# ORIGINAL PAPER

# Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never-cultivated field in a hot and arid ecosystem of southwest China

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Abstract We investigated the spore density, species composition, and diversity of arbuscular mycorrhizal fungi (AMF) in a cultivated land (CL), an old field (OF), and a never-cultivated field (NCF), which are located adjacently in a slope in the hot and arid ecosystem of southwest China. AMF spores in the rhizosphere soils of representative plants in the three habitats were extracted by wet-sieving and decanting. A total of 47 taxa of AMF including 31 taxa from the genus Glomus, 8 from Acaulospora, 6 from Scutellospora, 1 from Entrophospora, and 1 from Gigaspora were extracted and identified morphologically. The highest spore density occurred in NCF, slightly lower in OF and lowest in CL, and the Shannon-Wiener index of species diversity was reversed. The dominant species of AMF were different in the three habitats. OF resembled NCF more than CL in AMF spore density, species richness, and community composition, which means that AMF community in the OF has been developing from cultivated land to natural habitat. Cluster analysis based on the similarity in AMF community composition indicated that the distribution of AMF was not random over space and that AMF community composition associated with a given plant species was greatly habitat-convergence. Following

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L.-F. Li Faculty of Food Science and Technology, Yunnan Agriculture University, 650201 Kunming, People's Republic of China the cluster analysis, we hypothesized that the effect of habitats on AMF communities were greater than that of the host preference to AMF.

**Keywords** Arbuscular mycorrhizal fungi · Biodiversity · Community · Arid ecosystem · Ecosystem restoration

# Introduction

In recent years, there has been an increasing awareness by ecologists that the restoration and re-establishment of fragile and degraded ecosystems should be considered comprehensively and done systematically. The reason is that the restoration should include not only the aboveground systems but also the below-ground microorganisms which are associated functionally with plants. Arbuscular mycorrhizal fungi (AMF) are found to be essential components of sustainable plant-soil systems due to their symbiotic association with most of land plants to form arbuscular mycorrhizas (AM) and their multifunction. It is well known that AMF can influence the plant fitness, community structure, biodiversity, and ecosystem variability (van der Heijden et al. 1998b; Millner and Wright 2002). It has been suggested that the success of any ecosystem reforestation efforts are likely to depend on the establishment of mycorrhizas, and AM should receive special attention in indigenous tree seedling production and restoration (Wubet et al. 2003a).

Generally, AMF show little or no host specificity, as the roots of different host species are associated with different AMF species (Eom et al. 2000; Helgason et al. 2002; Lovelock et al. 2003). However, the existence of host preference for AMF has been suggested by non-random

associations of AMF with different host species in ecosystems (Vandenkoornhuyse et al. 2002; Gollotte et al. 2004). Daniell et al. (1999) suggested that fungal taxa might associate preferentially with particular plant taxa. Some studies have shown that different individuals of a plant species have distinct growth responses by inoculating different AMF species (van der Heijden et al. 1998b; Klironomos 2003). Other studies have indicated that a given AMF species originating from the same soils colonizes different plant species; its patterns of sporulation are distinctly different (Bever et al. 1996; Eom et al. 2000). Therefore, the composition of AMF in the soil would influence plant community structure and diversity (O'Connor et al. 2002; van der Heijden et al. 1998a). Contrarily, plant communities can also affect diversity and community composition of AMF (Johnson et al. 2004).

Conversion from natural habitats to agricultural lands has been referred as one of the leading causes for loss of biodiversity worldwide (Richter et al. 2002). In cultivated lands, AMF population, species composition, and diversity are often decreased compared to natural ecosystem (Helgason et al. 1998; Boddington and Dodd 2000). While Jansa et al. (2002) found that soil tillage had no significant effect on the diversity of AMF, as assessed by the diversity indices. In this paper, we preliminarily assessed AMF community structure and diversity in a cultivated land (CL), an old field (OF), and a never-cultivated field (NCF), which are located adjacently on a slope in a hot and arid ecosystem in southwest China. Our primary purpose was to elucidate the AMF patterns in species composition during conversion from NCF to CL and subsequently to OF and to test the hypothesis that the influence of the habitat on AMF community is greater than that of the host preference.

#### Materials and methods

#### Study site

The study site is located in Yuanmou  $(101^{\circ}35'-102^{\circ}06'E, 25^{\circ}23'-26^{\circ}06'N)$ , a typical hot and arid valley, southwest China. The mean annual temperature is 21.9°C, the highest up to 43°C. Mean annual rainfall is only 629 mm, mainly between June to October, and the evaporation is nearly six times (3,729 mm) its precipitation (data from Meteorological Station of Yuanmou County for 35 years statistics). Most of this area is covered with sparse bushes, only 6% with poor quality mixed forest and tree plantation, and 14% has no vegetation at all (Liu 2003). Human activities such as overgrazing and chopping have intensively disturbed this hot and arid ecosystem. Heavy rain in the short wet season accelerates the erosion of the susceptible soils. Thus, great efforts are required for ecological restoration in this area.

A project named as conversion of farmlands to forests and grasslands has been put forward and practiced by Chinese government in Yuanmou since 2001. Three habitats, representing cultivated land (CL), old field (OF), and never-cultivated field (NCF), which are located adjacently in a sloping field (about 5 × 8 km ) were chosen for the study. CL was converted from NCF about 30 years ago. CL remains as hand tilling and low-input soil management. Sweet potato, peanut, Chinese onion, and sorghum are cultivated in the CL in the wet season every year. OF was fallowed from CL since 2001, and there are still some residual crops (peanut, sorghum) naturally growing in it. Azadirachta indica A. Juss. and Cajanus cajan (L.) Millsp. (both are exotic species) were transplanted into the OF in 2001. Many indigenous grasses and herbs are now naturally re-colonizing in this habitat. The NCF is locally called savanna of valley type by plant ecologists (Jin and Ou 2000), is predominantly composed of grasses and bushes with a few trees. Heteropogon contortus Beauv. ex Roem. and Schult., Bothriochloa pertusa (L.) A. Camus., and Dodonea viscose Jacq. are prevalent plant species in NCF.

## Sample collection

Crops in CL, some residual crops, introduced plants, and naturally re-colonizing indigenous plants in OF and the predominant plants in NCF (Table 3) were sampled. Plant roots and about 500 g of their rhizosphere soils were collected to a depth of 5–30 cm in November 2004. Three replicates were randomly sampled from each of seven plant species in each habitat. The distance of three replicates is more than 30 m from each other. After clearing in 10% (w/v) KOH and staining with acid fuchsin (Li et al. 2005), the roots of all the surveyed plants were found to be typically arbuscular mycorrhizal. Soil samples were air-dried for 2 weeks and stored in sealed plastic bags at 4°C until they were treated. Physical and chemical characters of soils from the three habitats were given in Table 1.

## Spore isolation and treatment

Spores from the rhizosphere soil samples were isolated using the wet-sieving and decanting methods described by Zhao et al. (2001). All healthy AMF spores were collected manually and counted in the Petri dishes. More than 90% of spores or sporocarps were transferred onto glass slides containing polyvinyl lactic acid (PVA) with and without Melzer's reagent (Morton 1988) and identified under a compound microscope at up to 400× magnification. The identification was based on morphological characteristics with reference of Schenck and Pérez (1988) and other originally published papers and the descriptions provided

Habitat	Total N (g kg <sup>-1</sup> )	Total C (g kg <sup>-1</sup> )	Total P (g kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Total K (g kg <sup>-1</sup> )	Available K, (g kg <sup>-1</sup> )	pН
CL	0.8	9.4	0.4	10.4	15.0	132.0	6.56
OF	0.7	11.7	0.4	12.1	14.2	97.9	6.48
NCF	1.1	20.1	1.5	7.9	6.1	130.0	6.24

Table 1 Physical and chemical properties of the soils from the three habitats

Values in the table are the mean values of all samples in each habitat. CL Cultivated land; OF old field; NCF never-cultivated field

by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu).

# Statistical analyses

Spore density, species richness, isolation frequency (IF), relative abundance (RA), and Shannon–Wiener index of diversity were conducted as follows: spore density was defined as the number of AMF spores and sporocarps in 100 g soil; species richness was measured as the number of AMF species occurred per soil sample; IF=(the number of samples in which a given species was isolated/the total number of samples)×100%; RA=(the number of a given species spore/the total number of species)×100%; Shannon–Wiener index of diversity =  $-\sum_{i=1}^{s} p_i \ln p_i$ , where *s* is the number of species and  $p_i$  is the RA of the *i*th species.

Analysis of variance (ANOVA) and correlation analysis were carried out with SPSS software package (version 12.0). Data on spore densities were log transformed to fulfill the assumption of normality and homogeneity of variances before ANOVA. One-way ANOVA was performed to test the differences in spore densities between plant species from a given habitat or between habitats and to determine the differences in the number of AMF species between habitats. Multiple mean comparisons were performed using Duncan's multiple range test at the 0.05 level of probability within one-way ANOVA. The relationship between AM spore density and species richness was determined by Pearson's correlation analysis. Unweighted pair-group method of arithmetic averages (UPGMA) by the clustering program NTSYS (version 2.11a) was performed using simple matching coefficients (SM), and dendrograms were constructed to determine the relationship of AMF communities among host plants in all habitats or among the same host plant species in different habitats.

# Results

# AMF species composition

From 63 soil samples collected in the three habitats, 47 taxa of AMF were detected and identified. There were 31 taxa from the genus *Glomus*, 8 from *Acaulospora*, 6 from *Scutellospora*, 1 from *Entrophospora*, and 1 from *Giga*-

*spora* (Table 2 and Fig. 1). Among the 47 taxa, 10 belonged to *Glomus* and *Scutellospora* that were not identified to species level. The total number of species extracted from CL, OF, and NCF were 37, 35, and 34, respectively.

Spore density and species richness of AMF

AMF spore density varied greatly among different host plant species and among habitats (Table 3). It ranged from 565 for *Allium sativum* to 1782 for *Arachis hypogaea* in CL, from 1,208 for *H. contortus* to 1,837 for *B. pertusa* in OF, and from 1,060 for *C. cajan* to 2,352 for *H. contortus* in NCF, respectively. The mean spore density was highest in NCF, slightly lower in OF, and lowest in CL (Table 4). One-way ANOVA showed the significant differences in spore density between plant species in a given habitat or between three habitats (Table 5).

Different host plants harbored distinct AMF communities, but some AMF species were associated with all the replicates of a given plant in each habitat (Table 3). Contrary to spore density, the maximum of average species richness occurred in CL, and the minimum in NCF (Table 4). One-way ANOVA showed that there were no significant differences in AMF species richness in OF and NCF (Table 5). The total number of AMF species for each plant species was about 16 in each habitat and showed no significant difference in the three habitats. The number of the common species in the three replicates for each plant species in CL was significantly higher than OF and NCF (Tables 4 and 5).

Correlation analysis showed that when the habitats were considered separately, spore density was positively correlated with species richness only in CL (r=0.65, P<0.05), while when the habitats were considered together, there was no significant correlation between spore density and species richness.

#### IF, RA, and dominant species of AMF

*Glomus* and *Acaulospora* occurred most frequently, followed by *Gigaspora* and *Scutellospora*, and *Entrophospora* was detected least (Table 2). Dispersion and spore number should be considered simultaneously when determining the dominance of species in AMF community, so we defined the dominance of AMF species based on IF>50% and RA>5%. It is evident that *Glomus* and *Acaulospora* were the dominant genera, and the three species, *Acaulospora* 

Table 2 Isolation frequency (IF) and relative abundance (RA) of the identified AMF species in the different habitats

No.	AMF species	IF (%)				RA (%)			
		CL	OF	NCF	Mean	CL	OF	NCF	Mean
	Acaulospora	100.0	100.0	100.0	100.0	44.7	51.2	46.9	47.6
1	A. bireticulata	81.0	71.4	14.3	55.6	4.2	2.6	0.3	2.3
2	A. denticulata	23.8	4.8	19.1	15.9	0.6	0.1	0.6	0.4
3	A. foveata	14.3	52.4	_	22.2	1.6	13.3	_	6.0
4	A. laevis	-	9.5	_	3.2	-	4.1	_	1.7
5	A. mellea	14.3	19.1	14.3	15.9	5.3	1.3	0.7	2.2
6	A. scrobiculata	95.2	85.7	85.7	88.9	25.6	29.1	42.0	32.1
7	A. spinosa	47.6	14.3	19.1	27.0	2.0	0.6	0.3	0.9
8	A. tuberculata	76.2	23.8	28.6	42.9	5.6	0.3	3.0	2.6
	Entrophospora	4.8	19.1	4.8	9.5	0.1	0.4	0.1	0.2
9	E. infrequens	4.8	19.1	4.8	9.5	0.1	0.4	0.1	0.2
	Glomus	100.0	100.0	100.0	100.0	48.1	30.5	52.0	43.5
10	G. aggregatum	19.1	23.8	_	14.3	1.0	0.5	_	0.5
11	G. chimonobambusa	9.5	_	4.8	4.8	0.3	_	0.1	0.1
12	G. claroideum	90.5	76.2	85.7	4.0 84.1	8.9	4.5	8.9	7.0
12	G. clarum G. clarum	90.3 95.2	4.8	47.6	49.2	11.7	4.5 0.1	4.9	4.7
13	G. clavispora	4.8	-	-	1.6	0.1	-	ч.у —	0.1
14	G. constrictum	4.8 19.1	_ 9.5	9.5	12.7	0.1	0.4	0.2	0.1
15	G. fasciculatum	4.8	9.5	9.3 4.8	3.2	0.4	0.4	0.2	0.3
	5								
17	G. geosporum	14.2	9.5	14.3	12.7	0.5	0.5	0.3	0.4
18	G. intraradices	33.3	33.3	4.8	23.8	1.2	0.7	0.2	0.7
19	G. microaggregatum	9.5	_	-	3.2	0.2	-	_	0.1
20	G. microcarpum	-	4.8	9.5	4.8	-	0.1	0.3	0.1
21	G. monosporum	61.9	38.1	52.4	50.8	4.3	1.0	2.3	2.3
22	G. mosseae	90.5	57.1	47.6	65.1	11.0	8.8	1.3	7.1
23	G. multicaule	_	_	4.8	1.6	-	_	0.1	0.1
24	G. pansihalos	4.8	-	4.8	3.2	0.1	_	0.2	0.1
25	G. reticulata	9.5	—	_	3.2	0.2	_	_	0.1
26	G. rubiformis	4.8	—	4.8	3.2	0.1	_	0.1	0.1
27	G. sinuosa	66.7	47.6	57.1	57.1	4.6	2.5	1.2	2.7
28	Glomus sp 1	4.8	28.6	66.7	33.3	0.3	0.7	11.7	4.0
29	Glomus sp 2	_	19.1	19.1	12.7	-	1.4	2.1	1.3
30	Glomus sp 3	_	19.1	52.4	23.8	-	1.3	6.0	2.4
31	Glomus sp 4	38.1	23.8	9.5	23.8	2.0	3.5	1.4	2.4
32	Glomus sp 5	4.8	28.6	_	11.1	0.1	1.3	-	0.6
33	Glomus sp 6	_	14.3	28.6	14.3	_	2.2	2.5	1.7
34	Glomus sp 7	4.8	9.5	4.8	6.4	0.1	0.4	0.5	0.4
35	Glomus sp 8	14.3	14.3	66.7	31.8	0.8	0.3	6.0	2.2
36	G. spurcum	9.5	_	_	3.2	0.2	_	_	0.1
37	G. taiwanense	9.5	_	4.8	4.8	0.2	_	0.1	0.1
38	G. tortuosum	_	9.5	9.5	6.4	_	0.1	0.3	0.1
39	G. verruculosum	_	_	57.1	19.1	_	_	1.7	0.5
40	G. viscosum	4.8	19.1	_	8.0	0.1	0.5	_	0.2
	Gigaspora	71.4	61.9	33.3	55.6	5.6	0.5 7.6	0.7	4.6
41	G. gigantea	71.4	62. 0	33.3	55.6	5.6	7.6	0.7	4.9
.1	Scutellospora	52.4	66.7	19.1	46.0	1.6	10.3	0.7	4.1
42	S. calospora	9.5		-	3.2	0.2	-	0.5	0.1
	-			_					
43	S. heterogama	14.3	4.3		6.4 25.4	0.9	0.1	- 0.1	0.3
44	S. pellucida	14.3	57.1	4.8	25.4	0.3	3.2	0.1	1.5
45	Scutellospora sp 1	-	14.3	-	4.8	-	0.4	-	0.2
46	<i>Scutellospora</i> sp 2	19.1	9.5	14.3	14.3	0.3	0.2	0.3	0.2
47	S. verrucosa	—	47.6	-	15.9	_	6.5	—	2.7

CL Cultivated land; OF old field; NCF never-cultivated field





*scrobiculata*, *Glomus claroideum*, and *G. mosseae* were the dominant species in this hot and arid ecosystem (Tables 2).

The IF and RA of AMF species varied greatly among species and among habitats (Table 2). In CL, the spores of Glomus were the most frequent, and they accounted for about 48% of the total number of spores, followed by Acaulospora (45%). The dominant species were A. scrobiculata, A. tuberculata, G. claroideum, G. clarum, G. mosseae, and Gigaspora gigantea. Among them, A. scrobiculata, G. clarum, and G. mosseae accounted respectively for 26, 12, and 11% of the total number of spores. In addition, A. bireticulata, G. monosporum, and G. sinuosa were frequently observed, although their RA was less than 5%. In OF, the number of spores that belonged to Acaulospora, Entrophospora, Gigaspora, and Scutellospora were increased, and those of Glomus decreased to 31%. Acaulospora foveata, A. scrobiculata, G. mosseae, and Gi. gigantea were the dominant species. Although Scutellospora verrucosa occurred in less than 50% of the soil samples examined, they accounted for 6.5% of the total of spores. In NCF, the spores of Acaulospora were up to 47%, while those of Entrophospora, Gigaspora, and Scutellospora were

greatly reduced compared to CL and OF. *A. scrobiculata*, which accounted for 42% of spores, was the most common species, followed by *G. claroideum*, *G.* sp 1, *G.* sp 3, and *G.* sp 8. Additionally, *G. monosporum*, *G. sinuosa*, and *G. verruculosum* were frequently isolated in this habitat. It was remarkable that *A. scrobiculata* was the species which was common in each habitat, and it held a high percentage of the total of spores (Tables 2).

# Diversity of AMF communities

The AMF diversities, expressed by Shannon–Wiener index, were presented in Table 4. The maximum of AMF diversity occurred in CL and the minimum in NCF.

# Similarity of AMF communities

# Similarity of AMF community across the host plant species in all habitats

Cluster analysis of simple matching coefficients revealed three main groupings (Fig. 2). Group 1 contained all host

Table 3 Spore density, number of species, and AMF taxa presented in the rhizosphere soils of the sampled plants

Plants	Habitats	Abbreviations	SD (per 100 g soil)	Number of species			AMF taxa <sup>c</sup>	
				SR Sum <sup>a</sup>		Common <sup>b</sup>		
Allium sativum L.	CL	Asa (CL)	565.00± 117.51	$\begin{array}{c} 8.00\pm\\ 0.58\end{array}$	14	3	<b>1</b> 6 7 <b>12</b> 13 14 15 17 21 <b>22</b> 26 35 40 41	
Arachis hypogaea L.	CL	Ah (CL)	1,781.67± 116.24	$11.33 \pm 0.33$	17	6	1 <b>3 5 6</b> 7 <b>8 12</b> 13 17 18 22 <b>27</b> 28 31 36 41 46	
Cajanus cajan (L.) Millsp.	CL	Cc (CL)	1,525.00± 112.58	13.33± 1.20	19	9	<b>1</b> 2 <b>6</b> 7 <b>8</b> 10 12 <b>13</b> 18 19 <b>21 22</b> <b>27</b> 28 <b>31</b> 34 37 <b>41</b> 46	
Capsicum annuum L.	CL	Ca (CL)	850.00± 145.52	10.67± 0.33	15	8	<b>1 6</b> 7 <b>8</b> 10 11 12 <b>13</b> 18 <b>21 22</b> 24 27 <b>41 43</b>	
Helianthus annuus L.	CL	Ha (CL)	666.67± 33.46	10.00± 1.15	13	6	1 <b>6 8</b> 10 <b>12 13 22</b> 25 <b>27</b> 31 41 42 44	
Ipomoea batatas (L.) Lam.	CL	Ib (CL)	1,098.33± 227.20	12.33± 0.67	17	8	1 <b>2 6</b> 7 <b>8 12 13</b> 15 18 <b>21 22 27</b> 35 36 37 41 44	
Sorghum bicolor (L.) Moench	CL	Sb (CL)	1,445.00± 65.06	12.00± 0.58	19	7	<b>1 6 7 8</b> 9 11 <b>12 13</b> 15 16 18 19 21 <b>22</b> 27 31 <b>41</b> 42 43	
Arachis hypogaea L.	OF	Ah (OF)	1,393.33± 141.31	10.67± 2.91	18	4	1 <b>6</b> 8 9 10 <b>12</b> 15 18 21 22 <b>27</b> 31 32 34 35 38 40 <b>41</b>	
Azadirachta indica A. Juss.	OF	Ai (OF)	1,751.67± 281.98	$10.33 \pm 0.88$	20	3	<b>1</b> 3 4 5 <b>6</b> 7 10 12 15 17 21 <b>22</b> 27 28 29 30 41 44 45 47	
<i>Bothriochloa pertusa</i> (L.) A. Camus.	OF	Bp (OF)	1,836.67± 234.47	8.33± 0.33	13	5	<b>3 6</b> 7 <b>12</b> 13 17 28 30 33 41 <b>44</b> 46 <b>47</b>	
Cajanus cajan (L.) Millsp.	OF	Cc (OF)	1,573.33± 124.54	8.33± 1.76	16	3	1 <b>3</b> 4 5 <b>6</b> 7 8 12 18 20 29 30 41 <b>44</b> 46 47	
<i>Heteropogon contortus</i> Beauv. ex Roem. & Schult.	OF	Hc (OF)	1,208.33± 234.06	$10.33 \pm 1.20$	15	5	1 2 <b>3</b> 5 <b>6 8</b> 12 18 21 22 29 41 <b>44</b> 45 <b>47</b>	
Psoralea corylifolia L.	OF	Pc (OF)	1,800.00± 172.99	$10.33 \pm 0.33$	14	8	<b>1</b> 3 6 7 9 <b>12 18 22 27 31 32</b> 35 40 <b>41</b>	
Sorghum bicolor (L.) Moench	OF	Sb (OF)	1,395.00± 61.10	9.00± 0.00	14	4	<b>1</b> 6 9 10 12 18 <b>22 27</b> 30 31 32 33 40 <b>41</b>	
<i>Atylosia scarabaeoides</i> (L.) Benth.	NCF	Asc(NCF)	1,596.67± 362.28	9.67± 0.67	18	3	2 <b>6</b> 7 8 <b>12</b> 13 21 22 <b>27</b> 28 29 30 31 33 38 39 41 46	
Bothriochloa pertusa (L.) A. Camus.	NCF	Bp (NCF)	1,995.00± 78.10	9.33±	16	4	1 <b>6</b> 8 12 <b>13</b> 16 17 21 22 27 <b>28</b> 33 35 37 <b>39</b> 41	
Cajanus cajan (L.) Millsp.	NCF	Cc (NCF)	1,060.00± 176.92	$10.00 \pm 0.58$	17	3	2 <b>6</b> 8 <b>12</b> 13 15 17 22 24 <b>27</b> 28 29 30 33 35 39 41	
Capillipedium parviflorum Stapf	NCF	Cp (NCF)	1,601.67± 158.44	0.38 $9.33\pm$ 0.33	19	4	2 5 6 8 11 12 17 21 22 23 27 28 30 31 35 38 41 44 46	
Dodonaea viscosa Jacq.	NCF	Dv (NCF)	1,695.00± 135.77	0.33 9.33± 0.88	14	4	1 <b>6</b> 9 12 15 21 <b>22 27</b> 28 30 33 <b>35</b> 39 46	
<i>Heteropogon contortus</i> Beauv. ex Roem. & Schult.	NCF	Hc (NCF)	2,351.67± 223.13	9.00± 1.00	16	3	1 5 6 7 12 13 20 21 22 27 28 29 30 35 39 41	
Themeda caudata (Nees) A. Camus	NCF	Tc (NCF)	$1,843.33 \pm 185.52$	7.67± 0.67	13	4	5 6 7 12 13 18 21 26 28 30 33 35 39	

SD spore density; SR species richness; CL cultivated land; OF old field; NCF never-cultivated field

<sup>a</sup> The total number of species for three replicates

<sup>b</sup> The number of the common species existed in three replicates

<sup>c</sup> Numbers were the AMF codes in Table 1; the species with bold were the common species in three replicates.

plant species from CL and some from OF. Group 2 included all plant species from NCF. Group 3 only contained some plant species from OF. Results showed that the AMF communities associated with the plant species within the same habitat generally had a high degree of similarity.

# Similarity of AMF communities across the same plant species in different habitats

Cluster analysis showed that the AMF communities associated with *C. cajan*, which occurred simultaneously in the three habitats, divided into three distinct clusters,

Habitats	SD	Number of specie	es	Shannon-Wiener index of diversity	
		SR	Sum <sup>a</sup>	Common <sup>b</sup>	
CL	1,133.10±104.52 b	11.10±0.44 a	16.28±0.89	6.86±0.74 a	2.57
OF	1,621.43±94.04 a	9.86±0.54 ab	$16.57 \pm 1.07$	4.00±0.31 b	2.30
NCF	1,734.76±105.10 a	9.19±0.31 b	$16.14 {\pm} 0.80$	3.57±0.20 b	2.16

 Table 4 Diversity indices and the means±SE of spore density and the number of species

SD spore density; SR species richness; CL cultivated land; OF old field; NCF never-cultivated field

<sup>a</sup> The total number of species for three replicates

<sup>b</sup> The number of the common species existed in three replicates. Means followed by the different letters (a–b) in each column are significantly different within a given habitat according to Duncan's multiple range test at the 0.05 level of probability.

with each cluster containing three replicates only from each habitat (Fig. 3a). Likewise, the AMF communities associated with the replicates of the other four host plants, which occurred in two habitats, were more similar from a given habitat than from another habitat, respectively (Fig. 3b–e).

# Similarity of AMF communities across three habitats

Cluster analysis based on the similarity in AMF species composition among habitats indicated that the AMF community in OF resembled NCF more than that of in the CL (Fig. 4).

#### Discussion

Recently, molecular approaches have been applied to study the biodiversity of AMF in some plant species (Daniell et al. 2001; Wubet et al. 2003b; Gollotte et al. 2004). However, molecular identification is currently limited in the field researches as has been stated by Landis et al. (2004) and Oehl et al. (2004, 2005). In the present study, the AMF community composition and diversity were described based on morphological species. Clearly, spore populations do not exactly reflect the AMF community that is actually colonizing the plant roots because of the possible existence of some non-sporulating AMF species and also degradation of spore walls (Clapp et al. 1995; Daniell et al. 2001). However, we believe that using spores to identify AMF community and species richness is a valid approach for the purpose of our study as Oehl et al. (2005) proposed.

A total of 47 taxa representing five genera of AMF were extracted and identified directly from the soil samples in three habitats in this study. This is a considerably large number, given that only about 190 AMF species have been described worldwide so far, and that the samples were taken from three habitats, which arranged adjacently on a slope within a relatively small region of about 40 km<sup>2</sup> and with similar climatic conditions. The number of species detected from CL, OF, and NCF (37, 35, and 34, respectively) was relatively high compared to that usually reported from corresponding habitats (Oehl et al. 2004; Douds and Millner 1999). The relatively higher species numbers potentially due to the following reasons: (1) the high mycotrophic dependency of the sampled plant species. In the previous study, we have investigated the AMF colonization in the roots of these sampled plant species in the three habitats and found all surveyed plants formed AM and most plants were intensively colonized. We suggested that plants grown in this ecosystem might be highly dependent on AM (Li et al. 2007). (2) The hot and arid environmental conditions. It is known that high temperature

Table 5 F value from one-way ANOVA for spore density and the number of species

Habitats	df	SD	Number of species			
			SR	Sum <sup>a</sup>	Common <sup>b</sup>	
CL	6	11.80***	5.66**	_	_	
OF	6	3.71*	0.87	_	_	
NCF	6	3.75*	0.79	_	_	
Three habitats	2	9.95***	4.87*	0.06	14.07***	

SD spore density; SR species richness; CL cultivated land; OF old field; NCF never-cultivated field; df degrees of freedom

<sup>a</sup> The total number of species for three replicates

<sup>b</sup> The number of the common species existed in three replicates.

\*P<0.05

\*\*P<0.01

\*\*\*P<0.001

Fig. 2 Dendrograms of cluster analysis based on the similarity of AMF species composition across the host plant species in all habitats. The abbreviations of the host plant species were presented in Table 3. *Letters in parentheses* denoted habitats (*CL* cultivated land; *OF* old field; *NCF* never-cultivated field). Groups 1–3 indicated three main groupings formed by the cluster analysis



Fig. 3 Dendrograms of cluster analysis based on the similarity of AMF species composition across all samples of the host plant species, which occurred in at least two habitats. Arabic digitals (1-3) after abbreviations of the host plant species denoted the serial numbers of three replicates. The abbreviations of the host plant species were presented in Table 3. Letters in parentheses denoted habitats (CL cultivated land; OF old field; NCF never-cultivated field). a Cajanus cajan; b Arachis hypogaea; c Sorghum bicolor; d Bothriochloa pertusa; e Heteropogon contortus





Fig. 4 Dendrograms of cluster analysis based on the similarity of AMF species composition across three habitats. *CL* Cultivated land; *OF* old field; *NCF* never-cultivated field

and high light intensity may increase AMF sporulation (Cardoso et al. 2003; Koide and Mosse 2004). Additionally, spores are less susceptible to predation and parasitism in the arid environmental conditions, as levels of fungal predation and parasitism are lower than occur in locations with higher rainfall (Lovelock et al. 2003). (3) The sampling was done in November when the end of autumn and the beginning of the dry season. It has been reported that spore populations are typically greatest in the autumn in areas where there are marked warm/cold seasons (Douds and Millner 1999) and that substantially more spores are expected in the dry season (Guadarrama and Álvarez-Sánchez 1999; Lugo and Cabello 2002). The spores collected during this period not only have the greater spore density and population (Guadarrama and Álvarez-Sánchez 1999; Lovelock et al. 2003) but also are in better condition for identification (Douds and Millner 1999). Considering the seasonal nature of AMF, we believe that AMF diversity in this area would no doubt increase with longer-term sampling.

Spore density was found to be lowest in CL and highest in NCF, which corroborated the observation that agricultural practices decreased the AMF spore numbers (Boddington and Dodd 2000; Oehl et al. 2003). In contrast to spore density, the species richness and Shannon-Wiener index of diversity were found to be the highest in CL and the lowest in NCF. It could be easily explained from the nature of Shannon-Wiener index of diversity. The values of this index are determined by the species number, individuals of each species, and the evenness of their distribution (Magurran 1988). The distribution of AMF in CL are usually more even than that of the less disturbed or undisturbed systems (such as in the OF and NCF). Our research results conducted in the same sites showed that AMF species richness in the soils based on morphological identification was relatively lower, but the AMF molecular diversity in the corresponding roots was relatively higher in the NCF (unpublished data). Therefore, it seemed that the AMF species in CL are more likely to be strong sporulating species, while the species in NCF are possibly those of less or non-sporulating species.

The AMF community composition varied greatly across different land-use types in this hot and arid ecosystem. It was indicated that *Glomus* had the higher percentage of the spores (48%) isolated from CL. Jansa et al. (2002) generalized the prevalence of *Glomus* spp. in agriculturally

used soils, in contrast to rich AMF communities containing Gigaspora spp., Scutellospora spp., and Acaulospora spp. in uncultivated soils. It is generally believed that the species of Acaulospora and Glomus appear to be more tolerant to soil disturbance, as the formation of large spores from Gigasporaceae takes a longer time than that of small spores from other genera of AMF (i.e., the Glomaceae; Boddington and Dodd 2000), which leads to large spores having insufficient time to form and mature before soil disturbance. Compared to AMF community in CL, the relative abundance of Acaulospora, Entrophospora, Gigaspora, and Scutellospora increased while that of Glomus decreased in OF; this revealed that the AMF community has been changing from CL to NCF. Our results were in agreement with another study in which a trend towards increase in AMF belonging to the genera Acaulospora, Entrophospora, and Scutellospora was found under reduced tillage management (Jansa et al. 2002).

Cluster analysis based on the similarity in AMF species showed that AMF community in OF resembled NCF more than that of the CL. Cluster analysis based on the similarity in AM status (with respect to all colonization of different AM structures) also showed that OF were more similar to NCF than that in the CL (Li et al. 2007). These results indicated that disturbance from agricultural practices influenced AMF community composition, but through the combination of naturally re-colonizing of indigenous plants and transplanting exotic plants into the OF, AMF community had been showing a succession tendency from CL to NCF after 4 years. Therefore, we suggested that mixed (natural and artificial) restoration was an economic and effective way for conversion of farmlands to forests and grasses in this special ecosystem of hot and arid valley.

It is generally assumed that AMF do not show host specificity and are randomly distributed in natural ecosystems (Eom et al. 2000). In the present study, despite different host plants harboring a variety of AMF, there were some common species in all replicates of a given plant species, which showed some degree of host preference for AMF. Cluster analysis based on the similarity in AMF species composition indicated that the AMF communities in the rhizosphere soils of different plants that occurred within the same habitat generally had a high degree of similarity (Fig. 2). This result clearly demonstrated that AMF species not randomly distributed over space and that AMF communities seemed habitat-convergence. Similar result was found in another study on diversity of AMF across a fragmented forest in Panama, in which the convergence of AMF communities on islands and convergence of mainland AMF communities were found, regardless of geographic location (Mangan et al. 2004). In addition, the similarity analysis of a given host plant species, which occurred in two or three habitats, also

showed that the AMF communities from the same habitat had the greatest degree of similarity than those from another habitat (Fig. 3). This result also revealed that AMF community composition of a given plant species was greatly habitat-convergence. Based on these results, we hypothesized that AMF communities are more influenced by habitats compared to host preference.

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