Mycorrhiza (2012) 22:653–661
DOI 10.1007/s00572-012-0443-1

ORIGINAL PAPER

Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: a long-term experiment

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Received: 21 June 2011 / Accepted: 1 May 2012 / Published online: 15 May 2012

Abstract Cover crop species represent an affordable and effective weed control method in agroecosystems; nonetheless, the effect of its use on arbuscular mycorrhizal fungi (AMF) has been scantily studied. The goal of this study was to determine root colonization levels and AMF species richness in the rhizosphere of maize plants and weed species growing under different cover crop and weed control regimes in a long-term experiment. The treatment levels used were (1) cover of *Mucuna deeringian* (Muc), (2) "mulch" of *Leucaena leucocephala* (Leu), (3) "mulch" of *Lysiloma latisiliquum* (Lys), (4) herbicide (Her), (5) manual weeding (CD), (6) no weeding (SD), and (7) no maize and no weeding (B). A total of 18 species of AMF belonging to eight genera (*Acaulospora*, *Ambispora*, *Claroideoglomus*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, and *Scutellospora*) were identified from trap cultures. Muc and Lys treatments had a positive impact on AMF species richness (11 and seven species, respectively), while Leu and B treatments on the other hand gave the lowest richness values (six species each). AMF colonization levels in roots of maize and weeds differed significantly between treatment levels. Overall, the use of cover crop species had a positive impact on AMF species richness as well as on the percentage of root colonized by AMF. These findings have important implications for the management of traditional agroecosystems and show that the use of cover crop species for weed control can result in a more diverse AMF community which should potentially increase crop production in the long run.

Keywords Weeds · Herbicide · Fallow · Arbuscular mycorrhizal fungi · Cover crop · Mulch

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Introduction

In the state of Yucatan (Mexico), as in other regions of Mexico and Latin America, herbicides are commonly used to control weeds in maize agroecosystems. As a result, herbicide application has been selected for resistant individuals of many species of weeds which eventually reduce the effectiveness of weed chemical control (Caamal-Maldonado 1995). One alternative to chemical control is the use of mulch or cover crop species which reduce light penetration and temperature, limit weed establishment (Caamal-Maldonado et al. 2001; Castellano and Molina 1989), and favor the presence of herbivore insects which feed on their seeds (Pullaro et al. 2006).

The use of legumes as mulch or cover crops has been shown to promote a greater and more sustained maize

production in traditional agroecosystems in Yucatan (e.g., 700 kg/ha to >1,000 kg/ha) (Caamal-Maldonado et al. 2001). Nonetheless, the effect of cover crops on soil community diversity has been scantily studied. For instance, there are few studies on the effect of cover crop use on belowground mutualisms such as plant–arbuscular mycorrhizal fungi (AMF) interactions (Baumgartner et al. 2010; Boswell et al. 1998; Houngnandan et al. 2001; Kabir and Koide 2000), which may in turn also inhibit or reduce the establishment of weeds in crop fields (Jordan et al. 2000; Rinaudo et al. 2010; Veiga et al. 2011). Since most crop species cultivated in the tropics are associated to AMF (Schroeder and Janos 2004) and given the potential role of mycorrhizal interactions in maintaining crop diversity and productivity (Mohammad et al. 1998; Oehl et al. 2004), it is important to better understand the effect of cover crops on AMF–plant interactions.

Agroecosystem management practices have been shown to produce changes in AMF communities, generally causing a decrease in spore abundance and diversity (Oehl et al. 2004). The use of fertilizers or herbicides may contaminate the soil and also negatively impact on AMF communities (Abd-Alla et al. 2000; Allen and West 1993; Kurle and Pfleger 1994; Lekberg and Koide 2005; Mathimaran et al. 2007; Pasaribu et al. 2011). The use of mycorrhizal cover crop species such as *Mucuna pruriens* (L.) DC (Houngnandan et al. 2001) and *Trifolium repens* L. cv. Huia (white clover) (Deguchi et al. 2007) represents an alternative method which could circumvent such negative impacts while stimulating AMF root colonization in crops.

In spite of the importance of AMF in agroecosystems (Oehl et al. 2003, 2004) and their potential use in sustainable agricultural practices (Gianinazzi et al. 2010; Gosling et al. 2006; Leake et al. 2004) and for weed control (Bethlenfalvay et al. 1996; Jordan et al 2000, but see Daisog et al. 2011), few studies have looked at changes in soil AMF spore composition and mycorrhizal colonization levels under agricultural management regimes involving weed control. The main goal of the present study was to investigate the effect of different weed control methods on AMF species richness and mycorrhizal colonization levels in maize and species of weeds, in a long-term experiment in the state of Yucatan, Mexico. AMF species richness in the rhizosphere of maize and associated weeds was determined by taking soil samples and using trap plants to propagate spores and isolate the indigenous AMF.

Methods

Experimental design

The experimental site was located at the Campus de Ciencias Biológicas y Agropecuarias (CCBA) of the Universidad Autónoma de Yucatán (Yucatan, Mexico—20°51′57″ N, 89° 37′23″ W). The study region is of karstic geological origin, with abundant rock outcrops. The soil type is Leptosol, which ranges in depth from 0 to 25 cm (Díaz-Garrido et al. 2005). Climate is warm sub-humid with rains during summer and winter. Mean annual precipitation is of 900 mm and the mean annual temperature of 27.5°C (García 1973). The dominant vegetation type is low-height tropical deciduous forest (Flores and Espejel 1994).

The experimental site has been used for the last 13 years to grow maize under different conditions: (1) in association with a cover crop *Mucuna deeringiana* (Bort.) Merr. (Muc); (2) with the application of mulch of *Leucaena leucocephala* (Lam.) De Witt. (Leu); (3) with the application of mulch of *Lysiloma latisiliquum* (L.) Benth. (Lys); (4) with paraquat herbicide application (1, 1-dimethyl-4, 4-bipyridylium dichloride) (Her); (5) manual weeding (CD); (6) no weeding (SD); (7) without maize and no weeding (B). The experimental site consisted of a 35×60 m plot divided into 21 subplots (experimental units) each of 5×10 m, separated by 1 m, and with three replicate subplots per treatment (see Caamal-Maldonado et al. 2001).

Maize is cultivated in an annual cycle with planting in March (dry season) and harvesting in November (rainy season). Since 1990, the experimental site has received a traditional Mayan management regime each year which involves first the cutting down of arboreal vegetation, then the chopping of lower-height vegetation and, finally, burning of deposited plant material (Caamal-Maldonado 1995; Caamal-Maldonado et al. 2001).

Soil chemical characterization

During March 2003, eight soil samples were taken from each subplot to determine pH (KCI method), total organic carbon (TSBF colorimetric method), nitrogen (Kjeldhal method), and phosphorous (sodium hypobromite oxidation method) levels (Table 1). Analyses were conducted at the Laboratorio de Suelos y Plantas of the CCBA (Universidad Autónoma de Yucatán).

Soil sample processing, AMF propagation, spore extraction, and isolation

Because most AMF spores found in field soil samples are usually damaged, spores were isolated and identified by propagation pots using trap plants in nurseries to achieve a more reliable identification of AMF species (Brundrett et al. 1996; Douds and Millner 1999). In March of 2003, five soil samples were randomly collected at each replicate subplot to a maximum depth of 10 cm, and all samples from each subplot were mixed to give a total of 21 soil samples.

Table 1 Values for pH (n=8), total carbon content (C), nitrogen (N), and phosphorus (P) for soil samples of each treatment level used for weed control (values are means±SE)

Treatments	pH	C mg/100 g soil	N g/kg soil	P mg/kg soil
Muc	7.83±0.06	4,209.80±517.65	0.76±0.14	449.79±202.65
Leu	7.785±0.04	4,183.25±678.19	1.28±0.10	475.19±153.67
Lys	7.81±0.06	5,317.97±125.72	1.11±0.10	320.05±35.78
Her	7.72±0.1	6,541.49±1452.69	1.25±0.25	206.19±65.84
CD	7.73±0.06	5,067.58±85.62	0.90±0.10	192.33±37.18
SD	7.706±0.08	5,397.74±91.84	1.25±0.10	685.86±238.25
B	7.71±0.04	5,529.72±65.24	1.38±0.07	693.93±214.89

No differences were found between treatment levels for any of the measured variables

Muc—cover crop *Mucuna deeringiana*, *Leu*—mulch of *Leucaena leucocephala*, *Lys*—mulch of *Lysiloma latisiliquum*, *Her*—herbicide application, *CD*—manual weeding, *SD*—no weeding, *B*—no weeding and no maize

Each soil sample was 2 mm sieved, mixed with an equal volume of sterile sand (1:1 v/v), and placed in 2-l pots. Ten seeds of sorghum (*Sorghum vulgare* L.) were planted in each pot as trap plants (Sieverding 1991). Pots were randomly placed in a nursery (average temperature and relative humidity=29.1°C and 44.5 %, respectively) and were watered regularly until week 14, when watering was stopped in order to stimulate AMF sporulation (Brundrett et al. 1996). At the end of week 16, one 100-g sample was taken from each pot and AMF spores were extracted based on a modified of wet sieving and decanting technique (Gerdemann and Nicolson 1963). The soil was homogenized with solution of Tween 20 (0.05 %) in water, and the solution was then filtered across a series of sieves (600 μm, 400 μm, 190 μm, 122 μm, and 73 μm), and the retained fractions were decanted and AMF spores separated on a sucrose gradient (Sieverding 1983).

AMF species identification was based on spore morphological characteristics (shape, size, color, wall texture and layers, ornamentation, type of hyphae, auxiliary structures, germination shields, and spore configuration), according to published identification manuals (Schenck and Pérez 1990; Schüβler and Walker 2010; http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm, http://www.lrz.de/~schuessler/amphylo/). Species frequency of occurrence (FO) (Gaur and Adholeya 1994) was calculated as FO=(number of samples with a particular AMF species/total number of analyzed samples)×100.

Root samples, staining, and percent colonization

At the end of the agricultural management cycle (November 2003), secondary roots were collected from five maize plants (*Zea mays*) of each subplot (except for the B treatment) as well as from five individuals of the most abundant weed species: *Bidens pilosa* L., *Sanvitalia procumbens* Lam., *Sida acuta* Burm., *Digitaria insularis* (L.) Fedde, and *Priva lappulacea* (L.) Pers. In the case of the Her treatment, roots of the weed *Parthenium hysterophorus* L. were also collected as this species was highly abundant in plots belonging to this treatment level (representing more than 50 % of plant plot cover). Root samples were rinsed, labeled, transported to the laboratory, and stained using a modified Phillips and Hayman (1970) technique without phenol. Permanent preparations were made and the presence of AMF structures (hyphae, vesicles, arbuscules, coils, and spores) was determined using the magnified intersections method (McGonigle et al. 1990) in order to estimate the percent of root length containing AM fungal structures [percentage of colonization=(number of colonized fields/total number of fields) × 100].

Statistical analyses

Differences between treatment levels in pH, total organic carbon, total nitrogen, total phosphorous, and AMF species richness were analyzed with separate one-way ANOVAs (SigmaStat ver 3.1). When significant differences were found for each response variable, treatment level means were compared in a pairwise manner by means of a Student–Newman–Keuls test (P value significance level set at 0.05) (Zar 1999). Species composition values for each treatment were employed to obtain the Jaccard's similarity index (Magurran 1989), and the data were grouped based on a cluster analysis conducted in MVSP ver. 3.13.

The mean percentage of AMF root colonization was calculated for each treatment level as well as individually for each plant species across treatment levels. Overall differences between treatment levels and differences between treatment levels for each species were analyzed by means of non-parametric Kruskal–Wallis tests. When significant differences were found (P value set at 0.05), Dunn's test

was conducted to determine which treatment level means differed significantly using SigmaStat 3.1.

Results

Weed control

All the weed control methods used in this work showed to be effective at controlling weeds, particularly the cover crop *Mucuna deeringiana*, which cause as much as 50 % of the weed biomass reduction (see Caamal-Maldonado et al. 2001).

AMF spore identification and species richness

A total of 18 AMF species were identified. Species from the genus *Acaulospora* were the most abundant (28 % of total), followed by *Funneliformis* and *Rhizophagus* (17 %), *Claroideoglomus* and *Sclerocystis* (11 %), *Scutellospora* (6 %), and *Ambispora* and *Glomus* (5 %). Significant differences in species richness were found between treatments (F=8.51, df=6, P=0.0005). Treatments which showed the greatest AMF species richness average were Muc (10.67 species) and Lys (7.34), while the Leu and B treatments showed the lowest number of species (five each) (Fig. 1). Pairwise comparisons showed that only the Muc treatment level differed from the other treatments, showing a significantly greater average number of AMF species (P<0.001).

Similarity analyses indicated the existence of three groups of treatments based on species composition: (a) CD and Her (64 % of similarity), (b) B and SD (52 % of similarity), and (c) the remaining treatments (Fig. 2).

Frequency of occurrence of AMF spores

Scutellospora nigra and *Funneliformis geosporum* were found in all treatments (FO=100 %), followed by *Funneliformis mosseae* (FO=85.71 %), *Rhizophagus intraradices* (FO=71.42 %), and *Acaulospora scrobiculata*, *A. undulada*, and *Claroideoglomus claroideum* (57.14 % each). *Rhizophagus* (*aff. fasciculatum*), *Ambispora* (*aff. leptotichum*), *Rhizophagus* (*aff. manihotis*), and *Sclerocystis dussii* were present in only one treatment and thus showed the lowest frequency values (14.28 % in all cases; Table 2).

AMF root colonization

The overall percent of root colonization by AMF (all plant species together) was greatest for the Muc treatment (12.14± 8.54 %), while the lowest average value was observed for the Her treatment (2.55±3.03 %). Colonization levels in maize plants were highest for treatments SD (11.66±8.38 %) and CD (10.93±5.72 %), and lowest for treatment Her (2 %). In the case of weed species, greatest AMF colonization levels were observed for *S. procumbens* in the Muc (17.83 %), B (12.20 %), and Leu (9.26 %) treatments, while *P. lappulacea* showed highest colonization in the Lys treatment (8.65 %). In the case of the Her treatment, *P. hysterophorus* showed greatest mycorrhizal colonization (5.20 %) and was by far the most abundant species for this treatment (50 % of plant cover). Significant differences were observed between treatments for each species (all statistics values are presented in Fig. 3), as well as overall for all species between treatments (H=124.4, df=6, P<0.0001) (Fig. 4).

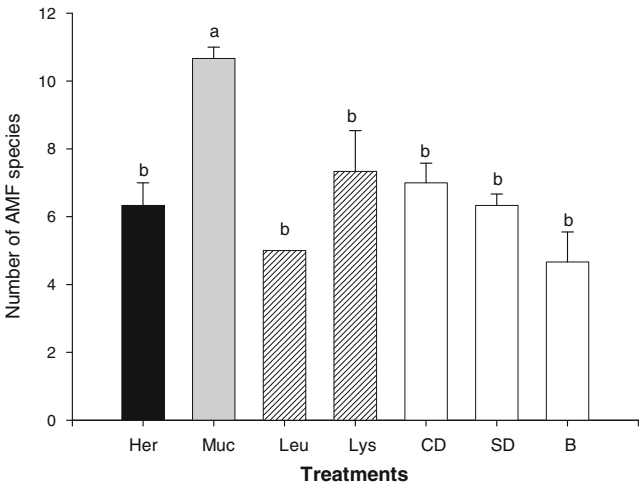

Fig. 1 Average number of AMF species (+1 SE) found for each treatment level used for weed control. *Muc*: cover crop *Mucuna deeringiana*; *Leu*: mulch of *Leucaena leucocephala*; *Lys*: mulch of *Lysiloma latisiliquum*; *Her*: herbicide application; *CD*: manual weeding; *SD*: no weeding; *B*: no weeding and no maize. Different *letters* indicate significant differences between treatment level means at P >0.05

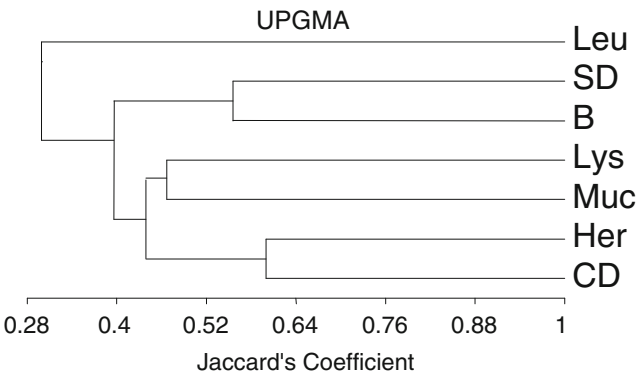

Fig. 2 Dendrogram resulting from the cluster analysis based on similarity in AMF species composition for each of the treatment levels used for weed control. *Muc*: cover crop *Mucuna deeringiana*; *Leu*: mulch of *Leucaena leucocephala*; *Lys*: mulch of *Lysiloma latisiliquum*; *Her*: herbicide application; *CD*: manual weeding; *SD*: no weeding; *B*: no weeding and no maize

Table 2 Frequency of occurrence (FO) of AMF species for each of the treatment levels used for weed control

AMF species	Cover crop	Mulch		Chemical	Traditional management			FO (%)
	Muc	Lys	Leu	Her	CD	SD	B	
Acaulospora delicata Waker, Pfeiffer & Bloss	+	+						28.57
Acaulospora laevis Gerdeman & Trappe		+	+					28.57
Acaulospora morrowiae Spain & Schenck	+			+	+			42.85
Acaulospora scrobiculata Trappe	+	+		+			+	57.14
Acaulospora undulata Sieverding	+	+		+	+			57.14
Rhizophagus (*aff. fasciculatum*) (Thaxt.) C. Walker & A. Schüßler	+							14.28
Ambispora (*aff. leptotichum*) (N.C. Schenck & G.S. Sm.) C. Walker, Vestberg & A. Schüßler					+			14.28
Rhizophagus (*aff. manihotis*) (R.H. Howeler, Sieverd. & N.C. Schenck) C. Walker & A. Schüßler	+							14.28
Glomus aggregatum Schenck & Smith emend. Koske			+			+		28.57
Claroideoglomus claroideum (N. C. Schenck & G. S. Sm.) C. Walker & A. Schüßler		+		+	+	+		57.14
Funneliformis constrictum (Trappe) C. Walker & A. Schüßler		+				+		28.57
Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler	+	+	+					42.85
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	+	+	+	+	+	+	+	100
Rhizophagus intraradices (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler	+		+	+		+	+	71.42
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	+	+		+	+	+	+	85.71
Sclerocystis dussii (Pat.) Höhn., Sber. Akad. Wiss.					+			14.28
Sclerocystis rubiformis Gerd. & Trappe	+					+	+	42.85
Scutellospora nigra (Redhead) Walker & Sanders	+	+	+	+	+	+	+	100

Muc—cover crop *Mucuna deeringiana*, *Leu*—mulch of *Leucaena leucocephala*, *Lys*—mulch of *Lysiloma latisiliquum*, *Her*—herbicide application, *CD*—manual weeding, *SD*—no weeding, *B*—no weeding and no maize

Discussion

Overall, 18 AMF species were detected by trapping and morphological spore identification in the present study of plots under maize cultivation, which is a value similar to that reported by Mathimaran et al. (2007) for mixed agricultural fields of maize–crotalaria in Kenya. Other studies conducted in maize monocultures have reported AMF richness values which range from 13 species in Minnesota (USA) (Johnson et al. 1991; Kurle and Pfleger 1996) to 15 species in Pennsylvania (USA) (Franke-Snyder et al. 2001). In Mexico, Guadarrama-Chávez et al. (2007) found 13 AMF species in maize fields in Oaxaca, including *A. scrobiculata*, *Funneliformis geosporum* (formerly *G. geosporum*), and *Sclerocystis dussii* (formerly *G. dussi*) which were also found in the present study. AMF species found in the present experimental site under maize belong to eight genera: *Acaulospora*, *Ambispora*, *Claroideoglomus*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, and *Scutellospora*. Although DNA-based molecular tools have the potential to provide a more complete picture of the AMF identities, we consider that identification based on trap cultures and spore morphology remains valid for the purpose of the present study.

Species belonging to what was previously considered the genus *Glomus* (including genera mentioned above) were most frequent (66.6 % of the total number of AMF species), and most common among the study subplots (FO=50–70 %). These results agree with previous reports from agricultural fields in Europe (Sjöberg et al. 2004), Africa (Duponnois et al. 2001), and the American Continent (Baumgartner et al. 2010; Johnson and Wedin 1997). It has been suggested that species belonging to this genus prevail after soil disturbances because their spores are resistant to the effects of both low- and high-impact practices in managed agroecosystems (Bethlenfalvay 1992). For example, *G. geosporum* found in all treatment levels in this study has also been reported as a dominant species of AMF communities from both natural (tropical deciduous forest—Allen et al. 1998; wetlands—Landwehr et al. 2002) and modified ecosystems (human-induced pastures—Johnson et al. 1992; agricultural fields—Baumgartner et al. 2005).

Kurle and Pfleger (1996) reported the genus *Sclerocystis* to be absent from tropical soils when inorganic fertilizers are

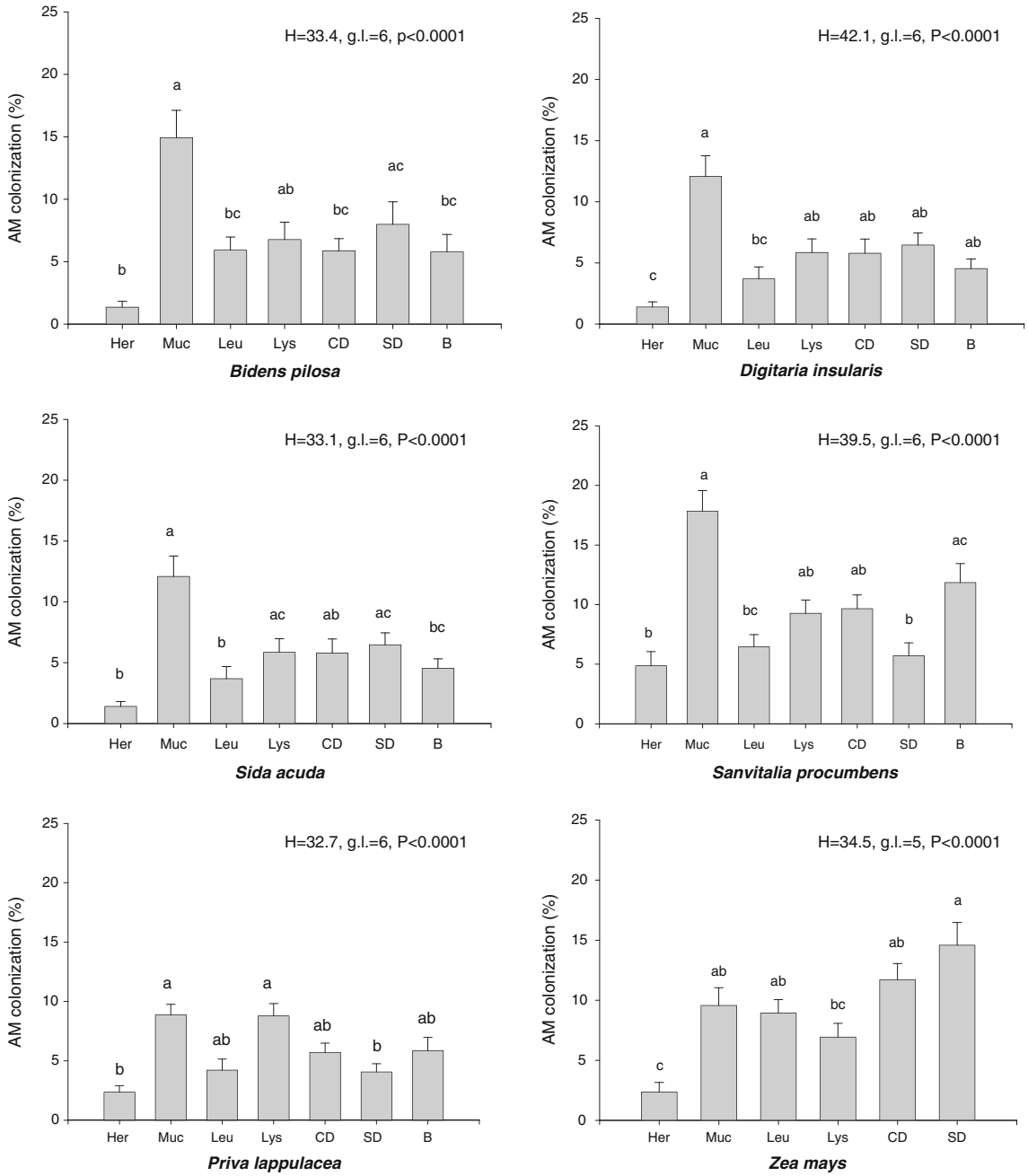

Fig. 3 Percent of AMF colonization in roots of maize and weed species under each of the treatment levels used for weed control (values shown are means+1 SE). *Muc*: cover crop *Mucuna deeringiana*; *Leu*: mulch of *Leucaena leucocephala*; *Lys*: mulch of *Lysiloma latisiliquum*; *Her*: herbicide application; *CD*: manual weeding; *SD*: no weeding; *B*: no weeding and no maize. Different *letters* indicate significant differences between treatment level means at $P > 0.05$

applied. Nonetheless, *Sclerocystis dussii* was found in the CD treatment under maize, and *Sclerocystis rubiformis* in the Muc, SD, and B treatments. It has been suggested that these species are sensitive to agricultural impacts and will only be found in low-impact agricultural systems such as those used here, while they will be absent from high-impact systems (involving the use of herbicides, insecticides, and/or mechanization) (e.g., Guadarrama-Chávez et al. 2007; Menéndez et al. 2001; Muthukumar and Udaiyan 2000; Oehl et al. 2003).

With respect to the *Acaulospora* genus, a total of five species were found (*A. delicata*, *A. laevis*, *A. morrowae*, *A. scrobiculata*, and *A. undulata*). This number is greater than that reported by Cuenca et al. (1998) for this genus in tropical agroecosystems. Also, species belonging to *Acaulospora* are generally more abundant in acid soils (Allen et

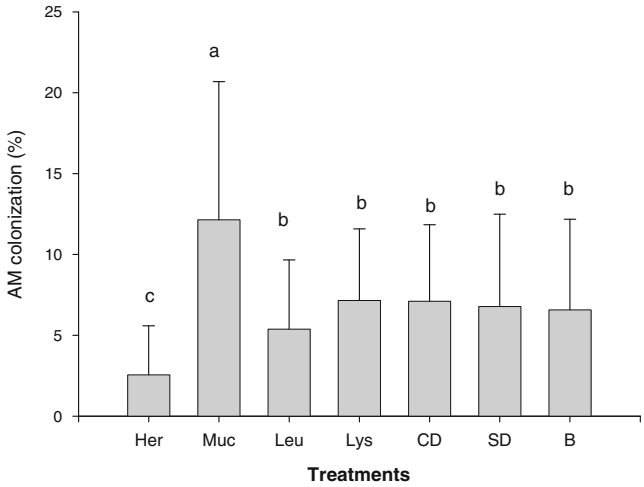

Fig. 4 Overall percentage of AMF colonization in roots of maize and weeds for each the treatment levels used for weed control (values shown are means +1 SD). *Muc*: cover crop *Mucuna deeringiana*; *Leu*: mulch of *Leucaena leucocephala*; *Lys*: mulch of *Lysiloma latisiliquum*; *Her*: herbicide application; *CD*: manual weeding; *SD*: no weeding; *B*: no weeding and no maize. Different *letters* indicate significant differences between treatment level means at $P > 0.05$. In all cases $n = 35$, except for treatment B where $n = 30$

al. 1995), while pH values in the subplots of the present experimental site were moderately alkaline (7.70–7.84). The absence of species of this genus from the SD treatment may be due to them being displaced by more competitive species such as those belonging to Glomaceae (Dodd 2000; Douds and Millner 1999).

In the present study, the only member of Gigasporaceae identified in the experimental subplots was *Scutellospora nigra*, which has also been reported in tropical forest soils in Costa Rica (Johnson and Wedin 1997) and in soils with different types of vegetation in Nigeria (Old et al. 1973). When a disturbance occurs, AMF species with larger spore sizes like those belonging to *Scutellospora* and *Gigaspora* may be more susceptible to physical damage (Allen et al. 2003). However, the low presence of Gigasporaceae in the present work may also relate to the relatively short time of trap culturing since members of this family generally exhibit a long vegetative phase before producing spores (de Souza and Declerck 2003).

The similarity between the SD and B plots in terms of AMF species composition may be related to the presence of roughly the same number and identity of weed species in both treatments which act as hosts for AMF. It has been argued that increasing plant diversity in agroecosystems may lead to an increased number of AMF species in the soil (van der Heijden 2002). Also, higher density and AMF species richness in undisturbed vs. disturbed soils has been reported in tropical monocultures with added organic matter (Boddington and Dodd 2000a, b).

The highest average AMF root colonization was observed for the Muc and Leu cover treatments. This agrees with reports that cover crops increase AMF colonization levels in crops with maize (Boswell et al. 1998; Kabir and Koide 2000). Differences in percent colonization between treatment levels involving cover crops may also have been due to differences in the effect each cover species had on the community of weed species which are potential AMF hosts (Baumgartner et al. 2005). The lowest AMF root colonization occurred in the Her treatment plots (2.61 %). Although it has been reported that normal rates of herbicide application do not have a negative effect on the establishment and function of arbuscular mycorrhiza (Smith et al. 1981), other studies suggest that herbicides can negatively affect the symbiosis (Allen and West 1993; Abd-Alla et al. 2000). Mujica et al. (1999) reported a negative effect of two herbicides on AMF colonization in weed species when they were applied at levels greater than those recommended. Herbicides can inhibit photosynthetic activity in plants, which causes a reduction in the sugar production and availability in the plant which may in turn limit the establishment of this interaction and directly affect AMF (Abd-Alla et al. 2000; Ocampo and Barea 1982; Trappe et al. 1984). The use of herbicides may also select for specific weed species which are resistant to them; this situation was observed for *Parthenium hysterophorus* in this study, which was by far the most dominant species in Her treatment subplots. The high abundance of this species may reduce both the abundance and diversity of AMF due to a lower number of alternative host plant species (van der Heijden 2002).

A low level of AMF colonization was expected for the CD treatment level due to the displacement of superficial soil cover during plant removal (Varma 1995). Nonetheless, because manual weeding is conducted only once a year (ca. 20 days after maize is planted) AMF species loss is not significant. On the other hand, greater percent colonization levels may be expected for the B treatment level compared to all other treatment levels because this treatment had the greatest diversity of plants. Nonetheless, because the fallow period is short, nutrient availability for plants is high throughout the cycle compared to what occurs in subplots subject to other treatment levels. This condition results in a reduced benefit for the plant from the interaction (Gavito and Miller 1998), which may even limit the establishment of AMF.

Some authors (e.g., Rosemeyer et al. 2000) have suggested that the use of cover crop species may favor the presence and abundance of AMF due to increased availability of organic matter and nutrients. The present study demonstrated that the use of cover crop species (both mulch and cover crops) in a long-term maize production system contributes to the maintenance of high levels of AMF species richness, which in turn results in high levels of AMF colonization in

maize (and weeds). These findings are pertinent to sustainable management practices in agroecosystems where non-conventional weed control methods should not only contribute to increase crop production but should also avoid negative effects on the beneficial biotic components of the agroecosystem which include AMF.

Acknowledgments The authors would like to thank Héctor Cetina for laboratory and field work. This study was financially supported by the PRIORI program of the Universidad Autónoma de Yucatán, through the projects PRIORI-FMVZ-02-015 and PRIORI-FMVZ-04-010.

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