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Diversity of arbuscular mycorrhizal fungi in greenhouse soils continuously planted to watermelon in North China

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Abstract In North China, watermelon is grown in commercial greenhouses in a continuous monoculture and with high application rates of manure or compost. The aim of this study was to determine how the diversity of arbuscular mycorrhizal fungi (AMF) in these soils changed over long periods (0 to 20 years) of monoculture. AMF in control soils (from fields not replanted with watermelon and located near the greenhouses) and in greenhouses (in Daxing, Beijing, and Weifang, Shandong) that had been continuously replanted with watermelon for 5, 10, 15, or 20 years (three greenhouses per year per location) were identified and quantified based on spore morphology and on denaturing gradient gel electrophoresis (DGGE). The total number of AMF species and genera were 13 and 3 in soils replanted for 5–20 years and 19 and 4 in control soils. AMF species richness (SR), the Shannon–Wiener index (*H*), and spore density declined as the number of years in which watermelon was replanted increased. The available phosphorus, potassium, and nitrogen in the soil increased as the number of years in which watermelon was replanted increased. Values for SR and *H* were higher when based on DGGE than on spore morphology. The results suggest that current greenhouse practices in North China reduce the AMF diversity in the soil.

Keywords Arbuscular mycorrhizal fungi · Denaturing gradient gel electrophoresis · Greenhouse · Watermelon · Diversities · Monoculture

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Introduction

Arbuscular mycorrhizal fungi (AMF) are important components of terrestrial ecosystems (Liu and Chen 2007; Smith and Read 1997). Because they have adapted to many different environments and plant hosts, AMF are represented by many diverse species (Peng et al. 2011; Antunes et al. 2009; Galván et al. 2009; Lugo et al. 2008), and Borstler et al. (2006) estimated that there could be 1,250 species of AMF in the world. In the past 10 years, about 113 AMF species in seven genera have been isolated in China, 70 species have been isolated in Africa, and 84 species have been isolated in the USA, France, and Germany (Liu et al. 2009).

AMF species richness (SR) and species diversity indices differ among ecosystems, and AMF SR and species diversity indices are usually greater in natural forests than in farmlands (Wang et al. 2003). There is relatively little information, however, about AMF community structure, composition, and distribution in commercial greenhouse systems (Sýkorová et al. 2007), and this is especially true for greenhouse systems that use continuous monoculture, as is the case for greenhouse watermelon production in North China. In commercial greenhouse production in North

China, the soil and watermelon plants are protected by plastic when the air temperature is <30°C but are uncovered when the air temperature is >30°C.

North China is the main region for greenhouse watermelon production in China. Beijing and Shandong Provinces, in particular, have temperate monsoon climates and fluvo-aquic soils that are optimal for greenhouse watermelon production. To reduce production costs in these systems, farmers typically plant successive watermelon crops in the same soil, i.e., watermelon is grown in continuous monoculture. Because the crop is not rotated and because of the relatively constant temperatures, high humidity, and overuse of pesticides and mineral fertilizers, soil chemical properties and soil quality are degraded, the soil microbial community is altered, and productivity declines (Yang and Zhang 2003; Li et al. 2010, 2003). These changes are obviously not conducive to sustainable watermelon production.

Previous studies have shown that mycorrhiza can be beneficial in agricultural ecosystems (Wang et al. 2005). Mycorrhizal symbiosis enhances the ability of plants to establish and to cope with the stresses of soil disturbance (Cuenca et al. 2003) and nutrient deficiency (Collier et al. 2003). And AMF should play an important role in watermelon production (Shi et al. 2006). Mycorrhizal inoculation can significantly increase nutrient acquisition, fruit yield, and quality of watermelon (*Citrullus lanatus*) under field conditions(Kayal et al. 2003; Li et al. 2004). We suspect that AMF fungi might be managed to improve the greenhouse soils that are repeatedly replanted with watermelon, but the AMF fungi in these soils have never been studied.

The aim of this research was to determine how AMF community composition changes over time in the continuous watermelon production systems practiced in greenhouses in North China. AMF were identified using PCR–denaturing gradient gel electrophoresis (DGGE) technology and traditional morphological methods. Information about how watermelon monoculture affects the AMF community could be useful for improving this production system.

Materials and methods

Sites and sampling

Sites were selected from two main watermelon production regions in China: one site was in Yudai Town in Daxing, Beijing (39° 07′N, 116° 03′E), and the other site was in Yaogou Town in Weifang, Shandong (36° 42′N, 118° 50′ E). About 105 m^3 of manure plus 19 kg of mineral fertilizer per ha were applied annually to greenhouse soil at the Daxing site, and about 75 m^3 of manure plus 26 kg of mineral fertilizer were applied annually at the Weifang site. The systemic fungicide carbendazim and the neonicotinoid insecticide imidacloprid were used at both sites.

In September 2009, 24 watermelon greenhouses (12 in Daxing and 12 in Weifeng) were selected where watermelon had been replanted continuously for 5, 10, 15, or 20 years (three replicate greenhouses for each length of time and location). Four plots (two in Daxing and two in Weifeng) were also designated in a nearby open farmland; soil from the open farmland, which served as a control, had been used for the production of watermelon and other crops but had not been used for continuous watermelon production. Three soil samples (5–30 cm in depth and about 500 g per sample) were collected from the root zone in each greenhouse during the replanting period. At the same time, three soil samples were collected from each control plot; at this time, maize had been recently harvested, and winter wheat seeds were germinating. Collected soil samples were divided into three subsamples for chemical analysis, spore isolation, and DNA extraction. Subsamples for chemical analysis and spore isolation were air-dried for 2 weeks and stored at 4°C. Subsamples for DNA extractions were stored at −20°C for 2 days. Greenhouse locations and soil characteristics are listed in Table 1.

Spore isolation and species identification

AMF spores were extracted from soil by routine wet sieving and decanting (Dalpe 1993). Fungal identification was based on spore morphology according to Schenck and Perez (1988), original descriptions of species, and detailed descriptions provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu).

AMF diversity based on morphological identification of species

Spore density (SD), SR, frequency (*F*), relative abundance (RA), importance value (IV), and Shannon–Wiener index (*H*) of diversity were calculated as follows: SD=the number of AMF spores per 100 g of soil; SR=the number of AMF species per 100-g subsample of soil; *F*=(the number of samples in which a given species or genus was detected/the total number of samples)×100; RA=(the number of spores of a given species/the total number of spores)×100; IV=(*F*+RA)/2; $H = -\sum_{i=1}^{S} (pi \ln pi)$, where *s* is the number of species and *pi* is the RA of the *i*th species.

DNA extraction and purification

Total DNA was extracted from a 10.0-g subsample of each soil sample as described by Yeates et al. (1998). Total DNA

Table 1 Locations of greenhouses and soil characteristics

Greenhouse locations	Crops	Codes	Years of watermelon replanting	pH	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Organic matter (g/kg)
Daxing	Maize/Wheat	CK1	0	6.4	60.13	25.81	196	16
	Watermelon	B5	5	5.8	91	18	142	30
		B10	10	6.4	72	13	113	32
		B15	15	6.2	126	47	250	17
		B20	20	6.1	151	59	291	11
Weifang	Maize/Wheat	CK2	0	6.4	65	21	239	19
	Watermelon	C5	5	6.6	53	18	161	15
		C10	10	6.3	74	34	274	11
		C15	15	6.4	124	70	326	9
		C20	20	6.3	178	75	355	9

B5, B10, B15, and B20 (and C5, C10, C15, and C20) were each represented by three replicate greenhouses. CK1 and CK2 were each represented by two plots in open farm fields that had not been continuously replanted with watermelon

was then purified using the gel-DNA-recovery kit (DP1702, Biotech, Beijing) as recommended by the manufacturer.

PCR amplification of AMF

Primers for PCR and citations are listed in Table 2. DNA extracted from soil samples was first amplified with fungal primers GeoA2 and Geo11. These are universal 18S rDNA fungal primers that have the potential to amplify all fungal DNA. The first-round PCR products were used as template in the second-round PCR, using primers AM1 and NS31-GC, which corresponds to NS31 described by Simon et al. (1992) plus a 5′ GC clamp sequence as described by Kowalchuk et al. (1997). The second-round PCR products were used as template in the third-round PCR, using primers NS31-GC and Glol. The third-round PCR produced a DNA fragment of approximately 250 bp.

PCR reactions were performed in a final volume of 20 μl containing 2 μl of 10× PCR buffer (Mg^{+}-free), 400 μM deoxynucleotide triphosphate mix, 0.2 μM primers, 1 unit of Taq DNA polymerase (TaKaRa *Taq*™), 2 mM $MgCl_2$, and 1.0 μl of template DNA. The products were amplified on an Eppendorf Master-Cycler Gradient (NY, USA). Conditions for the first-round PCR were 94°C for 4 min; 30×(94°C, 1 min; 54°C, 1 min; 72°C, 2 min); and 72°C for 7 min. Conditions for the second-round PCR were 94°C for 2 min; 30×(94°C, 45 s; 65°C, 1 min; 72°C, 45 s); and 72°C for 7 min. Conditions for the third-round PCR were 94°C for 2 min; 30×(94°C, 45 s; 55°C, 1 min; 72°C, 45 s); and 72°C for 7 min. Amplicon integrity and yield were analyzed by agarose (0.8% *w*/*v*) gel electrophoresis (80 V, 60 min) and ethidium bromide staining, and amplicons were stored at −20°C for subsequent DGGE analysis.

DGGE analysis

A 10-μl volume of PCR product was used for DGGE analysis. Gels contained 8% (*w*/*v*) polyacrylamide (37.5:1 acrylamide/bis-acrylamide) and 1× Tris/acetic acid/EDTA buffer (TAE), and were 1.5 mm thick (20×20 cm). The linear gradient used was from 30% to 60% denaturant, where 100% denaturing acrylamide was defined as containing 7 M urea and 40% (*v*/*v*) formamide. A 10-mL stacking gel containing no denaturants was added before

Table 2 Sequences of primers used in nested PCR for detection of AMF

Primer	Sequence	Sources
GeoA2	5′-CCAGTAGTCATATGCTTGTCTC-3′	Schwarzott and Schüßler 2001
Geo11	5′-ACCTTGTTACGACTTTTACTTCC-3′	Schwarzott and Schüßler 2001
AM1	5′-GTTTCCCGTAAGGCGCCGAA-3′	Helgason et al. 1998
NS31-GC	5′-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGG CACGGGGGTTGGAGGGCAAGTCTGGTGCC-3′	Simon et al. 1992; Kowalchuk et al. 1997
Glol	5′-GCCTGCTTTAAACACTCTA-3′	Cornejo et al. 2004

polymerization was completed (~2 h). DGGE analysis was run in the DCode system (Bio-Rad Laboratories, Hercules, CA, USA) at a constant temperature of 60°C. Electrophoresis was for 20 min at 75 V followed by 5 h at 120 V. Following electrophoresis, the gels were stained in 1× TAE containing 0.5 μg of ethidium bromide per ml of TAE, and gels were visualized by UV illumination. Gel images were digitally captured with the Alpha Imager System (Alpha Innotech, San Leonardo, CA).

Quantity-One software (Bio-Rad) was used to convert the DGGE banding patterns to optical density curves, in which the peaks represented individual taxonomic units. These units were differentiated by R*f* values and their peak areas, and both R*f* values and peak areas were used to calculate SR and *H*. Based on DGGE data, *H* was calculated as follows:$H = -\sum (ni/N)\ln(ni/N)$ where ni was the height of the peak and *N* was the sum of all peak heights of the optical density curve.

Data analysis

Data were analyzed by ANOVA. Differences in means were compared with Duncan's new multiple range test and considered significant at $P \leq 0.05$. Costat (CoHort Software, Berkeley, CA, USA) and 2003 Microsoft® Excel were used for statistical analyses.

Results

AMF species composition based on spore morphology

A total of 13 species of AMF were identified in the greenhouse soils that had been replanted with watermelon; these included eight taxa from *Glomus*, four from *Acaulospora*, and one from *Scutellospora* (Table 3). In contrast, control soils (from open field sites and areas that had not been replanted with watermelon) contained 19 AMF species belonging to 4 genera; these included *Glomus aggregatum*, *Glomus caledonium*, *Glomus claroideum*, *Glomus clarum*, *Glomus constrictum*, *Glomus etunicatum*, *Glomus geosporum*, *Glomus mosseae*, *Glomus pustulatum*, *Glomus reticulatum*, *Glomus versiforme*, *Acaulospora lacunosa*, *Acaulospora rehmii*, *Acaulospora nicolsonii*, *Gigaspora margarita*, *Scutellospora calospora*, *Glomus* sp1, *Acaulospora* sp1, and *Aculospora* sp2.

Frequency, relative abundance, and dominant species of AMF based on spore morphology

In replanted soils, *Glomus* and *Acaulospora* occurred most frequently, while *Scutellospora* occurred least frequently at both sites. Distribution and spore number were considered simultaneously to determine the dominant species in the AMF community. Thus, AMF with an IV greater than 50% were defined as dominant species. At Daxing, the IV of *G. mosseae* and *G. etunicatum* were 54% and 63% in soil that had been replanted with watermelon for 15 years and 5 years, respectively. At Weifang, the IV for *G. claroideum* and *G. etunicatum* were 71% and 63% in soil that had been replanted with watermelon 15 years and 5 years, respectively. In addition, *G. claroideum* was the most common species, with *F* values as high as 100% in some greenhouses (Table 3). In control soil, *G. clarum* and *Gi. margarita* were dominant species at Daxing, and *G. mosseae* was the dominant species at Weifang.

Diversity of AMF based on spore morphology

The compositions of AMF communities in the replanted soils (from greenhouses) and nonreplanted soils (from open fields) were compared based on spore morphology. In greenhouse soils, the SD at Daxing and Weifang and *H* at Daxing generally decreased with an increase in the number of years that watermelon had been replanted (Table 4). SD, SR, and *H* were significantly higher in control soils than in replanted soils (Table 4). SR values differed significantly between soils that had been replanted for 0 (control), 5, and 20 years at Daxing and for 0, 5, and 15 years at Weifang. Among replanted soils, the highest *H* value (1.91) and the lowest *H* value (1.07) was in soil that had been replanted at Daxing for 5 years and 20 years, respectively; the highest SD value (47) and highest SR value (10) was in soil that had been replanted for 5 years at Weifang. AMF *H* values, however, were higher in the control soils than in the soils that had been replanted with watermelon (Table 4).

DNA extraction and amplification of AMF

Total DNA, which was successfully extracted from all soil samples, was brown before purification but colorless after purification. Through PCR amplification, the target AMF fragments (Fig. 1) were amplified from all soil samples.

DGGE analysis

The compositions of AMF communities in the replanted soils (from greenhouses) and control soils (from open fields) were compared by PCR–DGGE. DGGE profiles of the AMF community differed between Daxing and Weifang (Fig. 2). In both Daxing and Weifang, SR and *H* values were higher in the control soils than in the replanted soils (Fig. 3). SR values generally declined as the number of

Table 3 Frequency (*F*), relative abundance (RA), and importance value (IV) of AMF detected in greenhouse soil that had been continuously replanted with watermelon for 5, 10, 15, or 20 years

Species of AMF		Years of continuous watermelon monoculture							
		At Daxing				At Weifang			
		5	10	15	20	5	10	15	20
Acaulospora									
A. excavata	*F*	67	0	33	33	67	33	0	0
	RA	10	0	8	20	19	7	0	0
	IV	38	0	21	27	43	20	0	0
A. lacunosa	*F*	67	33	0	0	33	0	0	0
	RA	10	23	0	0	4	0	0	0
	IV	38	28	0	0	19	0	0	0
A. nicolsonii	F	0	0	33	0	33	0	0	33
	RA	0	0	8	0	2	0	0	25
	IV	0	0	21	0	18	0	0	29
A. rehmii	F	33	33	0	33	0	33	67	0
	RA	5	4	0	40	0	11	17	0
	IV	19	19	0	37	0	22	42	0
Glomus									
G. aggregatum	F	67	33	33	0	67	0	0	0
	RA	10	12	8	0	4	0	0	0
	IV	38	23	21	0	36	0	0	0
G. caledonium	F	0	0	0	0	0	33	0	0
	RA	0	0	0	0	0	7	0	0
	IV	0	0	0	0	0	20	0	0
G. claroideum	F	33	33	67	33	100	67	100	33
	RA	20	20	25	20	19	37	42	50
	IV	27	27	46	27	57[a]	52[a]	71[a]	42
G. clarum	F	33	0	0	0	67	0	33	0
	RA	10	0	0	0	11	0	8	0
	IV	22	0	0	0	39	0	21	0
G. constrictum	F	67	0	0	0	33	33	0	0
	RA	3	0	0	0	2	7	0	0
	IV	35	0	0	0	18	20	0	0
G. etunicatum	F	100	67	33	0	100	33	67	33
	RA	25	28	8	0	26	26	25	27
	IV	63[a]	47	21	0	63[a]	30	46	30
G. geosporum	F	0	0	0	33	0	0	0	0
	RA	0	0	0	20	0	0	0	0
	IV	0	0	0	27	0	0	0	0
G. mosseae	F	33	67	67	33	67	67	0	33
	RA	8	20	42	38	11	4	0	25
	IV	20	43	54[a]	36	39	35	0	29
Scutellospora									
S. dipapillosa	F	0	0	0	0	33	0	33	0
	RA	0	0	0	0	2	0	8	0
	IV	0	0	0	0	18	0	21	0

Data are based on spore morphology

[a] Dominant species. The greenhouse soils sampled (as indicated by the number of years in which watermelon was replanted at Daxing or Weifang) correspond to those listed in Table 1.

years with continuous watermelon production increased at both Daxing (Fig. 3A) and Weifang (Fig. 3B). In soil samples from Daxing, *H* values declined slowly or remained stable as the number of years with continuous watermelon production increased (Fig. 3A). In soil samples from Weifang, *H* values declined and then increased as the

Table 4 Spore density, species richness, and species diversity indices of AMF in soils at Daxing and Weifang

Sampling sites	Replanting years	Spore density (No./100 g soil)	Species richness	Shannon–Wiener indexes, *H*
Daxing	0	73a	16a	2.22a
	5	40b	9b	1.91b
	10	25c	6bc	1.71c
	15	12d	6bc	1.21d
	20	5e	4c	1.07e
Weifang	0	73a	17a	2.13a
	5	47b	10b	1.89b
	10	27c	7bc	1.65c
	15	12d	5c	1.20d
	20	4e	7bc	1.14d

Data are based on spore morphology. Means in a column followed by different letters are significantly different ($P \leq 0.05$). The greenhouse and control soils sampled (as indicated by the number of years in which watermelon was replanted and Daxing or Weifang) correspond to those listed in Table 1

number of years with continuous watermelon production increased (Fig. 3B).

Discussion

The results of the current study indicate that AMF abundance, species richness, and species diversity decline as greenhouse soil is repeatedly planted with watermelon in North China. Possible reasons for this decline include the lack of diversity of host plants and the relative stability of environmental conditions. Compared to the watermelon production system studied here, systems with natural vegetation are rich in plant species diversity and environmental complexity, and such natural systems therefore support high AMF diversity (Zhang et al. 1999; Liu and Chen 2007). Other factors that could contribute to reduced diversity with continuous monoculture of watermelon in greenhouses include the application of fungicides and other pesticides and the application of large quantities of fertilizers and especially phosphorus. High phosphorus can inhibit the growth, development, and functioning of AMF (Tawaraya et al. 1994).

As AMF species diversity indices declined over time in soil that was repeatedly planted with watermelon, particular species of AMF became dominant. The dominant AMF species in the greenhouse soils were *G. etunicatum*, *G. claroideum*, and *G. mosseae*, although which species was dominant tended to change over time. Determining whether these dominant species ameliorate soil quality and improve plant growth requires additional research. In another study, however, the AMF species that become dominant during crop monoculture were associated with depressed crop yields, perhaps because the dominant AMF suppressed more beneficial AMF species (Johnson et al. 1992).

Zhao et al. (2007) and Lou et al. (2007)reported that pH values decreased as greenhouse soils were repeatedly planted, but that was not the case in the current study. Perhaps the application of substantial quantities of organic fertilizer in the current study balanced the acidity produced by mineral fertilizers. The pH values of the soils were

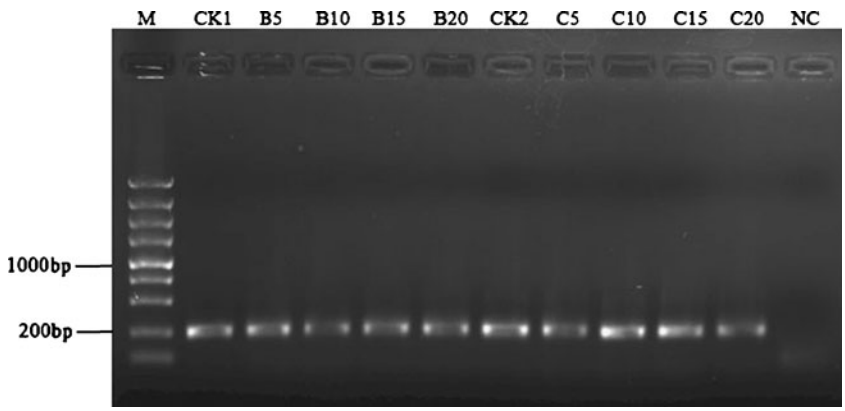

Fig. 1 PCR amplification of AMF fragments using primer pair NS31-GC and Glol. Sample codes are listed in Table 1. *M*=DS^{TM}5000 marker. NC (negative control) = PCR amplification when the template was replaced with water

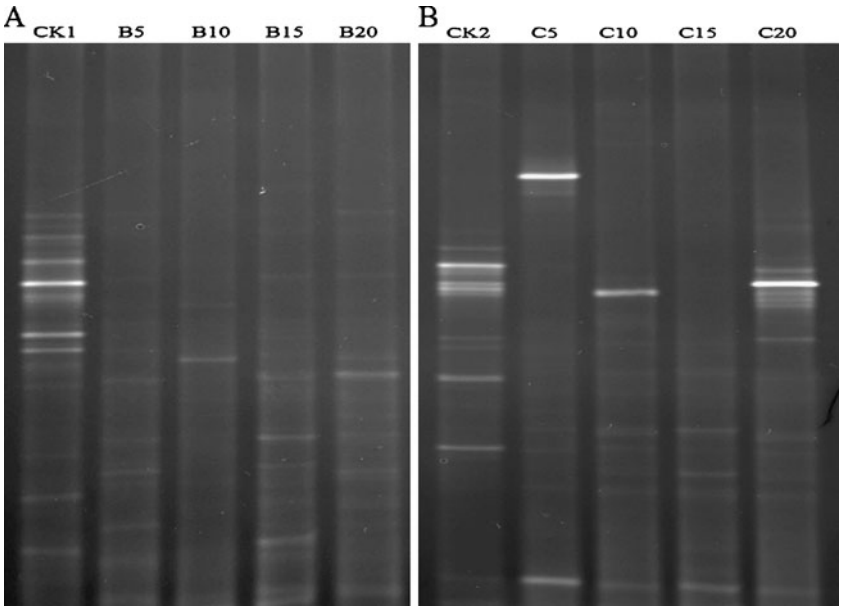

Fig. 2 DGGE analysis of AMF communities in soil samples from Daxing (**a**) and Weifang (**b**). Sample codes are listed in Table 1

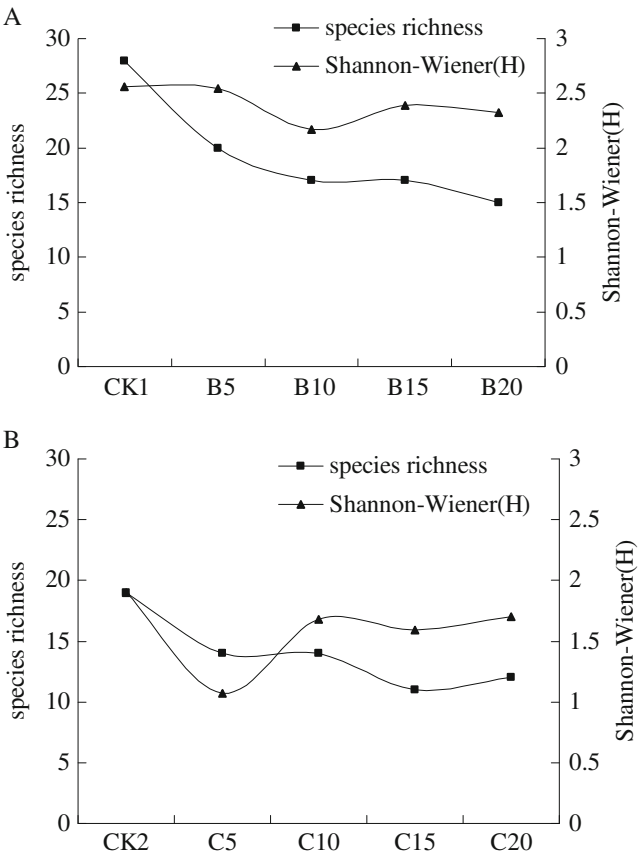

Fig. 3 Values for species richness and the Shannon–Wiener index of AMF in control soils (CK1 and CK2) that were not replanted with watermelon and in greenhouse soils that were continuously planted with watermelon for 5, 10, 15, or 20 years. Data are based on DGGE analysis. Sample codes on the *X* axis are listed in Table 1. Samples were from Daxing (**a**) or Weifang (**b**)

usually between 5.8 and 6.6, a range that favors *Glomus* (Porter et al. 1987; Wang et al. 1993). More acidic soils favor *Acaulospora* sporulation (Porter et al. 1987; Gai and Liu 2003).

The available phosphorus, potassium, and nitrogen increased with the increase in the number of years that a soil in the present study was continuously replanted with watermelon. It follows that spore density and species richness were negatively correlated with the available phosphorus, potassium, and nitrogen. The response of AMF to available phosphorus is variable (Jasper et al. 1989), and phosphorus application can either increase or decrease AMF spore production (Neumann and George 2004; Subramanian et al. 2006). As noted earlier, however, high phosphorus can inhibit AMF.

In conclusion, the results presented here indicate that in the greenhouse production system in North China, the replanting of a single crop (watermelon) year after year along with the application of large quantities of organic fertilizers reduces AMF diversity. These findings should be useful for understanding how to manage this greenhouse production system so that it is sustainable and productive. Future research is needed to determine whether sustainability and productivity in this system depend on high diversity and abundance of AMF fungi or on the abundance of particular species.

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References

Antunes PM, Koch AM, Dunfield KE, Hart MM, Downing A, Rillig MC, Klironomos JN (2009) Influence of commercial inoculation with *Glomus intraradices* on the structure and functioning of an AM fungal community from an agricultural site. Plant Soil 317:257–266

Borstler B, Renker C, Kahmen A, Buscot F (2006) Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. Biol Fertil Soils 42:286–298

Collier SC, Yarnes CT, Herman RP (2003) Mycorrhizal dependency of Chihuahuan desert plants is influenced by life history strategy and root morphology. J Arid Environ 55:223–229

Cornejo P, Azcón-Aguilar C, Barea JM, Ferrol N (2004) Temporal temperature gradient gel electrophoresis (TTGE) as a tool for the characterization of arbuscular mycorrhizal fungi. FEMS Microbiol Lett 241:265–270

Cuenca G, Andrade ZD, Lovera M, Fajardo L, Meneses E (2003) Mycorrhizal response of *Clusia pusilla* growing in two different soils in the field. Trees 17:200–206

Dalpe Y (1993) Vesicular–arbuscular mycorrhiza. In: Carter MR (ed) Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, pp 287–301

Gai JP, Liu RJ (2003) Effects of soil factors on AMF in the rhizosphere of wild plants. Chin J Appl Ecol 14:18–22 (in Chinese, with an English abstract)

Galván GA, Parádi I, Burger K, Baar J, Kuyper TW, Scholten OE, Kik C (2009) Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands. Mycorrhiza 19:317–328

Helgason T, Daniell TJ, Husband R, Fitter AH, Yong JPW (1998) Ploughing the word-wide-web. Nature 394:431

Jasper DA, Abbott LK, Robson AD (1989) Soil disturbance reduced the infectivity of external hyphae of vesicular–arbuscular mycorrhizal fungi. New Phytol 112:93–99

Johnson NC, Copeland PJ, Crookston RK, Pfleger FL (1992) Mycorrhiza: possible explanation for yield decline with continuous corn and soybean. Agron J 84:387–390

Kayal C, Higgs D, Kirnak H, Tas I (2003) Mycorrhizal colonisation improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. Plant Soil 253:287–292

Kowalchuk GA, Gerards S, Woldendorp JW (1997) Detection and characterization of fungal infections of *Ammophila arenaria* (marram grass) roots by denaturing gradient gel electrophoresis of specifically amplified 18 S rDNA. Appl Environ Microbiol 63:3858–3865

Li DC, Hua JM, Li ZP, Zhou X, Zhang TL, Velde B (2003) Changes in trace element contents in green-house soils and years of vegetable cultivation. Soils 35:495–499 (in Chinese, with an English abstract)

Li M, Liu RJ, Li XL (2004) Influences of arbuscular mycorrhizal fungi on growth and *Fusarium*-wilt disease of watermelon in field. Acta Phytopathol Sinica 34(5):472–473 (in Chinese, with an English abstract)

Li H, Sun AQ, Guo HJ (2010) Effects of different planting patterns on farmland soil quality in Yellow River alluvial plain of Shandong Province. Chinese J Appl Ecol 21:365–372 (in Chinese, with an English abstract)

Liu RJ, Chen YL (2007) Mycorrhizology. Science Press, Beijing (in Chinese)

Liu RJ, Jiao H, Li Y, Li M, Zhu XC (2009) Advances in the study of species diversity of arbuscular mycorrhizal fungi. Chinese J Appl Ecol 20:2301–2307 (in Chinese, with an English abstract)

Lou YL, Guan LZ, Wang LL, Ku KW, He L (2007) Changes of pH and enzyme activities in soils for different tobacco cropping years. Plant Nutr Fertilizer Sci 13:531–534 (in Chinese, with an English abstract)

Lugo MA, Ferrero M, Menoyo E, Estevez MC, Sineriz F, Anton A (2008) Arbuscular mycorrhizal fungi and rhizospheric bacteria diversity along an altitudinal gradient in south American puna grassland. Microb Ecol 55:705–713

Neumann E, George E (2004) Colonisation with the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) enhanced phosphorus uptake from dry soil in *Sorghum bicolor* (L.). Plant Soil 261:245–255

Peng J, Li Y, Shi P, Chen X, Lin H, Zhao B (2011) The differential behavior of arbuscular mycorrhizal fungi in interaction with *Astragalus sinicus* L. under salt stress. Mycorrhiza 21:27–33

Porter WM, Robson AD, Abbott LK (1987) Field survey of the distribution of vesicular- arbuscular mycorrhizal fungi in relation to soil pH. J Appl Ecol 24:659–662

Schenck NC, Perez Y (1988) Manual for the Identification of VA Mycorrhizal Fungi. Synergistic Publications, Gainesville, Florida

Schwarzott D, Schüßler A (2001) A simple and reliable method for SSU rRNA gene DNA extraction, amplification and cloning from single AM fungal spores. Mycorrhiza 10:203–207

Shi ZY, Diao ZK, Xu Q, Li M, Liu RJ (2006) Effects of media and arbuscular mycorrhizal fungi on growth and yield of watermelon. J Laiyang Agric College (Natural Science) 23(1):1–6

Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular–arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291–295

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Subramanian KS, Santhanakrishnan P, Balasubramanian P (2006) Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci Hortic 107:245–253

Sýkorová Z, Ineichen K, Wiemken A, Redecker D (2007) The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. Mycorrhiza 18:1–14

Tawaraya K, Saito M, Morioka M, Waqatsuma T (1994) Effect of phosphate application to arbuscular mycorrhizal onion on the development and succinate dehydrogenase activity of internal hyphae. Soil Sci Plant Nutr 40:667–673

Wang GM, Stribley DP, Tinker PB, Walker C (1993) Effects of pH on arbuscular mycorrhiza I. Field observations on the long-term liming experiments at Rothamsted and Woburn. New Phytol 124:465–472

Wang FY, Liu RJ, Lin XG, Zhou JM (2003) Comparison of diversity of arbuscular mycorrhizal fungi in different ecological environments. Acta Ecologica Sinica 23:2666–2671 (in Chinese, with an English abstract)

Wang MY, Diao ZK, Liang MX, Liu RJ (2005) Advances in the study of AM fungal diversity in agroecosystems. Acta Ecologica Sinica 25:2544–2549 (in Chinese, with an English abstract)

Yang JL, Zhang GL (2003) Quantitative relationship between land use and phosphorus discharge in subtropical hilly regions of China. Pedosphere 13:67–74

Yeates C, Gilling MR, Davison AD, Altavilla N, Veal DA (1998) Methods for microbial DNA extraction from soil for PCR amplification. Biol Proced Online 1:40–47

Zhang MQ, Wang YS, Xing LJ (1999) The relationship between the distribution of AM fungi and environmental factors. Mycosystema 18:25–29 (in Chinese, with an English abstract)

Zhao GX, Li XJ, Wang RY, Li T, Yue YD (2007) Soil nutrients in intensive agricultural areas with different land-use types in Qingzhou County, China. Pedosphere 17:165–171