

## Diversity of arbuscular mycorrhizal fungi in the Brazilian's Cerrado and in soybean under conservation and conventional tillage



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### ABSTRACT

The Cerrado is the largest savanna biome in the Neotropics and considered a major hotspot for world biodiversity. However, over recent decades the area has increasingly been converted to intensive agricultural ecosystems, primarily for soybean production. Conservation tillage systems have gained major importance in tropical America, especially in the Brazilian Cerrado. Long-term field experiments were established to evaluate the effects of soil tillage on soybean production in the Cerrado. The aim of our study was to determine arbuscular mycorrhizal fungi (AMF) diversity in three natural savanna forests and compare with AMF communities established in three field experiments focusing on soybean production. Since 2000, these experiments differed only in the crop rotation. In one experiment, the rotation was bi-annual with soybean and maize, in the second soybean was mono-cropped, and in the third the soybean/maize rotation was on a more intensive, annual basis. AMF spores were extracted from the soils, counted and morphologically identified. In total, 63 AMF species, belonging to 20 genera, were detected. Average spore densities and species richness decreased in conventionally tilled systems (3–4 spores  $g^{-1}$  and 12–17 species), when compared to no-tillage (4–6 spores  $g^{-1}$  and 15–18 species) and natural savanna (9–11 spores  $g^{-1}$  and 16–22 species), but AMF evenness (Pielou index) was higher under both tillage systems (0.65–0.77), than in the savanna forests (0.54–0.62). AMF community composition significantly differed between all systems. Indicator species were revealed for all three ecosystems: e.g. *Glomus macrocarpum* and *Sclerocystis sinuosa* (Cerrado), *Sc. coremioides* (no-tillage) and *Gigaspora margarita*, *Racocetra coralloidea* and *Ra. fulgida* (tillage). In conclusion, soil cultivation and fertilizer application lead to decreased AMF species richness but remarkably AMF diversity was maintained on similarly high levels in soybean-based crop production systems, even under intensive soybean mono-cropping. The changes in AMF community structure rather were linked to soil pH and potassium, calcium and magnesium than to phosphorus availability or the organic carbon contents. Several species were unrecoverable from either of the tillage systems following conversion from natural savanna forests to cropland.

### 1. Introduction

The Cerrado is the largest savanna in America (approx. 2 millions  $km^2$ ), extending from South Eastern Brazil up to Paraguay and Bolivia. It is the most species-rich savanna in the world, sheltering 5% of the world's and 30% of the Brazilian flora and fauna and constitutes the second largest tropical biome in South America (Myers et al., 2000;

Françoso et al., 2015; Françoso et al., 2015; Myers et al., 2000; Françoso et al., 2015). It has a large transition area with the Amazonian rainforest (ca. 6 million  $km^2$ , 60% in Brazil) in the North and the highly fragmented Atlantic Rainforest (formerly ca. 1.2 million  $km^2$ ) in the East, as well as a transition to the dry forest savanna biome in North Eastern Brazil, called the Caatinga (ca. 0.85 million  $km^2$ ). Due to its high species richness and significant endemism, the Cerrado is con-

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sidered as a hotspot for world biodiversity (Myers et al., 2000; Mittermeier et al., 2011; Marchese, 2015; Marchese, 2015; Myers et al., 2000; Mittermeier et al., 2011; Marchese, 2015; Marchese, 2015).

In recent decades, deforestation of the Cerrado has accelerated, mainly in favor of agricultural production (Klink and Machado, 2005; Carranza et al., 2014), especially for conversion to pastures for cattle production, and soybean and maize, but also for coffee or sugar cane production. More than 50% of the natural vegetation has already been lost (Costa and Pires, 2010), while further conversion, also in the Amazonian basin, will likely strongly affect the climate on regional and continental scales (Leite et al., 2012; Pires and Costa, 2013). However, despite major threats to biodiversity, climate change, water budgets under pronounced more dry seasons, there remains great concern for worst-case scenarios, which predict that the Cerrado will be completely deforested by 2030 (Malhado et al., 2010).

Soybean is, globally, the most widely grown legume (Ainsworth et al., 2012), cultivated primarily in large scale commercial systems in tropical and subtropical areas, such as in the USA, Brazil, Northern Argentina, China and India (FAO, 2016). Since 1970, worldwide soybean production has risen dramatically due, in part, to a massive increase in production area and to significant yield increases per unit area (Masuda and Goldsmith, 2009), even in temperate conditions (e.g. Schmidt et al., 2015). In 2014, soybean production reached 108 million tons in the USA and 86.7 tons in Brazil (FAO, 2016).

In the Brazilian Cerrado, the largest savanna biome in tropical America, soybean production has drastically intensified, both in terms of the increased area of production, but also in terms of the cultivars used, intense soybean mono-cropping, and the application of more effective nitrogen-fixing rhizobacteria (e.g. Hungria et al., 2006; Oya et al., 2004). However, soybean production has been identified as one of the underlying direct and indirect causes of tropical deforestation and soil degradation in Brazil (Barona et al., 2010). Moreover, intensified soybean production causes various soil fertility problems, such as loss of aggregate stability, decrease of soil organic matter, soil erosion and nutrient losses, such as observed in Brazil and Northeast China (Cunha et al., 2001; Liu et al., 2010).

Some alternatives to prevent soil erosion, fertility and yield losses and to minimize negative environmental impacts, have already been developed, for instance no-tillage practices, i.e. soybean seeding and management without any soil cultivation (Cunha et al., 2001; Yusuf et al., 1999). The re-introduction and promotion of beneficial micro-organisms, such as rhizobia and arbuscular mycorrhizal fungi (AMF) are other viable alternatives to optimize plant nutrition, especially plant nitrogen and phosphorus assimilation (Baum et al., 2015).

AMF form a mycorrhizal association with the majority of land plants (Smith and Read, 2008). These soil fungi deliver mineral nutrients to the plant roots, especially those of low mobility, such as P (e.g. Tchabi et al., 2010; Frosi et al., 2016). AMF also help protect host plants against pathogens and pests, including root nematodes, reducing damage (Baum et al., 2015; Tchabi et al., 2016), hydraulic (Armada et al., 2015) and saline (Yano-Melo et al., 2003) stresses, and increase plant tolerance against phytotoxic metals, such as manganese and aluminum (Zhang et al., 2015). Furthermore, AMF contribute to soil aggregation and stability, by producing a stable glycoprotein called glomalin (Wright and Upadhyaya, 1998).

Under various climatic conditions, several studies have investigated the impact of various agricultural management practices on the AMF diversity (Franke-Snyder et al., 2001; Oehl et al., 2003, 2004; Pontes et al., 2017), especially with respect to soil cultivation practices (e.g. Maurer et al., 2014; Wetzel et al., 2014; Säle et al., 2015). In the Cerrado, just a few pioneer studies have so far been conducted (e.g. Spain and Miranda, 1996; Miranda and Miranda, 2002; Carvalho et al., 2012), which have indicated that AMF spore densities and AMF species richness decreases following conversion from savannas to agricultural sites (Ferreira et al., 2012; Fernandes et al., 2016).

The aim of our current study was to determine AMF diversity and

community structure in soybean production systems subjected to conservation and conventional tillage practices within the Brazilian Cerrado, and to compare the AMF communities established with those of neighboring natural savanna forests. Our hypothesis was that land use for soybean production in the Cerrado undermines AMF diversity and that impact is greater when the soil is tilled. Furthermore, it was hypothesized that soybean monoculture leads to less diverse AMF communities when compared to soybean/maize crop rotation systems. Finally, we expected that pH and phosphorus availability have a greater impact on the AMF communities than the soil organic matter contents.

## 2. Material and methods

### 2.1. Study sites

The bulk of our study was performed using three established long-term field experiments of the Field Research Station of the “Centro de Pesquisa Agropecuária dos Cerrados” (Embrapa Cerrados) located in Planaltina-DF (near Brasília; latitude 15°38'S, 47°45'W; altitude: 1100 m asl), all established on Ferralsols (IUSS 2014). According to Köppen (Kottek et al., 2006), the climate is tropical humid with dry winters of about six months followed by six months of wet summer (Aw). Mean annual rainfall is about 1570 mm and the mean annual temperature is 21.3 °C. The original vegetation of the area is Cerrado, a typical Brazilian savanna forest according to Eiten (1972). In order to identify the naturally occurring AMF communities, three natural Cerrado forests were included in our study, each located adjacent to one of the three experiments.

The three field experiments are situated within approximately 1 km distance of each other. In the first experiment (47°43'3.92"W; 15°36'6.36"S), no-tillage and conventional tillage were established in 1979. Since 1995, either maize (*Zea mays* L.) or soybean (*Sorghum bicolor* (L.) Moench) was cultivated annually, and since 2000 a strict annual crop rotation between soybean and maize has been followed. In this experiment, the land was ploughed (25 cm depth) once annually before soybean or maize seeding. In the second experiment (47°44'12.09"W; 15°35'34.04"S), established in 1996, soybean has been cultivated in monoculture since 2000 and the same no-tillage and conventional tillage practices have been applied as in the first experiment. Also in the third experiment (47°42'24.93" W; 15°35'53.74"S), established in 2000, the main objective was to compare a no-tillage and conventional tillage system. Here, the annual crop rotation has been soybean followed by maize for 13 years, before soil sampling for the present study. In the cultivated treatments, soil was ploughed (25 cm depth) twice per year, once before soybean and once before maize seeding. Detailed information on the nine study sites, and especially on the management practices in the treatments of the three field experiments are provided in Table 1. Soybean and maize are cultivated for approximately 115 days and 120 days, respectively, per season until grain harvest. Herbicide application was consistent for both pre- and post-emergence of soybean and maize across all three experiments; weed growth was observed only before the first herbicide application, for approximately 3–14 days at the beginning of each rainy season, so that the emerging weeds cannot be considered as hosts for AMF species in the fields.

### 2.2. Soil sampling and soil analyses

Soils were sampled (0–20 cm sampling depth) twice in the field experiments and in the adjacent Cerrado sites, in triplicate. The first sampling set of soybean was conducted in November 2013 during the wet season, at 50 days after sowing, and the second sample set was removed in August 2014, during the dry season shortly after harvest, but well before the next growing season. At each site, three pseudo-replicate plots (7 m × 8 m) were sampled, 10 m distance from each other. Ten subsamples were removed from each replicate plot and

**Table 1**

Information on study sites: Ecosystems, crop rotation, age of current land use, fertilization level, plant protection strategies &amp; weed growth.

Study sites	(Agro-)Ecosystem	Current crop rotation (since)	Established in	Fertilization	Plant protection
Cerrado					
CS-I	Natural Savanna forest	–	–	–	–
CS-II	Natural Savanna forest	–	–	–	–
CS-III	Natural Savanna forest	–	–	–	–
I. Long-term field experiment					
NT-I	No-tillage	Bi-annual soybean/maize (2000)	1979	N: <i>Bradyrhizobium japonicum</i> & <i>B. diazoefficiens inocula</i> P: 35 kg ha <sup>-1</sup> K: 70 kg ha <sup>-1</sup>	Herbicides before and after seeding, repeatedly fungicides, insecticides & acaricides
CT-I	Conventional tillage	Bi-annual soybean/maize (2000)	1979		
II. Long-term field experiment					
NT-II	No-tillage	Soybean mono-cropping (2000)	1996	N: <i>Bradyrhizobium japonicum</i> & <i>B. diazoefficiens inocula</i> P: 35 kg ha <sup>-1</sup> K: 70 kg ha <sup>-1</sup>	Herbicides before and after seeding, repeatedly fungicides, insecticides & acaricides
CT-II	Conventional tillage	Soybean mono-cropping (2000)	1996		
III. Long-term field experiment					
NT-III	No-tillage	Annual soybean/maize (2000)	2000	N: <i>Bradyrhizobium japonicum</i> & <i>B. diazoefficiens inocula</i> P: 35 kg ha <sup>-1</sup> K: 70 kg ha <sup>-1</sup>	Herbicides before and after seeding, repeatedly fungicides, insecticides & acaricides
CT-III	Conventional tillage	Annual soybean/maize (2000)	2000		

Since 2000, glyphosate, S-Metolachlor and atrazine were the herbicide substances most frequently used in maize and soybean production. Most frequently used fungicide substances in soybean were carboxine and ziram (pre-emergence) and azoxystrobin, pyraclostrobin, cyproconazole and epiconazole (post-emergence), and at later stages tebuconazole (up to three applications including stubble treatments, e.g. against soybean rust).

combined into a pooled sample per replicate plot (~4 kg per field plot replicate). Physical and chemical soil analyses were conducted at the Soil Laboratory of Embrapa Cerrados, using standard methods (Silva et al., 1999).

### 2.3. Arbuscular mycorrhizal fungal trap cultures

A portion of the sampled soil was additionally used for establishing trap cultures (three trap culture pots per treatment) to obtain healthy glomerospores in sufficient quantity for morphological analysis. For each pot, 180 g of soil (60 g per field plot replicate) were placed in three lines on the surface of 1.5 kg of sterile substrate, placed in 2 kg plastic pots, and covered by another 500 g of the same sterile culture substrate. The substrate was a heat-sterilized soil/sand mixture (1:1), sterilized in an oven at 105 °C for 24 h. Five maize and 10 sorghum seeds were sown as hosts, on the top of the inoculum lines. The cultures were maintained in a greenhouse for eight months (day temperatures 26–33 °C, night temperatures 23–27 °C; relative air humidity approx. 70%) and watered every second day. After eight months, substrate samples were collected for spore extraction and morphological AMF species identification.

### 2.4. Arbuscular mycorrhizal fungal spore extraction and species identification

AMF spores were extracted from field and trap culture samples by wet sieving (Gerdemann and Nicolson, 1963), followed by water and sucrose centrifugation (Jenkins, 1964). For identification of the AMF species, the extracted spores were mounted on slides with PVLG (polyvinyl alcohol lactoglycerol) and PVLG + Melzer's reagent (1:1 v/v) and observed under a compound microscope. AMF species were identified according to Schenck and Pérez (1990), Błaszkowski (2012)

and all original and emended AMF genus and species descriptions available.

### 2.5. Arbuscular mycorrhizal fungal communities

To assess AMF community composition and structure, species richness, relative abundance, frequency, evenness and diversity were determined. Species richness was defined as the number of AMF species recorded in each study area. The frequency (F) of a given species (i) was estimated according to the equation:  $F = J_i/k$ , where F = frequency of occurrence of the species i,  $J_i$  = number of sites or field plot replicates, respectively, where the species i occurred, k = total number of sites or field plot replicates, respectively (Brower and Zar, 1984).

The relative abundance of AMF species (RA<sub>i</sub>) was calculated according to the equation:  $RA_i = A/\Sigma a \times 100$ , where RA<sub>i</sub> is the relative abundance of a given species i, A = abundance of the species i,  $\Sigma a$  = sum of abundances of all species.

The ecological indices were evaluated according to the Shannon index:  $H' = -\Sigma (P_i \ln [P_i])$ , where  $P_i = n_i/N$ ,  $n_i$  = number of individuals of the species i, and N = total number of individuals of all species (Shannon and Weaver, 1949); Pielou's evenness index ( $J$ ) =  $H'/\log(S)$ , where H' is the value obtained by the Shannon index and S is the total number of species (Pielou, 1975); Margalef's index, calculated based on:  $d = S-1/\log N$ , where S = number of species, N = total number of spores per sample (Margalef, 1958); and Sørensen's similarity index (Brower and Zar, 1984), which was used to compare AMF communities between different sites/treatments. The analyses, including the Cluster dendrogram, were performed using the PRIMER 6.0 program (Clarke and Gorley, 2006).

### 2.6. Data analysis

In general, the data obtained from the two collections for the different soil parameters, the AMF spore density, species richness and the different AMF diversity and community parameters were summarized for each of the four pseudo-replicates per site (=per treatment). The data were subjected to analysis of variance (ANOVA) and *t*-tests to compare between the sites. The means were additionally compared with Tukey test and Fisher's Least Significant Difference ( $p < 0.05$ ), using the program Assistat 7.6 beta (Silva and Azevedo, 2006). The data on the AMF communities (relative abundance) were used to perform Permutation multivariate analysis of variance (PerMANOVA), using Bray-Curtis distance, to test if the communities differ among land uses types. With these data linear regressions, canonical correspondence analysis (CCA) and redundancy analyses (RDA) were also calculated, to consider the ecological and soil parameters. The significance of axis and variables in CCA and RDA were determined by the Monte Carlo test ( $p < 0.05$ ). For the analyses of indicator species, the Monte Carlo test was used according to Dufre & ne and Legendre (1997). The species with indication value > 25% and  $p < 0.05$  were considered to be good indicators of the areas. All multivariate analyses (indicator species, RDA, CCA and PerMANOVA) were performed using the program PC-ORD version 6.0 (McCune and Mefford, 2006).

## 3. Results

### 3.1. Chemical and physical soil parameters

Soil organic carbon was 22–29 g kg<sup>-1</sup> in the natural Cerrado, 17–21 g kg<sup>-1</sup> in the no-tillage plots and 15–17 g kg<sup>-1</sup> in the tillage plots of the long-term field experiments (Table 2). Soil pH was 4.4–4.5 in the Cerrado, 5.4–5.5 in the no-tillage plots and 4.9–5.3 in the tillage plots. Soil available P was just 1.4–2.7 mg kg<sup>-1</sup> in the Cerrado, 27–42 mg kg<sup>-1</sup> in the no-tillage plots and intermediary (11–35 mg kg<sup>-1</sup>) in the tillage plots. Also the available nutrients K, Mg and Ca were higher in the fertilized field experiments than in the non-fertilized savannas (Table 2), while available Al was clearly higher in the natural savannas, but could drastically be reduced by soil nutrient, liming and pH management in the field experiments in the previous years. Sand contents were 41–49% in the whole study area, while silt contents were 18–23% and clay contents 29–38%.

### 3.2. Overall arbuscular mycorrhizal fungal species richness

In total, 10,871 AMF spores were identified from the field soil samples, belonging to 58 AMF species (Table 3). From the AMF trap cultures, five additional species were recovered, that were not unequivocally detected from the field soil samples. The 63 AMF species

Table 2

Chemical and physical soil parameter of the Cerrado Savanna, the No-Tillage and the Conventional Tillage sites under study.

		Cerrado			No-Tillage			Conventional tillage			LSD
		CS I	CS II	CS III	NT I	NT II	NT III	CT I	CT II	CT III	
Organic carbon	g/kg	28.5 a	23.6 ab	22.2 bc	20.6 bcd	17.3 de	18.5 cde	16.2 de	14.9 e	17.3 cde	3.0
pH	(H <sub>2</sub> O)	4.5 c	4.5 c	4.4 c	5.5 a	5.4 a	5.5 a	5.3 ab	5.3 ab	4.9 bc	0.3
P-avail	mg/kg	2.4 c	1.7 c	1.4 c	30.3 ab	42.4 a	27.5 ab	23.3 bc	34.5 ab	11.8 bc	14.7
K-avail	mg/kg	60.4 c	52.8 c	56.8 c	217.6 a	150.1 ab	148.8 ab	198.8 ab	138.4 b	152.0 ab	43.0
Mg-avail	mg/kg	19.4 c	17.6 c	16.8 c	165.9 a	145.3 a	143.7 a	169.8 a	159.4 a	82.3 b	35.5
Ca-avail	mg/kg	22.5 e	5.4 e	4.6 e	869.4 ab	636.2 bc	518.7 cd	907.4 a	712.4 abc	345.8 d	148.4
Al-avail	mg/kg	228.6 a	166.1 b	143.5 b	4.5 c	22.4 c	6.3 c	17.8 c	17.3 c	26.8 c	13.6
Clay	(%)	25.6 a	27.9 a	32.3 a	27.7 a	31.0 a	32.7 a	28.2 a	28.0 a	35.9 a	–
Silt	(%)	21.7 a	17.9 a	14.9 a	15.5 a	18.6 a	14.5 a	23.2 a	13.0 a	15.8 a	–
Sand	(%)	52.7 a	54.2 a	52.8 a	56.8 a	50.5 a	52.8 a	48.6 a	59.0 a	48.4 a	–

Data presented as means of three plot replicates. Means followed by the same letter in the same row do not differ by Tukey test at 5% after One-Way ANOVA. LSD is Fisher's Least Significant Difference. I, II, III refer to the three savannas and adjacent long-term field experiments investigated.

Table 3

r<sup>2</sup> and P-values obtained from linear regressions between the soil parameters affected by land use and management practices and AMF species richness.

	C <sub>org</sub>	pH <sub>H2O</sub>	P <sub>avail</sub>	K <sub>avail</sub>	Mg <sub>avail</sub>	Ca <sub>avail</sub>	Al <sub>avail</sub>
r <sup>2</sup>	0.10	0.16	0.05	0.24	0.14	0.17	0.16
P-value	0.11	0.04	0.24	0.01	0.05	0.03	0.04

belonged to 20 AMF genera and 11 families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Dentiscutataceae, Diversisporaceae, Entrophosporaceae, Gigasporaceae, Glomeraceae, Paraglomeraceae, Racocetraceae and Scutellosporaceae). The genera with the highest AMF species diversity were: *Acaulospora* (15 species), *Glomus* (8), *Racocetra* and *Rhizoglomus* (5), and *Archaeospora*, *Gigaspora*, *Funneliformis*, *Fuscutata* and *Dentiscutata* (3 species). Two species each were identified from *Cetraspora*, *Claroideoglomus*, *Sclerocystis* and *Scutellospora* (2), while one species was detected from *Ambispora*, *Bulbospora*, *Diversispora*, *Dominikia*, *Orbispora*, *Kuklospora* and *Paraglomus* (Table 3). Six species could not unequivocally be identified to species level and likely constitute new records for science (e.g. *Acaulospora* sp. CS1 and *Glomus* sp. CS5).

### 3.3. Arbuscular mycorrhizal fungal spore density and species richness

In the first sampling date (wet season), AMF spore density was between 9 and 14 spores g<sup>-1</sup> soil in the three Cerrado sites under study, 6–8 in the no-tillage, and 4–6 in the conventional tillage plots. In the second sampling date (dry season), densities were slightly lower: 7–11 spores g<sup>-1</sup> soil in the Cerrado, 3–5 in the no-tillage, and 2–3 in the conventional tillage plots. When summarized for both sampling dates, AMF spore density was 9–11 spores g<sup>-1</sup> soil in the Cerrado, 4–6 in the no-tillage, and 3–4 in the conventional tillage plots (Fig. 1A).

Average AMF species richness was 11–14 species in the three Cerrado sites at the first sampling date, while it was 10–14 species in the no-tillage, and 9–13 in the conventional tillage plots. In the second sampling date (dry season), AMF species richness was similar: 9–13 in the Cerrado, 11–13 in the no-tillage, and 8–11 in the conventional tillage plots. When summarized for both sampling dates, average AMF species richness decreased with the level of cultivation: 16–22 in the Cerrado, 15–18 in the no-tillage, and 12–17 in the conventional tillage plots (Fig. 1B). Total AMF species richness was 25–32 species in the Cerrado, 23–24 in the no-tillage, and 15–26 in the conventional tillage plots (Table 3).

### 3.4. Relationship between soil parameters and arbuscular mycorrhizal fungal species richness

Linear regressions between the chemical and physical soil parameters affected by the land use and management practices and the total

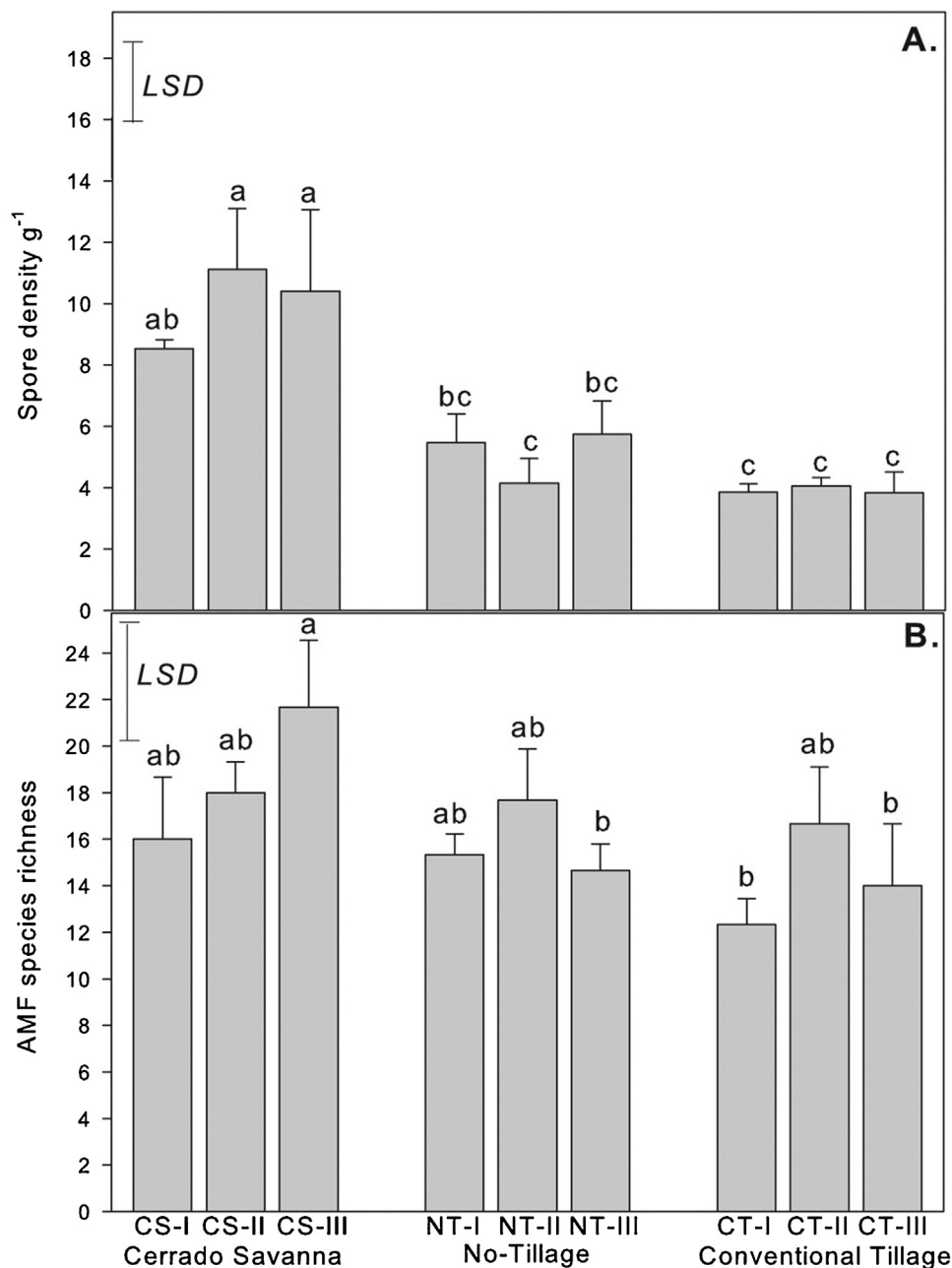


Fig. 1. AMF spore densities (A) and AMF species richness (B) in three Cerrado Savanna forests (CS), and the No-Tillage (NT) and the Conventional Tillage (CT) sites under study. Data presented as means of three plot replicates per site and depth. Means followed by the same letter in the same soil depth do not differ by Tukey test at 5% after One-Way ANOVA. *LSD* is the Fisher's Least Significant Difference.

AMF species richness at sites revealed negative correlations between the pH and cation nutrient availability (K, Mg and Ca) and the AMF species richness, while Al-availability was positively correlated with the number of species per site (Table 3). Remarkably, organic carbon and P availability was not correlated with the AMF species richness at sites.

### 3.5. Frequent, dominant, and rare arbuscular mycorrhizal fungal species

Twenty-one AMF species occurred frequently across ecosystems; ten species were recovered only in the natural Cerrado, and five species were found only in the savannas and in the no-tillage systems (Table 4). Seven commonly found species occurred only in the soybean based agro-ecosystems, and one other only in the conventional tillage system. Nineteen AMF species were only rarely found (Table 4).

The most frequently occurring AMF species in our study was *Glomus*

*macrocarpum*, recovered from all 9 sites and all 27 plots analyzed (Table 4). Also, *Gigaspora margarita* and *Acaulospora spinosa* were found at all nine sites, and in most plots. *Acaulospora mellea*, *Ac. scrobiculata*, *Claroideoglossum etunicatum*, *Gigaspora gigantea*, *Paraglossum occultum* and *Racocetra tropicana* were recovered from 8 sites (89%) and 33–85% of all plots analyzed.

The most dominant species in the field soil samples of the Cerrado were *Gl. macrocarpum*, *Gl. brohultii*, *Gl. microcarpum*, and *Gl. glomerulatum* (Table 4). In the no-tillage soybean production sites, the most dominant species were *Ac. mellea*, *Ac. scrobiculata*, *Gl. macrocarpum* and *Pa. occultum*. In the conventional tillage sites, *Gi. margarita* and *Ra. coralloidea* were the most dominant AMF species, and *Ra. fulgida* was exclusively associated with these agricultural ecosystems (Table 4).

Species, such as *Glomus* sp. CS6, *Acaulospora* sp. CS2, *Orbispora pernambucana*, *Bulbospora minima*, *Sclerocystis sinuosa* and *Scutellospora*

**Table 4**

Relative abundance and frequency (in %) of all AMF species found in three natural Cerrado savannas and each three adjacent no-tillage and conventional tillage systems under intensive soybean production.

Site	Cerrado			No-tillage			Convent. tillage			Frequency per 27 plots
	CS -I	CS -II	CS -III	NT -I	NT -II	NT -III	CT -I	CT -II	CT -III	
<i>Frequent species in all ecosystems under study</i>										
<i>Glomus macrocarpum</i>	59.6	47.4	48.4	39.9	22.1	36.1	36.2	39.1	35.2	100
<i>Gigaspora margarita</i>	0.2	0.7	0.9	2.0	0.8	2.4	3.9	6.8	9.8	89
<i>Acaulospora scrobiculata</i>	–	0.1	1.0	9.9	17.5	14.1	5.7	5.9	9.0	85
<i>Claroideoglomus etunicatum</i>	0.4	2.1	0.2	–	4.2	20.4	4.7	7.3	12.1	78
<i>Dentiscutata cerradensis</i>	0.1	0.4	0.2	1.6	1.3	7.4	–	0.6	–	78
<i>Gl. microcarpum</i>	6.6	25.1	7.7	11.2	9.4	–	36.8	18.6	–	78
<i>Ac. mellea</i>	1.1	0.9	3.5	4.7	5.7	–	0.4	6.2	0.1	70
<i>Ac. spinosa</i>	0.1	0.4	0.2	2.7	0.8	0.1	1.2	1.2	2.3	70
<i>Paraglomus occultum</i>	0.1	0.7	–	1.0	6.9	5.6	0.8	3.6	4.7	70
<i>Glomus brohultii</i>	13.0	11.1	6.6	–	13.4	4.6	–	3.0	5.9	67
<i>Gi. gigantea</i>	0.2	0.6	0.3	0.2	0.3	–	0.6	0.2	0.5	52
<i>Gl. glomerulatum</i>	5.3	2.3	5.3	–	3.1	1.0	–	0.8	1.8	52
<i>Ac. longula</i>	1.2	–	2.9	–	0.4	–	1.8	0.3	0.1	48
<i>Glomus sp. CS5</i>	0.1	1.5	–	–	1.3	–	2.5	1.5	–	48
<i>Ac. spinosissima</i>	0.2	–	0.9	0.2	–	0.8	–	–	0.4	33
<i>Racocetra tropicana</i>	0.1	0.2	0.1	0.1	–	0.1	0.4	0.2	0.1	33
<i>Scutellospora calospora</i>	0.1	0.6	3.3	–	–	–	–	0.3	–	30
<i>Rhizoglomus clarum</i>	0.2	0.4	–	–	–	–	–	0.2	0.1	26
<i>Funneliformis mosseae</i>	0.1	–	–	–	0.1	0.3	–	0.3	0.3	22
<i>Rh. intraradices</i>	0.1	–	0.1	1.1	1.4	2.1	–	0.2	–	22
<i>Species only found in the natural Cerrado savannas</i>										
<i>Glomus sp. CS6</i>	9.7	2.2	4.6	–	–	–	–	–	–	26
<i>Acaulospora sp. CS2</i>	0.1	0.2	0.8	–	–	–	–	–	–	22
<i>Orbispora pernambucana</i>	0.1	2.2	2.9	–	–	–	–	–	–	22
<i>Bulbospora minima</i>	–	0.2	1.9	–	–	–	–	–	–	15
<i>Cetranspora gilmorei</i>	–	–	0.2	–	–	–	–	–	–	11
<i>Sclerocystis sinuosa</i>	0.1	0.1	–	–	–	–	–	–	–	11
<i>Ac. foveata</i>	0.1	–	0.1	–	–	–	–	–	–	7
<i>Fu. halonatus</i>	0.1	–	0.1	–	–	–	–	–	–	7
<i>Archaeospora myriocarpa</i>	*	*	–	–	–	–	–	–	–	–
<i>Species only found in the natural Cerrado savannas and no-tillage systems</i>										
<i>Sc. coremioides</i>	–	–	0.1	0.2	0.3	0.5	–	–	–	26
<i>Ac. morrowiae</i>	–	0.3	6.2	–	1.3	–	–	–	–	15
<i>Dominikia sp. CS4</i>	0.8	–	0.6	1.0	–	–	–	–	–	15
<i>Ce. pellucida</i>	–	0.1	–	0.3	–	–	–	–	–	11
<i>Gl. ambisporum</i>	*	–	*	*	–	*	–	–	–	–
<i>Species only found in the agro-ecosystems</i>										
<i>Ac. denticulata</i>	–	–	–	21.2	2.9	0.7	0.8	0.5	–	44
<i>Ra. coralloidea</i>	–	–	–	1.1	–	0.1	2.2	0.6	6.9	44
<i>Acaulospora sp. CS1</i>	–	–	–	0.5	5.0	–	–	0.9	–	26
<i>Acaulospora reducta</i>	–	–	–	0.1	1.3	0.2	0.2	0.3	0.1	26
<i>Fuscutata rubra</i>	–	–	–	0.1	0.1	0.1	–	–	5.2	22
<i>Ambispora appendicula</i>	–	–	–	–	0.1	0.3	–	0.2	–	15
<i>Gi. decipiens</i>	–	–	–	0.3	–	0.2	–	–	0.7	11
<i>Species only found in conventional tillage systems</i>										
<i>Ra. fulgida</i>	–	–	–	–	–	–	2.0	0.2	–	15
<i>Rare found species, not attributed to an ecosystem</i>										
<i>Cl. claroideum</i>	–	0.1	0.1	–	–	–	–	0.8	–	15
<i>Ar. trappei</i>	–	–	0.1	–	–	–	–	–	1.3	7
<i>Rh. microaggregatum</i>	0.8	–	–	–	–	–	–	–	–	4
<i>Gl. diaphanum</i>	–	0.2	–	–	–	–	–	–	–	4
<i>De. scutata</i>	–	–	0.1	–	–	–	–	–	–	4
<i>Fu. geosporus</i>	–	–	0.1	–	–	–	–	–	–	4
<i>Ra. undulata</i>	–	–	0.1	–	–	–	–	–	–	4
<i>Sc. aurigloba</i>	–	–	0.1	–	–	–	–	–	–	4
<i>Acaulospora sp. CS3</i>	–	–	–	0.2	–	–	–	–	–	4
<i>Diversispora eburnea</i>	–	–	–	0.1	–	–	–	–	–	4
<i>Fu. heterogama</i>	–	–	–	0.3	–	–	–	–	–	4
<i>Ra. alborosea</i>	–	–	–	–	0.1	–	–	–	–	4
<i>Ac. sieverdingii</i>	–	–	–	–	–	0.2	–	–	–	4
<i>Ac. tuberculata</i>	–	–	–	–	–	*	–	–	–	–
<i>Fu. aurea</i>	–	–	–	–	–	0.1	–	–	–	4
<i>De. nigra</i>	–	–	–	–	–	0.1	–	–	–	4
<i>Rh. irregulare</i>	–	–	–	–	–	0.4	–	–	–	4
<i>Rh. natalense</i>	–	–	–	–	–	–	–	0.6	–	4
<i>Ac. laevis</i>	–	–	–	–	–	–	–	–	0.1	4

(continued on next page)

Table 4 (continued)

Site	Cerrado			No-tillage			Convent. tillage			Frequency per 27 plots
	CS -I	CS -II	CS -III	NT -I	NT -II	NT -III	CT -I	CT -II	CT -III	
<i>Ar. undulata</i>	–	–	–	–	–	–	–	*	–	–
<i>Kuklospora colombiana</i>	–	–	–	–	–	–	–	*	–	–
AMF species richness in field soil samples	27	25	32	23	24	24	15	26	21	
AMF species richness in field soil samples and trap cultures	29	26	33	24	24	26	15	28	21	

\* = only detected in AMF trap cultures but not in corresponding field soil samples.

Frequency per all 27 field plots might be classified as dominant (> 50%: D), very common (30.1–50.0%: M), common (10.1–30%: C), and rare (≤ 10%: R) species.

*calospora*, were exclusively found in the Cerrado soils (Table 4). Species found only in the natural Cerrado and no-tillage systems included *Sc. coremioides*, *Acaulospora morrowiae*, *Dominikia* sp. CS4 and *Cetraspora pellucida*. Species found only in the agro-ecosystems included *Ac. denticulata*, *Ra. coralloidea*, *Ac. reducta* and *Fuscutata rubra*.

Twelve of the rare AMF species were detected with one or two spores, such as *Acaulospora laevis*, *Dentiscutata scutata*, *De. nigra*, *Racocetra alborosea*, *Ra. undulata* and *Scutellospora aurigloba* (Table 4).

### 3.6. Arbuscular mycorrhizal fungal indicator species

In total, 18 AMF species were revealed as indicator species (Table 5). Ten species were indicative for the Cerrado sites, e.g. *Glomus macrocarpum*, *Gl. brohultii*, *Sc. sinuosa*, *Bu. minima* and *Or. pernambucana* (Table 5). Five species were indicators for the no-tillage systems, e.g. *Ac. denticulata*, *Ac. scrobiculata*, *Dentiscutata cerradensis*, *Pa. occultum*, and *Sc. Coremioides*. Three Gigasporales species were indicative of the conventional tillage systems (*Gi. margarita*, *Ra. coralloidea* and *Ra. fulgida*).

### 3.7. Arbuscular mycorrhizal fungal species composition in the ecosystems based on the multivariate analyses

In the Redundancy analyses (RDA) based on the AMF communities,

Table 5

Indicator species for the Cerrado savannas, no-tillage or conventional tillage in the present study.

	Indicator value (IV)	p-value
<b>Cerrado</b>		
<i>Glomus</i> sp. CS6	77.8	0.0002****
<i>Gl. glomerulatum</i>	66.4	0.0005****
<i>Scutellospora calospora</i>	73.2	0.0007****
<i>Acaulospora</i> sp. CS2	66.7	0.0009****
<i>Orbispora pernambucana</i>	66.7	0.0010***
<i>Gl. macrocarpum</i>	42.4	0.0030***
<i>Gl. brohultii</i>	53.7	0.0172**
<i>Bulbospora minima</i>	44.4	0.0217**
<i>Rhizoglomus clarum</i>	37.7	0.0502*
<i>Sclerocystis sinuosa</i>	33.3	0.0815*
<b>No-tillage</b>		
<i>Ac. denticulata</i>	84.0	0.0003****
<i>Ac. scrobiculata</i>	63.3	0.0005****
<i>Dentiscutata cerradensis</i>	69.7	0.0005****
<i>Sc. coremioides</i>	63.2	0.0012***
<i>Paraglomus occultum</i>	58.2	0.0296**
<b>Conventional tillage</b>		
<i>Racocetra coralloidea</i>	79.7	0.0001****
<i>Gigaspora margarita</i>	75.9	0.0002****
<i>Ra. fulgida</i>	44.4	0.0236**

The significance of these values was determined by the Monte Carlo test using 1000 permutations to (Dufrene and Legendre, 1997).

Significant \* on  $p < 0.1$ , \*\* on  $p < 0.05$ , \*\*\* on  $p < 0.01$ , \*\*\*\* on  $p < 0.001$  significance level.

Species with IV > 25% and  $p < 0.05$  were considered as good indicators of the areas.

the nine Cerrado sites clustered on the left side (Fig. 2A), while the 18 agricultural plots, belonging to the three long-term experiments, grouped with the other site. Several AMF species grouped together with the Cerrado sites. These were primarily the indicator species for these sites (e.g. *Bu. minima*, *Sc. sinuosa*, *Or. pernambucana* and several *Glomus* species), and also some less frequent species, such as *Cetraspora gilmorei*. Other AMF species clearly clustered with the soybean production sites (Fig. 2A). When the RDA analysis was applied on the six agricultural plots of the three field experiments only, the no-tillage and the conventional sites separated clearly from each other (Fig. 2B), but the field plot replicates of NT-I and CT-I (representing the oldest field experiment, subjected to the bi-annual soybean-maize crop rotation) also separated clearly from the field plot replicates of the two other experiments. Along the side representative for the no-tillage systems, several other species can be recognized, among them the indicator species *Ac. denticulata*, *Ac. scrobiculata* and *De. cerradensis*, and also other species like *Ce. pellucida* and *Fuscutata heterogama*. Species, such as *Fuscutata aurea*, *Fu. rubra* and *Archaeospora trappei* clustered within the sector representative for the conventional tillage systems together with the species found as indicators for these later sites (Fig. 2B; *Gi. margarita*, *Ra. coralloidea* and *Ra. fulgida*).

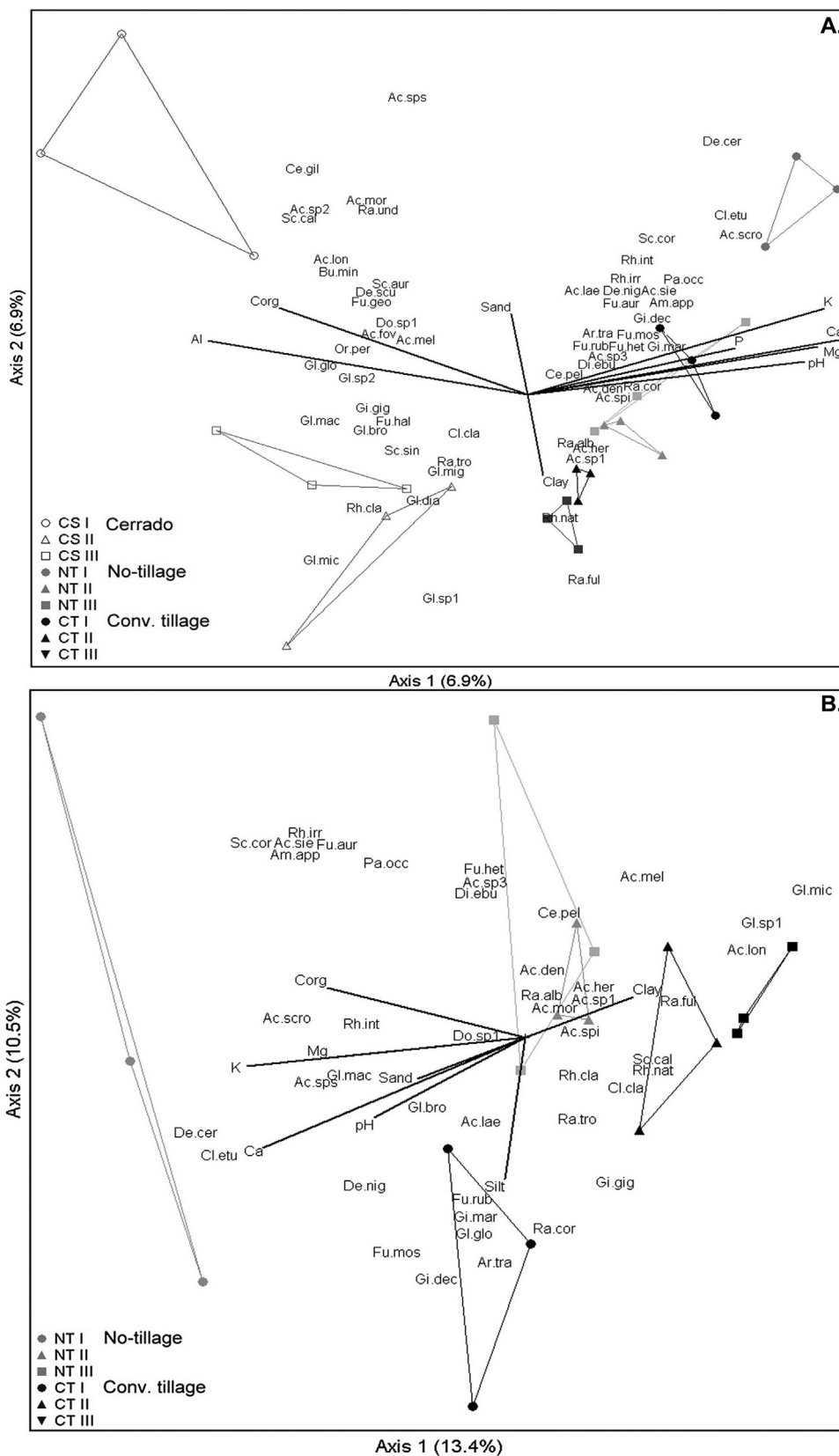
In the cluster analysis based on Sørensen similarity (Fig. 3), the three Cerrado sites formed one cluster, while the NT-I and CT-I separated slightly from the four other agricultural plots (NT-II, NT-III, CT-II and CT-III). The highest similarity was found between the no-tillage and conventional tillage plots of the soybean mono-culturing (NT-II and CT-II). The PerMANOVA analyses indicated that all ecosystems had a distinct AMF species composition ( $F = 5.71$ ;  $p = 0.0002$ ).

### 3.8. Arbuscular mycorrhizal fungal diversity indices

The Margalef diversity indices, based on the AMF species richness, were higher (3.3–4.6) in the three Cerrado sites than in the three no-tillage (2.8–3.6), and the three conventional tillage (2.5–3.3) plots of the experiments (Table 6). The Simpson dominance values were also higher in the Cerrado (0.27–0.31), when compared to the no-tillage (0.13–0.23) and the conventional tillage (0.18–0.26) sites. In contrast, the Pielou evenness was lower in the Cerrado (0.54–0.61) than in the no-tillage (0.65–0.75) and the conventional tillage (0.67–0.77) sites. The Shannon diversity considering both, species richness and evenness, was lowest in the Cerrado (1.57–1.87), slightly higher in the no-tillage (1.78–2.27) and intermediary in the conventional tillage (1.68–1.99) sites (Table 6).

## 4. Discussion

In the present study, we analyzed three Cerrado and three long-term experiments, based on soybean production, in order to understand if land use and especially tillage practices affect the diversity and community structure of AMF. In the agricultural sites, the soil pH had been increased by liming and fertilizer application from about 4.5 to 5.5, while organic carbon decreased; the availability of the plant nutrient elements P, K, Mg and Ca increased, and the availability of



(caption on next page)

plant toxic Al was drastically diminished. In the no-tillage systems some general goals could be reached: the organic soil carbon and plant nutrient levels were slightly higher than under conventional tillage.

Interestingly, organic carbon and available P, although clearly

changing upon land use change from savanna to soybean-based agricultural practices, had no significant impact on AMF species richness at the sites (*p*-values 0.11 and 0.24, respectively), while pH, cation nutrient and Al availability affected the richness (*p*-values



**Fig. 2.** Redundancy analysis (RDA) on the AMF species composition, considering A. the AMF spore populations of all nine study sites, and B. exclusively the six agricultural sites belonging to three soybean-based long-term tillage experiments (Table 1). Abbreviations of the species: *Ac.den* = *Acaulospora denticulata*, *Ac.fov* = *Ac. foveata*, *Ac.her* = *Ac. herreriae*, *Ac.lae* = *Ac. laevis*, *Ac.lon* = *Ac. longula*, *Ac.mel* = *Ac. mellea*, *Ac.mor* = *Ac. morrowiae*, *Ac.red* = *Ac. reducta*, *Ac.scr* = *Ac. scrobiculata*, *Ac.sie* = *Ac. sieverdingii*, *Ac.spi* = *Ac. spinosa*, *A.sps* = *Ac. spinosissima*, *Ac.tub* = *Ac. tuberculata*, *Am.app* = *Ambispora appendicula*, *Ar.myr* = *Archaeospora myriocarpa*, *Ar.tra* = *Ar. trappei*, *Ar.und* = *Ar. undulata*, *Bu.min* = *Bulbospora minima*, *Ce.gil* = *Cetranspora gilmorei*, *Ce.pel* = *Ce. pellucida*, *Cl.cla* = *Claroideoglossum claroideum*, *Cl.etu* = *Cl. etunicatum*, *Cl.lut* = *Cl. luteum*, *De.cer* = *Denticutata cerradensis*, *De.nig* = *De. nigra*, *De.scu* = *De. scutata*, *Di.ebu* = *Diversispora eburnea*, *Fu.geo* = *Funneliformis geosporus*, *Fu.hal* = *Fu. halonatus*, *Fu.mos* = *Funneliformis mosseae*, *Fu.aur* = *Fuscutata aurea*, *Fu.het* = *Fu. heterogama*, *Fu.rub* = *Fu. rubra*, *Gi.gig* = *Gigaspora gigantea*, *Gi.mar* = *Gi. margarita*, *Gl.amb* = *Glomus ambisporum*, *Gl.bro* = *Gl. brohultii*, *Gl.dia* = *Gl. diaphanum*, *Gl.glo* = *Gl. glomerulatum*, *Gl.mac* = *Gl. macrocarpum*, *Gl.mic* = *Gl. microcarpum*, *Ku.col* = *Kuklospora colombiana*, *Or.per* = *Orbispora pernambucana*, *Pa.occ* = *Pa. occultum*, *Ra.alb* = *Racocetra alborosea*, *Ra.cor* = *Ra. coralloidea*, *Ra.ful* = *Ra. fulgida*, *Ra.tro* = *Racocetra tropicana*, *Ra.und* = *Ra. undulata*, *Rh.cla* = *Rhizoglossum clarum*, *Rh.int* = *Rh. intraradices*, *Rh.irr* = *Rh. irregulare*, *Rh.mic* = *Rh. microaggregatum*, *Rh.nat* = *Rh. natalense*, *Sc.cor* = *Sclerocystis coremioides*, *Sc.sin* = *Sc. sinuosa*, *Sc.aur* = *Scutellospora aurigloba*, *Sc.cal* = *Sc. calospora*.

0.01–0.05). The relationship between AMF species richness with Ca content and pH was also reported on areas under various land uses in Estonia (Moora et al., 2014) and in areas cultivated with coffee in Mexico (Posada et al., 2016).

Both AMF spore density and AMF species richness were higher in the Cerrado. When summarized for two collection dates, there were 9–11 spores g<sup>-1</sup> soil and, including the results from the trap culture propagation, 29–26–33 species in the savannas, while 4–6 spores g<sup>-1</sup> and 24–24–26 species were revealed in the no-tillage and 3–4 spores g<sup>-1</sup> and 15–28–21 species in the conventional tillage systems. Also the Sørensen similarity and the CCA and RDA analyses revealed that larger differences existed for the AMF spore communities between the natural savannas and the agricultural sites. All these results provide an indication of the expected major impact on AMF spore communities following land use change, including soil cultivation, fertilizer application and liming practices. Higher spore densities and species richness in natural ecosystems, when compared to agricultural production systems, have also been reported from other climate zones (e.g. Treseder and Turner, 2007; Oehl et al., 2010); however, such differences were not always so clearly demarcated as in the present study (e.g. Säle et al., 2015; analyzing Central European agro-ecosystems on clay soils). Higher AMF diversity was also observed in a natural, rather than a cultivated site, of *Flemingia vestita* Benth ex Baker in India (Songachan and Kayang, 2013).

The data on AMF spore density and species richness, as well as the similarity and RDA analyses, indicate that in our study the differences in AMF community composition between the two tillage systems are rather low, when compared to the natural savannas. In our two tillage systems, investigated in three independent long-term field experiments, there was a relatively high AMF species richness [(15–)21–28 species], and in total 44 species were found in the soybean plots, indicating that in tropical savannas many species may be capable of adapting to the relatively short growth period of soybean production and may survive with just one to two host plant species in the crop rotation over years. In colder regions, where soybean and maize production have also become increasingly important, the degradation of AMF community diversity as a means of measuring biodiversity loss seems to be much more expressed than in the Cerrado (e.g. Brito et al., 2012; Fernandes

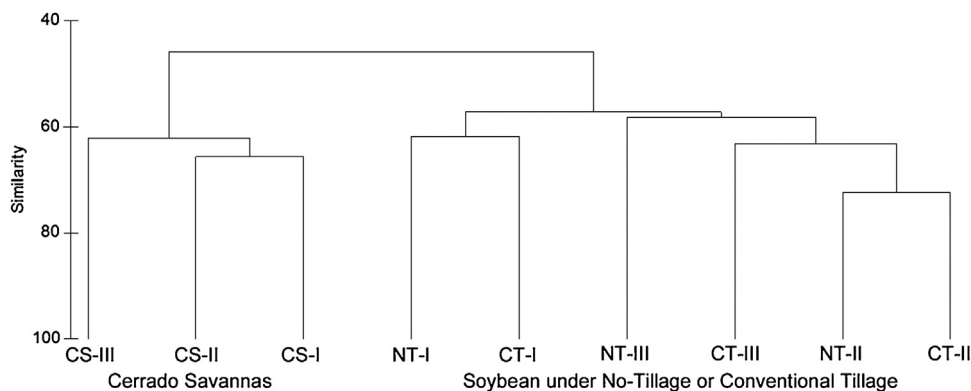
**Table 6**  
AMF diversity indices: Margalef and Simpson dominance (λ), Pielou Evenness (J'), and Shannon (H') diversity.

	Margalef (d)	Simpson dominance (λ)	Evenness (J')	Shannon (H')
<b>Cerrado</b>				
CS-I	4.5 a	0.27 ab	0.61 bc	1.87 ab
CS-II	3.7 ab	0.31 a	0.54 c	1.57 b
CS-III	3.3 ab	0.29 a	0.56 c	1.57 b
<b>No-tillage</b>				
NT-I	2.8 b	0.22 ab	0.69 abc	1.81 ab
NT-II	3.6 ab	0.13 b	0.79 a	2.27 a
NT-III	3.1 ab	0.23 ab	0.65 abc	1.78 ab
<b>Conventional tillage</b>				
CT-I	2.8 b	0.18 ab	0.77 ab	1.99 ab
CT-II	3.3 ab	0.21 ab	0.70 abc	1.96 ab
CT-III	2.5 b	0.26 ab	0.67 abc	1.68 b
LSD	1.0	0.10	0.10	0.35

Data presented as means of three plot replicates based on AMF spore populations obtained from two collection dates. Means followed by the same letter in the same row do not differ by Tukey test at 5% after One-Way ANOVA. LSD is Fisher's Least Significant Difference. I, II, III refer to the three savannas and adjacent long-term field experiments investigated.

et al., 2016; Oehl et al., 2005, 2010; Wetzel et al., 2014).

The AMF spore densities and species richness were slightly higher in the no-tillage than in the conventional tillage systems, and the multi-variate analyses, applied only on the agricultural sites, revealed a clear shift of the AMF community composition between the no- and the conventional tillage systems. The lowest AMF species richness was found in the conventional tillage system of the bi-annual crop rotation (CT-I), where a total of 15 species only were identified. In addition to the tillage system this result may be related to the age of the field experiment (established already in 1979) or the bi-annual crop rotation applied. Higher spore density and species richness, and shifts of the AMF community compositions, in no- or reduced conventional tillage were also observed e.g. by Bowles et al. (2016), Pereira et al. (2014), Säle et al. (2015), Wetzel et al. (2014), and also by Castelli et al. (2014) in maize and by Sheng et al. (2013) in soybean production, indicating



**Fig. 3.** Cluster analysis dendrogram based on Sørensen-Similarity among the three Cerrado Savanna forests (CS), the three No-Tillage (NT) and the three Conventional Tillage (CT) sites under study.

the negative effects of soil cultivation on the AMF propagules, such as the destruction of the mycelial network in the soils (Hu et al., 2015; Sheng et al., 2013). According to Alguacil et al. (2008), no-tillage systems also favor spore germination and mycorrhizal inoculum production, leading to both increased root colonization and spore formation (Wang et al., 2016). According to Mathew et al. (2012) and Murugan et al. (2014), the overall soil microbial biomass is more heavily affected by conventional than no-tillage systems, which leads to a loss of microbial propagules and species richness and may also change the physical-chemical soil characteristics, while no-tillage protects both organic carbon and the microbial biomass, including AMF (Avio et al., 2013; Dai et al., 2015; Mathew et al., 2012).

In accordance with the increased AMF species richness, the Margalef index and the Simpson dominance, which both consider species richness, were also from slightly to significantly higher in the Cerrado sites than in the agricultural soils. In contrast, the Pielou evenness as well as the Shannon diversity index, which considers for both richness and evenness, were significantly, albeit slightly, higher in the soybean sites than in the Cerrado savannas. This observation is somehow surprising, but may be reasoned by the fact that we found more dominant, indicative and also more rarely and exclusively occurring species in the three Cerrado forests than in the agricultural sites. According to Hu et al. (2015), no-tillage can contribute to the maintenance of high AMF diversity, while high fertilizer application and especially intensive soil cultivation might reduce both AMF richness and diversity in the long-term (Maurer et al., 2014; Sheng et al., 2013; Wetzel et al., 2014). However, we found lower species richness (15 species) only in the cultivated site (CT-1) with the bi-annual crop rotation, but even at this site only the Margalef, but not the other indices, decreased when compared to all other sites. When comparing soybean crops cultivated under different conditions, Kojima et al. (2014) also indicated that AMF diversity was more affected by long-term vegetation or environmental conditions than merely the previous crops.

In the dry season (second sampling date), AMF spore density and species richness were slightly lower than in the wet season. This was not only observed for the agricultural sites, where the crops had already been harvested some weeks prior to the second sampling, but also in the Cerrado sites. The absence of host plants and any spontaneous vegetation may possibly have reduced AMF spore density, richness and diversity (Bordoloi et al., 2015). Dodd et al. (1990) also observed the decreased AMF spore density in tropical savannas during dry seasons, confirming our results. However, the opposite was found for spore density in the dryer Caatinga savanna (Souza et al., 2016), and dryer areas of the more humid tropical rainforests (Silva et al., 2014) indicating that the sampling time within the wet and dry seasons may also affect the results, as spores in tropical soils are always exposed to harsh degradation processes, as soon as the soils become moist. Soil moisture changes may occur, however, with each single rain event, even during the dry season, when biotrophic AMF species without simultaneous plant growth may be exposed to microbial attacks.

The most frequent AMF species in our field soil samples, e.g. *Gl. macrocarpum*, *Gi. margarita*, *Ac. scrobiculata*, *Cl. etunicatum* and *Pa. occultum* are commonly found in tropical soils (Ferreira et al., 2012; Mergulhão et al., 2010; Tchabi et al., 2008) and can be considered as AMF generalists. Some of them even have a wider biogeographical distribution occurring in colder climates (e.g. Oehl et al., 2010). Some of the frequent species, however, also had a clearly higher and indicative abundance in specific ecosystems: e.g. *Gl. macrocarpum*, *Gl. brohultii* and *Gl. glomerulatum* for the savannas, *Pa. occultum* for the no-tillage, and *Gi. margarita* for the conventional tillage sites, as revealed by our indicator value analyses.

Other AMF were more clearly restricted to specific ecosystems (Table 4), which was well reflected by the indicator values (Table 5) and also in the RDA analyses (Fig. 2): e.g. *Bulbospora minima* and *Orbispora pernambucana*, which occurred exclusively in the Cerrados,

*Sc. coremioides*, which occurred mainly in the no-tillage systems, and *Ra. fulgida*, that occurred only in the conventional tillage systems. These AMF species, sometimes classified as AMF specialist species, may be especially sensitive after land use change to agricultural cropland and possibly irreversibly lost from the sites. The species found exclusively in the natural sites, might have already been lost from the agricultural sites following thirteen years of intensive soybean and maize production, e.g. *Bulbospora minima* and *Orbispora pernambucana*, for reasons that are not yet known (intensive fertilizer use, pH change, use of pesticides, lack of specific plant hosts, etc.). In a study from the Atlantic Rainforest biome in Pernambuco State, *Or. pernambucana* was found exclusively in the natural, but not in agricultural systems (Pereira et al., 2014), reflecting our study from the Cerrado biome. These findings indicate the need to protect such species and their natural habitats (Avio et al., 2013; Turrini and Giovannetti, 2012), which may otherwise become endangered or extinct, despite their rather wide biogeographical distribution in tropical Brazil (Carvalho et al., 2012).

Interestingly, the species richness ratio between Glomerales/Paraglomerales and Diversisporales/Gigasporales/Archaeosporales was > 1:1 for species that occur in all three ecosystems, while this ratio was < 1:4 for all species that were not detected in all three ecosystems. Many Diversisporales, Gigasporales and Archaeosporales species are known to have a strong seasonal life cycle (e.g. Oehl et al., 2009), and among them, especially *Acaulospora*, and several species in the Gigasporales and Archaeosporales appear to have clearly benefited from land use change to agricultural production, when compared to the natural savanna. For instance, *Ac. denticulata*, *Ac. scrobiculata*, *De. cerradensis* were indicative for the no-tillage, while *Gi. margarita*, *Ra. coralloidea* and *Ra. fulgida* were indicators for the conventional tillage systems. According to Silva et al. (2015), indicator species show that the environments in which they preferentially occur, represent ideal conditions for their growth, or in which they are most competitive, well-adapted to the local conditions. Our data also confirm the findings of Lekberg et al. (2007, 2008), who found under lower soil available P contents (1.3–6.2 mg kg<sup>-1</sup>), than in our soybean study sites, a series of gigasporalean species in agricultural, sandy soils in Zimbabwe, concluding that in sandy soils such species are less sensitive to soil disturbance (cultivation) than many other glomeromycotan taxa (see also Hart and Reader, 2004), as they may conclude their life cycle by forming large spores, while the disruption of their hyphal network before soybean or maize seeding cannot be harmful for the consecutive spore germination and plant root colonization.

Twelve AMF species were detected only with one or two spores and can be considered rare in this study. Rare species with low frequency occurrence, may be found in the environment under other forms, such as colonizing roots, or as auxiliary cells, may be inhibited by other AMF species, or may have been more common when favourable conditions were present (Buscot, 2015).

## 5. Conclusions

The Brazilian Cerrado is the largest savanna in tropical America, with major importance for tropical biodiversity and agricultural production. Soybean is one of the major crops in tropical and subtropical regions with intensive agricultural production. We found an astonishingly high AMF diversity in the Cerrado, but also in the two tillage systems under intensive soybean production. In the soybean fields, 44 AMF species were detected, among the highest records ever reported for an intensive annual agricultural crop. The conversion from the natural Cerrado forests to tropical cropland based on intensive soybean production, being in monoculture, annual or bi-annual rotations with maize led to a decrease in AMF spore densities and species richness and a clear shift in AMF species composition. In the two tillage systems, distinct AMF species communities and indicator species for tillage or no-tillage systems were identified, although a multitude of species appear to have been lost following agricultural production,

representing a substantial loss of biodiversity. Remarkably, through land use change, some gigasporalean species in particular became more abundant in the conventional tillage systems, while in no-tillage systems, some acaulosporacean and archaeosporalean species were more prominent, providing these systems with a wider range of characteristic higher level AMF taxa and ecological groups. These findings are special and clearly different from those known for other climatic regions, where many gigasporalean, acaulosporacean and several archaeosporalean are often the most sensitive AMF groups in agricultural production systems. Future studies are necessary to establish: (i) the degree to which such indicator species react to soil fertility levels; (ii) how such species, singly and/or combined, affect soil formation, aggregation and plant health and growth, and other ecosystem functions and agricultural services, such as resource-saving water, fertilizer and pesticide use.

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