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Promiscuous arbuscular mycorrhizal symbiosis of yam (*Dioscorea* spp.), a key staple crop in West Africa

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Abstract Yam (*Dioscorea* spp.) is a tuberous staple food crop of major importance in the sub-Saharan savannas of West Africa. Optimal yields commonly are obtained only in the first year following slash-and-burn in the shifting cultivation systems. It appears that the yield decline in subsequent years is not merely caused by soil nutrient depletion but might be due to a loss of the beneficial soil microflora, including arbuscular mycorrhizal fungi (AMF), associated with tropical "tree-aspect" savannas and dry forests that are the natural habitats of the wild relatives of yam. Our objective was to study the AMF communities of natural savannas and adjacent yam fields in the Southern Guinea savanna of Benin. AMF were identified by morphotyping spores in the soil from the field sites and in AMF trap cultures with *Sorghum bicolor* and yam (*Dioscorea rotundata* and *Dioscorea cayenensis*) as bait plants. AMF species richness was higher in the savanna than in the yam-field soils (18–25 vs. 11–16 spp.), but similar for both ecosystems (29–36 spp.) according to the observations in trap cultures. Inoculation of trap cultures with soil sampled during the dry season led to high AMF root colonization, spore production, and species richness (overall 45 spp.) whereas inoculation with wet-season soil was inefficient (two spp. only). The use of *D. cayenensis* and *D. rotundata* as baits yielded 28 and 29 AMF species, respectively, and *S. bicolor* 37 species. AMF root colonization, however, was higher in yam than in sorghum (70–95 vs. 11–20%). After 8 months of trap culturing, the mycorrhizal yam had a higher tuber biomass than the nonmycorrhizal controls. The AMF actually colonizing *D. rotundata* roots in the field were also studied using a novel field sampling procedure for molecular analyses. Multiple phylotaxa were detected that corresponded with the spore morphotypes observed. It is, therefore, likely that the legacy of indigenous AMF from the natural savanna plays a crucial role for yam productivity, particularly in the low-input traditional farming systems prevailing in West Africa.

Keywords Beneficial soil biota · Biodiversity · DNA extraction · Sustainable agriculture · Tropics · Tuber crop

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Introduction

Yam (*Dioscorea* spp.) is a tuber crop widely cultivated in the humid and subhumid lowland regions of sub-Saharan West Africa, the Caribbean, and Atlantic coastal lines of

tropical America and Asia (Onwueme and Haverkort 1991; Sotomayor-Ramirez et al. 2003; Suja et al. 2003; Baimey et al. 2006; Egesi et al. 2007a). In West Africa, yam is the most important tuber crop in terms of area coverage and a key staple food, particularly in Nigeria, Benin, and Togo (Kalu and Erhabor 1990; Olasantan 1999; Ile et al. 2006; Baimey et al. 2006). More than 90% of the global production (40 million tons fresh tubers per year) is produced in West Africa (Ravi et al. 1996; FAOSTAT 2007). Additionally, yam plays an important cultural role in the traditions of West Africa (Coursey 1984; Orkwor 1998). Regionally, yam production is relatively static despite the area under production steadily increasing (IITA 2006), indicating a gradual decrease in productivity. Major constraints for yam production were presumed to be low soil fertility, e.g., due to macro-and micronutrient deficiency (O'Sullivan and Ernest 2007) or damage by plant parasitic nematodes and virus diseases (Odu et al. 2004; Baimey et al. 2005; Egesi et al. 2007b). In terms of nutrient use, yam is a demanding crop, and consequently, it is planted traditionally at the beginning of the crop rotation cycle following slash-and-burn of forest respective savannas with significant tree components or long-term fallow vegetation (Carsky et al. 2001; O'Sullivan and Jenner 2006). However, such suitable land becomes scarce due to increasing land-use intensity driven by demographic pressure and dwindling land availability. Furthermore, inappropriately cultivated lands are exposed to erosion and soil degradation (Maduakor et al. 1984; Carsky et al. 2001; Salako et al. 2007). Inconsistent results were obtained in studies on the efficiency and economics of inorganic fertilizer application to yam. For example, Ferguson (1973) found that *Dioscorea alata* did not respond to phosphorus (P) fertilizer, particularly when seed yam sets of 100 g or more were used. He proposed that the accumulated P in the sets were sufficient for newly sprouting vines during early growth or, alternatively, that yam depends on arbuscular mycorrhizal fungi (AMF) for P acquisition. Similarly, trials in the Southern Guinea savanna of Benin showed that yam yields did not increase following application of ammonium super phosphate (Baimey 2005). Sotomayor-Ramirez et al. (2003) found that the application of micronutrients was most important for increasing yam (*D. alata* and *D. rotundata*) production, while a moderate application was adequate for macronutrients. Ahn (1993) attributed the moderate or limited need of P by yam to possible mycorrhizal benefits, as did Vander Zaag and Fox (1980), when observing that *D. esculenta* and *D. rotundata* did not respond to P fertilization in field experiments in Hawaii and Ghana but had decreasing levels of AM root colonization with increasing P fertilization. Valenzuela and deFrank (1995), in a review, also speculated that yam may depend on a mycorrhizal association to meet its P requirements.

Knowledge on the mycorrhizal status of yam has remained scarce so far with only limited information available (Ahulu et al. 2005; Oyetunji and Afolayan 2007), especially on *D. cayenensis* and *D. rotundata* that are preferably cuvltiated in sub-Saharan Africa. Micropropagated *D. rotundata* cvs. TDr131 and TDr179, however, were successfully inoculated with a mixture of AMF species when transferring from humidity chambers to field conditions (Uchendu 2000). Nevertheless, the extent to which yam is dependent on, or associated with AMF, has remained relatively unknown (Dare et al. 2007).

Recently, we studied the AMF diversity in soils from three ecological zones within the "yam belt" of West Africa (Tchabi et al. 2008) and found a high diversity for "natural" sites, with a relatively rapid decline in species richness observed with subsequent crops following yam in the crop rotation. In the present study, we focused further on the association of AMF with yam using data from sites in the Southern Guinea savanna (SGS) of Benin. Based on spore morphotyping, we determined the AMF species composition in three natural savanna sites and adjacent, recently cleared areas, under yam cultivation. Moreover, we set up trap cultures with soil inocula from individual sites using the host plants *Stylosanthes guianensis* and *Brachiaria humidicola* in a first trap culture system with wet season soil as AMF inoculum. In a second experiment, we used preferably dry season soil as AMF inoculum and each one cultivar of *D. cayenensis* and *D. rotundata* as bait plants to specifically identify the AMF symbionts associated with yam. In this trap culture experiment, trap cultures were also established with a commonly used bait plant, *Sorghum bicolor*. In all trap cultures, AMF root colonization, spore density, and species richness were determined as well as yam shoot and tuber weights. Furthermore, the AMF community colonizing *D. rotundata* roots in the field was assessed by molecular tools using a novel technique for DNA extraction and preservation.

Materials and methods

Study area and field sites

The SGS of Benin is situated between 7° and 9° N latitude in the sub-humid tropical savanna about 400 m asl. The soils are termed "ferruginous" and generally classified as Ferralsols according to FAO (2007). The area is characterized by a wet season between April and October and a dry season between November and March, with a variant towards the southern latitude, where a short dry spell occurs during the wet season around August. The mean annual rainfall is 1,000–1,200 mm, and the natural vegetation is composed mainly of Combretaceae, Mimosaceae,

Fabaceae, and grass layers of Poaceae (mainly *Andropogon gayanus*; Adjakidje 1984; Adjanohoun 1989).

Six sites were selected: three natural savannas (ns1–ns3), undisturbed for 25–30 years, and three fields cultivated with yam immediately following clearance of the natural vegetation (yf1–yf3). One important criterium for site selection dictated that the yam fields needed to be situated in close proximity to the adjacent savanna sites (Table 1).

Soil sampling and analyses

Soils were sampled twice at each site: first towards the end of the wet season (September–October 2004), when yam plants were aged approximately 5–6 months and close to harvest (harvest period occurs between September and January in the study area; Ile et al. 2007). The second sampling occurred during the dry season (February 2005) shortly after yam harvest when the vegetation was barren. For each sampling occasion and site, four replicate quadrant plots (100 m^2) were determined, with six soil cores per quadrant randomly removed using a 6-cm diameter corer, to a depth of 20 cm. The six soil core samples were combined into a composite sample (replicate) per site for each occasion. Samples were air-dried on an open bench in the greenhouse for 72 h at the International Institute of Tropical Agriculture (IITA) station in Abomey-Calavi, Benin, then maintained in the refrigerator at 4°C for 2 weeks and transferred to the Institute of Botany in Basel, Switzerland.

Each air-dried soil sample was divided into three equal subsets which were used to: (a) determine selected soil chemical parameters (pH, organic carbon, and available P; Table 1), (b) establish AMF trap cultures, and (c) isolate and identify AMF spores. The two methods used for describing available P represent two standard methods ("P-Morgan" (Na-acetate), and "P-Citrate") that are often used to measure easily respective slowly available P in slightly acidic soils (Oehl et al. 2005; Tchabi et al. 2008). For three sites (ns1, ns2, and yf3), the spore data from the wet season field samples have been reported previously (Tchabi et al. 2008).

Source and acclimatization of yam plantlets

Tissue culture plantlets of *D. rotundata* and *D. cayenensis* were obtained from IITA-Ibadan, Nigeria. Plantlets were multiplied under in vitro conditions by subculturing nodal segments from established in vitro plantlets in culture-tubes containing specific yam propagation media (Ng 1988, 1992, 1994). Plantlets were regenerated in a culture room with a 12 h photoperiod, 3,000 lx light intensity, at 27±1°C and 70±5% relative humidity.

The in vitro plantlets were conveyed to Basel, Switzerland, where they were planted out in 50 cm^3 pots containing a 2:1:1 *w*/*w*/*w* substrate mixture of sterilized peat, Vermiculite (GERMEX, Vermica AG, Switzerland), and quartz sand (Quartz d'Alsace, 5% of free silica; Smurfit Company, France). The substrate pH (H_2O) was 5.0 and the substrate contained 4.5% organic matter, 220 and 703 mg g^{-1} easily and slowly available P (P-acetate and P-citrate, respectively), and 717 mg g^{-1} easily available potassium (K-acetate). The plantlets were acclimatized for 3 weeks in trays covered initially with a plastic sheet to retain a high relative humidity (70%). Plantlets were maintained in the greenhouse with a day/night regime of 12/12 h and 25/21°C and irrigated once or twice per day. After 3 days, the plastic sheet was progressively removed for increasingly longer periods (6–12 h) per day over the following week, before the cover sheet was removed completely at three weeks. One week later, the plantlets were transferred into the trap culture pots inoculated with AMF.

Table 1 Geographic position of study sites and selected chemical soil parameters: natural savannas (ns1-3) undisturbed for at least 25-30 years and adjacent yam fields (yf1-3) sampled during the first year following forest clearance

Sampling sites	Geographic position	pH (H_2O)		Organic C g kg^{-1}		Available P (Na-acetate) mg kg^{-1}		Available P (citrate) mg kg^{-1}	
		w.s.	d.s.	w.s.	d.s.	w.s.	d.s.	d.s.	w.s.
Natural Savanna 1 (ns1)	07°45.739′ N; 002°27.519′ E	6.7	6.8	24.9	26.1	52.8	74.2	83.0	144.1
Natural Savanna 2 (ns2)	07°57.217′ N; 002°26.935′ E	7.2	7.2	44.1	37.7	27.3	27.0	37.6	35.8
Natural Savanna 3 (ns3)	08°19.661′ N; 001°51.340′ E	6.5	6.9	20.3	23.8	28.8	21.8	34.9	30.6
Yam field 1 (yf1)	07°49.114′ N; 002°14.519′ E	6.1	6.5	9.3	9.9	8.7	6.1	8.7	8.7
Yam field 2 (yf2)	07°55.111′ N; 002°10.507′ E	6.7	6.7	16.8	15.1	10.9	8.7	13.1	13.1
Yam field 3 (yf3)	08°19.730′ N; 001°51.332′ E	6.2	6.3	6.4	7.5	6.5	3.9	8.7	13.1

Soil samples were taken both during the wet season and dry season

w.s. wet season, *d.s.* dry season

First trap culture experiment for AMF

Trap cultures were inoculated with aliquots of each of the replicate wet season soil samples per site. Four trap culture pots (pots 20×20×30 cm) were established for each site, plus four non-inoculated control pots totaling 28 pots. For each pot, 4 kg of substrate was used, consisting of a sterilized 3:1 (*w*/*w*) mixture of Terragreen® (a calcined granular Attapulgite clay mineral, American aluminum oxide, oil dry US special, type III R, >0.125 mm; Lobbe Umwelttechnik, Iserlohn, Germany) and quartz sand (Alsace quartz sand, 5% of free silica, Smurfit Company, France), respectively. The chemical composition of the substrate was 0.3% organic matter, 10 and 1,480 mg kg^{-1} easily and slowly available P (extracted with sodium acetate and citrate), respectively, 191 mg kg^{-1} easily available potassium (extracted with sodium acetate), and a pH equal to 5.8. A 180-g sample of soil inoculum, divided into three equal sub-portions, was added to each pot, placed along three lines on the surface of 3 kg substrate and subsequently covered with the remaining 1 kg substrate. A 20-mm thick drainage mat (Enkadrain ST; Colbond Geosynthetics, Arnhem, The Netherlands) was placed at the bottom of each pot. As bait plants, five *S. guianensis* and four *B. humidicola* plantlets were planted alternately and equidistantly above the three lines of inoculum. Automated watering systems (Tropf-Blumat; Weninger GmbH, Telfs, Austria) were used to irrigate the trap cultures, which were maintained in a greenhouse in Basel for 24 months under a day/night regime of 25/21°C, with a photoperiod of at least 12 h (with supplementary artificial light depending on the season) and a mean relative humidity of 65±5%. Trap plants were trimmed to ~4 cm above the substrate level at 2-month intervals during the first year and 6-month intervals during the second.

Second trap culture experiment: including yam species as trap plants

In order to specifically detect those AMF associated with yam, a second trap culture experiment was set up using the dry-season soil samples as inocula and yam (*D. rotundata* cv TDr89/02461 and *D. cayenensis* cv TDc98-136) as well as *S. bicolor* as bait plants. The trap pots were established as above with a slight modification. Pots (1 L volume), were established using 800 g of substrate and 50 g of soil inoculum, and one plant per pot, to unequivocally attribute the sporulating AMF species to its host. In order to compare the two trap culture experiments, four additional pots inoculated per bait plant with wet season soil samples of field site yf2, were also included in this second experiment. Including four nonmycorrhizal controls per plant species, a total of 96 trap culture pots were established and maintained for 8 months, as above, with the exception that the bait plants were not trimmed during the experiment.

After 4, 6, and 8 months, and for the first trap culture experiment after 24 months, two separate soil cores (of 1.5 cm diameter, 10 cm depth) were removed from each pot to extract AMF spores and determine root colonization. For the trap cultures using yam as bait plants, yam shoot and tuber weights were recorded at 8 months.

Isolation and identification of AMF spores from field sites and trap cultures

AMF spores from field samples or from trap cultures were extracted by wet sieving and sucrose density gradient centrifugation after Oehl et al. (2003). For this purpose, 25 g air-dried field soil samples or 60 ml trap culture substrate were suspended in 300 ml of water using a 500-ml beaker. The soil suspension was passed through 1,000-, 500-, 125-, 80-, and 32-µm nested sieves. The 1,000- and 500-µm sieves were checked for sporocarps, spore clusters, and large spores and also on any roots present. The contents of the 125-, 80-, and 32-µm sieves were resuspended in a centrifuge tube, layered onto a water-sucrose solution (70% *w*/*v*) and centrifuged at 2,000 rpm for 2 min. After centrifugation, the floating spores were collected by passing the supernatant through the 32-µm sieve and rinsed with tap water. Spores, spore clusters, and sporocarps obtained from all sieves were transferred to Petri dishes and counted using a dissection microscope (Olympus SZ12) at up to ×90 magnification. The spore density (= spore abundance) in a field sample was expressed as the number of AMF spores per gram soil (field samples) or per milliliter substrate (trap cultures).

For species identification, healthy spores were mounted on glass microscope slides with polyvinyl alcohol-lactic acid-glycerol (PVLG) or PVLG mixed 1:1 (*v*/*v*) with Melzer's reagent (Brundrett et al. 1994). The spores were examined under a compound microscope (Zeiss, Axioplan) at up to ×400 magnification. Identification was based on current species descriptions, identification manuals (Schenck and Pérez 1990, International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, INVAM: http://invam.caf.wvu.edu), and own analyses on the type specimens of the large majority of AMF species (>95%) deposited in the mycological herbaria of major relevance for the Glomeromycota (e.g., OSC, FH, Z+ZT, PDD, IBACC La Habana-Cuba, DPP). The relative abundance of each species was recorded on a scale: <3% (rare); 3–20% (frequent); >20% (abundant).

Determination of AMF root colonization

AMF root colonization was determined from trap cultures after 4 months of growth. The roots obtained upon wet

sieving for spore isolation were collected with a forceps from a 50-µm sieve and stained with Trypan blue according to Brundrett et al. (1994). After careful mycorrhizal root observation under a compound microscope at up to 200-fold magnification, the gridline-intersect technique (after Giovannetti and Mosse 1980; Brundrett et al. 1994) was used to assess root colonization using a stereo microscope at up to 90–150-fold magnification.

Molecular identification of AMF in *D. rotundata* roots at field sites

Roots of *D. rotundata* were sampled from the field in the SGS in April 2007 to identify the AMF species actually colonizing yam in the field using a novel molecular technique. The roots analyzed derived from single plants of *D. rotundata* cv. *kokoro* and *D. rotundata* cv. *kpakala* cultivated near Toui Vap (08°43.452′ N; 02°40.047′ E), and from a *D. rotundata* cv. *kokoro* plant near Kilibo (08° 42.106′ N; 02°40.544′ E). Newly formed roots were removed from freshly excavated mature *D. rotundata* tubers, carefully washed and chopped into ca. 1 cm length pieces all at site in the field. The pieces were then immediately crushed onto FTA® cards (PlantSaver WB120065, Whatman International Ldt, Kent, UK) according to the manufacturer's indications and air-dried for about 30 min. They were conveyed to the laboratory in Basel and stored at room temperature until further analyses.

In the laboratory, the cards were placed on a cutting mat, and single 2 mm diameter disks were removed from an area containing the absorbed sap that had been squeezed out from the roots, using a Harris Micro Punch™ (Whatman Inc. UK). Each disk was transferred to a 0.2-ml PCR tube and supplemented with 200 µl of FTA® Purification Reagent (Whatman International Ltd). The tube was sealed, inverted twice, and incubated for 5 min at room temperature. As much of the reagent as possible was then removed and the washing procedure with the FTA® Purification Reagent repeated, followed by two washes with 200 µl of $TE_{0.1}$. The disks were then dried for 20 min at 56°C.

The dry disks were immersed into a PCR mix containing Taq Polymerase from Amersham (Basel, Switzerland), 2 mM $MgCl_2$, 0.5 µM primers, 0.13 mM of each desoxynucleoside triphosphate, and bovine serum albumin (BSA) at a concentration of 0.4 µg μL^{-1}. PCR was carried out in a nested procedure as described by Redecker (2000). The first round of amplification was performed using the universal eukaryote primers NS5 and ITS4 in a 25 µl assay. The cycling parameters were 94°C for 5 min, 30 cycles of 94°C for 30 s, 51°C for 45 s, 72°C for 2 min, and a final 5 min at 72°C. The products were diluted 1:10 with sterilized double-distilled water and used as templates for the second PCR, which was conducted with Glomerales-specific primers (Redecker 2000). Annealing temperature was 61°C and PCR reactions were preheated to 61°C during sample loading to prevent nonspecific amplification. Positive PCR products were purified using the QuickClean 5 M PCR Purification Kit (GenScript Coporation, Piscataway, NY, USA) and cloned into a PCR®4-TOPO®-Vector (Invitrogen, Basel, Switzerland). Representative clones were reamplified in a sequencing reaction, precipitated using BigDye X-Terminator (Applied Biosystems, Rotkreuz, Switzerland) and sequenced on a GeneticAnalyzer 3130 (Applied Biosystems, Rotkreuz, Switzerland). Sequences were edited in Sequence Navigator (version 1.0).

Partial Sequences of the 18S ribosomal DNA were aligned to previously published sequences in PAUP*4b10 (Swofford 2001). The glomeromycotan origin of the sequences was initially tested with Blast (Altschul et al. 1997). Phylogenetic trees were obtained primarily by distance analysis (neighbor-joining algorithm) with PAUP* 4b10 using the Kimura two parameter model and a gamma shape parameter of 0.5. Results were verified by performing parsimony analyses. Since it is not possible for species in the Glomeromycota to assign a single sequence to a certain species or fungal isolate, sequence types were defined in a conservative manner as consistently separated phylogenetic clades/groups in the phylogenetic trees. The sequence phylotypes (st) were designated after their origin: West Africa (WA), the genus they belonged (e.g., *Glomus*=Gl; *Acaulospora*=Ac; *Gigaspora*=Gi; *Scutellospora*=Sc), and a numerical index of the sequence types within each genus (1–4), e.g., resulting in 'stWAGi1'.

Calculations and statistical analysis

For the field sites, AMF spore density (number of spores per gram soil), and species richness (number of species detected) were recorded as average and total numbers per field site and sampling date and—if useful—as a sum of both dates. In the trap cultures, AMF spore density (number of spores per milliliter soil) and species richness were also recorded as average and total numbers per culture. The numbers of AMF species were determined from those that produced spores in the trap cultures inoculated with dry season field soils. For AMF root colonization, the mean percentage of colonized root length was recorded per site. Prior to further analysis, in order to provide homogeneity of variances, data on spores per gram soil (field) or per milliliter substrate (trap cultures) were $\log(x+1)$ transformed and AMF colonization $\arcsin(x/100)$ transformed. Differences in spore density, species richness, and AMF root colonization between field sites were separated using Fisher's least significant difference (LSD) at $P \leq 0.05$ after one-way ANOVA analysis with SAS program, version 9.1 package (SAS Institute 2007).

Results

AMF spores and species at field sites

The AMF spore density varied between 2–13 and 3–17 spores g^{-1} soil in samples collected during the wet and the dry season, respectively (Fig. 1a, b). The spore density varied less between sites in the natural savannas than yam fields (6–11 versus 2–17 spores g^{-1}, respectively). No correlation was found between the spore density and any of the edaphic factors determined (pH, available P, and organic C; data not shown).

Overall, 40 AMF species belonging to ten genera and eight families were identified by morphotyping of spores isolated from the field samples (Table 2). Of these 40 species, 19 belonged to the Glomeraceae, nine to the Acaulosporaceae, five to the Racocetraceae (Oehl et al. 2008), and two each to Dentiscutataceae and Gigasporaceae (Oehl et al. 2008); the Entrophosporaceae, Paraglomeraceae, and Ambisporaceae were each represented by one species. Seven additional morphotypes could not be attributed to a described species (Table 2) and may represent new undescribed species (*Glomus* spp. WAGl1, WAGl2, WAGl3, WAGl4, *Acaulospora* sp.WAAc1, *Racocetra* sp. WARa1, and *Racocetra* sp. WARa2).

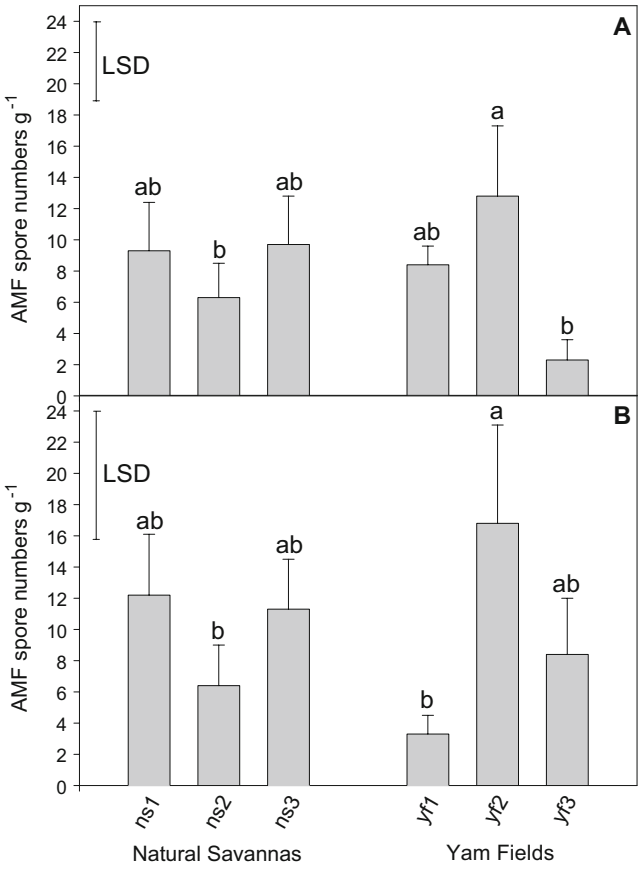

Fig. 1 AMF spore density (presented as spore numbers per gram in field soil samples) collected from natural savannas (*ns1–3*) and adjacent yam fields (*yf1–3*) in the Southern Guinea savanna of Benin in the wet season (September–October 2004, **a**) and in the subsequent dry season (February 2005, **b**). Data are reported as averages and standard deviations for four replicate plots per field site. Nonsignificant differences between sites are shown by *identical letters*, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA

The AMF species richness observed in the soil samples of the savannas was similar in the wet and dry season, whereas in the yam fields, it was higher in the wet than in the dry season samples (Fig. 2, Table 2). The cumulative species richness, comprising all the species detected in both the wet and dry season samples, was lower in yam fields than under natural savannas, indicating a change in the AMF community structure within the first year after slash-and-burn (Fig. 2c). The species richness was not correlated with any of the edaphic factors analyzed (pH, available P and organic C; data not shown). Of the 40 species identified, 12 were found exclusively in the savanna samples and eight exclusively in the yam field samples. *Glomus etunicatum* and *Acaulospora scrobiculata* numerically dominated the spore populations in the yam fields, while various sporocarpic *Glomus* spp., such as *Gl. sinuosum*, *Gl. clavisporum*, *Gl. taiwanense*, and *Gl. pachycaulis* were more frequently recovered from savanna soil than from yam fields and also *Acaulospora* species.

First trap culture experiment with wet season soil as AMF inoculum

In the trap cultures inoculated with wet season soils, AMF root colonization in *S. guianensis* and *B. humidicola* remained on an extremely low to insignificant level (0–5%) during 8 months. *Gl. etunicatum* was almost the only species producing spores during the 8 months (sites ns1, yf1, and yf3) and in one yf3 pot, a few *Gigaspora gigantea* spores were additionally recovered, while in pots from ns2, ns3, and yf2, no sporulation was detected.

Second trap culture experiment preferably performed with dry season soil

Also in the second trap culture experiment, by using *D. cayenensis*, *D. rotundata*, and *S. bicolor* as bait plants, AM root colonization remained extremely low (0–5%) after initial inoculation with wet-season soils (tested of yf2) and almost no spores were produced neither on the two yam species and *S. bicolor* nor with *S. guianensis* and *B. humidicola* as bait plants (data not shown). In contrast, all AMF communities established well in the trap cultures inoculated with dry-season soils and sporulated abundantly on all three bait plants used. Interestingly, despite a comparatively low AMF root colonization (10–20%), *S. bicolor*

Table 2 AMF species identified in soil sampled from three natural savannas (ns1–3) and three yam fields (yf1–3) during the wet season and subsequent dry season

AMF species	Natural savannas						Yam fields					
	ns1		ns2		ns3		yf1		yf2		yf3	
	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.
Glomeraceae												
Glomus etunicatum	●	●	●	●	●	●	▲	▲	▲	▲	▲	▲
Gl. macrocarpum	●	●	●	●	●	●	●	●	●	●	●	●
Gl. intraradices	●	●	●	●	●		●	●				
Gl. sinuosum	●	●			●				○			○
Gl. brohultii	●	●			●					●		
Gl. constrictum	●		●		●				○		○	
Gl. clavisporum			●	●	●				○			
Gl. taiwanense			●			●			○			
Gl. sp. WAGl2[a]	●										○	
Gl. sp. WAGl4[b]	●		●		○							
Gl. pachycaulis	●	●										
Gl. versiforme				○		○						
Gl. hoi				○		○						
Gl. mosseae				○		○						
Gl. sp. WAGl3					○	○						
Gl. ambisporum											○	
Gl. fasciculatum										○		
Gl. claroideum											○	
Gl. sp. WAGl1[c]												○
Acaulosporaceae												
Acaulospora sp. WAAc1[d]	●	●		●			●		●	●		
Ac. scrobiculata		●	●		●		▲	▲	▲	▲	▲	▲
Ac. spinosa			●	●	●	●			●	●		
Ac. mellea						○	●		●	●		
Ac. laevis		○			○							
Ac. excavata			●	●								
Ac. elegans				●		●						
Ac. morrowiae				○								
Kuklospora colombiana	●			●	●			●				
Entrophosporaceae												
Entrophospora infrequens			○		○		○					
Gigasporaceae												
Gigaspora decipiens							○					
Gi. gigantea							○					
Racocetraceae												
Racocetra fulgida	●	●	●	●			●	●	●		●	
Racocetra sp. WASc1	●	●	●		●		●	●	●	●	●	●
Racocetra sp. WASc2	○	○				○	○	○				
Ra. verrucosa		○		○		○						
Cetraspora pellucida				○								
Dentiscutataceae												
Fuscutata heterogama							○					
Fu. savannicola							○					

Table 2 (continued)

AMF species	Natural savannas						Yam fields					
	ns1		ns2		ns3		yf1		yf2		yf3	
	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.
Paraglomeraceae												
Paraglomus occultum		○				○		○				
Ambisporaceae												
Ambispora gerdemannii				○	○					○		
Total species numbers	14	14	13	17	16	13	14	9	12	10	9	6
Total species numbers per site	18		23		25		16		15		11	
Total species numbers per system	32						28					
Total species numbers in field samples	40											

Please note that according to the authors' analyses, *Gl. pachycaulis* and *Gl. rubiforme* are not conspecific relying on the type specimens and original species descriptions and illustrations of both species. The relative abundance of each AMF species was recorded following the scale: (rare): ○, <3% or only a few specimens found; (frequent): ●, 3–20%; (abundant): ▲, >20%

ns natural savanna, *yf* yam field, *w.s*. wet season, *d.s*. dry season

[a] Resembling *Gl. halonatum*

[b] Resembling *Gl. aureum*

[c] Resembling *Gl. rubiforme*

[d] Resembling *Ac. rehmii*

induced a much higher spore production in the trap cultures (12–94 spores mL^{-1}) than the highly AMF-colonized yam (*D. cayenensis* and *D. rotundata*, 70–95%), which produced 0.1–17 and 1–34 spores mL^{-1}, respectively (Figs. 3 and 4).

In the trap cultures inoculated with dry season soils, 45 AMF species, representing ten genera and eight families were identified (Table 3). The majority of the species belonged to the *Glomeraceae* (22 spp.) followed by the *Acaulosporaceae* (16 spp.), *Racocetraceae* (two spp.), *Gigasporaceae*, *Ambisporaceae*, *Archaeosporaceae*, *Entrophosporaceae*, and *Paraglomeraceae* (one sp. each). Of the 45 species (or morphotaxa) recorded, 16 could not be identified from the field samples (Table 2). These were mainly species forming small and rapidly degraded spores, such as *Gl. eburneum*, *Ac. undulata*, and *Intraspora schenkii* or species such as *Gl. aggregatum*, *Kuklospora kentinensis*, and *Ku. colombiana,* which are usually difficult to distinguish from similar looking species such as *Gl. intraradices*, *Ac. scrobiculata*, and *Ac. dilatata*, respectively) in field samples. Of the species detected in the field samples, 11 were not found in the trap cultures, especially sporocarpic *Glomus* spp., such as *Gl. pachycaulis* and above all, several *Gigaspora*, *Racocetra*, and *Fuscutata* spp. Twenty-nine species were detected in trap cultures from both savannas and yam fields, whereas eight species were exclusively from savanna and eight exclusively from yam field trap cultures (Table 3). The AMF species sporulating fastest and most abundantly were *Gl. etunicatum* and *Ac. scrobiculata* in the majority of the trap cultures. These two species had also produced a greater absolute and relative spore density in the yam fields than in the savannas (Table 2).

AMF species associated with yam

After 8 months trap culturing with yam, AMF spores of 37 spp. were detected in total (Table 3). Of these, 29 spp. were associated with *D. cayenensis* and 28 with *D. rotundata,* with 20 spp. common to both plant species. From the trap cultures using *S. bicolor*, a total of 37 spp. were found associated, of which four were not recovered from the yam cultures. The mean species richness detected per site was also higher in the trap cultures with *S. bicolor* (8–11) than in those with *D. cayenensis* (4–7) or *D. rotundata* (3–8; Fig. 5).

Trap cultures using yam as bait plants produced AMF spores from seven families. Approximately half of these were Glomeraceae (18 *Glomus* spp.), followed by the Acaulosporaceae (12 *Acaulospora* spp. and one *Kuklospora* sp.). Furthermore, spores of *Racocetra fulgida*, *Gi. gigantea*, *Entrophospora infrequens*, *Paraglomus occultum*, and *Intraspora schenckii* were recovered (Table 3).

Overall AMF detected by spore morphotyping from both field and trap culture samples

Considering both field and trap culture results, a total of 56 species were detected across sites (Table 4) with a similar

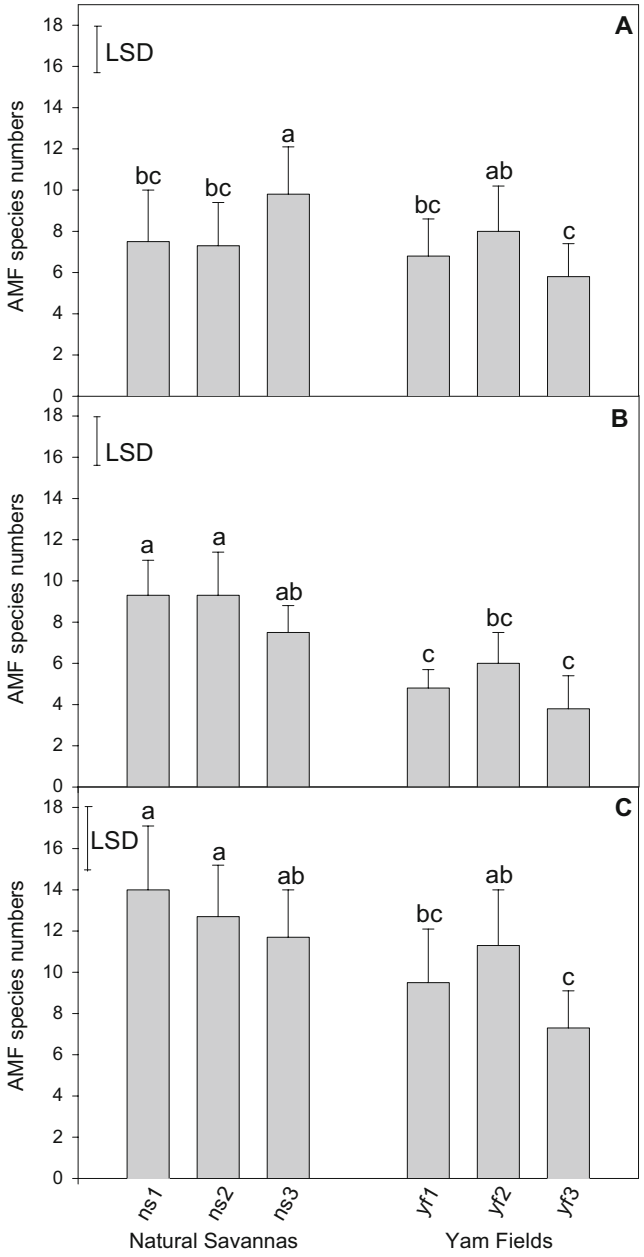

Fig. 2 AMF species richness in field soil samples collected from natural savannas (*ns1–3*) and adjacent yam fields (*yf1–3*) in the Southern Guinea savanna of Benin in the wet season (September–October 2004, **a**) and in the subsequent dry season (February 2005, **b**) and cumulative from both seasons (**c**). Data are reported as averages and standard deviations for four replicate plots per field site. Nonsignificant differences between sites are shown by *identical letters*, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA

total species richness recovered from savanna (29, 33, and 36; overall 45) and yam sites (29, 30, and 35; overall 45). The mean species richness was, however, slightly higher in the savanna (20, 21) than the yam sites (17–19) a result which, however, became statistically significant only when the data of all three savanna respective yam sites were pooled together.

Molecular identification of AMF species colonizing yam roots collected at field sites

The molecular analyses, performed on roots sampled from the field in the SGS in April 2007, were based on partial

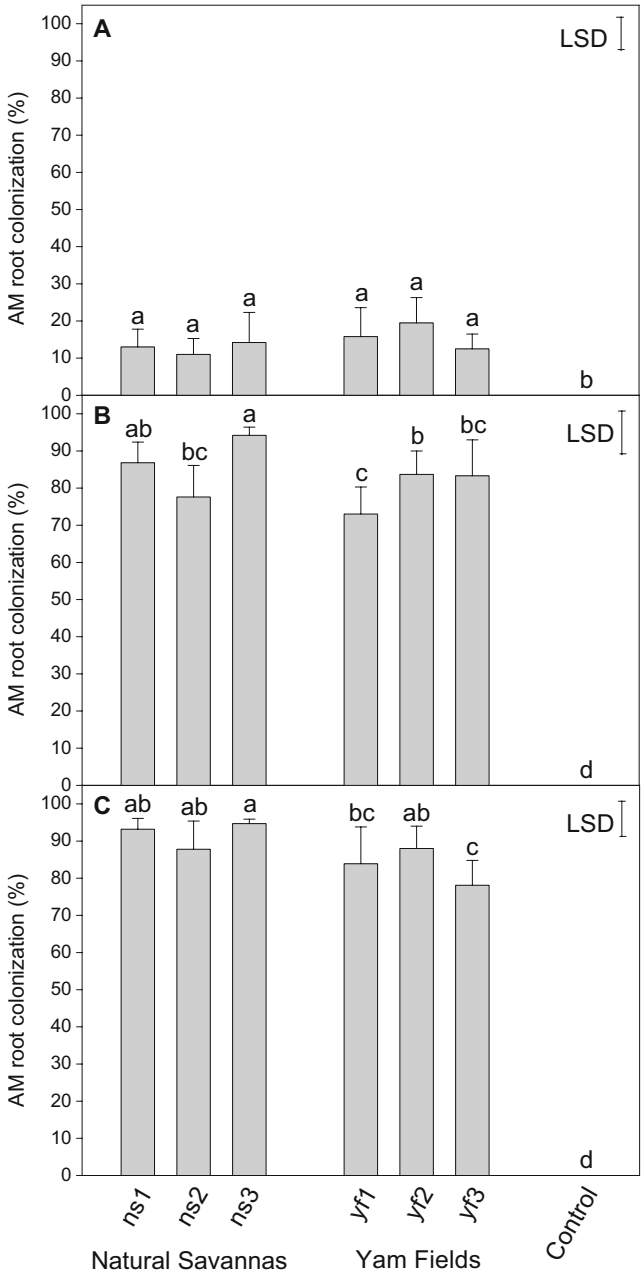

Fig. 3 AMF root colonization (%) of *S. bicolor* (**a**), *D. cayenensis* (**b**), and *D. rotundata* (**c**) following 4 months cultivation in trap cultures using dry season field soils from three natural savannas (*ns1–3*) and adjacent yam fields (*yf1–3*) as inocula and including a nonmycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Nonsignificant differences between sites are shown by *identical letters*, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA

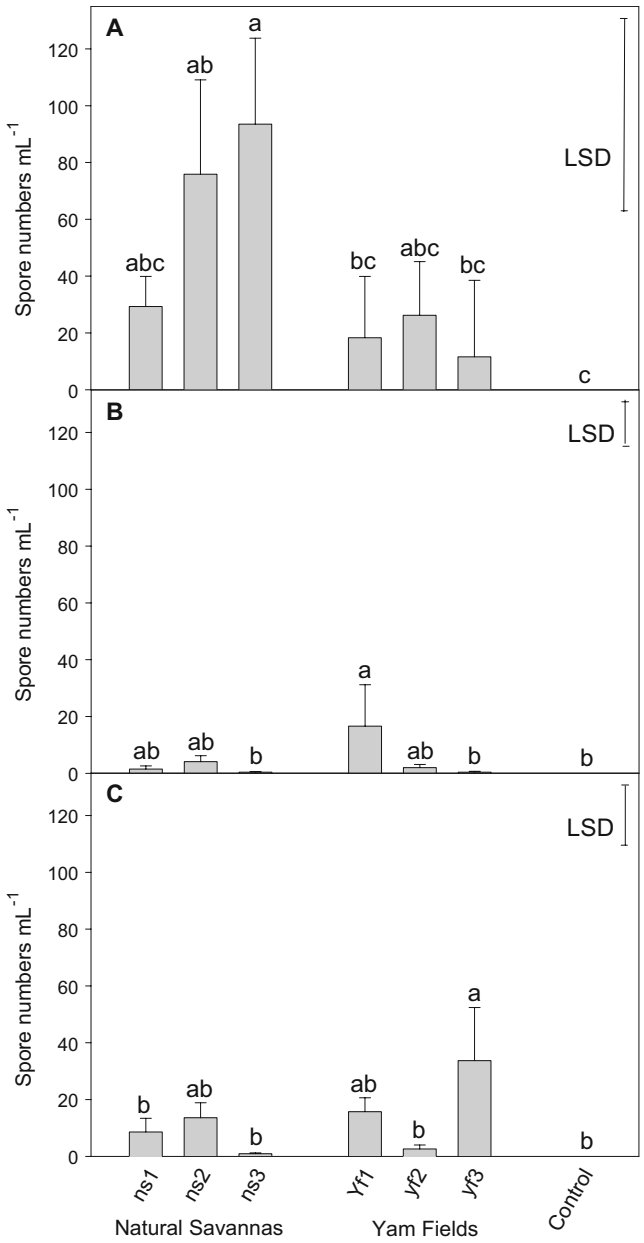

Fig. 4 AMF spore density (presented as spore numbers per milliliter of substrate) on *S. bicolor* (**a**), *D. cayenensis* (**b**) and *D. rotundata* (C) following four months cultivation in trap cultures using dry season field soils from three natural savannas (*ns1–1*) and adjacent yam fields (*yf1–3*) as inocula, and including a nonmycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Nonsignificant differences between sites are shown by *identical letters*, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA

18S ribosomal gene sequences analyzed in field collected yam roots (*D. rotundata*) using a novel technique for DNA extraction and preservation. The technique has the advantage that DNA can be extracted from the roots and concentrated on filter cards directly in the field, and the immediately air-dried DNA can easily be preserved on the cards for several months under room temperature before further analyses will be performed. Phylogenetic grouping revealed that the AMF sequence types (st) detected fell into the five AMF genera that dominated the spore populations assessed from the field sites: *Glomus*, *Acaulospora*, *Cetraspora*, *Racocetra*, and *Gigaspora* (Fig. 6). Most st were closely related to sequences of species already found by spore morphotyping from the six field sites under study, e.g., stWAGl1 related to *Gl. constrictum*, stWAGl2 related to *Gl. clarum*, stWAGl3 to *Gl. sinuosum*, stWAAc1 related to *Ac. spinosa* and related species, stWAGi1 related to *Gi. gigantea* and related species, stWACe1a and stWACe1b related to *Ce. pellucida* and related species, and stWARa4 related to *Ra. fulgida* sequences (Fig. 6). However, the sequence types obtained could also belong to unknown species related to known species; a clear attribution is not even allowed for stWAGl3, forming a clade with *Gl. sinuosum* with a high bootstrap support (96, Fig. 6). One st (stWARa2) could not at all be related to sequences of a known species, but we suspect that these sequences belong to *Racocetra* sp. WARa1 or *Ra.* sp. WARa2 (Table 2) or a closely related, described, or undescribed *Racocetra* species, since this st groups within sequences of this new genus. However, the data clearly show that AMF species of at least five different genera actually colonized the yam roots in the field.

Effect of the soil inocula on yam development

The different soil inocula originating from the savanna and yam field sites did not influence yam shoot growth in the trap cultures compared with non-mycorrhizal controls (Fig. 7). However, *D. rotundata* tuber weights were, on average, 40% heavier than the controls in pots inoculated with soil derived from three sites (ns1, ns2 and yf3; Fig. 8b). In the case of *D. cayenensis*, tuber weights were approximately 20% heavier than non-inoculated controls across treatments, although only for the ns1 site was the effect significant ($P<0.05$; Fig. 8a).

Discussion

From only a relatively small number of sites in the SGS of Benin in West Africa, under natural savanna and adjacent yam fields, extensive spore morphotyping resulted in the detection of totally 56 AMF species. Such species diversity in a restricted area indicates a huge AMF species richness in West African soils, especially when compared with similar studies from other tropical ecosystems (e.g., Sanginga et al. 1999; Dalpé et al. 2000; Lekberg et al. 2007; Mathimaran et al. 2007; reviews by Öpik et al. 2006 and Gai et al. 2006). Using molecular identification tools,

Table 3 AMF species recovered from trap cultures using *Sorghum bicolor*, *Dioscorea cayenensis*, and *Dioscorea rotundata* as bait plants and inoculated with soil sampled during the dry season from natural savanna and yam field sites

AMF species	*Sorghum bicolor*						*Dioscorea cayenensis*						*Dioscorea rotundata*					
	ns			yf			ns			yf			ns			yf		
Months after trap culture establishment	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8
Glomeraceae																		
Glomus etunicatum		●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Gl. macrocarpum										●					●			
Gl. intraradices	●	●	●	●	●	●		●	●		●	●			●		●	●
Gl. sinuosum		●	●		●	●						●						●
Gl. brohultii			●															
Gl. constrictum	●																●	●
Gl. clavisporum			●						●								●	●
Gl. taiwanense						●						●						
Gl. sp. WAGl2[a]																		
Gl. sp. WAGl4[b]		●	●						●		●	●	●					●
Gl. pachycaulis																		
Gl. versiforme			●				●										●	●
Gl. hoi		●	●	●	●	●			●	●					●	●		
Gl. mosseae	●			●	●	●						●	●			●		
Gl. sp. WAGl3																		
Gl. ambisporum						●												
Gl. fasciculatum				●			●				●	●						
Gl. claroideum	●	●	●	●	●	●	●											
Gl. sp. WAGl1[c]																		
Gl. tortuosum			●															
Gl. eburneum	●									●			●			●	●	●
Gl. sp. WAGl7[d]			●			●		●	●		●	●						
Gl. diaphanum			●															
Gl. aggregatum	●	●	●	●	●	●	●			●	●		●			●		
Gl. sp. WAGl5[e]						●										●		
Gl. sp. WAGl6[f]			●							●								
Acaulosporaceae																		
Acaulospora sp. WAAc1[g]		●	●	●			●	●	●	●	●	●	●	●	●	●	●	●
Ac. scrobiculata	●	●	●		●	●	●	●	●	●	●	●	●	●	●		●	●
Ac. spinosa			●			●	●	●	●									●
Ac. mellea							●								●	●		
Ac. laevis									●									
Ac. excavata																		
Ac. elegans	●																	
Ac. morrowiae						●									●			
Ac. rehmii										●			●	●	●		●	●
Ac. dilatata						●												
Ac. sp. WAAc3[h]	●		●						●									
Ac. longula									●	●			●					
Ac. undulata										●								
Ac. sp. WAAc2			●												●			
Kuklospora colombiana			●	●	●				●									
Ku. kentinensis				●	●	●						●	●					●
Ku. sp. WAKu1				●														

Table 3 (continued)

AMF species	*Sorghum bicolor*						*Dioscorea cayenensis*						*Dioscorea rotundata*					
	ns			yf			ns			yf			ns			yf		
Months after trap culture establishment	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8
Entrophosporaceae																		
Entrophospora infrequens		•	•				•	•	•				•	•	•			
Gigasporaceae																		
Gigaspora decipiens																		
Gi. gigantea				•														•
Racocetraceae																		
Racocetra fulgida	•									•								
Racocetra sp. WASc1																		
Racocetra sp. WASc2																		
Ra. verrucosa																		
Cetraspora pellucida															•			
Dentiscutataceae																		
Fuscutata heterogama																		
Fu. savannicola																		
Paraglomeraceae																		
Paraglomus occultum	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Ambisporaceae																		
Ambispora gerdemannii				•														
Archaeosporaceae																		
Intraspora schenckii	•															•		
Number of species per system and trap plant	29			23			19			21			20			22		
Number of species per plant species	37						29						28					
Total number of species in trap cultures	45																	

Spores were identified at 4, 6, and 8 months of trap culturing. •—the sampling period at which spores of respective species were detected

[a] Resembling *Gl. halonatum*

[b] Resembling *Gl. aureum*

[c] Resembling *Gl. rubiforme*

[d] Resembling *Gl. tenue*

[e] Resembling *Gl. tortuosum*

[f] Resembling *Gl. arborense*

[g] Resembling *Ac. rehmii*

[h] Resembling *Ac. elegans*

Husband et al. (2002) recorded 30 AMF taxa from three sites in tropical savannas of Panama. The high AMF species richness demonstrated in our study could partly be attributed to the sampling of soils at two separate occasions in different seasons. It is also probably a result of the combination of the methods employed to detect spores, including direct spore detection in fresh field samples, trap cultures using different host plants, and use of molecular techniques. In a previous AMF study of multiple field sites under different land use intensities within two eco-climatic zones, the Southern and Northern Guinea savannas in Benin, 35 and 39 AMF species, respectively, were detected in the soils sampled during the wet season (Tchabi et al. 2008), reflecting present field sample results (40 species). In this respect, it is remarkably that several AMF species—so far known only from the tropical Americas—have been confirmed from Benin, which is in accordance with the facts that AM fungi are much older (about 400–450 Ma) than the separation of Southern and Central America from Western Africa (about 100 Ma) and that important migrations of people and transfer of plant species took place between these continents during the last centuries (Oehl et al. 2007).

The trap cultures inoculated with soil collected during the dry season yielded a multitude of AMF species,

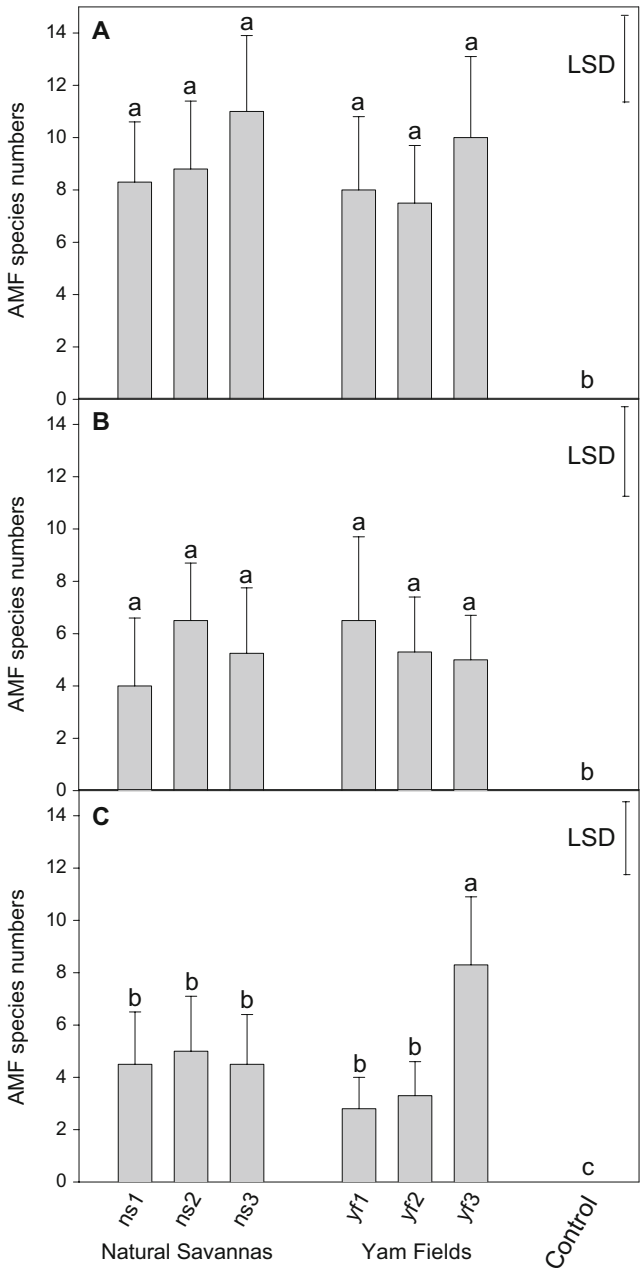

Fig. 5 AMF species richness on *S. bicolor* (**a**), *D. cayenensis* (**b**), and *D. rotundata* (**c**) following 4 months cultivation in trap cultures using dry season field soils from three natural savannas (*ns1–3*) and adjacent yam fields (*yf1–3*) as inocula and including a nonmycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Nonsignificant differences between sites are shown by *identical letters*, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA

compared with the very poor yield obtained with trap cultures inoculated with wet season soils. This could result from increased exposure of AMF spores to fungivorous nematodes, colembollans, and microbial antagonists under humid soil conditions in wet season soils (Klironomos et al. 1999; Bakhtiar et al. 2001; Tiunov and Scheu 2005; Tchabi 2008). Moreover, many AMF species may complete their life cycle with sporulation only towards the end of the wet season, for survival during the "off" (dry) season, with newly formed spores often becoming dormant. Consequently, our study indicates and strongly recommends that for successful trapping of AMF from such tropical ecosystems, soil sampling should be performed during the dry season. Nevertheless, despite our intensive search with repeated sampling and trap culturing, we assume that there remain additional AMF species in these ecosystems, undetected through limited or no sporulation, both in the field soils and in the trap cultures.

The current study documents for the first time that yam is associated with a plethora of AMF species and that when using yam as bait plants in trap cultures, the roots rapidly become highly colonized by a multitude of AMF species. With *D. cayenensis* and *D. rotundata*, the number of AMF species detected by spore morphotyping steadily increased over time in the trap cultures, with one species (*Gl. etunicatum*) appearing already 2 months after inoculation and up to 29 species after 8 months. Similarly, Oehl et al. (2003) and Chaurasia and Khare (2005) observed an increasing number of species sporulating in trap cultures over a period of 20 months. In the present study, some species consistently sporulated faster than others in trap cultures, particularly *Gl. etunicatum* and *Ac. scrobiculata,* on both yam cultivars and sorghum. These two AMF species also tended to dominate the spore populations observed in the soil samples from the yam field sites. The fact that especially *Gl. etunicatum* belonging to Glomus group B sensu Schuessler et al. was not detected through molecular analyses might be due to the fact that such species might be able to rapidly reproduce spores but might not necessarily colonize the roots on a significant respective detectable level (e.g., Oehl et al. 2003, 2004).

From the sorghum trap cultures several AMF species were recovered which were not detected with yam, whereas other species were recovered exclusively with yam. Even among the yam cultivars, some AMF species were recovered only with *D. rotundata* and others only with *D. cayenensis.* This could be indicative of a certain host specificity of these AMF spp, although we did not additionally assess such a possible specificity during this study. AMF host specificity, or rather preference, has been observed with other crops (e.g., Bever et al. 1996, 2001; Sýkorová et al. 2007). Lovelock et al. (2003) discovered differences in relative spore density of AMF communities depending on the host plants used, and Vandenkoornhuyse et al. (2002) observed that the AMF community colonizing *Trifolium repens* differed from that in neighboring *Agrostis capillaris*. Such host preferences have obvious implications for establishing efficient and comprehensive AMF trap

Table 4 AMF species richness for three natural savanna sites (ns1–ns3) and three adjacent yam fields (yf1–yf3) identified by spore morpho-typing

	Natural savannas			Yam fields			*LSD*
	ns1	ns2	ns3	yf1	yf2	yf3	
AMF species richness (mean of four replicate plots)[a]	20.5a	20.3a	20.0a	19.3a	17.3a	16.8a	3.9
AMF species richness (sum of four replicate plots)	29	36	33	29	35	30	–
Total AMF species richness per (agro-)ecosystem	45			45			
Total AMF species richness in study area	56						

Data combined for field site and trap culture results

[a] Average of four replicate plots per field site. Non-significant differences between sites are shown by identical letters and were determined by Fisher's Least Significant Difference (*LSD*) at the 5% level after one-way ANOVA

cultures, especially for studying AMF communities in ecosystems (Oehl et al. 2003).

AMF root colonization in the trap cultures was markedly higher for yam than for sorghum. A high level of AMF colonization of yam has also been reported from the field, although this varied with yam genotype (IITA 2005). The high level of yam root colonization could be associated with low yam root density, compared to the extensive root

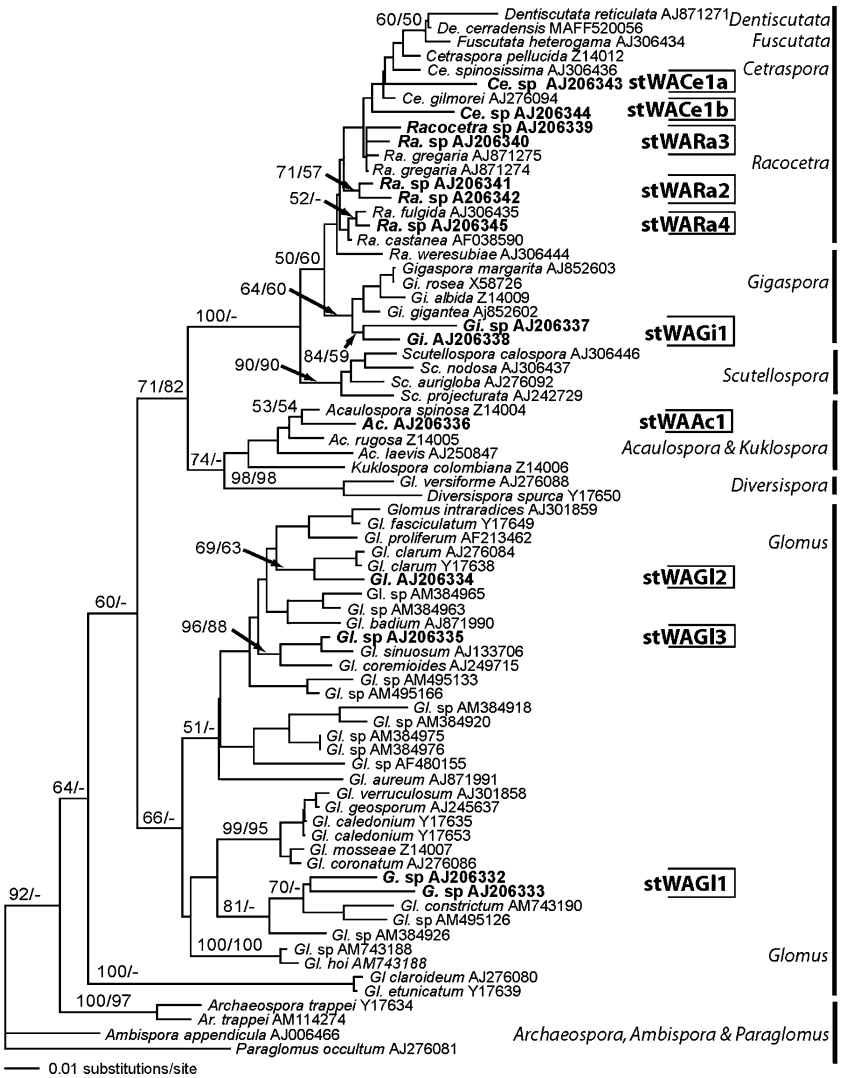

Fig. 6 Phylogenetic tree of Glomeromycota obtained by neighbor-joining analysis of 310 characters of the 18S rDNA subunit. *Numbers above branches* denote bootstrap values from 1,000 replications for neighbor-joining and for parsimony. The tree was rooted with *Pa. occultum*. Sequences obtained in the present study from *D. rotundata* roots are shown in *boldface*. They are attributed to AM fungi on the genus level (e.g., *Gl.* sp. for *Glomus*) and are additionally labeled with the database accession number (e.g., AJ206343). The sequence types (*st*, e.g. stWAGl1) are labeled with the continent of origin (*WA*=West Africa), the genus abbreviation (e.g. *Gl* for *Glomus*) and the numerical index of the st within the genus (1–4). The *brackets* show the delimitation of the nine (ten) sequence types

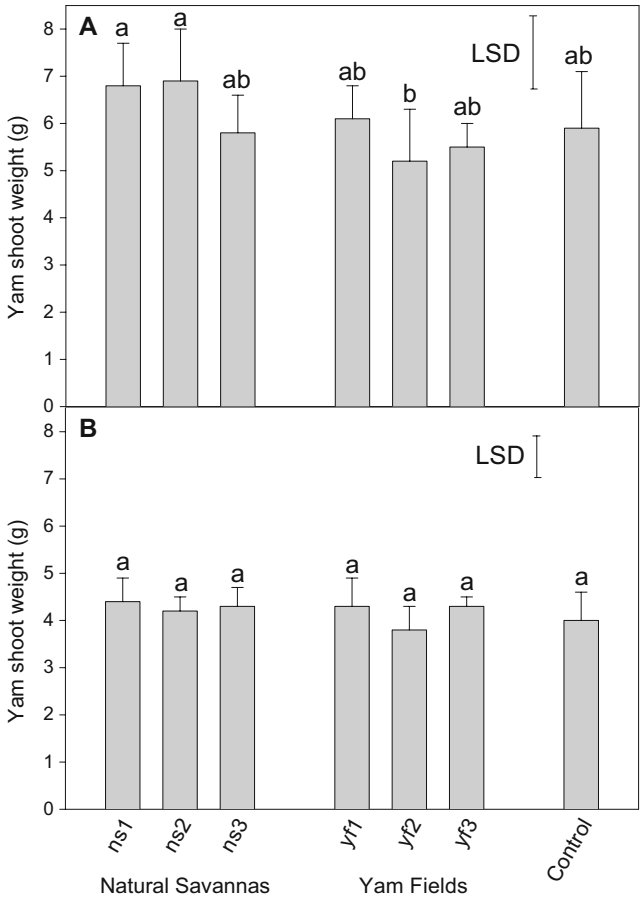

Fig. 7 Shoot dry weight of *D. cayenensis* (**a**) and *D. rotundata* (**b**) following 8 months cultivation in trap cultures using dry season field soils from three natural savannas (*ns1–3*) and adjacent yam fields (*yf1–3*) as inocula and including a nonmycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. *Columns with the same letter* are not significantly different at $P=0.05$. LSD denotes the least significant difference between sites

system of sorghum. The current findings are indicative that yam is a highly mycotrophic plant and suggest significant AMF dependency. In this context, it is interesting to note that despite the much higher level of AMF root colonization of yam than of sorghum in the trap cultures, much less spores were produced with yam than with sorghum. Similar observations were also made at the ecosystem level in Central Europe (Oehl et al. 2003), where AMF communities present in seminatural grassland soil inocula colonized bait plant roots faster and more densely but with less abundant spore production than the soil inocula containing AMF communities from neighboring arable lands. Further investigations are needed to understand these mutual life history strategies.

AMF root colonization of yam in trap cultures was enhanced upon inoculated with soil from savannas as compared to inoculation with soil from adjacent yam fields. This might be related to the observed reduction in AMF species richness in the course of the first year following clearance of the natural vegetation for yam cultivation (Tchabi et al. 2008). The current study reveals how the decline in AMF species richness following the land use change from natural savannas to yam cultivation results in reduced AMF infection potential, even in the traditional low-input farming system. This decline further illuminates the loss of biodiversity following land use change, even in the first year following clearance of the natural vegetation. It provides greater evidence concerning the loss of beneficial microorganisms (AMF richness) than was observed in the previous related study (Tchabi et al. 2008), where a dramatic decrease of AMF species richness was found with increasing land use intensity in the Sudan and Guinea savannas but not evidently during the transition to yam in the first year after slash-and-burn.

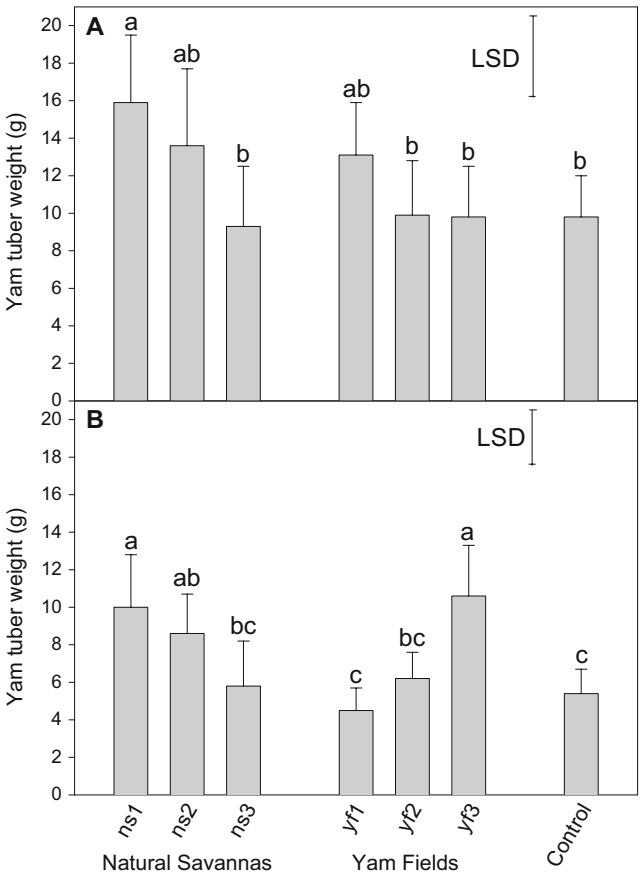

Fig. 8 Tuber dry weight of *D. cayenensis* (**a**) and *D. rotundata* (**b**) following 8 months cultivation in trap cultures using dry season field soils from three natural savannas (*ns1–3*) and adjacent yam fields (*yf1–3*) as inocula, and including a nonmycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Nonsignificant differences between sites are shown by *identical letters*, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA

While yam shoot growth in the trap cultures was unaffected following inoculation with the soils from field sites, yam tuber development was substantially enhanced in some cases, compared with controls. Although other intrinsic factors related to in vitro plantlets could interfere with tuber development, the increased tuber production is more likely influenced by AMF given the outstanding high AMF root colonization. This further indicates that AMF may play an important role in yam nutrition and tuber production and explain the good production performance of yam immediately following savanna clearance, when AMF abundance is high as compared to subsequent years (Tchabi et al. 2008). Plant growth promotion by AMF is a well-established phenomenon across crops and climatic zones (e.g., Chaurasia and Hare 2005; Caglar and Akgun 2006; Smith and Read 2008). Further studies on yam growth promotion by AMF should now focus on the specificity and functionality of the symbiotic interaction and the influence of inocula levels on AMF-yam interactions under controlled and field conditions. A particular emphasis should focus on the selection of indigenous AMF isolates that hold promise for outstanding benefits for sustainable yam production.

Conclusion

By extensive spore morphotyping of field samples and, in addition, of samples obtained from prolonged trap cultures using different bait plant species, an outstanding AMF species richness was revealed in ecosystems of the SGS. To establish successful trap cultures for AMF present in such tropical ecosystems, use of soil inocula collected during the dry season should be considered essential, as the wet season inocula virtually failed. By using yam as bait plants in trap cultures followed by morphological identification of the newly formed AMF spores and by molecular assessment of the AMF community composition colonizing yam roots in the field, we could clearly show that yam engages in promiscuous symbiotic interactions with a wide range of AMF species. Spores of *Gl. etunicatum* and *Ac. scrobiculata* were most frequently detected in yam fields, and these AMF species also sporulated most rapidly and profusely in the trap cultures with yam. It will be challenging to investigate whether these, or other, more slowly sporulating species are more important, or alternatively, whether combinations of AMF species are better. Consequently, it will be useful to elucidate the role of AMF for the high yam productivity observed in the first year after savanna clearance and whether the later decline of yam productivity is directly associated with the decline of AMF species richness that is taking place in parallel.

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