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ScienceDirect
Journal of Integrative Agriculture
2013, 12(11): 2103-2113
November 2013
RESEARCH ARTICLE

Effect of Different Vegetation Types on the Rhizosphere Soil Microbial Community Structure in the Loess Plateau of China

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Abstract

The Loess Plateau in China is one of the most eroded areas in the world. Accordingly, vegetation restoration has been implemented in this area over the past two decades to remedy the soil degradation problem. Understanding the microbial community structure is essential for the sustainability of ecosystems and for the reclamation of degraded arable land. This study aimed to determine the effect of different vegetation types on microbial processes and community structure in rhizosphere soils in the Loess Plateau. The six vegetation types were as follows: two natural grassland (*Artemisia capillaries* and *Heteropappus altaicus*), two artificial grassland (*Astragalus adsurgens* and *Panicum virgatum*), and two artificial shrubland (*Caragana korshinskii* and *Hippophae rhamnoides*) species. The microbial community structure and functional diversity were examined by analyzing the phospholipid fatty acids (PLFAs) and community-level physiological profiles. The results showed that rhizosphere soil sampled from the *H. altaicus* and *A. capillaries* plots had the highest values of microbial biomass C, average well color development of carbon resources, Gram-negative (G^-) bacterial PLFA, bacterial PLFA, total PLFA, Shannon richness, and Shannon evenness, as well as the lowest metabolic quotient. Soil sampled from the *H. rhamnoides* plots had the highest metabolic quotient and Gram-positive (G^+) bacterial PLFA, and soil sampled from the *A. adsurgens* and *A. capillaries* plots had the highest fungal PLFA and fungal:bacterial PLFA ratio. Correlation analysis indicated a significant positive relationship among the microbial biomass C, G^- bacterial PLFA, bacterial PLFA, and total PLFA. In conclusion, plant species under arid climatic conditions significantly affected the microbial community structure in rhizosphere soil. Among the studied plants, natural grassland species generated the most favorable microbial conditions.

Key words: soil microbial biomass, microbial community structure, PLFA, community-level physiological profiles, vegetation types

INTRODUCTION

Knowledge of natural variations in ecosystems is essential for the sustainability of ecosystems as well as for the reclamation of degraded arable land. Soil microorganisms contribute to soil quality and play key roles in soil ecosystem processes, including nutrient cycling, organic matter decomposition, and bioremediation (Chen M M *et al*. 2007). Variations in soil microbial communities attributed to ecosystem management and global change can significantly affect the ecosystem balance (Bossio and Scow 1995). Soil microorganisms are closely related with

Received 23 October, 2012 Accepted 15 Januray, 2013
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doi: 10.1016/S2095-3119(13)60396-2

their surroundings, rapidly responding to changes and environmental stresses. Thus, these microorganisms are used as sensitive indicators of soil stresses and of soil recovery (Winding *et al*. 2005).

Rhizosphere is defined as the soil adjacent to and influenced by plant roots; a zone of usually high microbial activity and clearly distinct from bulk soil in terms of nutrient availability, pH, and redox potential (Hinsinger *et al*. 2009). Plant roots release a high amount of exudates, such as sugars, amino acids, organic acids hormones, and enzymes (Grayston *et al*. 1997), most of which are available to the microbial community (Nguyen 2003). Consequently, numerous microorganisms are found in this zone. Many studies have investigated the microbial community structures in rhizospheres, ranging from a purely agricultural point of view to a more environmental perspective (Esperschütz *et al*. 2009; Hamer and Makeschin 2009). The most common methods of characterizing microbial communities in soil are the phospholipid fatty acid (PLFA) and community-level physiological profiles (CLPPs). For instance, Innes *et al*. (2004) assessed the effects of individual plant species on the microbial communities in rhizosphere soils of different fertility properties using a microcosm experiment in the semi-fertile temperate grasslands of northern England. A greenhouse pot experiment was conducted by Chen *et al*. (2007b) to investigate the influence of soil moisture content on the soil microbial community structure of white clover and ryegrass using PLFA and CLPP methods. Tscherko *et al*. (2004) quantified the effect of *Poa alpina* on the soil microbial community in a primary succession of alpine ecosystems, and determined whether these effects are controlled by the successional stage. The plant-specific growth of soil microbes can reportedly exert a positive or negative effect on a plant, thereby altering the relative performance of individual species within plant communities (Klironomos 2002).

The Loess Plateau in China, covering approximately 58×10^4 km^2, is known for its long agricultural history and serious soil erosion (Chen L D *et al*. 2007). Vegetation destruction resulting from long-term poor land use practices, such as deforestation, overgrazing, and over-reclamation, has accelerated soil erosion (Fu *et al*. 2009) and deteriorated the ecological environment. In 1999, a project named Grain for Green was launched by the Chinese government to control soil erosion and improve land quality by converting large areas of sloping cropland to forestland and grassland in the loess hilly area of the Loess Plateau. These conversions have resulted in improved soil conditions, including physical properties (Zhu *et al*. 2010), nutrient status (Cao *et al*. 2008; Zhang *et al*. 2011a), and microbial properties (An *et al*. 2009; Zhang *et al*. 2012; Xiao *et al*. 2013). However, changes in soil microbial communities during the conversion of slope cropland to other land use in this region, particularly at the root-soil interface (rhizosphere) where microorganismal metabolic activities frequently develop, are rarely reported. Therefore, this study aimed to determine the effect of six revegetation types (two each of artificial shrublands, artificial grasslands, and natural grasslands) on the soil microbial communities in the Loess Plateau using PLFA profiles and CLPP analysis. We hypothesized that the vegetation type significantly affected the microbial community structure and functional diversity, and that soil sampled from the natural grassland plots had the highest PLFA biomass, microbial diversity, and functional diversity.

RESULTS

Soil microbial biomass C, basal respiration, and metabolic quotient

The content of microbial biomass C, basal respiration, metabolic quotient, and pH significantly differed among the vegetation types (P<0.05, Table 1). The highest microbial biomass C was found in the soil of *Heteropappus altaicus* and *Artemisia capillaries*, followed by *Astragalus adsurgens*, *Panicum virgatum* and *Caragana Korshinskii* (Fig. 1-A). Similar basal respiration values were found for *C. korshinskii*, *A. adsurgens*, *P. virgatum*, and *H. altaicus* which were significantly higher than those for *Hippophae rhamnoides* and *A capillaries* (Fig. 1-B). The maximum metabolic quotient was found in the rhizosphere soil of *P. virgatum*, followed by *C. korshinskii* and

A. adsurgens. The lowest metabolic quotient was recorded for *A. capillaries* (Fig. 1-C). *H. altaicus* and *A. capillaries* also showed lower soil pH values than the other four plants, although it did not remarkably change, ranging from 8.52 to 8.68 (Fig. 1-D).

Table 1 Statistical analysis of the microbial properties

Sample no.	Properties[1)]	*df*	*F* value	*P*
1	Microbial biomass C	5	18.406	<0.001
2	Basal respiration	5	9.517	<0.001
3	Metabolic quotient	5	16.245	<0.001
4	pH	5	2.527	0.034
5	G^- bacterial PLFA	5	12.786	<0.001
6	G^+ bacterial PLFA	5	25.809	<0.001
7	Bacterial PLFA	5	9.185	<0.001
8	Fungal PLFA	5	21.598	<0.001
9	Total PLFA	5	26.279	<0.001
10	Fungal: bacterial PLFA ratio	5	10.028	<0.001
11	H_{CLPP}	5	44.854	<0.001
12	E_{CLPP}	5	48.273	<0.001
13	H_{PLFA}	5	30.256	<0.001
14	E_{PLFA}	5	27.196	<0.001

[1)] H_{CLPP}, Shannon richness of CLPP; E_{CLPP}, Shannon evenness of CLPP; H_{PLFA}, Shannon richness of PLFA; E_{CLPP}, Shannon evenness of PLFA.

Microbial community structure assessed by the PLFAs

Total concentrations of PLFA can be used to indicate the total biomass of soil microbial communities. Table 1 shows that the Gram-negative (G^-) bacterial, Gram-postive (G^+) bacterial, bacterial, and fungal PLFA, as well as the fungal:bacterial PLFA ratio in the rhizosphere soils significantly differed among the six plants ($P<0.05$). A similar trend was found for G^- bacterial, bacterial, and total PLFA, which were significantly higher in the rhizosphere soil of *H. altaicus* and *A. capillaries* than in that of the other four plants (Fig. 2-A, C and E). G^+ bacterial PLFA can be grouped into three categories (Fig. 2-B): the highest value for *H. rhamnoides*; the middle for *A. adsurgens*, *H. altaicus* and *A. capillaries*; and the lowest for *C. korshinskii*. The highest fungal PLFA was found in *A. capillaries* soil, followed by *A. adsurgens*

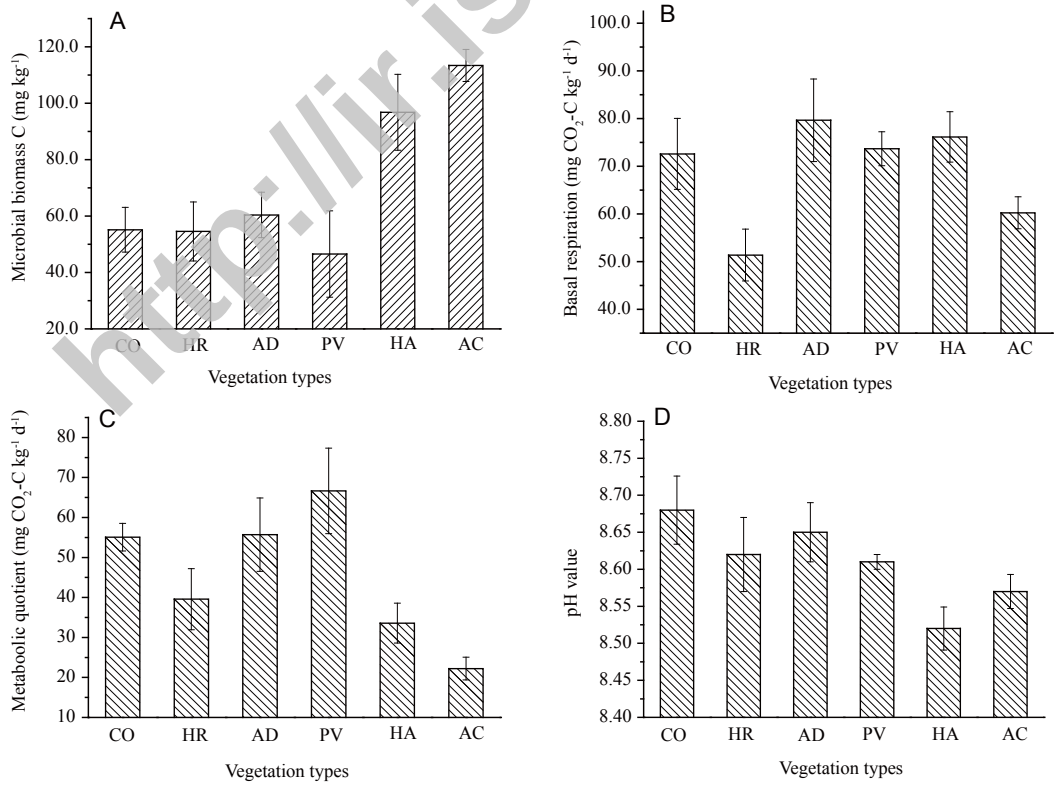

Fig. 1 Rhizosphere soil microbial properties under the different vegetation types. Results are given as mean±SD. CO, *C. korshinskii*; HR, *H. rhamnoide*; AD, *A. adsurgens*; PV, *P. virgatum*; HA, *H. altaicus*; AC, *A. capillaries*. The same as below.

soil. No remarkable difference was observed for *H. rhamnoides*, *C. korshinskii*, *P. virgatum*, and *H. altaicus* (Fig. 2-D). *A. adsurgens* and *A. capillaries* showed higher fungal:bacterial ratio in rhizosphere soil than that in the other plants (Fig. 2-F).

Highly significant differences between the PLFA richness and evenness of different plant treatments were observed ($P<0.05$, Table 1). A higher PLFA richness value was observed in the rhizosphere soils of *H. altaicus*, *A. capillaries*, and *H. rhamnoides*, followed by *A. adsurgens*, *P. virgatum*, and *C. korshinskii*. Except for the lower PLFA evenness in the rhizosphere soil of *P. virgatum*, no significant difference was found among the other five plants (Fig. 3-A and B).

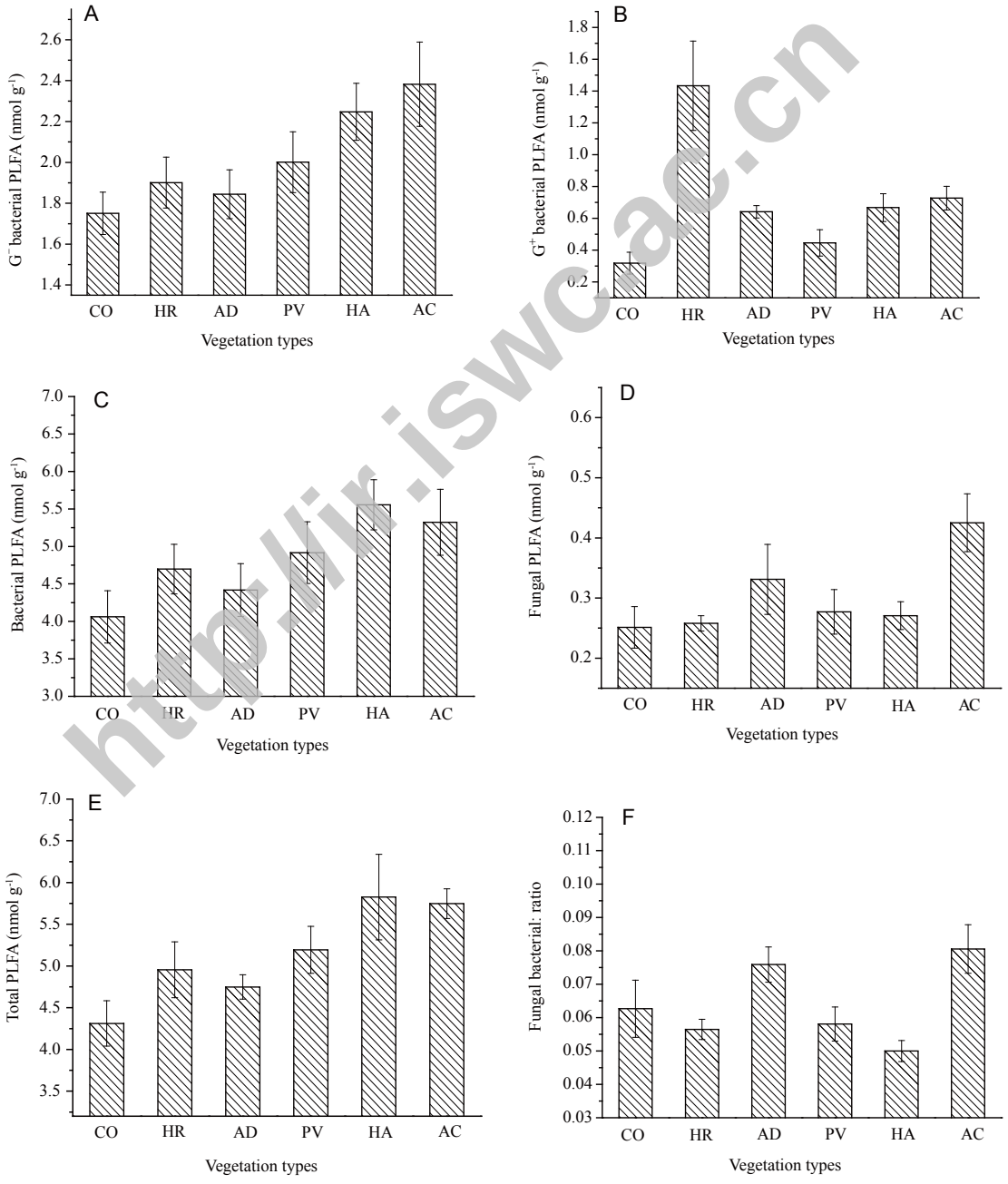

Fig. 2 Fatty acids of the microbial communities from rhizosphere soil of different plants.

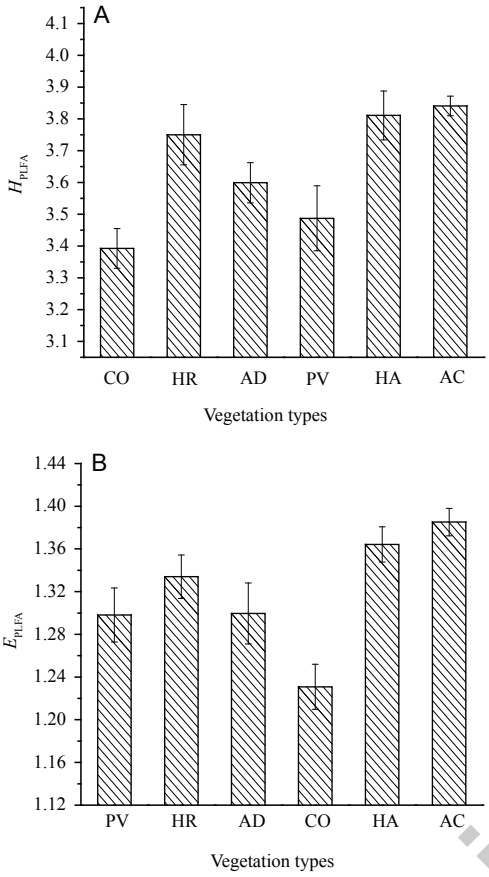

Fig. 3 Shannon richness and evenness index of PLFA in the rhizosphere soil of different plants. H_{PLFA}, Shannon richness of PLFA; E_{PLFA}, Shannon evenness of PLFA.

Community-level physiological profiles (CLPPs)

CLPPs were used to determine the substrate utilization potential of fast-growing, heterotrophic bacteria. Average well color development (AWCD) generally followed a sigmoidal pattern with the incubation time, but the rate of increase varied with the plant species (Fig. 4). Within the first 24 h, no significant AWCD difference was found among the rhizosphere soils of the six plants; thereafter, the AWCD increased with time. After 240 h, AWCD ranked in the following order: *H. altaicus*>*A. capillaries*>*C. korshinskii*>*P. virgatum*>*H. rhamnoides*>*A. adsurgens*. Table 1 shows significant differences among the CLPP richness and evenness in response to different plant treatments evaluated with the data from 240

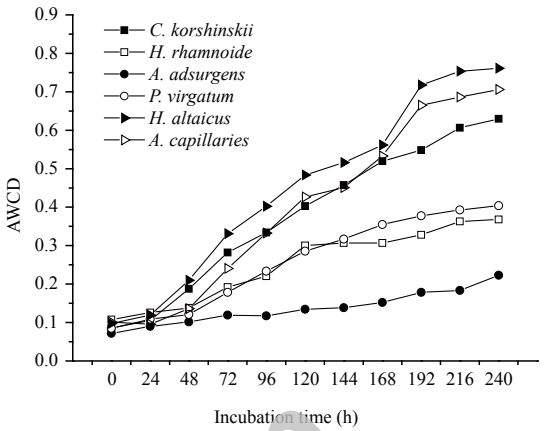

Fig. 4 Average well color development profiles (AWCD) for the microbial communities in rhizosphere soil of different vegetation types.

h incubation. The CLPP richness and evenness similarly behaved, higher in the soil of *H. altaicus* and *A. capillaries*, followed by *A. adsurgens*, *H. rhamnoides*, *C. korshinskii*, and *P. virgatum* (Fig. 5).

Correlation among the rhizosphere microbial properties of the different plant species

Table 2 showed the relationships among the rhizosphere soil microbial properties of the different plants. Microbial biomass C was positively correlated with the G^- bacterial PLFA, bacterial PLFA, total PLFA, and H_{CLPP}, but negatively correlated with the metabolic quotient and pH (P<0.05 or P<0.01). Basal respiration was positively correlated with the metabolic quotient and negatively correlated with the G^+ bacterial PLFA (P<0.05 or P<0.01). The metabolic quotient was negatively correlated with the G^- bacterial PLFA, bacterial PLFA, total PLFA, and H_{CLPP}. For the microbial community structure, a significant positive correlation was found among the G^- bacterial PLFA, bacterial PLFA, total PLFA, and H_{CLPP}. The bacterial and total PLFA were also positively correlated with H_{PLFA} (P<0.05). A positive correlation also existed between the fungal PLFA and fungal:bacterial ratio (P<0.05 or P<0.01), but no significant relationship was observed between the fungal PLFA and other properties.

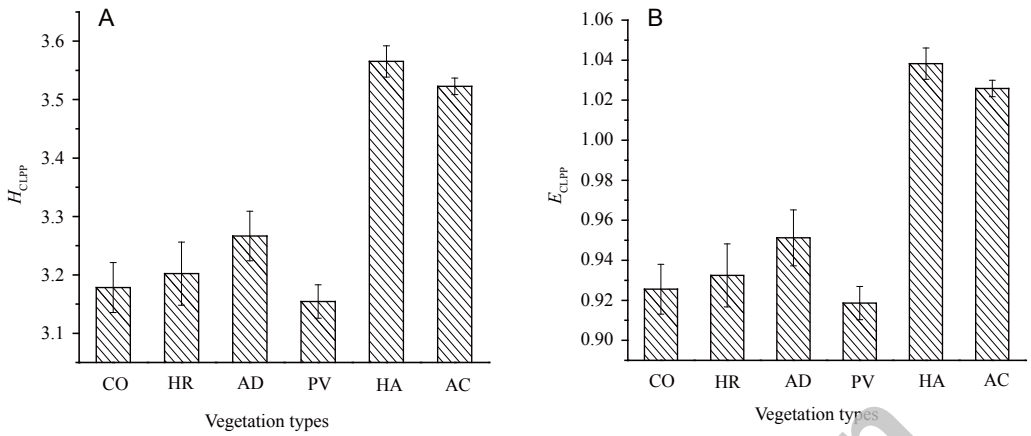

Fig. 5 Shannon richness and evenness index of CLPP in the rhizosphere soil of different plants.

Table 2 Correlation matrix between the different properties determined

Properties	Microbial biomass C	Basal respiration	Metabolic quotient	pH value	G^- bacterial PLFA	G^+ bacterial PLFA	Bacterial PLFA	Fungal PLFA	Total PLFA	F:B ratio	H_{PLFA}	E_{PLFA}	H_{CLPP}
Microbial biomass C	1.000	-0.052	-0.848**	-0.597**	0.666**	-0.052	0.694**	0.274	0.614**	0.025	0.259	0.276	0.867**
Basal respiration		1.000	0.498*	0.036	-0.121	-0.708**	-0.167	-0.131	-0.177	-0.024	-0.082	0.025	-0.012
Metabolic quotient			1.000	0.517*	-0.591**	-0.290	-0.607**	-0.153	-0.573*	0.133	-0.319	-0.287	-0.756**
pH value				1.000	-0.365	-0.176	-0.114	-0.059	-0.118	0.023	-0.255	-0.236	-0.642**
G^- bacterial PLFA					1.000	0.103	0.678**	0.458	0.711**	0.055	0.309	0.239	0.545*
G^+ bacterial PLFA						1.000	0.047	-0.035	0.042	-0.054	0.170	0.093	-0.059
Bacterial PLFA							1.000	0.169	0.995**	-0.371	0.506*	0.393	0.409
Fungal PLFA								1.000	0.271	0.840**	-0.179	-0.199	0.224
Total PLFA									1.000	-0.273	0.476*	0.363	0.424
F:B PLFA ratio										1.000	-0.495*	-0.454	-0.028
H_{PLFA}											1.000	0.975**	0.203
E_{PLFA}												1.000	0.217
H_{CLPP}													1.000

*, correlation is significant at the 0.05 level (2-tailed); **, correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Soil microbial biomass C, basal respiration, and metabolic quotient

Previous studies have shown that the microbial biomass C significantly differed among different species (Sinha *et al.* 2009), which was consistent with our results. Among the plants studied, *H. altaicus* and *A. capillaries* showed the highest microbial biomass C values. This finding can be attributed to the release of metabolites through root exudates which may favor microbial population growth. Basal respiration reflects the actual microbial activities in soil. In this study, no significant correlation was observed between microbial biomass C and basal respiration. This result was inconsistent with that of Garcia *et al.* (2005), who investigated the ability of different plant species to promote microbiological processes in semiarid rhizosphere soil in southeast Spain. The reason for this discrepancy may be the special environment of the rhizosphere zone. The rhizosphere is a microbiosphere with chemical, physical, and biological properties different from those of bulk soils, where biochemical reactions and energy flows occur much frequently. The rhizosphere microclimate of the plants differed from one another because of the plant cover, which can result in different temperatures and moisture levels across the rhizospheres, thus affecting microorganism metabolic activity (Richard *et al.* 2004). No significant correlation was found between microbial biomass C and respiration, which suggested that part of the mi-

crobial biomass C was not directly correlated with the microbial activity in the Loess Plateau (Zhang *et al*. 2011b). A high metabolic quotient value indicates that soil microorganisms are living under environmental stress (Anderson and Domsch 1993). In the Loess Plateau, the arid climate is a stress to microbial activity; thus, microorganisms require high energy to alleviate the damages inflicted by drought stress. Consequently, the metabolic quotient was higher than the results of other researchers. The higher metabolic quotient of *H. rhamnoides* soil suggested its poor and unstable rhizosphere microenvironment. Furthermore, compared with the other four plants, *H. altaicus* and *A. capillaries* presented lower pH values. This finding can be attributed to the higher amounts of root exudates of organic anions with a concomitant release of H^+ as well as respiration in alkaline soils, cation-anion exchange balance by roots, and redox-coupled processes.

Microbial community structure assessed by PLFAs

Differences among the rhizosphere soil microbial communities of the six plants under the same climatic and soil conditions in the field were observed. *A. capillaries* and *H. altaicus* rhizospheres showed the highest G^- bacterial PLFA, bacterial PLFA, total PLFA, Shannon richness, and Shannon evenness. This finding was also supported by the measurement of microbial biomass using the chloroform-fumigation-extraction method. Ridder-Duine *et al*. (2005) concluded that the pool of rhizo-competent microbial populations available in bulk soil initially determines the rhizosphere microbial community structure, they demonstrated that the microbial community in the rhizospheres is largely determined by the bulk soil conditions rather than plant species. Tscherko *et al*. (2004) also reported that rhizosphere microbiota in the early stage is determined by the available microorganism resources in bulk soil rather than by the host plant. A high microbial PLFA is commonly observed in soils with high C and N contents, which provide sufficient nutrient resources for microorganisms (Zelles 1998). Zhang *et al*. (2011a) revealed that abandoned cropland for natural recovery has a better capacity for improving soil quality than shrubland and grassland planted by humans in the Loess Plateau. Based on the above views, the higher microbial PLFA in the two natural grassland species in the present study can be related to their favorable bulk soil conditions. Several researchers have pointed out that leguminous plant species are usually characterized by a greater abundance of microbial community in rhizospheres (Montealegre *et al*. 2002; Hamer and Makeschin 2009). However, in our study, the two leguminous species, *C. korshinskii* and *A. adsurgens*, showed lower values of G^- bacterial, bacterial, and total PLFA than *A. capillaries* and *H. altaicus.* The contrasting results can be partly explained by the differences among the environmental conditions and soil properties in these studies, and more importantly, the difference among the natures of the plants. Compared with artificial shrubland and grassland (where the species are selected by humans), natural vegetation occurs through spontaneous natural succession without any anthropogenic influence. Natural vegetation depends on the natural factors of the broader ecosystem development context of plant associations, soil, animals, and particularly soil organisms (Whisenant 1995). Thus, natural vegetation has a stronger adaptability to natural environment conditions. Natural vegetation can allow more species to colonize because of the creation of high habitat diversity, which is difficult or impossible to achieve through planting (Florgard 2004).

G^- bacterial are more frequent in the rhizosphere, preferably growing on plant labile C, whereas G^+ bacteria may be dominant in bulk soil where the available C is relatively less (Paterson *et al*. 2007; Bird *et al*. 2011). Thus, the higher G^- bacteria PLFA in the rhizosphere of *H. rhamnoides* suggested a lower rhizodeposition by the roots. The significant differences among the fungal:bacterial PLFA ratios of the rhizospheres of the six plants indicated that significant changes in the abundance of bacterial fatty acids can result in a broad-scale change in microbial community. This finding was consistent with that of Hamer and Makeschin (2009), who determined the effects of plant species on the rhizosphere soil microbial community composition in set-aside arable land in northeast Germany. In our study, the fungal:bacterial PLFA ratio in the rhizosphere soil of *A. adsurgens* and *A. capillaries* was significantly higher than that of the other plants. Thus, more fungal

communities were induced by the root exudates in their rhizospheres, in accordance with the result of fungal PLFA.

Community-level physiological profiles

The differences found among the CLPPs of the microbial communities from the three grassland types supported our hypothesis that the vegetation type significantly affected the functional diversity of a microbial community. The diversity of CLPPs and AWCD were higher in natural grassland than in artificial shrubland and grassland. This finding can be due to the release of C and N resources by natural plant species roots, which offered available substrates for microorganisms and allowed numerous microorganisms to be active in the rhizosphere. The higher total carbon utilization by microbial communities of *H. altaicus* and *A. capillaries* may indicate complexity in the microbial community structure. Bacteria reportedly have a higher metabolic activity than fungi and more readily available carbon present in natural grasslands, which stimulate bacterial growth (Anderson and Domsch 1975). This result agreed with that of the CLPPs but was contrary to that of fungal PLFA. This discrepancy can be attributed to the characteristics of these two methods. The Biolog method essentially targets bacterial communities that can grow fast in microtiter plate wells, and the contribution of fungi is not measured for their slow growth. This method mainly shows the capacity of microorganisms to grow on certain substrates (Chapman *et al*. 2007). On the other hand, PLFA analysis determines the bacteria, fungi, and actinomycetes community by measuring the phospholipid fatty acid levels in the membrane of live cells, reflecting changes in community composition. Different pH values also significantly affect microbial communities (Grayston *et al*. 2001). Meharg and Killham (1990) reported that a relatively high pH favors bacterial growth both directly and indirectly because a high pH increases exudation from plant species in grasslands. However, in our study, a significant negative correlation was found between the pH and Shannon evenness of CLPPs. This difference can be due to the different experimental field conditions such as climate, soil texture, and precipitation, as well as the different plant species. The result also suggested that low pH values can promote the capacity of bacterial communities for carbon substrate utilization in the arid Loess Plateau.

CONCLUSION

Different vegetation types significantly affected the microbial community structure and functional diversity in the rhizosphere soil in the Loess Plateau. Compared with the rhizosphere soils of artificial shrubs (*C. korshinskii* and *H. rhamnoides*) and grasses (*A. adsurgens* and *P. virgatum*), the soil of natural grassland species (*H. altaicus* and *A. capillaries*) had higher microbial biomass C, AWCD, G^- bacterial PLFA, bacterial PLFA, total PLFA, Shannon richness, and Shannon evenness, as well as the lowest metabolic quotient. The rhizosphere soil of *H. rhamnoides* had the highest metabolic quotient and G^+ bacterial PLFA, and *A. adsurgens* and *A. capillaries* had the highest fungal PLFA and fungal:bacterial PLFA ratio. Therefore, natural grassland species generated the most favorable microbial conditions among the studied plants.

MATERIALS AND METHODS

Study sites

The study site was located in the Dunshan watershed of the Ansai Soil and Water Conservation Station in the northern Loess Plateau (109°19′23′′E, 36°51′30′′N), China, which is affiliated with the Chinese Academy of Sciences. The annual mean temperature in the area is 8.8°C, and the annual mean precipitation is 510 mm. The soil type is Huangmian soil (Calcaric Cambisols, FAO) deposited by wind and characterized by a yellow color, absence of bedding, silty texture, and looseness (Zhu *et al.* 2010). These features make the soil particularly susceptible to wind erosion. After two decades of vegetation restoration, vegetation in the region predominantly comprises shrubland (e.g., *C. korshinskii* and *H. rhamnoides*) and grassland (e.g., *A. adsurgens* and *P. virgatum*) species. The cropland has also been abandoned for natural recovery.

Experiment design and soil sampling

In 2000, six vegetation types were established on the slope

cropland: two artificial shrubland (*C. korshinskii* and *H. rhamnoides*), two artificial grassland (*A. adsurgens* and *P. virgatum*), and two natural grassland (*A. capillaries* and *H. altaicus*) species. The six locations were similar in terms of their aspect, gradient, elevation, and previous farming practices. Three plots (20 m×20 m) with uniform hill slopes of 20° were established for each vegetation type. All plants grew under semiarid conditions, without irrigation, fertilization, or disturbance after planting.

In September 2008, the predominant species was *A. capillaries* in one natural grassland and *H. altaicus* in another. Soil samples were collected from three sample plots within each vegetation type (Table 3) (Zhang *et al*. 2011b). These plots were considered to be true replicates of the total experimental area because the distance among them exceeded the spatial dependence (<13.5 m) of most soil chemical and microbial variables (Mariotte *et al.* 1997). Five randomly selected plants of each species were removed from their respective plots. Soil that strongly adhered to the roots and accumulated within the space covered by the roots was considered to be rhizosphere soil (Garcia *et al.* 2005). Fresh soil samples (stored at 4°C) were used to determine the microbial biomass C, respiration, and functional CLPP. Soil for PLFA analysis was immediately frozen at -20°C for about 2 d, and then the soil was freeze-dried. The soil properties and microbial community characteristics were then determined as described below.

Table 3 Detailed information for the sample sites

	Vegetation types	Slope aspect	Slope (°)	Altitude (m)	Coverage (%)	Minor herbaceous
Artificial shrublands	*C. korshinskii*	N	20	1 257	72.5	*A. sacrorum*, *C. chinensis* (Maxim)
	H. hamnoides	N	22	1 220	60.6	*A.argyi*, *S. bungeana*, *A. sacrorum*
Artificial grasslands	*A. adsurgens*	NE 10°	20	1 235	68.5	*L. davurica*, *L. indic*
	P. virgatum	NW 25°	24	1 282	75.2	*P. annua*, *H. altaicus*
Natural grasslands	*H. altaicus*	NW10°	24	1 311	70.5	*S. bungeana*, *A. sacrorum*
	A. capillaries	N	22	1 298	64.5	*L. davurica*, *P. bifurca*

Soil type is loess soil.

Laboratory analysis

Microbial biomass and respiration Microbial biomass C was measured by the fumigation extraction method (Vance *et al*. 1987). Basal respiration was estimated through CO_2 evolution at 25.8°C in samples incubated for 14 d and adjusted to 50% of the field water-holding capacity (Jenkinson and Powlson 1976). Any CO_2 respired was trapped in NaOH, and the residual NaOH was titrated with HCl. The metabolic quotient was calculated as the basal respiration per unit of microbial biomass C (Anderson and Domsch 1993). Soil pH was measured in a soil-water suspension (1:2.5 soil:water) using an automatic acid-base titrator.

PLFA analysis The three-step procedure involving the extraction, fractionation, and quantification of soil phospholipids was based on the method of Bligh and Dyer (1959) and modified by Bardgett *et al*. (1996). The separated PLFAs were subjected to mild alkaline methanolysis at 50°C, and the resulting fatty acid methyl esters were detected using an Agilent 7890 gas chromatograph equipped with a flame ionization detector. A mixture of bacterial fatty acid methyl esters (FAMEs) (Supelco UK) that ranged from C11 to C20 was used as a qualitative standard to identify the separated FAMEs. The concentrations of single FAMEs were calculated using the internal standard (19:0) peak as a reference. The fatty acid nomenclature was used as described by Frostegard *et al.* (1993). The polyenoic, unsaturated PLFA 18:2*w*6 was used as the indicator of fungal biomass (Federle 1986). The branched, saturated PLFAs i15:0, a15:0, i16:0, i17:0, and a17:0 were chosen to represent G^+ bacteria. On other hand, 16:1*w*9, cy17:0, 18:1*w*9, cy19:0, and saturated fatty acids containing an -OH group were used to represent G^- bacteria. The fungal:bacterial PLFA ratio was used as an indicator of changes in the relative abundance of these two microbial groups (Bardgett *et al.* 1996), which constituted 95% of the total heterotrophic metabolism of soil (Petersen and Luxton 1982).

Community-level physiological profiles Microbial carbon substrate utilization was measured by the method of Chen *et al.* (2007b) using Biolog® ECO-plates (Biolog, Hayward, USA), which contained 31 different carbon sources and one control well (containing no substrate). An initial 10^{-1} soil dilution was performed by suspending the wet soil equivalent to 10 g of dry soil in 100 mL of sterile physiological saline (0.85%). Serial dilutions were carried out to 10^{-3} dilution. A 20 mL aliquot of 10^{-3} soil dilution was shaken for 10 min and left undisturbed for 15 min to minimize the amount of soil in the microbial suspension. This dilution was chosen because pre-tests had shown that the lowest dilution did not cause interference (unspecific turbidity and absorbance) in the assay with the co-extracted soil components. A 150 mL aliquot of the supernatant was added to each well of the plates. The plates were incubated at 25°C for 10 d, and color development in the wells was measured as absorbance at 590 nm every 24 h using a plate reader. The absorbance of the control well was subtracted from the absorbance of each well.

Diversity index of the microbial community structure To determine the effect of vegetation types on the microbial community structure, the diversity of fatty acids was calculated using the Shannon index H (H_{PLFA}) (Tscherko *et al*. 2004):

$$H=-\sum_{i=1}^{n} pi \ln pi$$

Where *pi* is the relative abundance of each fatty acid in the total sum and *n* is the number of detected fatty acids. The equitability of the fatty acids was calculated with Shannon's evenness *E* (E_{PLFA}):

$E=H/\ln(S)$

Where *S* is the total number of fatty acids tested in the community (Shannon 1948). The diversity of the CLPP (H_{CLPP}, E_{CLPP}) was calculated using the same formula, where *pi* is the relative AWCD of each carbon source in the total sum of AWCD, and *S* is the total number of available carbon source.

Statistical analysis

All results are reported as the mean±standard deviation. Differences between mean values were evaluated by one-way ANOVA. Pearson's test was used to evaluate the relationship between microbial properties. SPSS 15.0 software was used for all analyses.

Acknowledgements

This work was supported by the Strategic Technology Project of Chinese Academy of Sciences (XDA05060300) and the Science and Technology Research and Development Program of Shaanxi Province, China (2011KJXX63). The authors thank two anonymous reviewers and editors of the journal for their valuable comments, suggestions, and revisions of this manuscript.

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(Managing editor SUN Lu-juan)