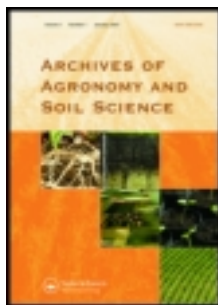


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## Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan

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A survey was conducted in 13 selected crops in the White Nile state, Central Sudan, to assess root colonization, spore densities and species richness of arbuscular mycorrhizal fungi (AMF) based on morphological aspects. For each crop, four samples were taken from one field. Additionally, an attempt was made to study the relationship between soil physico-chemical properties and AMF colonization rates, spore densities, species richness and other selected AMF diversity indices. The mean percentage of AMF colonization across all crops was 34%, ranging from 19% to 50%. The spore densities retrieved from the different rhizospheres were relatively high, varying from 344 to 1222 spores per 100 g dry soil with a mean of 798. There was no correlation between AMF spore densities in the soil and the root colonization rates. A total of 42 morphologically classifiable species representing 14 genera of AMF were detected without correlation between species richness in the soil and the spore densities. A hierarchical cluster analysis based on the similarity among AMF communities with respect to crop species overall showed that species compositions were relatively similar to the highest dissimilarities of about 25% dividing three of the four mango samples and the four sorghum samples from all other samples.

**Keywords:** AMF community; ecosystem; soil properties; spore extraction; AMF infection

### Introduction

As widespread and copious members of the soil biota, the obligately symbiotic arbuscular mycorrhizal fungi (AMF) are a generally acknowledged key factor in agricultural ecosystem functioning and sustainability (Verbruggen & Kiers 2010). The AMF have recently been placed into a new monophyletic phylum, the Glomeromycota (Schüßler et al. 2001), and they form symbiotic relationships with over 80% of all terrestrial plants (Brundrett 2002). The bidirectional exchange of nutrients between plants and AMF often results in a nutritional benefit for both partners. The host plant provides the fungus with carbohydrates, while in return the plant obtains rather immobile mineral nutrients such as phosphorus (P) from the fungus (Smith & Read 2008). AMF may also enhance host growth and survival by improving tolerance to drought (Yamato et al. 2009) and to some root pathogens and nematodes (Azcón-Aguilar & Barea 1997). They contribute to soil aggregate stability (Rillig & Mummey 2006) and may help in reducing salinity effects

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(Evelin et al. 2009). Lower AMF species richness has been found in arable fields, while species-rich AMF communities were detected in different natural ecosystems and perennial communities such as tropical forests (Snoeck et al. 2010). It has been suggested that the occurrence of AMF species could alter plant communities by influencing the relative abundance and diversity of plant species (van der Heijden et al. 1998).

Sudan is one of the largest countries in Africa, covering about 1.8 million km<sup>2</sup>. However, only about 15% of the land area is currently under cultivation due to limited water and P availability. Under such environmental conditions, symbioses between plants and AMF may be an important factor for plant adaptation and survival. However, there are almost no data available describing AMF occurrence and diversity in Sudan. Therefore, a survey was performed in 2009 in the White Nile state, Central Sudan, to assess AM root colonization and AMF spore densities and species richness in nine fields planted with 13 different important crop species. The White Nile state was selected for this study as it encompasses a high diversity of agro-ecologies and cropping systems. In addition, an attempt was made to study the relationship between soil physico-chemical properties and AMF colonization rates, spore densities and species richness and selected AMF diversity indices. The AMF species were identified and classified based on spore morphology.

## Materials and methods

### *Study sites and sampling*

The study area is located in the White Nile state in the centre of Sudan and about 300–320 km south of Khartoum. The region has a semi-arid climate with three main seasons, namely warm winter (October–February), a hot dry summer (March–June) and a hot rainy summer (July–September) with annual precipitation varying from 300 to 600 mm, with maximum rainfall peaks in August. The mean annual temperature is about 28°C with a maximum of 32.2°C in May and a minimum of 23°C in January.

Field soil samples were taken from Kosti province (western bank of the White Nile River), El Jelebein province and Rabak province, which are situated on the eastern bank of the White Nile River. The Calcaric Vertisol soils of the study sites have developed in a hot, semi-arid climate under a savanna/scatter acacia shrub vegetation (Robinson 1971), and the soil textures were classified as heavy montmorillonitic clay. The uppermost layers (0–30 cm) of the soils have a quite uniform brownish colour (10 R 4/2 to 4/3), containing about 60% clay and are moderately alkaline (pH 7.5–8.5). They are non-saline, non-sodic and characterized by a high cation exchange capacity (>50 cmol kg<sup>-1</sup>), low available P (<8 mg kg<sup>-1</sup>) and adequate K levels (>150 mg kg<sup>-1</sup>). The region accommodates both rainfed and irrigated farming. The major irrigated areas are confined along the branches of the White Nile River.

Root and soil samples were collected from 13 crops in May 2009 (Table 1). The mango, grapefruit, lemon and sweet orange trees, and the bananas were 3–4 years old. Mango and banana were at the flowering stage, while citrus were at the vegetative stage. The annuals Jews mallow, sorghum, sugarcane, mint and pigeon pea were growing vegetatively; onion and okra were flowering. Per crop, approximately 1–2 kg of soil (including roots) were collected from a depth of 0–30 cm using an auger and stored in the refrigerator until processing. There were four sampling points in each field near the field corners. At each sampling point, four 250 g subsamples were collected within about 5 m<sup>2</sup> each, mixed and pooled to produce composite soil samples. A total of 52 soil samples were thus collected. From these samples, the roots of the respective crop species were carefully freed from adhering soil and immediately fixed in 50% ethanol.

Table 1. Plant species and coordinates where sample were taken.

No	Common name	Scientific name	Family	Soil origin
Latitude	Banana	<i>Musa spp</i> L.	Musaceae	14° 54' 911" N 49° 85' 710" E
Longitude				
2	Grapefruit	<i>Citrus paradisi</i> L.	Rutaceae	14° 48' 718" N 49° 04' 738" E
3	Lemon	<i>Citrus limon</i> L.	Rutaceae	14° 48' 726" N 49° 21' 063" E
4	Sweet orange	<i>Citrus sinensis</i> L.	Rutaceae	14° 51' 367" N 49° 21' 063" E
5	Mango	<i>Mangifera indica</i> L.	Anacardiaceae	14° 42' 270" N 47° 83' 330" E
6	Jews mallow	<i>Corchorus olitorius</i> L.	Tiliaceae	14° 42' 647" N 47° 84' 373" E
7	Mint	<i>Mentha spp.</i> L.	Lamiaceae	14° 60' 132" N 46° 25' 513" E
8	Okra	<i>Abelmoschus esculentus</i> L.	Malvaceae	14° 60' 435" N 46° 39' 575" E
9	Onion	<i>Allium cepa</i> L.	Liliaceae	14° 40' 302" N 47° 99' 328" E
10	Sorghum forage	<i>Sorghum bicolor</i> L. var Abu Sabeen	Poaceae	14° 60' 426" N 46° 90' 210" E
11	Sugar cane	<i>Saccharum officinarum</i> L.	Poaceae	14° 49' 757" N 50° 02' 575" E
12	Alfalfa	<i>Medicago sativa</i> L.	Fabaceae	14° 56' 160" N 46° 43' 735" E
13	Pigeon pea	<i>Cajanus cajan</i> L.	Fabaceae	14° 59' 358" N 46° 38' 190" E

### Soil analysis

For physico-chemical analyses, soils were air-dried, ground and passed through a 2-mm mesh sieve. Particle size analysis was based on the gravimetric pipette-method (Day 1965), and textural classes were identified according to the United States Department of Agriculture (USDA) triangle system. Soil pH in a suspension 1:1 (soil:water) was determined according to McLean (1982). Electrical conductivity ( $\text{dS m}^{-1}$ ) was measured at room temperature in 1:5 w/v soil suspension using a conductivity meter. The cation exchange capacity (CEC) was estimated at pH 7 using ammonium acetate as extractant (Richards 1954). Oxidizable organic carbon (C) (%) was estimated according to Walkley (1947). Total organic carbon was obtained by multiplying oxidizable organic carbon with the recovery factor of 1.334. Subsequently, C rates were multiplied by 1.72 to obtain soil organic matter (Walkley 1947). Total N was measured by the Kjeldahl method (Bremner & Mulvaney 1982). Available P was determined according to the sodium bicarbonate method (Olsen et al. 1954). Hydraulic conductivity ( $\text{cm h}^{-1}$ ) was determined using positive head measurement. Soil analyses were performed at the laboratory of the Land and Water Research Center (LWRC), Agriculture Research Corporation (ARC), Wad Medani, Sudan.

### Staining and estimation of mycorrhizal root infection

Fixed roots in ethanol were rinsed repeatedly in tap water, cut into small segments (1 cm) and cleared at approximately 90°C in 10% KOH for 1 h; darker roots were bleached in 3% alkaline  $\text{H}_2\text{O}_2$  for 20 min (Kromanik & McGraw 1982). Thereafter, the roots were washed with tap water and stained with glycerol-trypan blue solution (0.05%) at 90°C for 30 min

(Phillips & Hayman 1970). Percentage of root length colonized by AMF was evaluated by the grid-line intersection methods described by Giovannetti and Mosse (1980).

### ***Extraction and estimation of arbuscular mycorrhizal fungal spores***

Spores were isolated from their substrate using a mix of wet sieving and sucrose gradient technique as described in Oehl et al. (2003). A sample of 25 g of air-dried field soil was mixed for 3 min with 100 ml of distilled water using a household blender. The resulting mixture was passed through 500, 250, 150 and 40  $\mu\text{m}$  sieves. The fraction retained in the 500- $\mu\text{m}$  sieve was checked for large spores, spore clusters, sporocarps and organic matter debris. Soil materials retained by the 250, 150 and 32  $\mu\text{m}$  sieves were recovered from each sieve, suspended in a water–sucrose solution (70% (w/v)) gradient and centrifuged at 484 g for 2 min. The supernatant was passed through the 32  $\mu\text{m}$  sieve, washed with tap water and transferred to new Petri dishes. Spores, spore clusters and sporocarps were recovered and counted at 40 $\times$  magnification. For identification, spores were picked under the dissecting microscope with a glass micropipette and subsequently mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or polyvinyl-lactic acid-glycerol mixed with Melzer's reagent (1:1 (v/v); Brundrett 2002) to get permanent slides for spore observation and identification under a compound microscope (Zeiss; Axioplan) at up to 400 $\times$  magnification. Taxonomic identification was based on identification manuals (Schenck & Perez 1990), on species descriptions since 1990 and on own analyses of about 95% of the type specimens deposited in the public mycological herbaria (Oehl et al. 2008, 2011b). Classification follows Oehl et al. (2011a).

### ***Ecological AMF diversity indices***

To determine differences in the structure of the AMF communities on different crops, the following parameters were calculated: The isolation frequency (IF) of occurrence was calculated as the percentage of samples in which a genus or species occurred among all samples, and it reflects the distribution status. Relative spore density (RD) was defined as the ratio between the spore densities of a particular genus or species to the total AMF spore densities and it shows the degree of sporulation ability of different AMF in a given soil. The importance value (IV) was used to evaluate the dominance of AMF species based on IF and RD and was calculated as  $IV = (IF + RD)/2$ . An  $IV \geq 50\%$  indicates that a genus or species is dominant;  $10\% < IV < 50\%$  applies to common genera or species; an  $IV \leq 10\%$  indicates that a genus or species is rare (Chen et al. 2012). Diversity indices were computed using statistical package 'Diversity Excel add-in' of the University of Reading (SCC 2012). Species richness ( $S$ ) is the number of species present in a community; the Shannon–Weaver index ( $H'$ ) of diversity was calculated with the formula:  $H' = -\sum [(n_i/n) \ln (n_i/n)]$ , where  $n_i$  is the number of individuals of species  $i$  and  $n$  is the number of all individuals of all species; Simpson's dominance index ( $D$ ) was calculated with the formula  $D = \sum (n_i/n)^2$ ; Evenness ( $E$ ) was calculated by dividing Shannon diversity by the logarithm of the species richness.

### ***Statistical analysis***

Data on spore densities and root colonization rates were logarithmically and arcsine transformed, respectively, to meet assumptions of the Pearson correlation. Pearson correlation analysis was used to detect the relationship between spore densities 100  $\text{g}^{-1}$  soil,

percent root colonization and AMF species richness using SAS 9.1 statistical package (SAS Institute Inc., Cary, NC, USA). A cluster analysis was run to construct a dendrogram showing similarity among AMF communities from different host plants. The grouping was done with the Ward's group linkage using squared Euclidean distance making use of the software SPSS, version 18.0.

## Results

### *Soil analyses*

The physico-chemical soil analyses are summarized in Table 2. The pH was uniformly moderately alkaline, ranging from 7.9 to 8, and electrical conductivity (EC 1:5) values ranged from 0.2 to 0.7 dS m<sup>-1</sup> indicating that salinity effects were mostly negligible (data not shown). However, cation exchange capacity (CEC) values varied from 36 to 67 cmol kg<sup>-1</sup> indicating that the soil has a high resistance to changes in soil chemistry, while total amount of nitrogen (N) and available P contents were low. Organic carbon present in the soil was adequate. The C:N ratio ranged from 10 to 19 indicating that the decomposition of organic matter under the given environmental conditions was rather slow (Table 2).

### *AMF root colonization and spore density in the soil*

All root samples surveyed in this study were colonized by AMF. The mean percentage of AMF colonization was 34%, ranging from 19% to 50% (Figure 1(a)). Percent AMF root colonization was highest in roots collected from jews mallow (50%) and lowest in okra (19%).

The spore densities (expressed as per 100 g dry soil) retrieved from different rhizosphere crops were relatively high, varying from 344 on jews mallow to 1222 on sorghum with a mean of 798 (Figure 1(b)). There was no correlation between spore densities in the soil and the root colonization rates (data not shown).

### *AMF species richness and diversity*

A total of 1745 AMF spores were identified and classified from the 52 rhizosphere soil samples. A total of 42 morphologically classifiable species representing eight families and 13 genera of AMF were detected with no correlation between the species richness found in a soil sample and the spore density (data not shown). The most abundant family and the most abundant genus were Glomeraceae (19 species) and *Glomus* (14 species), respectively. All other families and genera were represented by one to six and one to three species, respectively (Table 3). The RDs of AMF ranged from 0.1% to 12.6%, the IF from 1.9% to 98%, and the importance values (IVs) ranged from 0.99 to 55.3 (Table 3). RD was positively correlated with IF ( $r = 0.85$ ,  $P < 0.001$ ) (data not shown).

With respect to crop species, the genera *Claroideoglomus*, *Glomus*, *Funneliformis* and *Paraglomus* were encountered in almost all crops. The genus *Kuklospora* was recovered only in sorghum (Figure 2). *Glomus intraradices* was the most frequently occurring AMF species (98.1%) with an RD of 12.6%, followed by *Cl. etunicatum* (IF = 92.3%, RD = 12.0), *Paraglomus occultum* (IF 80.1%, RD 9.0), *Glomus* sp NIL31 resembling *Gl. aureum* (IF 76.9%, RD 13.6%), *Fu. mosseae* (IF 69.2%, RD 6.9%), *Cl. claroideum* (IF 55.8%, RD 3.6%) and *Septoglomus constrictum* (IF 53.9%, RD 3.5) (Table 3).

Table 2. Chemical and physical soil characteristics of the surveyed fields in White Nile state, Sudan (means per field based on four samples are shown).

Site	Crops	Chemical							Physical			
		pH	CaCO <sub>3</sub> g kg <sup>-1</sup>	CEC cmol kg <sup>-1</sup>	P mg kg <sup>-1</sup>	OC g kg <sup>-1</sup>	C/N	N g kg <sup>-1</sup>	HC cm h <sup>-1</sup>	Sand	Silt	Clay
Kenana A1	Banana	8	57	67	3	13.0	15	0.90	0.81	7	27	66
Kenana A2	Grapfruit	8	19	58	6	12.3	13	0.96	0.47	12	24	64
Kenana A2	Lemon	8	19	61	7	11.5	11	1.13	0.46	13	25	62
Elmansori	Mango	8	24	53	8	10.4	10	1.11	1.22	22	24	54
Kenana A4	Orange	8	23	60	8	10.8	14	0.79	0.46	12	25	63
Elmansori	Jews Mallow	8	38	58	4	12.1	19	0.65	0.49	7	25	69
Abaisland	Mint	8	80	36	3	9.4	12	0.82	1.33	38	10	52
Assalayia	Okra	8	80	36	2	13.3	10	1.39	1.70	42	13	45
ElMakhada	Onion	8	19	52	3	8.5	13	0.64	0.48	5	26	69
Assalayia	Sorghum	8	97	36	2	12.5	15	0.84	2.43	46	9	45
Kenana A3	Sugarcane	8	86	63	3	13.4	16	0.85	0.85	7	25	69
Kosti	Alfalfa	8	73	67	6	8.3	10	1.00	0.45	9	21	70
Abaisland	Pigeon pea	8	76	54	2	11.9	14	0.87	0.62	16	18	66
	Min	8	19	36	2	8.3	10	0.64	0.45	5	9	45
	Max	8	97	67	8	13.4	19	1.39	2.43	46	27	70
	Mean	8	53	54	4	11	13	1	0.9	18	21	61
	Sd±	0.2	29	11.3	2.3	1.7	2.7	0.2	0.6	14.4	6.2	9

Note: CaCO<sub>3</sub>, calcium carbonate; CEC, cation exchange capacity; P, available phosphorus; OC, organic carbon; C:N, carbon to nitrogen ratio; N, total nitrogen; HC, hydraulic conductivity.



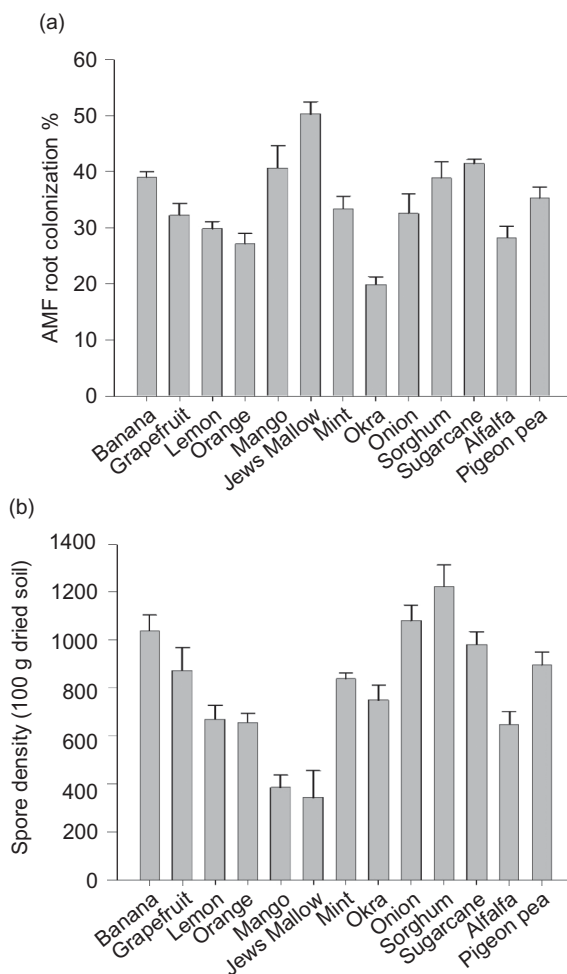


Figure 1. (a) Average AMF root colonization rates (%) on different host plant species by AMF; (b) Mean spore density 100 g<sup>-1</sup> air-dried soil from different host plant species. Means of four samples are shown. Error bars are standard error of the mean.

The AMF diversity expressed by the Shannon–Weaver index was highest in sorghum ( $H' = 2.27$ ) and Jews mallow ( $H' = 2.13$ ) and lowest in alfalfa ( $H' = 1.4$ ). AMF species evenness ranged between 0.7 and 1.0 with a mean of 0.9. The least even distribution was in mint, the highest in forage sorghum. Species dominance ( $D$ ) was in parallel with evenness and highest in Jews mallow ( $D = 0.92$ ), followed by sorghum ( $D = 0.89$ ) and lowest in mint ( $D = 0.67$ ) (Figure 3).

#### **Relationship between soil physico-chemical properties, crops and parameters of AMF diversity**

Total N levels in the soils varied among crops from a mean of 0.6 to 1.4 g kg<sup>-1</sup> soil but varied relatively little within crop (Figure 4(a)). The apparent decrease of the percentage root colonization with increasing N levels in the soil can thus not be interpreted. C:N

Table 3. Absolute number of spores identified per species, relative spore density, isolation frequency and importance value of arbuscular mycorrhizal fungi (AMF) identified from 52 soil samples from 13 crops in Central Sudan.

AMF species	Site <sup>a</sup> where found	S <sup>b</sup>	RD% <sup>c</sup>	IF% <sup>d</sup>	IV% <sup>e</sup>
<b>Acaulosporaceae</b>					
<i>Acaulospora morrowiae</i>	5, 6, 8, 10, 11,	15	0.85	15.38	8.12
<i>Ac. sieverdingii</i>	4	1	0.06	1.92	0.99
<i>Acaulospora</i> sp. NIL3 resembling <i>Ac. spinosa</i>	1, 4, 7, 8, 9, 11	25	1.42	17.31	9.36
<i>Kuklospora kentinensis</i>	10, 11	6	0.34	3.85	2.09
<b>Ambisporaceae</b>					
<i>Ambispora gerdemannii</i>	1, 3, 4	15	0.85	13.46	7.16
<i>Ambispora</i> sp NIL6	2	2	0.11	1.92	1.02
<i>Ambispora</i> sp NIL7	2, 5, 10	13	0.74	11.54	6.14
<b>Archaeosporaceae</b>					
<i>Archaeospora trappei</i>	5, 6, 9	3	0.17	5.77	2.97
<i>Archaeospora</i> sp	6, 11	2	0.11	1.92	1.02
<i>Archaeospora</i> sp NIL10	2, 4, 10, 11	10	0.57	13.46	7.01
<b>Diversisporaceae</b>					
<i>Diversispora aurantia</i>	11	1	0.06	1.92	0.99
<i>Diversispora eburnea</i>	2, 13	5	0.28	4.77	2.06
<i>Diversispora</i> sp NIL13	5	1	0.06	1.77	0.99
<b>Entrophosporaceae</b>					
<i>Claroideoglo mus claroideum</i>	1, 2, 3, 4, 5, 6, 10, 13	63	3.57	55.77	29.67
<i>Cl. etunicatum</i>	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13	177	10.04	92.31	51.17
<i>Cl. sp</i> NIL16 resembling <i>Cl. luteum</i>	5, 9, 11	69	3.91	15.38	9.65
<i>Entrophospora infrequens</i>	13	1	0.06	1.92	0.99
<i>Entrophospora</i> sp NIL18, resembling <i>En. nnevadensis</i>	5, 10	3	0.17	3.85	2.01
<i>Entrophospora</i> sp NIL19	9, 10, 11	15	0.85	15.38	8.12
<b>Glomeraceae</b>					
<i>Funneliformis coronatus</i>	3, 7	7	0.40	9.62	5.01
<i>Fu. geosporus</i>	5, 10	14	0.79	19.23	10.01
<i>Fu. mosseae</i>	1, 2, 3, 4, 5, 6, 7, 9, 11, 12	122	6.92	71.15	39.04
<i>Glomus aggregatum</i>	10, 11	5	0.28	1.92	1.10
<i>Gl. ambisporum</i>	1, 5	8	0.45	9.62	5.03
<i>Gl. diaphanum</i>	2, 5, 6, 9	16	0.91	7.69	4.30
<i>Gl. fasciculatum</i>	9, 13	11	0.62	11.54	6.08
<i>Gl. intraradices</i>	1, 2, 3, 5, 6, 9, 11, 12, 13	222	12.59	98.08	55.33
<i>Gl. liquidambaris</i>	4, 10, 11	15	0.85	1.92	1.39
<i>Gl. sinuosum</i>	1, 2	208	11.80	26.92	19.36
<i>Glomus</i> sp OMAN5	5, 6, 10, 11, 12, 13	50	2.84	44.23	23.53
<i>Glomus</i> sp. NIL31 resembling <i>Gl. aureum</i>	1, 2, 3, 4, 5, 6, 7, 9, 13	240	13.61	76.92	45.27
<i>Glomus</i> sp. NIL32 resembling <i>Gl. coreomioides</i>	1, 7, 10, 11, 12	20	1.13	1.92	1.53
<i>Glomus</i> sp. NIL33 resembling <i>Gl. spinuliferum</i>	4, 8, 9, 13	11	0.62	9.62	5.12
<i>Glomus</i> sp. NIL34 resembling <i>Gl. tortuosum</i>	6, 11	19	1.08	13.46	7.27
<i>Glomus</i> sp. NIL35	1, 9, 10	23	1.30	23.08	12.19
<i>Glomus</i> sp NIL36 closely resembling <i>Gl. taiwanense</i>	4	10	0.57	1.92	1.25
<i>Septoglo mus constrictum</i>	1, 2, 3, 5, 6, 7	61	3.46	53.85	28.65

(continued)

Table 3. (Continued).

AMF species	Site <sup>a</sup> where found	S <sup>b</sup>	RD% <sup>c</sup>	IF% <sup>d</sup>	IV% <sup>e</sup>
<i>Simiglomus hoi</i>	1, 2, 3	32	1.82	13.46	7.64
<b>Pacisporaceae</b>					
<i>Pacispora</i> sp. NIL40	2, 6, 9	16	0.91	19.23	10.07
<b>Paraglomeraceae</b>					
<i>Paraglomus bolivianum</i>	10	1	0.06	1.92	0.99
<i>Pa. occultum</i>	1, 2, 3, 4, 5, 6, 7, 9, 12, 13	158	8.96	80.77	44.87
<i>Paraglomus</i> sp. NIL42	1, 5, 6	63	3.57	23.08	13.33
<b>Total AMF species richness:</b>		1763	100%		
<b>42 species</b>					

Notes: <sup>a</sup>Sites (species) at which the species were found. For site numbers, see Table 1.

<sup>b</sup>S = Absolute number of spores identified per species.

<sup>c</sup>RD = Relative spore density.

<sup>d</sup>IF = Isolation frequency.

<sup>e</sup>IV = Importance value.

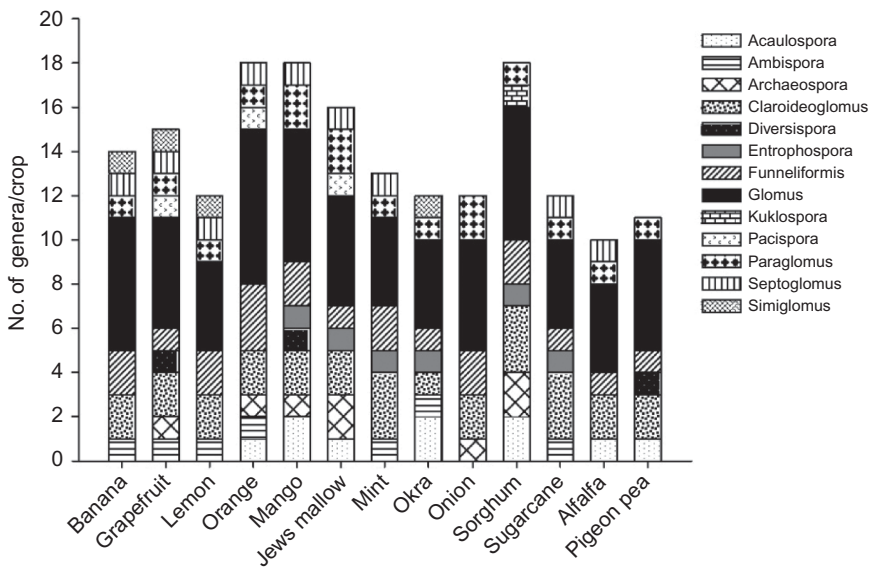


Figure 2. Glomeromycota genera occurring in different crops in Sudan.

ratios were almost inverse to the N levels in soils, i.e. with increasing N levels the C:N ratio decreased (data not shown). While N levels were highest in the Okra field and lowest in Jews mallow, C:N ratios were lowest in Okra and highest in Jews mallow.

In contrast to the total N levels, available P levels were more variable within and among fields. Especially in some of the perennial crops, banana, grapefruit, lemon, mango and alfalfa the variation was large (Figure 4(b)). The spore densities in the soil in these crops were rather uniform, however. For example, in lemon, P availability in the samples varied five-fold, ranging from 2 to 10 mg kg<sup>-1</sup>, while spore density varied only between 528 and 784 per 100 g of soil. For banana, available P ranged from 1 to 10 mg kg<sup>-1</sup> and spore density was about 900–1200 per 100 g. Thus, the apparent negative correlation between available P level and AMF spore density in the soil could be an artefact and may

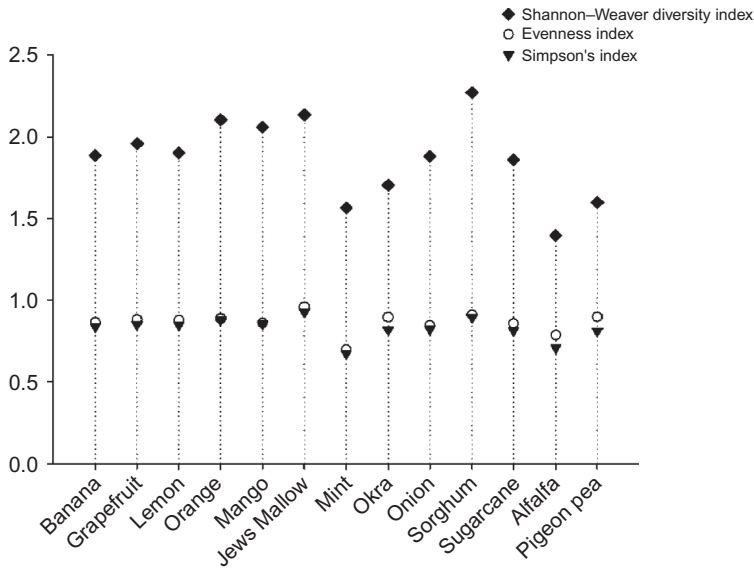


Figure 3. Ecological diversity measures used to describe AMF communities collected from different host plant species.

be overridden by the influence of the host crop. Similarly, it was not possible to determine meaningful relationships among other soil physico-chemical parameters and AMF communities.

The hierarchical cluster analysis based on the similarity among AMF communities with respect to the crop species overall showed that species compositions were relatively similar with the highest dissimilarity of about 25% dividing three of the mango samples and the four sorghum samples from all other samples (Figure 5). Otherwise no clear cluster emerged according to crop species or species group.

## Discussion

Overall, a total of 42 AMF fungal species were detected from the 52 soil samples representing 14 genera of AMF. This is an astonishingly high number, as only about 250 species have been classified worldwide for the phylum *Glomeromycota* (Oehl et al. 2011a). In general, relative spore density was found to be positively correlated with IF.

Despite sometimes large variability in soil environment, especially P availability for banana, lemon, grapefruit and mango, the spore density in the soil was very uniform for these crops. This finding supports the idea that crops do influence AMF community.

The relatively high spore densities of 344–1222 in 100 g rhizosphere soil in our study sites could be due to the prevalent hot and arid environment throughout the year. It is known that AM spore density is influenced by temperature (Cardoso et al. 2003) and high sporulation may be a result of stress in the study area. In the present study, samplings were carried out in the dry summer season (19–29 May 2009) when increased spore densities can be anticipated (Verbruggen & Kiers 2010).

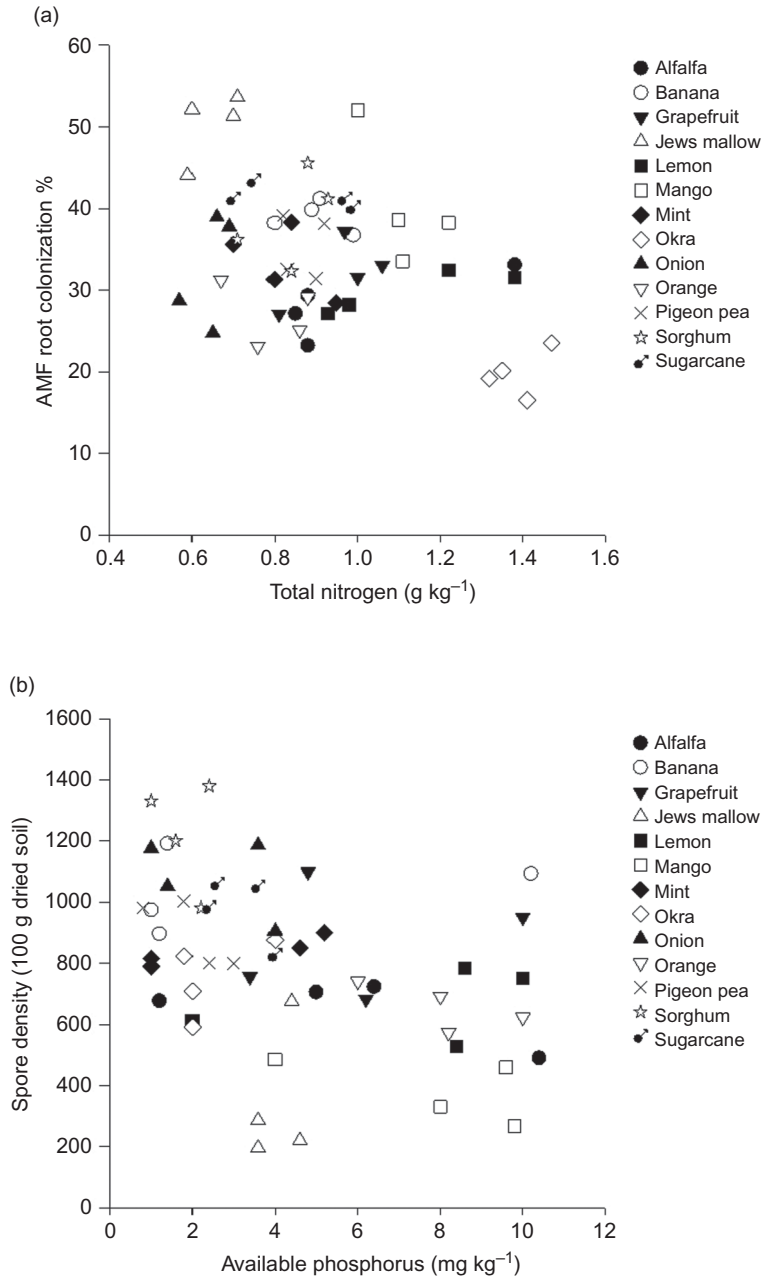


Figure 4. (a) AMF root colonization (%) on 13 different crops as influenced by total N in the soil and (b) AMF spore density on these crops as influenced by available P.

The highest spore densities were recorded in rhizosphere soils of forage sorghum, followed by onion and banana. This could possibly be attributed to the differences in rooting habits between monocots and dicots (Douds et al. 2005).

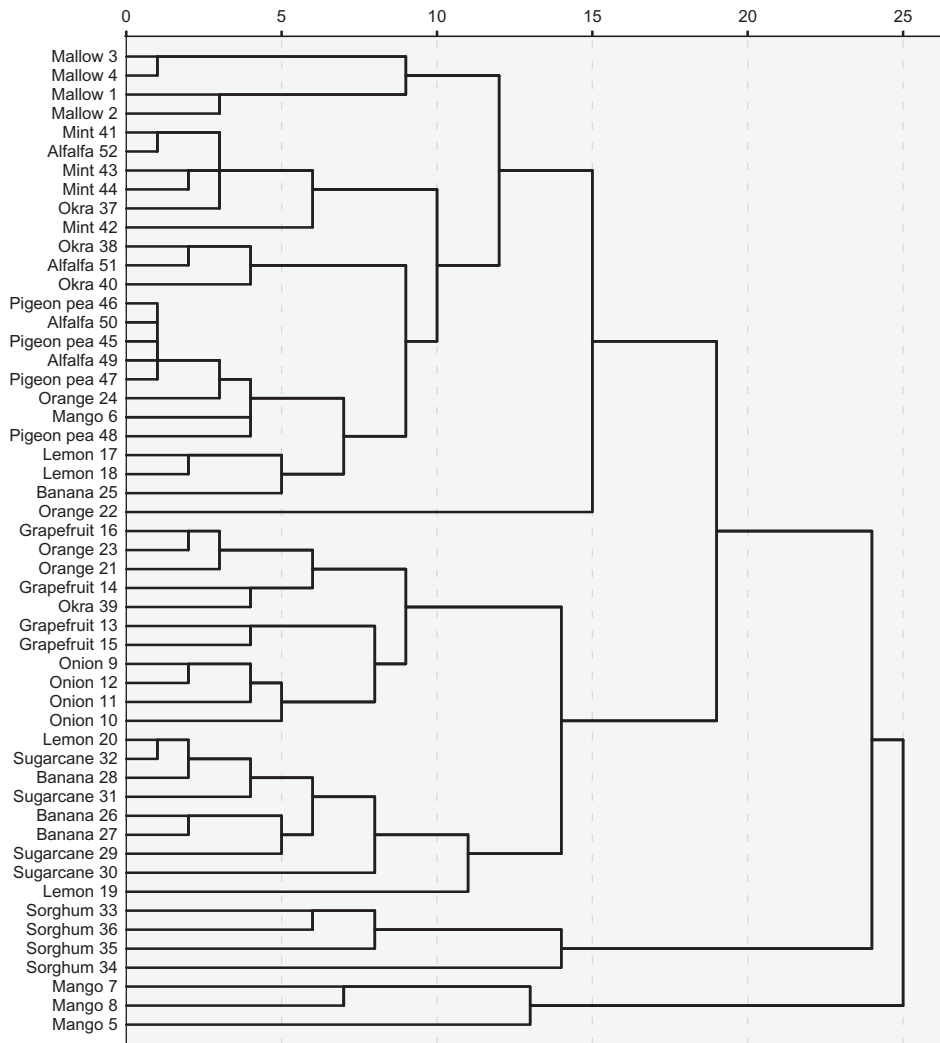


Figure 5. Dendrogram of the cluster analysis based on the similarity of AMF species composition in 52 soil samples taken from 13 different crops (Ward linkage rescaled distance cluster).

Although high levels of AMF colonization rates appeared to be associated with lower spore densities, for example, Jews mallow and mango, no clear correlation between spore density and root colonization existed. Lack of correlation could be due to the fact that there are AMF species that rely more on extensive formation of hyphal networks instead of survival through spore formation as primary infective propagules (Biermann & Linderman 1983).

In this study, there was a prominent divergence in the proportional distribution of AMF genera among crop species. Spores from all orders of *Glomeromycota* were found, except Gigasporales (including *Gigaspora* and *Scutellospora*). The absence of Gigasporales might be due to the soil disturbance associated with cultivation practices, especially soil tillage and fertilization. This has been shown by others before Muthukumar and Udaiyan (2002); Johnson (1993) reported that long-term fertilization (8 years)

resulted in a significant reduction in relative spore densities of *Gigaspora* as well as *Scutellospora* with a concomitant proliferation in *Glomus intraradices*. Furthermore, it has been reported that the spore densities of *Gigaspora* and *Scutellospora* species were reduced upon conventional tillage and disturbance (Boddington & Dodd 2000) and *Gigasporaceae* have been shown to rely solely on spores as their primary infective propagules. This might be the reason why the *Gigasporales* cannot cope well with disturbance. Moreover, soil texture might also play a key role for the occurrence of *Gigasporales* spp. in tropical soils (Lekberg et al. 2007). These authors found species of this order abundantly in sandy soils but not in clayey Vertisols that per definition modify their soil aggregates permanently through repeated expanding and shrinking processes that might harshly affect the hyphal networks of *Gigasporales* (De la Providencia et al. 2005).

The genus *Glomus* was the most dominant and widely distributed followed by *Acaulospora*. This is not surprising since it was previously shown that *Glomus* species are most abundant among the glomeromycotan genera in tropical areas (Snoeck et al. 2010), regardless of the type and intensity of disturbance in the different ecosystems. Moreover, *Glomus* species have the potential to produce a relatively high number of spores within a very short period of time (Oehl et al. 2009). Douds and Schenck (1990) reported that *Glomus intraradices* has been found to be less sensitive to P and N fertilization, suggesting that the intracortical sporulation by *G. intraradices* may reduce the path of carbon flux and could contribute to the relative insensitivity of this species to fertilization. However, the possibility of involvement of more subtle interactions related to site and time of sampling cannot be ruled out.

Species richness was highest on sorghum followed by mango and orange, the lowest number of species was observed in alfalfa. Similarly, it has been shown that AMF community composition depends on host plant species and, therefore, plant species may have varying degrees of selectivity on AMF species that range from selective specialists to non-selective generalists (Oehl et al. 2003; Scheublin et al. 2004). The low number of AMF species in alfalfa as well as pigeon pea could be related to the fact that nodulation in legumes may systematically suppress AMF root colonization via alteration of root exudates and C allocation strategy (Catford et al. 2003).

## Conclusion

Overall, the results of the study indicate that AMF abundance and diversity are high in the area studied with *Glomus* as the most common genus in all surveyed crops. The relationship between soil physico-chemical parameters and AMF communities remains unclear; however, while it appears that plant species play a role in regulating mycorrhizal colonization and spore density this would have to be verified by a more extensive study as well as, the role of crop rotations and soil management on AMF communities.

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