

# Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol

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## Abstract

Arbuscular mycorrhizal fungi (AMF) represent a functionally important component of soil microbial community, being of particular significance for plant mineral nutrition in tropical agroecosystems. The effects of crop rotation of maize (*Zea mays* L.) with crotalaria (*Crotalaria grahamiana* Wight & Arn.) versus continuous maize and phosphorus (P) fertilization on AMF spore community composition and diversity were studied in a long term field experiment in western Kenya. The spores were isolated from the soil, identified according to their morphologies, and enumerated. Trap pots using soil from the maize–crotalaria rotation were sown with four different plant species, sunflower (*Helianthus annuus* (L.) Merill.), leek (*Allium porrum* L.), maize, and crotalaria. The spores isolated from the traps were identified according to their morphology and by sequencing of their large ribosomal subunits (LSU). Ten AMF species were isolated from the field soil and 16 species from the traps. Altogether, 18 species were recorded in the field site. The spore communities in the field soil were dominated by *Scutellospora* and *Acaulospora* species. The species diversity of AMF spores in the soil was affected neither by crop rotation nor by P fertilization. However, the composition (relative species abundances) of AMF spore communities was significantly affected by crop rotation. The abundance of *Acaulospora scrobiculata* and *Scutellospora verrucosa* spores was significantly higher in soil under maize–crotalaria rotation than under continuous maize. Both the composition and diversity of spore communities, as well as spore densities in the traps were strongly affected by species identity of the host plant, whereas the P fertilization history of the soil only affected diversity of the spore communities. Functional consequences of changing composition of AMF communities through agricultural management practices are discussed.

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**Keywords:** Crop rotation; Crotalaria; Maize; Phosphorus fertilization; Spores; Trap pots

## 1. Introduction

Continuous cropping with inadequate external inputs has caused depletion of nutrients such as phosphorus (P) and nitrogen (N) in African soils (Smithson and Giller, 2002; Bünemann et al., 2004c). Improved fallows are currently being adopted for rapid replenishment of soil fertility (Sanchez, 1999). It has been shown that introduction of leguminous fallows for a season into a conventional system of continuous maize (*Zea mays* L.) cultivation increases

microbial diversity and biomass (Bünemann et al., 2004a; Bossio et al., 2005). A substantial part of microbial communities in the soil belongs to the arbuscular mycorrhizal fungi (AMF), an ancient group of fungi that establishes mutualistic symbiosis with a great majority of plant species (Leake et al., 2004). They play an important role in P uptake and growth of many cereals, legumes, and other crop plants (Sieverding, 1990; George et al., 1995). This process of enhancing P absorption by plants appears to be particularly important in highly weathered, fine textured, and acid tropical soils, where great proportions of applied P fertilizer are not available to plants due to strong fixation of P on iron and aluminium oxides (Jama et al., 1997; Bünemann et al., 2004c).

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Agricultural management practices might affect AMF communities both qualitatively and quantitatively (Sieverding, 1990; Miller et al., 1995). This was documented in previous studies showing that crop rotation, fertilization, and tillage affect the composition and diversity of AMF communities as well as spore and mycelium densities in temperate and tropical agroecosystems (Sieverding, 1990; Jansa et al., 2002; Oehl et al., 2003). Information about species composition of AMF community appears important to understand mycorrhizal function in agroecosystems (Johnson and Pfleger, 1992). Traditionally, studies aiming at characterizing AMF communities in field soil employ spore surveying, which is sometimes complemented by trap culturing (Douds et al., 1993; Jansa et al., 2002; Oehl et al., 2004). These spore-based surveys are considered to be a baseline for assessing the impact of agricultural practices on the AMF communities (Douds and Millner, 1999). However, it has become clear that morphological characterization of the AMF spore community and its diversity might not reflect the actual functional symbiosis that refers to active fungal structures within and outside roots (Clapp et al., 1995; Jansa et al., 2003). These active structures such as fungal hyphae and arbuscules in the roots and the soil can only be properly identified by means of molecular or immunological approaches (Treseder and Allen, 2002; Redecker et al., 2003; Sanders, 2004), which may require calibration for each specific field site (Jansa et al., 2003). Therefore, the application of such tools is heavily constrained in fields with no preliminary information available.

This study was aimed at characterization of the effects exerted by crop rotation of maize with a leguminous shrub *Crotalaria grahamiana* Wight & Arn. (crotalaria) and P fertilization on indigenous AMF communities in a long term field experiment in western Kenya. Observations of AMF spores directly isolated from the field soil as well as trap culturing with four different plant species were employed. Spore morphology surveys were complemented by large ribosomal subunit (LSU) sequencing from monospecific AMF cultures.

## 2. Materials and methods

### 2.1. Site and experimental design

The field experiment was conducted between March 1997 and January 2002 in Central Kisa, Butere-Mumias District, in western Kenya (0°09'N, 34°33'E). The experiment was situated at an elevation of 1485 m on a farmer's field with a history of maize cultivation without mineral fertilization. Mean annual rainfall at this site was 1705 mm. The rain falls in two rainy seasons between March and May and between October and November. This results in two growing seasons, the long rainy seasons between March and August and the (less reliable) short rainy season from September through February. The soil type at this site was a Ferralsol (FAO

classification) containing 39% clay, 24% silt, and 37% sand in the top 15 cm, with pH (H<sub>2</sub>O) of 5.0. The experimental design was a randomized complete block with four replications. Two crop rotation treatments, continuous maize (COM) and maize–crotalaria fallow rotation (MCF) were studied at two levels of P fertilization, supplying either none (P–) or 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> (P+) in the form of triple superphosphate at the beginning of each long rainy season. At the same time, all plots received KCl application of 100 kg K ha<sup>-1</sup> yr<sup>-1</sup>. Plot size was 6 m × 12 m during the first two seasons and 6 m × 6 m thereafter, because the plots were split before long rains 1998 in order to study an intermediate P fertilization level, which was not included in this study. At the beginning of each long rainy season, the soils were manually tilled down to 15 cm. Fertilizers were incorporated into the top 2 cm by manual harrowing and maize was sown between mid-March and mid-April at 0.75 m × 0.25 m spacing. One to 2 months later, crotalaria was sown between maize rows at the spacing of 0.75 m × 0.50 m. Maize was harvested from all plots in August and all maize residues were removed so as to minimize termite attraction to the fields. COM plots were then tilled and planted with maize between end August and mid-September. Maize plots were harvested in January and the fallows were cut in February or March. Crotalaria wood was then removed from the MCF plots and the litter was left in the fields. Maize plots were manually weeded one to three times per seasons, as necessary. Lowest weed biomass was recorded in COM during 2 out of 3 monitored long rainy seasons, indicating that the crotalaria fallow did not reduce weed pressure in the field experiment. Pesticides to control stalk borer and termites were applied once per season. The maize yield forgone during growth of the crotalaria was compensated by higher post-fallow yields, but the cumulative total yield of maize in MCF (14.6 Mg ha<sup>-1</sup> during 5 yr) was not significantly different from the COM (12.4 Mg). In both COM and MCF, P fertilization doubled total maize yields, increased N removal by maize from the field by 60% and 49% (COM and MCF, respectively) as compared to the respective P–plots, and remained without effect on amounts of recycled biomass. P fertilization significantly increased both total P content of the soil (720 and 835 mg kg<sup>-1</sup> in P– and P+ soil, respectively, as of January 2000) and P availability in top 15 cm (resin-extractable P being 1.7 and 6.6 mg kg<sup>-1</sup> in P– and P+ soil, respectively, as of January 2000). Mineral N availability (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> combined) remained unaffected (18.3 mg kg<sup>-1</sup>) by the P fertilization. Total P content of the soil remained unaffected, whereas P availability was sometimes slightly (but significantly) affected by crop rotation: For example, resin-extractable P being 1.2 and 0.9 mg kg<sup>-1</sup> in COM and MCF (both P–) soils, respectively, as of January 2002. Higher levels of available N (21.9 mg kg<sup>-1</sup>) were encountered under MCF than under COM (15.5 mg kg<sup>-1</sup>). Significantly higher levels of soil organic and microbial carbon (C), N, and P were found under MCF than under COM, indicating higher microbial activity under the crotalaria rotation treatment. Further details about the field experiment

and soil properties can be found elsewhere (Bünemann et al., 2004b,c).

## 2.2. Soil sampling and AMF spores in field soil

Soil was sampled in January 2002 from both P– and P+ plots under both COM and MCF treatments. At the time of soil sampling, the maize in COM fields had been harvested and fallows were still standing. Fifteen soil cores were randomly collected from the depth 0–15 cm from each experimental plot and pooled to obtain one composite sample per plot. The samples were sieved (4 mm) to remove plant debris and stored at 4 °C during transport to the lab. The AMF spores were isolated from 50 g (fresh weight) soil samples using wet sieving and sucrose density gradient centrifugation method (Jansa et al., 2002). The soil was washed through 500 and 40 µm sieves with tap water. The materials collected on the 40 µm sieve were blended at high speed for 5 s in Warring blender to release AMF spores from the mycelium. The suspensions were then washed again on 40 µm sieve and transferred into 50 ml centrifuge tubes and brought to a final volume of 20 ml with distilled water. Fifteen millilitre of 2.5 M sucrose solution were then slowly added to the tubes and centrifuged at  $1000 \times g$  for 5 min. Viable spores (settled at transition between sucrose solution and water) were washed on a 40 µm sieve, transferred to Petri dishes, and observed using a stereomicroscope (Olympus SZX12). Different spore types (according to size, color, opacity, and shape of hyphal attachment) were enumerated. A 5–10 spores of each spore type were mounted in polyvinyl-lacto-glycerol (PVLG) and PVLG-Melzer reagent (1:1, v:v) on a glass slide; the spores in Melzer reagent were crushed in order to observe staining of different layers of spore wall. Spores were examined using a compound microscope (Olympus AX70) with Nomarski optics. The spore morphological features were compared with original descriptions of AMF species (Schenck and Perez, 1990) and with the online reference culture database published at <http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm>. Then, names were assigned to each spore type. Soil humidity was estimated after drying soil samples at 105 °C for 24 h.

## 2.3. Trap pots

Field soil from both the P+ and P– plots under MCF rotation was used as inoculum for trap pots. Unsterile field soil from each of the plots was mixed with autoclaved quartz sand (grain size 0.7–1.2 mm) and calcinated Montmorillonite (grain size 0.3–0.7 mm, Oil Dri Chem-Sorb IIIIR, Maagtechnic, Dübendorf, Switzerland) in a ratio of 1:2:2 (v:v:v). This mixture is referred to as substrate throughout the rest of this paper. The substrate was used to fill 800 ml pots (approximately 750 g substrate dry weight per pot). Sixteen pots were prepared with substrate from each of the eight field plots. The pots were each planted with one of the following plant

species: crotalaria, leek (*Allium porrum* L. cv. Dubouchet), maize cv. Corso, and dwarf sunflower (*Helianthus annuus* (L.) Merrill. cv. Gelber Knirps). Four replicate pots were established per treatment. The plants were grown for a total of five months in a growth chamber (Conviron PGV36, Winnipeg, Canada) under following conditions: photoperiod 16 h, 28/18 °C, 65/80% relative aerial humidity (day/night, respectively), photosynthetically active radiation flux during daytime was  $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Since maize and sunflower completed their life cycle within 3 months, their shoots were removed and the pots (with the substrate left undisturbed) were replanted with the same plant species. Pots were watered to approx. 60% substrate water holding capacity with deionized water using tensiometer-controlled irrigation units (Blumat, Austria) and fertilized once every two week with 25 ml of eightfold concentrated Hoagland nutrient solution (Sylvia and Hubbell, 1986) with low (80 µM) P concentration. Plant shoots were removed from all pots after 5 months of cultivation, substrate was removed from the pots and mixed, and AMF spores were isolated and identified from 20 g (fresh weight) of substrate from each trap pot as described above. Abundances of spores of each AMF species were estimated on the following semi-quantitative scale: (0) not detected, (1) 1–5 spores, (2) 6–20 spores, (3) 21–50 spores, (4) 51–200 spores, and (5) 201–500 spores per sample. AMF spores from the pots were further used to establish monospecific cultures using leek as a plant host. For this, one to ten AMF spores of the same AMF species obtained from the trap pots were placed on the germination root of a leek seedling, which was then grown for 6 months in a pot filled with a sterile substrate. Details of sterile substrate preparation can be found elsewhere (Jansa et al., 2002).

## 2.4. Molecular identification

Spores from both monospecific cultures and the trap pots were used for sequencing of part of the large ribosomal subunit (LSU) in order to further confirm species identification based on spore morphology. Four spores were collected from each of five AMF species successfully subcultured in monospecific cultures (*Gigaspora gigantea*, *Scutellospora nigra*, *S. verrucosa*, *Acaulospora mellea*, and *Glomus microaggregatum*) along with three species from the trap pots (*Scutellospora heterogama*, *Acaulospora denticulata*, and *A. scrobiculata*). DNA was isolated separately from each single spore according to Jansa et al. (2002) with a few modifications given below. Spores were crushed by a flamed glass pipette tip in 10 µl of Tris-HCl (100 mM, pH 8.0), heated at 95 °C for 1 min and then cooled on ice. Five microlitre of the extract were directly used as template for a nested polymerase chain reaction (PCR) with eukaryotic specific primers (ITS3 and NDL22) and then with fungal specific primers (LR1 and FLR2) as described previously (Jansa et al., 2003). PCR product of the second PCR (700–760 bp long, depending on the AMF species) was purified from the gel with a Gel extraction kit (Qiagen AG,

Hombrechtikon, Switzerland), ligated into a pDrive cloning vector (Qiagen), heat-shock transformed into DH5 $\alpha$  competent cells, and selected on ampicillin media (100 mg l<sup>-1</sup>). Plasmids containing inserts were isolated from overnight cultures by using a plasmid purification kit (Qiagen) and sequenced at Microsynth (Balgach, Switzerland). Five clones per AMF species were sequenced. All sequences were manually edited and blasted against the GenBank sequence database (<http://www.ncbi.nlm.nih.gov>) to ensure affinities with glomalean sequences. The sequences successfully read in full length were deposited in the Genbank under accession numbers AY900494 through AY900517. Those sequences were aligned with a selection of sequences previously published in the Genbank by using Clustal X (Version 1.83), and used for construction of a phylogenetic tree.

### 2.5. Calculations and statistical analysis

Spore abundances estimated on a fresh weight basis of field soil were adjusted to dry weight using the soil humidity values. Spore abundance classes in the trap pots were replaced with class means. AMF spore densities in each sample were calculated by summing abundances of all species recorded in the sample. Species richness was calculated as a number of species recorded in each sample. Shannon–Wiener diversity index ( $H'$ ) was calculated for each field sample/trap pot using the Eq. (1), where  $p_i$  was the relative spore abundance of the  $i$ -th species among all  $N$  identified species in a sample.

$$H' = -\sum_{i=1}^N p_i \times \ln(p_i) \quad (1)$$

Two- and one-way analyses of variance (ANOVAs) were performed in Statgraphics (Version 3.1) so as to assess the effects of crop rotation and P fertilization in the field as well as the effects of soil P fertilization history and trap plant species identity in trap pots on AMF spore densities, species richness and diversity. These ANOVAs were performed with nontransformed data after ensuring conformity of the data with ANOVA assumptions. Following significant ANOVAs, differences between treatment means were analyzed by multiple range comparison based on least-significant difference (LSD). Further, the effects of crop rotation and P fertilization in the field as well as the effects of soil P fertilization history and trap plant species identity in the traps were assessed on AMF spore community composition (relative abundances of the AMF species) by a multivariate redundancy analysis (RDA) in CANOCO (Version 4.5). Upon identification of factors, or their interactions, which significantly affected community composition of the AMF spores, the data were further analyzed by nonparametric Kruskal–Wallis test because significant deviations from ANOVA assumptions were noted in the datasets. Differences between treatment means

were analyzed as above. Mean values with accompanying standard errors of means are reported unless specified otherwise. Significance of results refer to  $p < 0.05$ , unless specified otherwise.

## 3. Results

### 3.1. Spore densities in the field soil and in the traps

In field soil, low density of AMF spores was generally observed ( $0.94 \pm 0.08$  per g dry weight soil). Spore densities were affected neither by crop rotation ( $p = 0.14$ ) nor by P fertilization ( $p = 0.34$ ) nor by interaction of the two above factors ( $p = 0.31$ ). In trap pots, a mean of  $7.40 \pm 0.71$  spores per g fresh weight substrate was found. The spore densities were significantly affected by plant species identity in the trap pots ( $p < 0.001$ ), but not by P fertilization of field soil used for setting up the traps ( $p = 0.76$ ) nor by the interaction of plant species and P fertilization of the soil ( $p = 0.96$ ). Significantly more spores were found under crotalaria ( $14.2 \pm 1.75$  per g fresh weight substrate) than under any other plant species in the traps. Maize in trap pots supported significantly higher spore densities ( $8.97 \pm 1.16$  g<sup>-1</sup>) than sunflower ( $3.66 \pm 0.89$  g<sup>-1</sup>) or leek ( $2.75 \pm 0.54$  g<sup>-1</sup>).

### 3.2. Identification of AMF species

Identification based on spore morphologies indicated presence of 10 AMF species in the field soil and 16 species in the trap pots (see [electronic supplementary materials for photos](#)). Altogether, 18 AMF species were recorded either in the field soil or in the traps (Table 1). Some of these species were not detected in field soils, but were found exclusively in the trap pots (*Acaulospora lacunosa*, *A. laevis*, *Archaeospora trappei*, *Glomus lamellosum*, *G. microaggregatum*, *Scutellopora heterogama*, *S. pellucida*, and *S. persica*). Spores of two species (*Glomus fasciculatum* and *Scutellopora dipurpurascens*) previously observed in field soil could not be detected in the traps. DNA sequences of six out of eight AMF species subjected to PCR and cloning of LSU showed similarities with glomalean sequences and were thus included into the phylogenetic analysis. All sequences from spores of *A. denticulata* and *A. scrobiculata* proved to belong to asco and basidiomycetous fungi and, therefore, were excluded from the phylogenetic analysis. The LSU from any of the six AMF species from which we obtained the sequence data has never been sequenced before (or sequences are not yet available in public databases). Therefore, species affiliations were not possible to compare with other datasets, although affinities of the sequenced AMF species to their genera could be broadly confirmed (see [electronic supplementary materials for the phylogram](#)). The LSU sequences from spores of *S. nigra*, *S. heterogama*, *S. verrucosa*, and *G. gigantea* clustered together with



Table 1  
AMF species recorded in the Kenyan ferralsol

Species	P+ <sup>a</sup> , COM <sup>b</sup>	P+, MCF <sup>c</sup>	P– <sup>d</sup> , COM	P–, MCF
<i>Acaulospora denticulata</i> Sieverding & Toro	F <sup>e</sup>	F, T <sup>f</sup>		F, T
<i>Acaulospora lacunosa</i> Morton		T		T
<i>Acaulospora laevis</i> Gerdemann & Trappe		T		T
<i>Acaulospora mellea</i> Spain & Schenck	F	F, T	F	F, T
<i>Acaulospora scrobiculata</i> Trappe	F	F, T	F	F, T
<i>Acaulospora spinosa</i> Walker & Trappe	F	F, T	F	T
<i>Archaeospora trappei</i> (Ames & Linderman) Morton & Redecker		T		T
<i>Gigaspora gigantea</i> (Nicol. & Gerd.) Gerd. & Trappe	F	F, T	F	F, T
<i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker & Koske	F	F		F
<i>Glomus lamellosum</i> Dalpe, Koske & Tews		T		T
<i>Glomus microaggregatum</i> Koske, Gemma & Olexia		T		T
<i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders	F	T	F	F, T
<i>Scutellospora heterogama</i> (Nicol. & Gerdemann) Walker & Sanders		T		T
<i>Scutellospora nigra</i> (Redhead) Walker & Sanders	F	F, T	F	F, T
<i>Scutellospora dipurpurascens</i> Morton & Koske			F	
<i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders		T		
<i>Scutellospora persica</i> (Koske & Walker) Walker & Sanders		T		T
<i>Scutellospora verrucosa</i> (Koske & Walker) Walker & Sanders	F	F, T	F	F, T

<sup>a</sup> Soil receiving phosphorus (P) fertilization (50 kg P ha<sup>-1</sup> yr<sup>-1</sup>).

<sup>b</sup> Continuous maize cropping.

<sup>c</sup> Maize–crotalaria fallow rotation.

<sup>d</sup> Soil receiving no P fertilization.

<sup>e</sup> Detected in field soil.

<sup>f</sup> Detected in trap pots.

previously published sequences of other *Scutellospora* and *Gigaspora* spp. Sequences from spores of *A. mellea* clustered together with published sequence of *Acaulospora laevis* and sequences of *G. microaggregatum* showed affinity to published sequences of *Glomus intraradices* (see electronic supplementary materials for the phylogram).

### 3.3. AMF species richness and diversity

The species richness of AMF spore communities in the field soils (mean value  $5.19 \pm 0.34$ ) was not affected by either crop rotation ( $p = 0.87$ ), P fertilization ( $p = 0.42$ ), or by their interaction ( $p = 0.87$ ). Likewise, the diversity of the spore communities (mean value  $1.35 \pm 0.06$ ) was unaffected by crop rotation, P fertilization of the field soil and the interaction of the two factors ( $p = 0.44, 0.22, \text{ and } 0.96$ , respectively). Species richness of AMF spore communities in trap pots was significantly affected by plant species identity ( $p < 0.001$ ), but only marginally by P fertilization of the field soil used for setting up the pot cultures ( $p = 0.059$ ). The interaction between plant species identity and P fertilization of the field soil was also significant ( $p = 0.006$ ). The richness of AMF spore communities was significantly higher under crotalaria and maize ( $4.75 \pm 0.27$  and  $4.56 \pm 0.18$ , respectively) than under sunflower and leek ( $3.13 \pm 0.26$  and  $2.88 \pm 0.25$ , respectively). The interaction between P fertilization of field soil and plant species identity in trap pots was significant because the species richness of AMF spore communities under leek plants was lower in previously fertilized than in the

unfertilized soil ( $1.94 \pm 1.66$  and  $3.81 \pm 0.70$ , respectively,  $p < 0.001$ ), whereas the richness was unaffected by P fertilization of the field soil under other plant species. Diversity of AMF spore communities was significantly affected by both plant species identity ( $p < 0.001$ ) and P fertilization of the soil used for establishment of trap pots ( $p = 0.008$ ) as well as by their interaction ( $p < 0.001$ ). The diversity of AMF spore communities in trap pots was consistently higher in previously unfertilized than in P fertilized soils ( $1.05 \pm 0.05$  and  $0.87 \pm 0.06$ , respectively). The diversity was higher under crotalaria and maize ( $1.16 \pm 0.08$  and  $1.15 \pm 0.05$ , respectively) than under sunflower and leek ( $0.79 \pm 0.08$  and  $0.73 \pm 0.08$ , respectively). The interaction between P fertilization of field soil and plant species identity in trap pots was significant because of significantly different AMF diversity in pots with previously P unfertilized and fertilized soil planted with leek ( $1.09 \pm 0.07$  and  $0.38 \pm 0.09$ , respectively). At the same time, there were no differences in AMF diversity with respect to previous soil P fertilization under any other plant species.

### 3.4. AMF community composition

The AMF communities both in the field soil and in trap pots were dominated by non-*Glomus* genera such as *Scutellospora*, *Acaulospora*, and *Gigaspora* (Fig. 1). AMF community composition (relative abundances of the different AMF species) in the field soil was significantly affected by crop rotation, which explained 20.1% of the

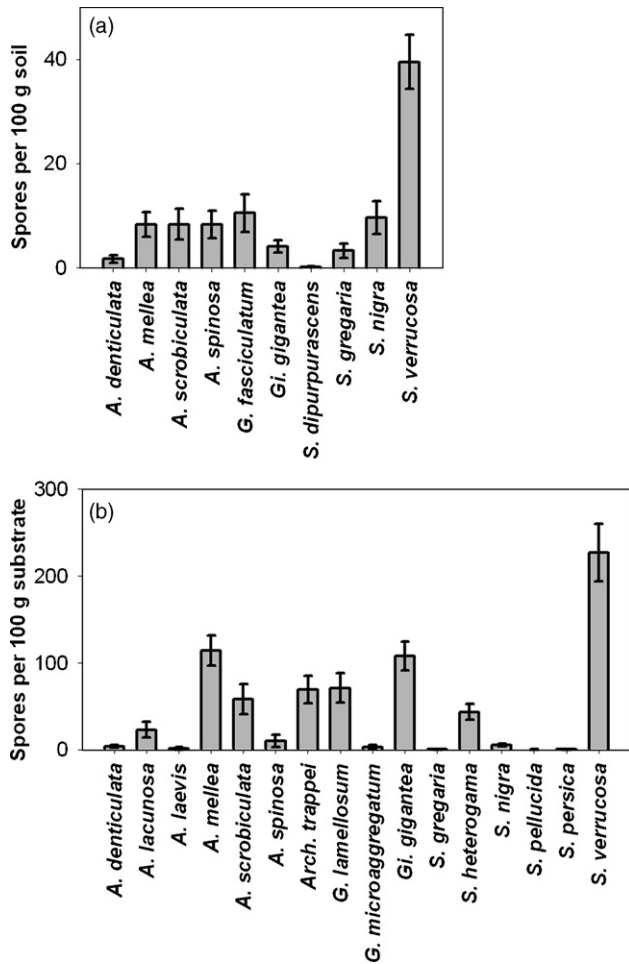


Fig. 1. Composition of AMF community in (a) field soil and (b) trap cultures. Grand means and associated standard errors of means are shown for 16 and 128 replicates, respectively. Spore abundances are expressed on a dry weight basis for field soil and on a fresh weight basis for trap pot substrate.

variability in the dataset (RDA,  $F = 3.52$ ,  $p = 0.005$ ; Fig. 2). The composition of AMF community was not affected by P fertilization (RDA,  $F = 0.91$ ,  $p = 0.48$ ) or the interaction between soil fertilization and crop rotation (RDA,  $F = 1.56$ ,  $p = 0.18$ ). The abundance of spores of both *Acaulospora scrobiculata* and *Scutellospora verrucosa* were higher in MCF than COM soils ( $p = 0.037$  and  $0.021$ , respectively;

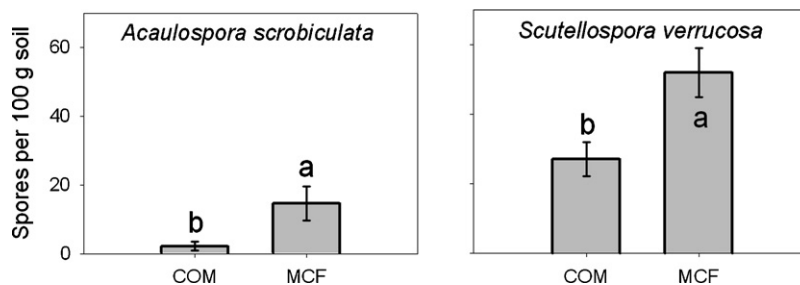


Fig. 3. Abundances of spores of two AMF species in the field soil as affected by crop rotation (continuous maize, COM and maize–crotalaria rotation, MCF). Spore abundances are expressed on a dry weight basis of the soil. Means and standard errors of means are shown for eight replicates. Different letters indicate significant differences between the means as determined by LSD multiple range comparison ( $p < 0.05$ ).

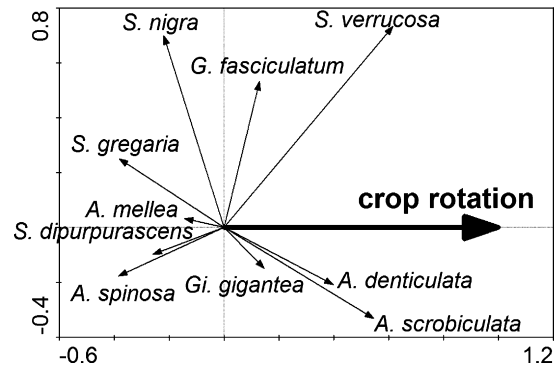


Fig. 2. Effect of crop rotation on the community composition (relative species abundances) of the AMF in the field soil. Results of redundancy analysis are shown using the spore abundances of the different AMF species. Size and orientation of the vectors represents correlation among them and with the axes. The smaller the angle between the vectors (or a vector and an axis) and the longer the vectors, the more correlated are the variables represented by the vectors. Axis 1 represents the effect of the crop rotation (a gradient from continuous maize to maize–crotalaria rotation), axis 2 is the most explanatory axis pooling all other environmental effects on composition of the AMF community.

Fig. 3). The community composition of AMF in the trap pots was not affected by P fertilization history of the soil used for establishment of the pots (RDA,  $F = 1.62$ ,  $p = 0.14$ ). However, it was significantly affected by the identity of plant species in the pots (RDA,  $F = 6.37$ ,  $p < 0.001$ ; Fig. 4), which explained 13.3% of variability in the dataset. Abundances of 7 out of 16 of the AMF species were affected by the identity of plant species in the trap pots (Fig. 5). The significance of interaction between the identity of crop plant species (four levels, coded as four dummy variables) and P fertilization of the field soil was not possible to test directly because of software limitations. The significance of the interaction between P fertilization and plant species identity was thus tested within all possible pairs of species, where plant species identity could be coded as one dummy variable (values 0 and 1 for absence and presence of a particular species). This set of analyses did not indicate any significant interaction between the identity of plant species and P fertilization of the field soil on the composition of AMF spore community in trap pots (analyses not shown).

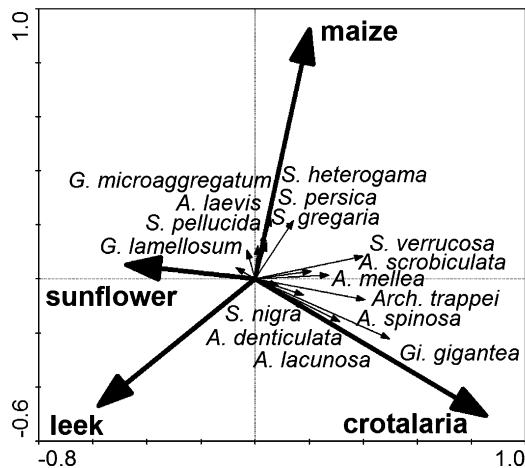


Fig. 4. Effect of identity of plant species in trap pots on the community composition (relative species abundances) of the AMF in the substrate. Results of redundancy analysis are shown using the spore abundances of the different AMF species. Vectors representing different plant species are shown in bold. Size and orientation of the vectors represent correlation among them and with the axes.

## 4. Discussion

### 4.1. Identification of the AMF

Different approaches were followed in this study to identify AMF isolated from the field experimental area. Observation of fresh AMF spores after trapping in pot cultures and subsequent LSU sequencing from monospecific cultures gives much greater confidence in the results from the field, where spore identification is often difficult due to low density, age, and destruction by predators and/or parasites (Jansa et al., 2002; Landis et al., 2004). The density of AMF spores was several times higher in our traps compared to the field soil, indicating an important advantage of trap culturing in stimulating AMF sporulation. We are aware of ongoing discussion about the AMF species concept and its relevance for phylogenetic and functional diversities within this fungal group (Schüßler et al., 2001; Sanders, 2004). Given the lack of a sound and generally accepted species concept and well recognized limitations of alternative (PCR-based) strategies for assessing AMF diversity in natural ecosystems (Redecker et al., 2003; Sanders, 2004), we consider our approach to be a justified method of choice for uncovering the effects of agricultural management practices on soil AMF communities.

### 4.2. AMF in different agroecosystems

The total of 18 recorded AMF species in this study (Table 1) is similar to the results of previous studies from the temperate zone, where Bever et al. (1996), Franke-Snyder et al. (2001), Jansa et al. (2002), Oehl et al. (2003), and Oehl et al. (2004) reported species richness of 23, 15, 17, 25, and 35 in single field sites, respectively. These results all indicate

generally higher species richness of AMF communities in agricultural soils than previously believed (Johnson, 1993; Helgason et al., 1998; Daniell et al., 2001). One of the reasons for these different outcomes is certainly the sampling intensity in the different studies (Morton et al., 1995). On the other hand, different identification approaches are likely to contribute to the differences among the different studies. This is because PCR-based approaches may inadvertently miss or underestimate presence of some species simply because of their rarity or absence of conserved priming sites (Redecker et al., 2003). Spore surveys from field soil alone may also underestimate presence of some AMF species that do not frequently sporulate under given environmental conditions (Sanders, 2004). This advocates for employing more than one approach when aiming at a thorough description of AMF diversity and species occurrence at a field site.

We showed here that AMF communities in tropical ferralsol under simple crop rotation were not dominated by *Glomus* spp. This finding was rather unexpected because several other studies from temperate zone (Central Europe and the USA) were all showing a dominance of *Glomus* spp. in agricultural soils (Helgason et al., 1998; Franke-Snyder et al., 2001; Jansa et al., 2002; Oehl et al., 2003). Likewise, AMF spore surveys in tropical soils in Venezuela and Indonesia indicated absence of genera such as *Gigaspora* and *Scutellospora* upon soil disturbance imposed either through agricultural use or by heavy landscaping machinery. The AMF communities in those disturbed soils were dominated by *Glomus*, *Acaulospora*, and *Entrophospora* spp. (Cuenca et al., 1998; Boddington and Dodd, 2000). AMF species surveys from Africa comparable with our study are scarce. One study from Senegal indicated presence of diverse AMF communities in sand dunes (including *Scutellospora* and *Acaulospora* spp.), but the communities were still dominated by *Glomus* spp. (Diallo et al., 1999). Likewise, AMF communities in the Namibian desert were exclusively composed of *Glomus* and *Acaulospora* spp. (Stutz et al., 2000).

As expected, not all of the AMF species, whose spores were observed in the field soil, could also be found in the trap pots. Additionally, some species not observed in the field soil were detected in the traps (see Table 1). Occurrence of additional AMF species in the traps is a well documented phenomenon, justifying the use of trap cultures for more complete AMF surveys than direct isolation of spores from the field soils (Brundrett et al., 1999; Jansa et al., 2002; Oehl et al., 2004). Given the different environmental conditions in trap pots in comparison to the fields, some of the AMF rarely sporulating in the field soil might start forming spores in the pots. This was probably the case of 8 out of 18 AMF species in this study, which were recorded exclusively in the trap pots. Similarly high proportions of additional species appearing exclusively in the trap cultures were reported in other studies. For example, Jansa et al. (2002) reported 3 out of 17 and Oehl et al. (2004) reported 14 out of 30 AMF

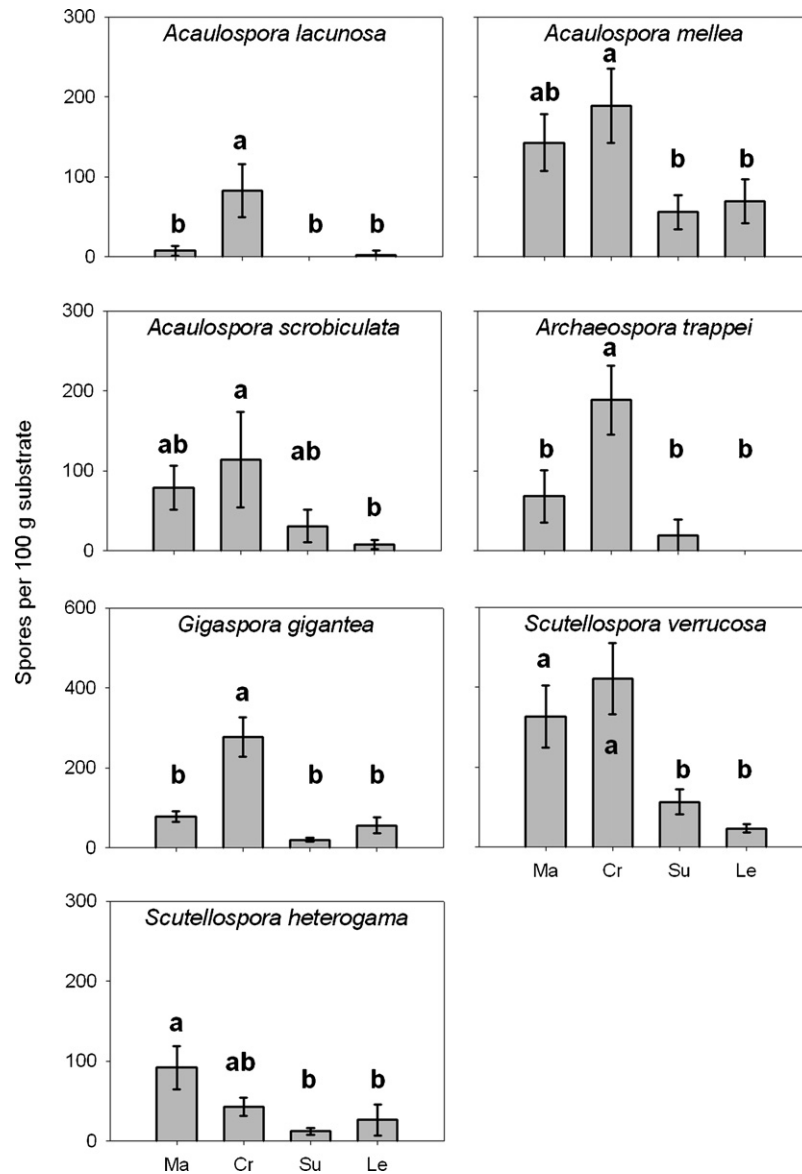


Fig. 5. Abundances of seven AMF species in trap pots as affected by the identity of plant species in the pots (maize, Ma; crotalaria, Cr; sunflower, Su; leek, Le). Spore abundances are expressed on a fresh weight basis of the substrate. Means and standard errors of means are shown for 32 replicates. Different letters indicate significant differences between the means as determined by LSD multiple range comparison ( $p < 0.05$ ).

species, which could only be detected in their trap pots and not in the original field soil. On the other hand, some AMF species frequently forming spores in the field soil may not be detected in the traps either because the conditions in the pots are less favorable for their sporulation or because those species are outcompeted by others (Brundrett et al., 1999). In this study, this was probably the case of the two AMF species, *G. fasciculatum* and *S. dipurpurascens*, both of them exclusively detected in the field soil and not in the traps. Similarly, Oehl et al. (2004) reported three out of 35 species recorded at a single field site failing to produce spores in the traps. It has, however, been noted previously that relative species abundances and diversity of AMF communities in the field soil may be quite different from those in the traps because of different environmental

conditions and different composition of the plant cover (Bever et al., 1996; Jansa et al., 2002). Therefore, the measures of diversity estimated in the pots must be treated cautiously as they are of limited relevance to the field situation (but could be used for comparison of different treatments of the trap pots).

#### 4.3. Impact of crop rotation

Our results revealed that crop rotation of maize with crotalaria significantly affected AMF spore community composition compared to maize monocropping. The crop rotation had, however, no significant influence on the density and species diversity of AMF spore communities in the field soil. The shifts in species composition and sometimes in



diversity of both soil microbial and AMF communities due to incorporation of legumes into the crop rotation with cereals have been reported before (Lupwayi et al., 1998; Alvey et al., 2003; Oehl et al., 2003). The reasons for the microbial shifts are primarily due to the different amounts and qualities of organic C inputs to the soil (either root exudates or litter), as well as soil temperatures and moisture dynamics in the differently cropped soil. In addition to the above factors, the effects on AMF are likely driven by preferential associations between certain plant and AMF species (Dhillion, 1992; Hendrix et al., 1995; Bever, 2002). Specifically, preferential association of legumes versus non-leguminous plants with different AMF species was recently documented with molecular techniques (Scheublin et al., 2004). Our study also contributes direct evidence for preferential associations between certain AMF and plants species by showing that plant species identity significantly affected both the AMF community composition and diversity in the trap pots. Surprisingly, highest species richness and diversity of AMF spore communities as well as highest spore densities were detected in trap pots planted with maize or crotalaria. This probably means that the indigenous AMF in the field soil used as inoculum were previously 'selected for' by the two plants sown in the field experiment (or were simply compatible with them, having highest fitness as expressed by their sporulation rate in association with these plant species).

Higher spore abundances were observed here of one *Acaulospora* and one *Scutellospora* spp. in the MCF than in the COM soils. Our results are thus corroborating previous field observations of Johnson et al. (1991) followed by An et al. (1993) in North American agroecosystems, who reported preferential associations between soybean and *Gigaspora* spp., and between maize and *Glomus* spp. The fact that the abundance of *G. gigantea* was not affected by crop rotation in our system is possibly due to geographic distance, different soil and climatic context as well as due to different AMF functional properties in the different field experiments. The questions of which AMF species are dominant in different agroecosystems, which factors determine the composition of AMF communities, and how functional properties vary among isolates of the same AMF species of different geographical origin are certainly legitimate but would deserve a more coherent research effort than seen so far. Since we only performed one field sampling here, no conclusions about temporal dynamics of the AMF communities in the differently cropped soils could be made. However, as plant phenologies and the dynamics of soil processes are likely to be different in the differently cropped soils, it appears important for the future also to look for the dynamics in the AMF communities in the differently cropped soils.

#### 4.4. Impact of P fertilization

This study provides evidence for limited effects of P fertilization on the diversity of AMF communities and

clearly shows no effects of fertilization on AMF community composition. Neither the spore density nor the diversity and composition of AMF spore communities in the field soil samples were affected by fertilization. On the other hand, the richness and diversity (but not the composition) of AMF spore communities was higher in traps established from P<sup>-</sup> than from P<sup>+</sup> soils. The absence of a strong effect of P fertilization on the diversity and composition of AMF spore communities in this study was in accord with previous observations of only a limited effect of fertilization on the composition of AMF communities, in contrast to the effects induced by crop rotation (Johnson, 1993; Kiers et al., 2002). However, the absence of fertilization effect on spore densities in this study was surprising, as previously it has repeatedly been shown that P fertilization resulting in increases of available soil P levels reduced spore densities and/or root colonization by AMF (Jensen and Jakobsen, 1980; Douds and Schenck, 1990; Miller et al., 1995; Kahiluoto et al., 2001; Allison and Goldberg, 2002), noting that most of the previous studies were performed in temperate soils that were not severely P deficient. Our soil was, however, strongly P deficient. The P fertilization of this soil at levels of 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> resulted in a dramatic increase of P availability (1.7 and 6.6 mg kg<sup>-1</sup> resin extractable P in P<sup>-</sup> and P<sup>+</sup>, respectively) and in doubling maize yields (Bünemann et al., 2004b). Therefore, it is likely that under these specific conditions the potential negative P fertilization effect on AMF spore density was offset by increased C supply to the fungi by more vigorously growing crops. It has indeed been shown that application of moderate amounts of P fertilizer into P deficient soils may stimulate the levels of mycorrhizal colonization of roots and increase the magnitude of mycorrhizal benefits for the plants, following a bell-shaped curve with a maximum under low-to-moderate, but not extremely low P availabilities (Bolan et al., 1984; Picone, 2002). Since the soils in the tropics are commonly depleted in available P pools and continuing removal of P with crop export further aggravates this problem (Smithson and Giller, 2002), some application of P fertilizer will be needed to offset loss of fertility in such soil (Buerkert et al., 2001; Bünemann et al., 2004c). Application rates may have to be, however, carefully tuned within the frames of integrated soil fertility management so as not to dramatically decrease continuing benefits of plants from mycorrhizal symbiosis.

Interestingly, the influence of P fertilization on the diversity of AMF spore communities in the traps was strongly modulated by the identity of the trap plant: The effect of soil P fertilization history was only apparent in leek-planted pots and remained insignificant for any other plant species. This interdependency between different factors such as plant species identity and P fertilization of soil reveals a multidimensional nature of impact of agricultural practices on the indigenous AMF communities. This presents particular challenges for design of

future field experiments and for approaches to identify key players affecting AMF communities in them.

#### 4.5. Functional implications

The results presented above raise the question: Does more diverse AMF community imply a better function in terms of contributing to nutrient acquisition by the crops? Some authors argued that the diversity may not necessarily relate to the function but rather to sustainability of an agroecosystem (Sieverding, 1990), whereas others suggested that the greater the diversity, the more benefits will be conferred to the crops, because the mycorrhizal community will span broader range of functions (Koide, 2000). The question also arises which diversity is important in this context, whether it is the diversity of species or diversity of functions such as the capacity of AMF to promote plant growth and nutrient acquisition. It is known that species identity does not necessarily carry with it information about the fungal functions since only some of the functions are conserved on the species level, whereas others vary both among and within species, i.e. among different isolates of the same AMF species (Munkvold et al., 2004; Jansa et al., 2005). Therefore, it may be necessary to characterize functional rather than the taxonomic diversity of the AMF community (and possibly adjust the species concept of the AMF to be more functionally relevant), although this may prove to be more difficult than previously thought (Sanders, 2004). As suggested previously (Sanders, 2002), it is possible that due to co-evolution between plants and AMF species, the plants would preferentially establish mycorrhizal symbiosis with fungi (species or genotypes) that are more beneficial (or are cheaters pretending to be more beneficial) for them than the others. In accordance with this notion, we showed in this study that *S. verrucosa* was more frequent under a rotation involving the crotalaria. In another study, *S. verrucosa* was indeed shown to promote growth of *Cajanus cajan*, another tropical woody legume. On the other hand, it did not promote growth of maize (Diederichs, 1991). Probably, this particular AMF species is functionally better symbiont with woody leguminous plants than with maize in a tropical agroecosystem, but further research is needed to throw more light on these diverse plant-AMF interrelationships.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.agee.2006.06.004.

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