacia* sample collected in Liudaogou watershed; **ZA,** *A. fruticosa* sample collected in Zhifanggou watershed; **ZH,** *H. rhamnoides* sample collected in Zhifanggou watershed; **ZR,** *R. pseudoacacia* sample collected in Zhifanggou watershed; **YA,** *A. fruticosa* sample collected in Yangling plant nursery; **YH,** *H. rhamnoides* sample collected in Yangling plant nursery; **YR,** *R. pseudoacacia* sample collected in Yangling plant nursery.

initial denaturing step of 94°C for 4 min, then 30 cy cles consisting of 30 s at 94°C, 30 s at 50°C and 2 min at 72°C, follo wed by a final extension step of 72°C for 7 min. Thermal cycling of AMF included an initial denaturing step of 94°C for 2 min, 30 cyc les consisting of 45 s at 94°C, 1min at 65°C and 45 s at 72°C, follow ed by a final extension step of 72°C for 7 min.

The PCR products were examined by 1% agarose gel electrophoresis and purified using gel-DNA-recovery-kit (DP1702, Bioteke, Beijing).

Restriction enzyme digestion and gene scan analysis

The restriction enzyme *Hha Ⅰ* (TaKaRa, Japan) was chosen for purified bacterial PCR product and *Mbo Ⅰ* (TaKaRa, Japan) was chosen for purified AMF PCR product. Each restriction digestion reaction, containing 2 µl 10×buffer, 7 µl dH_2O, 1 µl restriction enzyme and 10 µL purified PCR product, was incubated for 4 h at 37°C, followed by an inactivation step at 65°C for 15 min. Digested DNA was scanned by Shanghai Gene Core BioTechnologies Co., Ltd.

Statistical analysis

Each terminal restriction fragment (T-RF) in the profiles was regard as an operational taxonomic unit (OTU), peaks with peak height <100 fluorescent units were excluded from the analysis. Based on the number and abundance of OTU in the profiles, the Shannon diversity index (*H*) and the Evenness index (*E*) were measured (Zak et al., 1994):

Relative peak height (*Pi*) : $Pi = ni / N$ (1)

Shannon diversity index (*H*) : $H = -\sum Pi \ln Pi$ (2)

Evenness index (*E*) : $E = H / \ln S$ (3)

Here, n is one T-RF peak height, and N is cumulative peak height.

Statistical analysis was performed by Microsoft Excel (2003) and SPSS version 18.0 and Canoco for windows 4.5.

RESULTS

DNA purification and amplification

After purification, the total DNA extracted from soil samples was clear and pure. The size of the genome fragments averaged about 20 kb. The 16S rDNA gene fragments amplified by using bacterial universal primers formed a single band on agarose gel with a length of 1500 bp, whereas amplified gene fragments of 18S rDNA were about ≈ 550 bp.

Diversity of bacteria and AMF

The T-RFLP fingerprinting of bacteria was performed using the restriction enzyme *Hha Ⅰ*. According to the number of T-RFs and the relative peak heights, the species richness, Shannon diversity index, and Evenness index of the nine samples were calculated (Table 2). The bacterial community diversity indices in the rhizosphere of *A. fruticosa*, *H. rhamnoides* and *R. pseudoacacia* revealed that bacterial diversity differed greatly between regions and native plants species. The species richness and Shannon diversity index of *A. fruticosa* in Yangling plant nursery were significantly higher ($p<0.05$) than in the Zhifanggou watershed and in the Liudaogou watershed. The species richness and Shannon diversity index of *R. pseudoacaci* and *H. rhamnoides* decreases significantly ($p<0.05$) from the Zhifanggou watershed to the Yangling plant nursery and the Liudaogou watershed

Table 2. Diversity indices of bacteria in three regions on the Loess Plateau.

Sample ID	Species richness(S)	Shannon diversity index(H)	Evenness index(E)
LA	67e	2.76c	0.66ab
LH	82cd	2.74c	0.62ab
LR	91c	4.01ab	0.89a
ZA	49f	2.1d	0.54b
ZH	127ab	4.3a	0.89a
ZR	136a	4.39a	0.89a
YA	139a	4.4a	0.89a
YH	74de	3.68b	0.86ab
YR	115b	3.9ab	0.82ab

LA, *A*. *fruticosa* sample collected in Liudaogou watershed; **LH,** *H*. *rhamnoides* sample collected in Liudaogou watershed; **LR,** *R*. *pseudoacacia* sample collected in Liudaogou watershed; **ZA,** *A*. *fruticosa* sample collected in Zhifanggou watershed; **ZH,** *H*. *rhamnoides* sample collected in Zhifanggou watershed; **ZR,** *R*. *pseudoacacia* sample collected in Zhifanggou watershed; **YA,** *A*. *fruticosa* sample collected in Yangling plant nursery; **YH,** *H*. *rhamnoides* sample collected in Yangling plant nursery; **YR,** *R*. *pseudoacacia* sample collected in Yangling plant nursery.

Table 3. Diversity indices of AMF in three regions on the Loess Plateau.

Sample ID	Species richness(S)	Shannon diversity index(H)	Evenness index(E)
LA	14e	1.44f	0.56a
LH	65a	2.53a	0.61a
LR	46abc	2.27bc	0.6a
ZA	18de	1.21f	0.43b
ZH	58ab	2.41ab	0.6a
ZR	40bc	2.12cd	0.58a
YA	36cd	1.79e	0.5ab
YH	47abc	1.99de	0.52ab
YR	58ab	2.39ab	0.59a

LA, *A*. *fruticosa* sample collected in Liudaogou watershed; **LH,** *H*. *rhamnoides* sample collected in Liudaogou watershed; **LR,** *R*. *pseudoacacia* sample collected in Liudaogou watershed; **ZA,** *A*. *fruticosa* sample collected in Zhifanggou watershed; **ZH,** *H*. *rhamnoides* sample collected in Zhifanggou watershed; **ZR,** *R*. *pseudoacacia* sample collected in Zhifanggou watershed; **YA,** *A*. *fruticosa* sample collected in Yangling plant nursery; **YH,** *H*. *rhamnoides* sample collected in Yangling plant nursery; **YR,** *R*. *pseudoacacia* sample collected in Yangling plant nursery.

(the lowest). In the rhizosphere of *R*. *pseudoacacia*, the diversity indices were higher than the other two plants in three regions.

The T-RFLP fingerprints of AMF were performed using the restriction enzyme *Mbo I*. The species richness, Shannon diversity index and Evenness index of the nine samples of AMF were calculated (Table 3). The diversity of AMF was lower than that of bacteria. The species richness and Shannon diversity index of *A*. *fruticosa* in Yangling plant nursery were significantly higher than in the Zhifanggou watershed or in the Liudaogou watershed (p<0.05), while the difference of between the Zhifanggou watershed and the Liudaogou watershed was not significant. *H*. *rhamnoides* decreases from the Liudaogou watershed and Zhifanggou watershed to the Yangling plant nursery (the lowest), but *R*. *pseudoacaci* was contrary, and the difference was not significant. Overall, the community diversity in rhizosphere of *A*. *fruticosa*, *R*. *pseudoacaci* and *H*. *rhamnoides* in Zhifanggou watershed approached or exceeded that in Yangling plant nursery, demonstrating that microbial community in rhizosphere soils has improved significantly.

Principal component analysis (PCA)

PCA was performed to analyze the microbial community in rhizosphere soils. Results of bacteria (Figure 1A) showed that the first principal component's contribution rate was 25.60% and nine samples were divided into two groups, with samples 3 and 7 in one group and the others in a second group; the second principal component of the contribution rate was 21.72% and the two parts of that samples were further divided into three categories, distributed into three quadrants. PCA of AMF (Figure 1B) showed that the first principal component's contribution

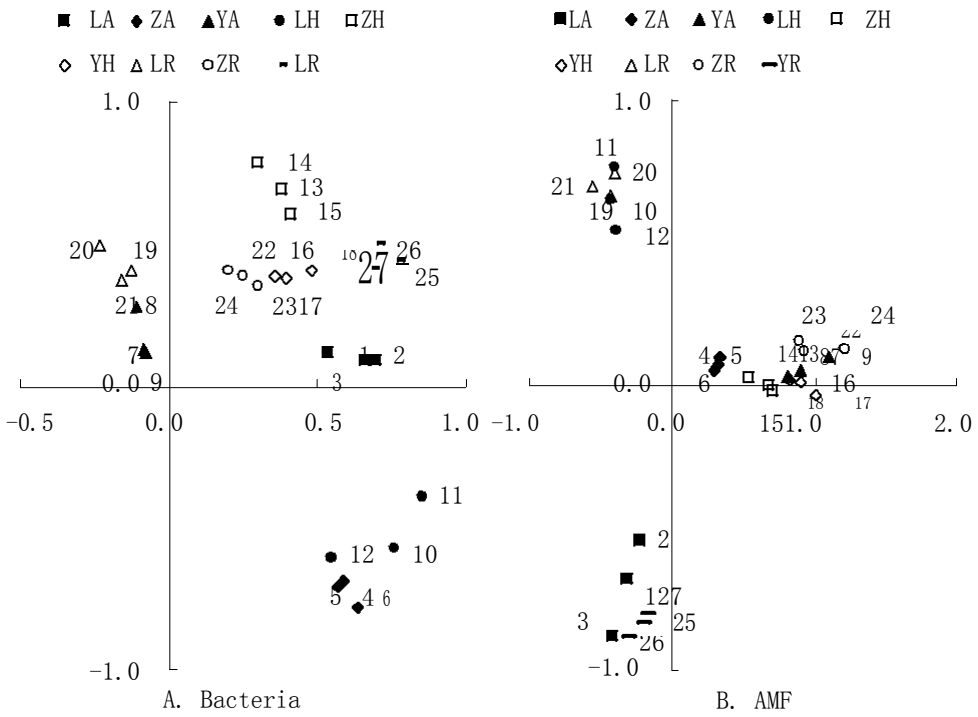

Figure 1. PCA analysis of rhizosphere bacteria and AMF. **A,** soil bacteria; **B,** AMF.The bacterial first principal component's contribution rate was 25.60%, and second principal component of the contribution rate was 27.72%. The AMF first principal component's contribution rate was 39.40%, and second principal component of the contribution rate was 22.70%. **LA,** *A. fruticosa* sample collected in Liudaogou watershed; **LH,** *H. rhamnoides* sample collected in Liudaogou watershed; **LR,** *R. pseudoacacia* sample collected in Liudaogou watershed; **ZA,** *A. fruticosa* sample collected in Zhifanggou watershed; **ZH,** *H. rhamnoides* sample collected in Zhifanggou watershed; **ZR,** *R. pseudoacacia* sample collected in Zhifanggou watershed; **YA,** *A. fruticosa* sample collected in Yangling plant nursery; **YH,** *H. rhamnoides* sample collected in Yangling plant nursery; **YR,** *R. pseudoacacia* sample collected in Yangling plant nursery.

rate was 39.40%, and nine samples were divided into two parts, with samples 1, 2, 3, and 9 in one group and the rest in another group; the second principal component of the contribution rate was 22.70% and the two parts of the sample further divided into three categories, distributed into three quadrants. These results indicate that host plant had no specificity with both AMF and bacterial community diversity, while the AMF community diversity showed regionalism obviously.

Correlation analysis

To reveal the relationship between AMF and bacterial diversity and soil characteristics, correlation analysis were performed. Results revealed that AMF and bacterial diversity were positively correlated (r=0.88, p<0.01).

Redundancy analysis (RDA)

RDA was performed to analysis the relationships between host plant and community diversity (Figure 2A). The results show that the samples in the Yangling plant nursery (samples 19-27) exhibited the highest bacterial diversity followed by the Zhifanggou watershed (Samples 10-18) and the Liudaogou watershed (samples 1-9) (the lowest). These results indicate that bacterial diversity in the rhizosphere was mainly influenced by environment. The species abundance and the Shannon index of AMF in the rhizosphere of *R. pseudoacacia* of the three regions were higher than the other plants.

The relationships between environmental conditions and community diversity were analyzed (Figure 2B). The results show that organic matter content (OM) has greatest influence on the community diversity followed by pH; total nitrogen (TN), available phosphorus (AP) and available potassium (AK) have little influences. OM was positive correlated with the bacterial community diversity indices except evenness index of AMF. The pH was positive correlated with the community diversity indices of AMF but was negative correlated with the bacterial community diversity indices. TN, AP and AK were positive correlated with the bacterial community diversity indices

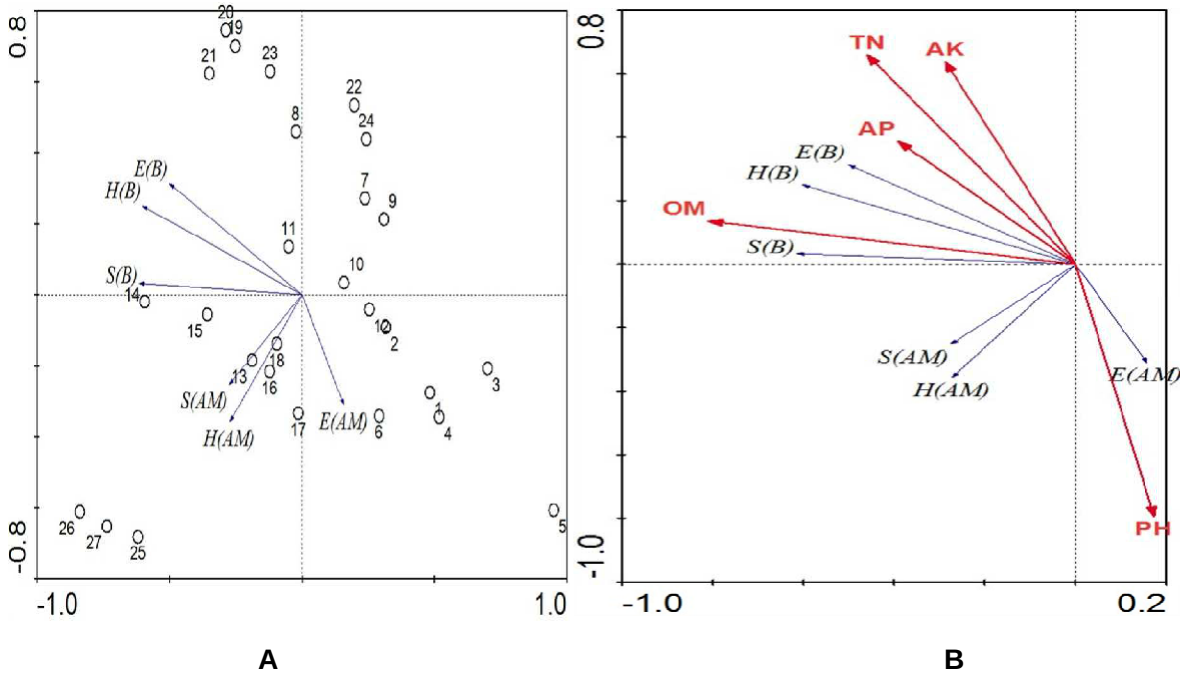

Figure 2. RDA analysis of rhizosphere bacteria and AMF. **A,** The multi-axial figure of host plant and community diversity; **B,** the multi-axial figure of environmental conditions and community diversity.**1-24,** sample plot and host plant; **1-3,** *A. fruticosa* sample collected in Liudaogou watershed; **4-6,** *H. rhamnoides* sample collected in Liudaogou watershed; **7-9,** *R. pseudoacacia* sample collected in Liudaogou watershed; **10-12,** *A. fruticosa* sample collected in Zhifanggou watershed; **13-15,** *H. rhamnoides* sample collected in Zhifanggou watershed; **16-18,** *R. pseudoacacia* sample collected in Zhifanggou watershed; **19-21,** *A. fruticosa* sample collected in Yangling plant nursery; **22-24,** *H. rhamnoides* sample collected in Yangling plant nursery; **25-27,** *R. pseudoacacia* sample collected in Yangling plant nursery; **E(B),** bacterial evenness index; **H(B),** bacterial Shannon diversity index; **S(B),** bacterial species richness index; **E(AM),** Evenness index of AMF; **H(AM),** Shannon diversity index of AMF; **S(AM),** species richness index of AMF; **TN,** total nitrogen; **AP,** available phosphorus; **AK,** available potassium; **OM,** organic matter.

but were negative correlated with the community diversity indices of AMF. And the diversity of AMF showed the specificity with the host plants which was consistent with the results of PCA.

DISCUSSION

The extraction of total DNA from soil is one of the key technologies of modern ecology research. Ideally, the extraction method should be able to extract DNA samples from all microbial species present. A number of studies have examined general community structure by DNA extraction methods (Krsek and Wellington, 1999; Leff et al., 1995; Kehrmeyer et al., 1996; Yeates et al., 1998). In general, DNA extraction methods for soil/sediments can be divided into two categories, isolation of cells from sediments and subsequent lysis and DNA extraction (Jacobsen and Rasmussen, 1992), and cell lysis and extraction directly from the sediments (Tsai and Olson, 1992). The latter is more widely used for soil DNA extraction because of its simplicity and higher yield (Cullen and Hirsch, 1998). In this study, total DNA was extracted by the enzymatic hydrolysis method consistent with previous studies (Zhang et al., 2010; Ren et al., 2006). Our results reveal that the extracted DNA was of sufficient quality for T-RFLP analysis. Total DNA extracted from soil contains humic acid that first needs to be eliminated (Teng et al., 2004). Li et al. (2008) found that PVPP column chromatography was a simple and fast method to purify DNA from soil extraction but Tsai and Olson (1992) reported the recovery rate of large fragments was low and that the purified samples had lower yield after microcolumn purification. In this study, we did not employ further purification steps; however, the total DNA purified by the gel-DNA-recovery-kit was colorless, suggesting that most of the humic acids were removed.

The choice of primers and restriction enzymes are crucial in the success of T-RFLP analysis. Only when a variety of target microbial fragments are amplified efficiently can T-RFLP analysis truly reflect the microbial diversity of the sample. We used the primers 8-27F and 1492R to amplify the conserved bacterial V3 fragment.

Although the AM1 primer site was not well conserved in certain divergent lineages of AMF, such as the *Archaeosporaceae* and the *Paraglomaceae* (Redecker, 2000; Aldrich-Wolfe, 2007), the AM1-NS31 primer pair has been widely used to amplify AMF (Mummey et al., 2005; Zhang et al., 2009). Indeed, AM1 reliably amplifies three traditional AMF families (*Glomaceae*, *Acaulosporaceae*, and *Gigasporaceae*), while excluding known non-target plant and fungal DNA. The choice of restriction enzymes may affect the ability of T-RFLP to distinguish species. Dunbar et al. (2001) found that fewer terminal restriction fragments (T-RFs) were obtained from double digestion than by combining results from separate single enzyme digests. Therefore, the restriction enzyme *Hha I* was chosen for bacterial analysis. Mummey et al. (2005) found that the restriction enzymes *Hinf I*, *Mbo I* and *Nla III* yielded the most unique T-RFs. Tests of both *Hinf I* and *Mbo I* on a subset of the samples used in their study revealed that *Mbo I* yielded a greater diversity of T-RF sizes and was thus chosen for all subsequent experiments.

There were great differences of the AMF and bacterial community diversity between regions and native plants species. The community diversity indices showed that Ecological Restoration on the Loess Plateau was effective. Species richness and the Shannon diversity index of bacteria and AMF in rhizosphere of *R. pseudoacacia* were higher than that of *H. rhamnoides* and *A. fruticosa* in the three regions; probably *R. pseudoacacia* can promote ability of soil aggregate preferably, and reduce the pH (Du et al., 2008).

PCA and RDA showed that bacterial community diversity had no strict specificity with the host plants, in agreement with Miniaci et al. (2007), Pivato et al. (2007) and Brodie et al. (2002), because the bacterial community diversity depended on not only host plant, but also the environmental conditions such as soil type (Berg and Smalla, 2009), growth stage (Van Overbeek and Van Elsas, 2008; Cavaglieri et al., 2009), cropping practices (Watt et al., 2006), the other rhizosphere biological (Rosenberg et al., 2009) and so on. But some other studies have indicated that bacterial population structure can be species-dependent (Roesti et al., 2006; Long et al., 2008). Because some studies evaluates root exudates are more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities (Dennis et al., 2010; Haichar et al., 2008). Host plant had no strict specificity with AMF community diversity while environmental condition did great influence on AMF community diversity. This was consistent with Zhang et al. (2010). The reasons maybe was AMF is more sensitive to ambient environmental condition compare with host plant, therefore, the community diversity of AMF was species-dependent inconspicuous. However, Börstler et al. (2006) discovered that the AMF species composition was also related to above ground plant biodiversity in two differently managed mountain grasslands in Germany. Panwar and Tarafdar (2006) found the most significant determinant of AMF community structure was the host species.

Based on RDA, OM and pH were primary influencing factor. Previous work has demonstrated that soil properties such as pH, OM and nutrient availability are strong determinants of microbial community structure (Grayston et al., 2004; Jesus et al., 2010, Ibekwe et al., 2010; Lovieno et al., 2010).

Correlation analyses showed that the AMF community diversity in the rhizosphere was positively correlated with the bacterial community, indicating that AMF may have promoted and sustained the bacterial community. Indeed, Du et al. (2008) found that AMF in rhizosphere of *R. pseudoacacia* can promote microbial community stability and increase functional diversity. Zhang et al. (2010) also suggested that the bacterial and AMF diversity in *H. rhamnoides* and *C. microphylla* rhizosphere were positively correlated. However, Pivato et al. (2009) indicated that enhancement of arbuscular mycorrhiza development was only induced by a limited number of bacteria.

The T-RFLP technique is widely used in mycorrhizal ecology (Dickie and FitzJohn, 2007). It was first developed by Liu et al. (1997) as a tool for assessing bacterial diversity and comparing the community structure of bacteria in different environmental samples. The technique has the significant advantage of low cost and relative simplicity, permitting sufficient replication to address important ecological questions. Although limited by the fidelity of DNA extraction and quality, it may be the most sensitive molecular method for the study of fungi (Brodie et al., 2002; Singh et al., 2006; Hao et al., 2009).

Conclusion

In this study, T-RFLP technique was extended to study the diversity of bacteria and AMF of three plants in three regions on the Loess Plateau. The host plants do not show strict specificity with the community diversity of bacteria and AMF. The environmental conditions exhibit great influence on the community diversity of AMF and it is worth noticed that organic matter and pH of soil, rather than other environmental factors, are more indicative of the change of the community diversity of bacteria and AMF.

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