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Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China

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Abstract Knowledge about the presence and diversity of arbuscular mycorrhizal fungi (AMF) in a specific area is an essential first step for utilizing these fungi in any application. The community composition of AMF in intensively managed agricultural soil in the Sichuan Province of southwest China currently is unknown. In one set of samples, AMF were trapped in pot cultures from 40 fields growing legumes in the Panxi region, southeast Sichuan. In a second set of samples, the MPN method with four-fold dilutions and maize as host was used to estimate infective propagules in soil from another 50 agricultural sites throughout the province. Soil types were heterogeneous and were classified as purple, yellow, paddy and red. Crops at each site were either maize, wheat or sweet orange. From this set of soil, AMF spores were also extracted and identified. Including all ninety soils, thirty glomeromycotan species in *Glomus* (20 species), *Acaulospora* (four species), *Scutellospora* (three species), *Ambispora* (one species), *Archaeospora* (one species) and *Paraglomus* (one species) were identified. Yellow, red and purple soils yielded similar numbers of AMF species, while AMF species diversity was clearly lower in paddy soil. In trap culture soils, the most frequent species were *Glomus aggregatum* or *Glomus intraradices*, *Glomus claroideum* and *Glomus etunicatum*. The species *Acaulospora capsicula*, *Acaulospora delicata*, *G. aggregatum* (or *intraradices*), *G. claroideum*, *Glomus epigaeum*, *G. etunicatum*, *Glomus luteum, Glomus monosporum*, *Glomus mosseae* and *Glomus proliferum* were successfully cultured as single-species pot cultures in *Plantago lanceolata*. The three most frequent species in field soils were *G. mosseae*, *Glomus caledonium* and *Glomus constrictum*. MPN values varied between 17 and 3334 propagules 100 g soil^{-1} among the fifty field sites sampled. Regression analysis, including host&soil, log(P) and pH as explanatory variables explained 59% of the variation in log(MPN). The highest MPN estimates were found in purple soil cropped with maize and citrus, 324 and 278 propagules 100 g soil^{-1}, respectively. The lowest MPN value, 54 propagules 100 g soil^{-1}, was measured in wheat in purple and yellow soil. Despite intensive agricultural management that can include often repeated tillage, our examination of 90 agricultural sites revealed that soils of the Sichuan region have moderate to high numbers of infective AMF propagules as well as a high

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AMF species diversity. This opens possibilities for further studies and utilization of AMF in agriculture and horticulture in the Sichuan province, People's Republic of China.

Keywords Agricultural soil · Arbuscular mycorrhizal fungi · *Glomeromycota* · MPN · Soil type · Species richness · Species distribution

Introduction

The arbuscular mycorrhizal (AM) symbiosis is the most commonly occurring underground symbiosis in plants. It can be found in a large majority of terrestrial plants (Newman and Reddell 1987) and in almost a quarter of a million plant species (Gadkar et al. 2001). The AM symbiosis plays an important role in natural ecosystems as well as in agroecosystems.

Chinese studies concerning AMF community composition and species distribution have been reviewed by Wang and Liu (2001) and Gai et al. (2006), and more than a hundred species of AMF among nine genera, including 12 described as new species, have been identified during the last 20 years. Early work in China focused mainly on cultivated crops, but more recently, a wide range of natural ecosystems have been surveyed for AMF (Gai et al. 2006). In natural ecosystems, the diversity of fungi in the phylum *Glomeromycota* (Schüßler et al. 2001) has often been very high. In a recent study from three relatively undisturbed habitats in Dujiangyan of the Sichuan Province, Zhang et al. (2003) found 47 taxa belonging to five genera of *Glomeromycota*. Members of *Glomus* and *Acaulospora* appeared to dominate these sites. Also Tao et al. (2004) studying valley-type savannas of the Jinsha river in southwest China, found very high AMF diversity with *Glomus* and *Acaulospora* also as the dominant genera. AMF spores were abundant and colonisation of plant roots was high, indicating that the plants grown in this dry valley might be highly dependent on the symbiosis. In another study conducted in the Xishuangbanna tropical rainforest of southwest China, again *Glomus* and *Acaulospora* were dominant, although the species richness, 1–7 per soil sample, was low compared with other studies from subtropical southwest China (Zhao et al. 2003). Wang et al. (2003) found considerable AMF diversity even in spoiled or degraded ecosystems such as the saline soils of Yellow River Delta (33 taxa), coal-mine spoil heaps of south western Shadong Province (21 taxa) and degenerated grasslands of inner Mongolia (14 taxa).

In cultivated soils of China, AMF species richness understandably can be quite variable. Samples collected from alkaline field soil of the Shandong and Hebei Provinces in the north of China contained 20 species distributed amongst *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus* and *Scutellospora* (Gai et al. 2004) with the highest proportion from *Glomus* (13 species) and *Acaulospora* (four species). Conversely, in a study performed on several field crops in the Shanxi Province, Ye et al. (2003) found sporulation by only seven *Glomus* species.

The Sichuan Province of the People's Republic of China is situated in the southwest part of the country. It is located between 97°22′ and 108°32′ east longitude and 26°03′ and 34°19′ north latitude, with a total area of 485,000 km^2. The topography is complicated: there are plateau and mountain regions in the west which gradually merges into a basin in the east. Most areas of the province are subtropical. In the eastern basin, the average annual temperature is generally between 16 and 18°C, and annual precipitation varies between 1,000 and 1,200 mm. Summer in this area of Sichuan is hot and rainy and winter is dry and mild. Climate and soil are optimal for agricultural production, making the basin with its surrounding hilly area one of the most important farming areas in Sichuan, and indeed in the whole of China. In the basin, two or even three yields per year can be harvested. The southwest mountain region of Sichuan is also subtropical with good insolation levels. Compared with the basin area the diurnal difference in temperature is greater in the mountains. The coldest mean monthly temperature is 10–12°C. This area is the centre for production of subtropical fruits and vegetables for the whole province. The plateau of the northwest of Sichuan has a monsoon type climate, and the annual variation in temperature is low, but with a distinct separation of rainy and dry seasons. This area is the main forest and pastoral area of the province, so that only a small proportion is cultivated.

The occurrence and diversity of the *Glomeromycota* in cultivated soils of Sichuan Province has not been studied. However, the high AMF diversity in undisturbed natural habitats of southwest China indicates that species richness also may be high in agricultural soils. The aim of this investigation was to estimate viable propagule density of AMF in some intensively managed agricultural soils of Sichuan in relation to soil type, standing crop and altitude. Additional aims were further to identify species of AMF directly from soil and from trap cultures, and to establish single-species cultures for potential use as inoculants of selected crops in the region.

Materials and methods

Two sets of soil were collected for studying various traits of AMF in soils of the Sichuan region. Soil set 1 was used to study the distribution of AMF species. Soil set 2 was used to study both AMF species distribution and densities of infective propagules.

Soil types studied

Soil types of Sichuan are characterised as purple, paddy, yellow, red and sandy. These soil groupings are widely used and understood in agronomic terms within the area, but the soils have not been characterised in a formal scheme such as that in http://soils.usda.gov/technical/classification/taxonomy/ and no such classification currently is available. Each soil type has certain basic properties, which can be changed with human activity. Purple soils are found mainly below 800 m above mean sea level (AMSL) in the low mountains and hills of the Sichuan Basin and encompass about 9,113,300 ha. It is one of the most common soil types in Sichuan. Purple soils can be acidic, neutral or alkaline, but the majority of purple soils in our study were acidic. They generally are characterised as productive farmlands with good water permeability, high in N and low in available P. Paddy soils are concentrated mainly in the hills and plains in the bottom of the basin. This soil type covers 4,601,000 ha and accounts for 41.3% of cultivated lands in Sichuan. Paddy soils contain high organic matter (Org M) and N, and are relatively low in total P and K. In our study, however, paddy soils had the highest levels of available P. Yellow soils are distributed in the mountains surrounding the basin and on terraces and hills on the cross-river in the basin and the plain in western Sichuan. General altitude is 1,000–1,200 m AMSL over an area of 4,521,700 ha. These soils usually are acidic and low in available P. Red soils are distributed throughout the hills and valley area of the south western Sichuan at an altitude of 1,100–1,800 m AMSL; they are found in over 1,109,900 ha. Red soils often are slightly acidic with heavy viscosity and are deficient in P and other nutrients. They are high in clay, so water availability can be very low with concomitant cultivation problems (Liu et al. 1997).

Soil set 1

Study area and sampling

To examine distribution of native AMF species by trap culture methods, 40 soil samples were collected from small-scale field plots of the Panxi region of southeast Sichuan between 1st and 10th August 2003 (Fig. 1). Samples consisted of 1 kg of soil collected each from four adjacent plots at a depth of 0–20 cm. These samples were pooled, thoroughly mixed and then used for establishing pot cultures at SAGU.

Samples were collected with a spade close to leguminous standing crops. These were cowpea (*Vigna unguiculata*; 17 sites), garden pea (*Pisum sativum*; ten sites), kidney bean (*Phaseolus vulgaris*; nine sites), mung bean (*V. radiata*; one site), pigeon pea (*Cajanus cajan*; one site) and silver wattle

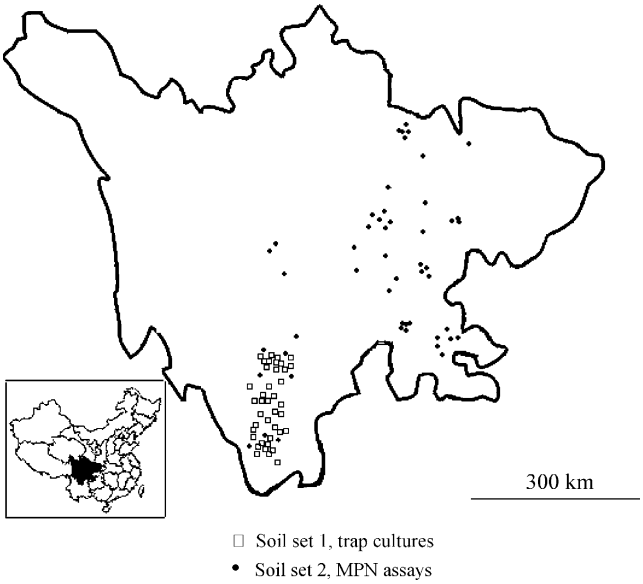

Fig. 1 Study area of Sichuan. Sites marked with an open square (set 1) and a filled square (set 2) indicate sites for AMF trapping and MPN assays, respectively

(*Acacia dealbata*; two sites). Soils were classified as red (19 sites), yellow (17 sites) or sandy (four sites). Soil samples were used only for trap cultures to measure species diversity at these locations.

Trap culture setup

Trapping of AMF was performed at SAGU, China by diluting field soil with sterile river sand and sowing with white clover. Few AMF sporulated in these cultures. Therefore those results are not included here. Cultures of AMF from Sichuan were established at Agrifood Research Finland (MTT) in mid-November 2003 with approximately 20 ml soil substrate from failed trap cultures first established at SAGU. Soil inoculum was sandwiched in an approximate of 0.5–1 cm layer in the middle of a 300-ml pot between layers of steam sterilised sand+vermiculite (3 V, Vermipu Oy, Finland) 4:1, fertilized with 1 g l^{-1} Osmocote Plus (16N–3P–11K, 8–9 month longevity, Sierra Chemicals, The Netherlands) and limed with 5 g l^{-1} Dolomite lime (Saxo Oy, Finland). Pots were maintained in a growth chamber at 20/18°C (day night), 50–60% relative humidity, under warm white (approximately 100–120 μmol m^{-2} s^{-1}) artificial light and a daylength of 14 h. *P. lanceolata* was used as trap plant.

Spore extraction

Small samples (approximately 15 ml) from each trap culture were suspended in water and thereafter sieved using 500 and 50 μm sieves. Contents from each sieve were washed with water into a dish for examination under the

dissecting microscope. Further microscopic studies of spore morphology were carried out by examining features of spores mounted in PVLG (Omar et al. 1979) with (PVLG/M) and without the addition of Melzer's reagent (5:1 *v/v*, Walker et al. 1993).

Establishment of AMF pot cultures

Newly formed spores of AMF from trap cultures were used to establish second generation multispore or single-spore culture attempts in open 300-ml pots containing a dis-infested substrate of attapulgite clay (Agsorb 8/16, Oil-Dri, Wisbech, UK) and sand (1:1). For multispore culture attempts, between 20 and 30 spores were placed on the roots of three-week old seedlings of *P. lanceolata* as they were transplanted to a pot. The cultures were maintained at 20/18°C (day/night), 50–60% relative humidity, in a growth chamber with warm white artificial light (approx. 100 μmol m^{-2} s^{-1}). Details of original collection and isolation, resultant cultures and subcultures, and herbarium specimens have been recorded in a database developed by C. Walker (Walker and Vestberg 1998). In this database, each culture pot is given an 'Attempt' number (unique to each culture attempt made from any of the original samples) and culture number (sequential for subcultures from a particular attempt). Voucher specimens of fresh material from both trap cultures and from pure cultures have been accessed as semi-permanent microscopic slides in the personal herbarium of M. Vestberg, with each individual collection assigned an accession number.

Soil set 2

Soil samples collected from a larger area of Sichuan (Fig. 1) were used to determine infective inoculum density (MPN) in agricultural soils of the area. AM fungi were also identified directly from these soils. Attempts to establish viable trap cultures were not successful. The study was carried out at SAGU, Yaan, China.

Study area and sampling

Fifty soil samples were collected 17–20 February 2004 from various parts of Sichuan (Fig. 1). The sampling procedure was identical to that for the first set of soils, except that the resultant 2-kg sample was divided into four equal parts for separate analyses: (1) pH (0.5 mol^{-1} $NaHCO_3$), extractable P (0.5 M $NaHCO_3$, pH 8.5, Olsen et al. 1954), organic matter percentage (OM%) and soil type, (2) MPN assay (Porter 1979), (3) AMF spore extraction directly from field soil by decanting and wet sieving (Gerdemann and Nicolson 1963) and (4) to establish trap cultures which failed and is not discussed further. Wheat and maize root samples were also collected where present and stained with methyl blue (replacing Trypan blue in the method of Phillips and Hayman 1970) and percentage root colonisation estimated with the gridline intersect method (Giovannetti and Mosse 1980).

Nineteen samples were collected from the basin area of Sichuan (300–1,300 m AMSL). From the hills (300–1,300 m AMSL), the mountain (1,000–5,000 m AMSL) area and the high plateau (2,000–2,800 m AMSL), 21, seven and three samples were collected, respectively. Soils were classified as purple (27 sites), yellow (13 sites), paddy (eight sites) and red (two sites). The standing crops were maize (*Zea mays*; 24 sites), common wheat (*Triticum aestivum*; 13 sites), sweet orange (*Citrus sinensis*; ten sites), sugar cane (*Saccharum officinarum*; two sites) and a soft fruit plantation (one site). At the time of soil collection, maize was at an early developmental stage (approximately 30-cm-high shoots) and the other standing crops were close to maturity.

MPN assay

MPN assays of each of the 50 soil samples were set up under natural light in a greenhouse at SAGU, Yaan. Soil was passed through a 20-mm sieve and mixed with autoclaved river sand to make nine successive dilutions (4^0–4^{-9}) of five replicates each and placed in 400-ml pots as described by Sieverding (1991). Three seeds of maize were sown in each pot and thinned to one seedling after emergence. All MPN assays were set up contemporaneously and maintained between March 3 and May 13, 2004. Plants were watered according to need with no extra fertilizers applied. At harvest, roots were cut from the centre of each pot. Absence or presence of AMF colonisation was recorded after staining of roots with methyl blue. MPN number estimates and confidence limits for each soil sample were calculated according to Sieverding (1991) and Table VIII of Fisher and Yates (1970). Results were expressed as number of infective propagules per 100 g oven dry soil.

Spore extraction

AMF spores were extracted by soaking 50 g of soil in 1 l of water. The suspension was then passed through a 100 and 400-μm sieve. Contents from both sieves were washed with water into separate dishes for examination under the dissecting microscope. Further microscopic studies of spore morphology were carried by examining features of spores mounted in PVLG and PVLG/M.

Statistical methods

The data of natural log-transformed MPN were analysed by regression analysis. The MPN measurements were log-

transformed to correct for skewness in data distribution. Host and soil were combined into a combination variable with eight levels and soil P data were log-transformed. The combination variable of host and soil (host&soil) was created because not all combinations of the two original variables were observed. Applicable host-by-soil interactions could be examined by constructing contrasts using the combination variable. Log-transformation of P measurements linearised the relationship between P and log(MPN).

The associations between log(MPN) and the potential explanatory variables were first examined through scatter diagrams. From the diagrams, it was deduced that the starting point for the model building should be adding the host&soil variable as an explanatory variable because there seemed to be clear differences between the host&soil classes in log(MPN) measurements. Scatter diagrams also indicated potential associations of log(MPN) with log(P) and pH. Therefore, log(P) and pH were the second variables to be examined during the model construction. Also, Org M and Altitude as well as two-way interactions of host&soil with log(P), pH, Org M and Altitude were examined during the modelling process. Variables were added one by one into the model that already included previously sustained explanatory variables. The criterion for choosing whether the added variable should be sustained in the model was the change in coefficient of determination (R^2) and the P value of the type III F test.

The statistical modelling was performed using the GLM procedure of SAS 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA). The assumptions of the model were checked by graphical methods: residuals were plotted against the fitted values and the normality of the residuals was checked through box plots.

Table 1 Species of the *Glomeromycota* identified from field soil, trap cultures and pure cultures originating from the Sichuan Province, People's Republic of China

Fungal name	Field soil Frequency/50	Trap culture Frequency/40
*Acaulospora capsicula**		1
*A. delicata**		2
A. scrobiculata	4	1
A. tuberculata	3	
Ambispora callosa		1
Archaeospora trappei		6
*Glomus aggregatum/intraradices**		12
G. caledonium	7	
*G. claroideum**		12
G. constrictum	5	
G. coronatum	5	
*G. epigaeum**		6
*G. etunicatum**	2	10
G. fragilistratum		1
G. geosporum	13	4
G. hoi		1
G. lamellosum		1
*G. luteum**		2
G. magnicaule	1	
G. manihotis	2	
G. microcarpum	1	
*G. monosporum**		5
*G. mosseae**	19	7
*G. proliferum**		1
G. sinuosum	1	
G. tortuosum	2	
Paraglomus occultum		6
Scutellospora calospora	1	
S. heterogama	1	
S. projecturata	1	

AMF established as single-species cultures are indicated with a * after the species name

Results

AMF fungal species composition

From both sets of soils encompassing 90 sites, thirty species of the *Glomeromycota* were identified (Table 1). *Glomus* dominated (20 species), followed in descending order by *Acaulospora* (four species), *Scutellospora* (three species), *Ambispora* (one species), *Archaeospora* (one species) and *Paraglomus* (one species). Only five AMF species were identified from both trap culture soils (soil set 1) and field soils from set 2. These were *Acaulospora scrobiculata*, *Glomus etunicatum*, *Glomus mosseae* and *Glomus tortuosum*. *Glomus aggregatum/intraradices*, *Glomus claroideum* and *G. etunicatum* were found most frequently in trap cultures of soils from set 1 while *G. mosseae*, *Glomus caledonium* and *Glomus constrictum* were recovered most frequently from field soils of set 2. The reason we have combined the names *G. intraradices* and *G. aggregatum* is that they are not readily separable morphologically, and indeed, there is some evidence that they may be synonymous (discussed in the INVAM website at http://invam.caf.wvu.edu).

Sixteen, 15 and 14 AMF species were identified from yellow, red and purple soils, respectively. Paddy and sandy soils yielded only three and six AMF species, respectively, but the study also included fewer samples of these soil types. The percentage occurrence of *G. mosseae* and some other commonly found AMF species in the five soil types of the study are indicated in Table 2. Species richness per individual soil sample was highly variable. Species numbers varied between none present to seven in the MPN soils and between none and six in the trap culture soils. In MPN soils, mean AMF species richness was 3.4 in maize and 2.1 and 1.4 in sweet orange and wheat, respectively.

Table 2 Distribution of some commonly found AMF species across 90 sites in Sichuan, People's Republic of China

AM fungus	Proportion (%) of soil samples with occurrence of AMF				
	Yellow soil (*N*=30)	Purple soil (*N*=27)	Red soil (*N*=21)	Paddy soil (*N*=8)	Sandy soil (*N*=4)
Acaulospora scrobiculata	3.3	11.1	–	–	25.0
Glomus aggregatum/intraradices	20.0	–	19.0	–	25.0
G. caledonium	6.7	18.5	–	–	–
G. claroideum	16.7	–	19.0	–	25.0
G. constrictum	–	–	19.0	12.5	–
G. etunicatum	20.0	3.7	19.0	–	25.0
G. geosporum	6.7	25.9	9.5	25.0	–
G. mosseae	30.0	33.3	4.7	37.5	–

AMF species successfully cultured as single species in pot cultures with *P. lanceolata* as host are indicated with an asterisk in Table 1. Several unidentified *Glomus* spp with minute hyaline to slightly coloured spores borne singly or in loose clusters, with or without a Melzer's reaction, were detected in some trap cultures. Four of these *Glomus* species have been successfully cultured. One or more of them may be new and will be the subject of further study. Among the AMF species identified, eleven have not been reported previously in China; *Acaulospora capsicula*, *Acaulospora delicata*, *Ambispora callosa*, *Glomus coronatum*, *Glomus epigaeum*, *Glomus fragilistratum*, *Glomus lamellosum*, *Glomus luteum*, *Glomus microcarpum*, *Glomus proliferum* and *Scutellospora projecturata.* We are aware of the opinion that *G. epigaeum* and *Glomus versiforme* are synonymous (Berch and Fortin 1983), but there is some evidence to suggest this may not be the case (C. Walker, unpublished). The specimens we found agreed in morphology with the original species description of the former (Daniels and Trappe 1979) and we therefore take a conservative point of view in this matter.

Infective AMF propagule densities (MPN)

Soil properties

OM% in soils varied between 0.5% and 4.4%. Soil pH varied from acid (4.8) to neutral (6.9). The amount of extractable P varied between 7.1 and 178 mg P kg soil^{-1} with the average higher in paddy soil (70.8 mg kg soil^{-1}) than in yellow (45.5 mg kg soil^{-1}) and purple soil (25.7 mg kg soil^{-1}). Soil with the main crops maize, wheat and citrus had an average P concentration of 23.3, 51.6 and 61.9 mg kg soil^{-1}, respectively.

MPN assays

The numbers of infective propagules (MPN) of AMF in 100 g of dry soil varied between 17 and 3,334 across the fifty sites in soil set 2. Regression analysis was used to determine the extent to which how much of this high variation was dependent on host, altitude, soil type, soil properties or combinations of these variables. The final statistical model included host&soil, log(P) and pH as explanatory variables. The R^2 for the final model was 59%, i.e. 59% of the variation in log(MPN) is explained by the final model. The effects of Organic matter and Altitude, as well as two-way interactions of host&soil with log(P), pH, Organic matter and Altitude were not statistically significant in the model where the effects of host&soil, log(P) and pH were already taken into account. Significance of terms included in the final model of log(MPN) is shown in Table 3.

Estimated means and their 95% confidence intervals for the host&soil combinations are shown in Table 4. The highest estimated mean MPN numbers were found in purple soil cropped with maize and citrus, 324 and 278 propagules 100 g soil^{-1}, respectively. Pairwise comparisons showed that these levels were significantly higher than the propagule numbers found with wheat, 54 propagules, in the same soil (P=0.01 and 0.001, respectively). In yellow soil, the numbers of MPN in maize also was higher than in wheat (181 vs. 54; P=0.02). A nearly significant difference between MPN in paddy and purple soil with citrus as host was also observed (P=0.05) (Table 4).

Table 3 Statistical significances of the terms included in the final model of log(MPN)

Model term	*df*[a]	*F* value	*P* value	Estimate of slope	S.E. of slope
Host&soil	7	4.97	<0.001	–	–
log(P)	1	3.36	0.08	−0.34	0.19
pH	1	3.45	0.07	−0.60	0.32

[a] The error degrees of freedom were 36.

Table 4 Estimated means and 95% confidence intervals at log(P)=3.3 and pH=6.0 (at their means) of host & soil combinations

Host & soil	*N*	Estimated mean[a]	95% confidence interval for the mean[a]
Citrus & paddy	2	71	(23–221)
Citrus & purple	4	278	(126–614)
Citrus & yellow	3	108	(40–287)
Maize & purple	18	324	(216–486)
Maize & yellow	5	181	(89–366)
Wheat & paddy	6	93	(45–191)
Wheat & purple	3	54	(21–141)
Wheat & yellow	5	54	(27–110)

[a] Logarithmic values transformed back to the original scale of measurement

Root colonisation

Mycorrhizal colonisation of maize and wheat generally was low. Maize and wheat in purple soil showed the highest colonisation, averaging 21.6% and 9.4%, respectively. Maize was colonised at levels of 12.1% and 10.9% in yellow and paddy soil, respectively, while colonization of wheat was 5.4% in this soil type (results not shown).

Discussion

AMF fungal species composition

A total of 30 AMF species representing the genera *Acaulospora*, *Ambispora*, *Archaeospora*, *Glomus*, *Paraglomus* and *Scutellospora* were identified from trap cultures (soil set 1) and field soils (soil set 2). Generic diversity is half of that currently listed (December 2007) in *Glomeromycota* (http://www.lrz-muenchen.de/~schuessler/amphylo/). In their comprehensive review of twenty years of AMF research in China, Gai et al. (2006) report that 104 AMF species within nine genera have been discovered in different plant species and various ecological habitats since the 1980s. Thus it appears that Chinese soils generally have high AMF diversity. This is not surprising, since China embraces a large diversity of climatic conditions and soil types that support a wide range of ecosystems and vegetation structures (Gai et al. 2006). According to Gai et al. (2006), China has about 30,000 plant species, which comprise about one eighth of the plant species known worldwide.

In this study, species of *Glomus* were the most widespread and abundant followed by *Acaulospora* and *Scutellospora*. Prevalence of *Glomus* species in agricultural soils of China and elsewhere is widely reported (Zhang et al. 1994; Vestberg 1995; Zhang et al. 1998; Oehl et al. 2003; Gai et al. 2004). However, *Acaulospora* dominated in some Chinese natural ecosystems (Zhao et al. 2003; Li et al. 2003; Tao et al. 2004). Similar to the agricultural soils, a study of various natural ecosystems in the Sichuan province revealed more species of *Glomus* than of *Acaulospora* (Zhang et al. 2003). Among *Glomus* spp. identified, *G. mosseae*, *G. etunicatum* and *Glomus geosporum* were found in both sets of soils with their different standing hosts. These AMF species can thus be regarded as "generalists" for intensively managed arable lands in Sichuan Province. This finding is partly consistent with the work of Oehl et al. (2003), who, however, also listed *Glomus diaphanum*, *G. constrictum* and *Scutellospora calospora* as "generalist" AMF for temperate agroecosystems of Central Europe. Some studies also mention certain AMF species as "specialists" for certain habitats. For example, in China, *Pacispora scintillans* was readily found in regions of high altitude such as Tibet and Xinjiang (Qiao et al. 2005) while *Glomus formosanum* have been predominantly reported from the subtropical or tropical regions of China (Zhang et al. 2004). In Switzerland, Oehl et al. (2003) found sporocarpic *Glomus* spp., such as *Glomus sinuosum*, much more commonly in grasslands than in arable lands. Our study included only intensively tilled arable land, so naming of one or several AMF "specialists" for a certain habitat type was not possible. No member of the genus *Gigaspora* was discovered in this study, but this genus has been found in other studies in cultivated soils in eastern, central southern and northern China (Gai et al. 2006). The absence of *Gigaspora* is not surprising because *Gigaspora* is not always detected in surveys of AMF. For example, *Gigaspora* was only recently found for the first time in Europe (Jansa et al. 2002), where its existence in Switzerland was verified by means of morphological and molecular biological tools (Jansa et al. 2003).

There was a distinct difference in AMF diversity in the two sets of soil. Only five species out of thirty were detected both by direct extraction (soil set 2) from soil and by trap culturing (soil set 1). Such a difference in AMF species composition between trapping and direct spore extraction has also been found in several other studies (Brundrett et al. 1999; Clapp et al. 2002; Oehl et al. 2003). Brundrett et al. (1999) found that most species of *Scutellospora*, *Acaulospora* and *Gigaspora* were obtained primarily from field-collected spores, but only 50% of these culture attempts were successful. With *Glomus*, they found the opposite pattern. Several *Glomus* spp. rarely detected in field soil were dominant in trap cultures. The differences between isolation methods often is pronounced with prolonged (Oehl et al. 2003) or repeated trapping (Stutz and Morton 1996). Due to failure in trap culturing of soil set 2 and also to the lack of resources, we have no data showing the impact of isolation method on AMF species outcome when using the same soil. However, it is clear that several isolation methods should be used simultaneously to

obtain a more complete picture of the AMF species composition in soil. Brundrett et al. (1999) studied four different techniques for isolating AMF, i.e. (1) spores separated from soil, (2) soil trap cultures, (3) root samples or (4) transplanted seedlings. They found that all these techniques complemented each other because they often produced cultures dominated by different species from the same soil sample.

Infective AMF propagule densities (MPN)

MPN estimates of AMF infective propagules varied considerably amongst the 50 soil samples collected from agricultural soils of Sichuan Province. The lowest single value was 17 and the highest more than 3,300 propagules per 100 g soil DW. Such great variation in MPN is not surprising because many variables are at work over a broad range of environments. Causation clearly cannot be identified in our study because of the multitude of variables over such a broad geographic range. Regression analysis revealed that the extent variation in MPN results could be explained by external variables. Host&soil, P and pH explained 59% of variation in MPNs.

MPN numbers in purple soil cropped with maize and citrus were about 300 per 100 g soil, while only about 50 AMF propagules were measured under wheat in the same soil type. Adelman and Morton (1986) also showed dramatic differential effects of reciprocal host and soil environments on infectivity of native AMF communities. Troeh and Loynachan (2003) found that the MPN number of propagules in soil depended on both cropping management and soil type being higher in continuous maize than in continuous soybean. Other studies also show that plant composition can greatly affect infectivity, as shown by variation in MPN values and AMF spore numbers with crop rotations (Harinikumar and Bagyaraj 1989; Arihara and Karasawa 2000; Vestberg et al. 2005). Estimated means of MPN (Table 2) suggest that soil type impacted on fungal infectivity. Few studies show impacts of soil type on AMF traits. Rathore and Singh (1995) found a significant negative correlation between MPN of AMF and the clay content of six soil types in India. Low AMF spore diversity in heavily textured clay soil also has been found (Mathimaran et al. 2005). Land and Schönbeck (1991) observed faster initial AMF colonisation in silt soil than in clay soil, but the maximum spore density was not affected by soil type.

Extractable P in the soil samples varied between 7 and 178 mg kg soil^{-1}. Soil P almost significantly (Table 3) explained the variation in MPN. It is documented that root colonisation (Thomson et al. 1986) and AMF sporulation (Douds and Millner 1999; Kahiluoto et al. 2001) can decrease with increasing levels of extractable soil P. Also other studies showing differences between soils in MPN of AMF usually have higher MPN values at lower than at higher P levels (Scheltema et al. 1987; Chelius and Triplett 1999; Troech and Loynachan 2003). Soil pH was one of the factors explaining variation in MPN numbers. The relationship between soil pH and MPN was slightly negative (Table 3). The soils were slightly acid with the majority of them having a pH between 5.5 and 6.5. This range of pH has been found to favour *Glomus* (Porter et al. 1987, Wang et al. 1993), while *Acaulospora* has been found to sporulate more abundantly in acid soils (Porter et al. 1987; Gai and Liu 2003). Except for sporulation, pH has also been reported to affect AMF spore germination (Daniels and Trappe 1980), hyphal growth and root colonisation (Medeiros et al. 1994). AM fungal structures themselves have also been shown to affect substrate pH. In a study of monoxenic *Glomus intraradices* culture, Bago et al. (1998) found pH decreases in zones of the medium in which a high number of arbuscular mycorrhizal fungal spores were formed.

In comparisons with other MPN studies of infective AMF propagules in tropical crops, our values (54 to 324 propagules 100 g soil^{-1}) are close to those measured by Michelsen and Rosendahl (1989) in mixed herb vegetation in Somalia, but about half of the densities observed in finger millet (1,000 propagules per 100 g soil), ground nut (1,080 propagules per 100 g soil), cowpea (1,000 propagules per 100 g soil) and sunflower (800–890 propagules per 100 g soil) in India (Harinikumar and Bagyaraj 1989). In some studies, extremely high MPN values of 10,000 or more propagules per 100 g soil have been observed (Scheltema et al. 1987; Land et al. 1993).

There has been some debate about the scientific accuracy of the MPN assay, although at the very least, they provide indicator measurements on the relative colonisation potential of AMF. The estimates are very dependent on the experimental conditions used. From this point of view, comparisons of MPN levels of studies carried out in highly different ecological situations can be seen as only indicative. Wilson and Trinick (1982) listed several factors which have a high impact on the result. Such factors include host, temperature, time of harvest, pot size, soil (whether dry of moist), nutrient status of diluent, root density and measurement of all roots exposed to fungal inoculum—especially at higher dilutions. The confidence limits of the MPN method are a reflection of dilution factor and replicate number (Cochran 1950). The four-fold dilution factor used by us sacrificed some precision in the data but allowed a greater number of samples for comparison.

Conclusion

Agricultural soils of the Sichuan province, China are managed relatively intensively including at least two

harvested crops per year. As a result of frequent tillage and high mean yearly temperature, the content of soil organic matter is low. This was found also in our study in which the OM% varied between 0.5% and 4.4%. Despite the intensive management, our study of 90 agricultural sites revealed that soils of this region have moderate to high numbers of viable AMF propagules as well as a high AMF species diversity. This provides good possibilities for further studies and utilization of AMF in agriculture and horticulture in the Sichuan province. The AMF species established as pure cultures have a potential use as inoculants for selected crops of the area.

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