Morphotype-based characterization of arbuscular mycorrhizal fungal communities in a restored tropical dry forest, Margarita island-Venezuela

Laurie Fajardo[1], Milagros Lovera[1], Pauline Arrindell[1], Victor Hugo Aguilar[2], Zamira Hasmy[3] & Gisela Cuenca[1]

1. Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Apartado 20632, Caracas 1020-A, Venezuela; lfajardo@ivic.gob.ve; mlovera@ivic.gob.ve; parrinde@ivic.gob.ve; giscuenca@yahoo.es
2. Facultad de Humanidades y Educación, Universidad Central de Venezuela; Caracas 1051, Venezuela; vhal999@gmail.com
3. Instituto Nacional de Salud Agrícola Integral; Maracay 2102, Venezuela; zamhas@gmail.com

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Abstract: The mycorrhizal component of revegetated areas after ecological restoration or rehabilitation in arid and semiarid tropical areas has been scarcely assessed, particularly those made after mining disturbance. We evaluated and compared the presence of arbuscular mycorrhizal fungi of a small area of restored tropical dry forest destroyed by sand extraction, with a non-restored area of similar age, at the peninsula of Macanao, Margarita Island (Venezuela). Our study was undertaken in 2009, four years after planting, and the mycorrhizal status was evaluated in four restored plots (8 x 12.5 m) (two were previously treated with hydrogel (R2 and R2'), and two were left untreated (R1 and R1'), and four non-restored plots of similar size (NR1 and NR1' with graminoid physiognomy with some scattered shrubs; and NR2 and NR2', with a more species rich plant community). Apparently the restoration management promoted higher arbuscular mycorrhizal fungi (AMF) species richness and diversity, particularly in restored soils where the hydrogel was added (R2 treatment). Soil of the NR1 treatment (with a higher herbaceous component) showed the highest spore density, compared to samples of soils under the other treatments. Considering species composition, *Claroideoglomus etunicatum* and *Rhizophagus intraradices* were found in all treatments; besides, *Diversispora spurca* and *Funneliformis geosporum* were only found in non-restored plots, while members of the Gigasporaceae (a family associated with little disturbed sites) were commonly observed in the plots with restored soils. Mycorrhizal colonization was similar in the restored and non-restored areas, being a less sensitive indicator of the ecosystem recovery. The trend of higher richness and diversity of AMF in the restored plot with hydrogel suggests that this management strategy contributes to accelerate the natural regeneration in those ecosystems where water plays an essential role. Rev. Biol. Trop. 63 (3): 859-870. Epub 2015 September 01.

Key words: arbuscular mycorrhizal fungi richness, ecological restoration, mycorrhizal colonization, natural succession, tropical dry forest.

The restoration of degraded areas involves not only the aboveground system but also the belowground microorganisms functionally associated with plants (Li, Li, & Zhao, 2007a). Soil microorganisms are crucial for soil ecosystem function and most of the observed aboveground patterns are driven by belowground processes (Allen et al., 1999). For this reason, the successful restoration of disturbed areas requires careful consideration of all plant-soil system components (Moynahan, Zabinski, & Gannon, 2002). Arbuscular mycorrhizal fungi (AMF), phylum Glomeromycota, have been found to be essential components of this system due to their symbiotic association with over 80 % of terrestrial plants species to form arbuscular mycorrhizas (AM) (Smith & Read, 2008). It is well known that AMF can influence

plant fitness, community structure, biodiversity and ecosystem variability (van der Heijden et al., 1998). However, there is little available information, especially in the dry tropics, about AMF and their ability to form AM under stressful disturbed conditions compared with the information on these processes from wet ecosystems.

Tropical dry forest (TDF) is considered one of the most endangered tropical ecosystems (Janzen, 1988; Murphy & Lugo, 1995). From an ecological point of view, the restoration of TDF has become a priority due to their high biological diversity together with a remarkable concentration of endemic species, and a high diversity of both life-forms and functional groups of plants and animals (Dirzo, Young, Mooney, & Ceballos, 2011).

Most of the studies found in the literature about the use of AMF in the restoration of degraded TDF are related to the use of different AMF species for producing mycorrhizal native plants in a greenhouse, to be planted later in the field (Allen, Allen, Egerton-Warburton, Corkidi, & Gómez-Pompa, 2003; Allen, Allen, & Gómez-Pompa, 2005). Other studies have assessed the effects of different types of disturbance on the mycorrhizal status of native tree species in a tropical deciduous forest (Allen, Rincon, Allen, Pérez-Jimenez, & Huante, 1998) or have explored the limitations for the establishment of mycorrhizal associations in disturbed tropical dry ecosystems (Gavito, Pérez-Castillo, González-Monterrubio, Vieyra-Hernández, & Martínez-Trujillo, 2008). Additionally, Camargo-Ricalde, Dhillion, & Jiménez-González, (2003) evaluated the mycorrhizal status of perennial xeric plant species occurring in an arid tropical shrub and tropical deciduous forest; Guadarrama, Castillo-Arguero, Ramos-Zapata, Camargo-Ricalde and Álvarez-Sánchez (2008) determined the mycorrhizal inoculum potential of the soil under secondary vegetation at different stages of development, and Leal, Siqueira and Stürmer (2013) evaluated how AMF community composition was affected by conversion of tropical amazon forest to pasture. The mycorrhizal status of revegetated areas after ecological restoration or rehabilitation has been little studied. The diversity of AMF species was evaluated in a gradient of environmental restoration belonging to riparian areas of Brazilian Atlantic forest (Bonfin, Vasconsellos, Stürmer, & Cardoso, 2013). Nevertheless, to our knowledge, the mycorrhizal component of restored areas, particularly after mining disturbance in arid and semiarid tropical areas, has not been assessed. It is well known that mining disturbance negatively affects soil ecosystem components, and as AMF are beneficial to both plant and soil health, and they have been considered a very important tool for the restoration of degraded ecosystems (Cuenca, De Andrade, & Escalante, 1998; Li, Zhang, & Zhao, 2007b).

In general, the disturbance of plant communities degrades key physical, chemical and biological soil properties (structure, nutrient availability, organic matter content and/or microbial activity). This degradation limits the potential for the reestablishment of native plants species, thus accelerating erosion and desertification.

In recent decades, sand mining extraction in the lowlands of the Macanao peninsula, Margarita island in Venezuela has led to the total destruction of plant communities associated with ephemeral water courses, which represent the most developed, complex and species rich communities in this region (González, 2007). This activity has not only destroyed many of the dry shrublands and forests, but has also had other undesirable consequences, such as for example, the topographic disturbance of the ephemeral creeks, the removal of topsoil and plant species important for the diet and refuge of fauna (including some endangered species, e.g. *Amazona barbadensis*), an increase in erosion and the invasion of exotic plant species, amongst others (Fajardo, Rodríguez, González, & Briceño-Linares, 2013). Based on the evaluation of the successional dynamic that occurs once sand extraction is stopped, we conducted a pilot restoration assay of affected areas which gave us a good opportunity to assess the effect

of this management strategy on AMF communities (Fajardo et al., 2013).

Sand extraction produces a total elimination of the superficial layer of soil, which drastically reduces the infective propagules of AMF, planting native trees could modify the spores number, intraradical colonization and produce changes in the AMF composition, richness and diversity, compared with successional areas where the herbaceous component is predominant. The aim of this study was to evaluate the mycorrhizal status of restored sand mining areas and compare them to non-restored areas of similar age. Specifically, we evaluated: 1) the richness, diversity and composition of AMF assemblages in an area restored five years ago, and compared them with the AMF assemblages present in a non-restored area of similar age; and 2) compared the AM colonization in restored and non-restored plots. Mycorrhizal status was related with variables like spore number, mycorrhizal colonization and composition, richness and diversity of the AMF assemblages.

MATERIALS AND METHODS

Study site: The study was carried out in Quebrada La Chica, located in the North of the Macanao peninsula (11º01'19'' N - 64º16'36" W), on the Western side of Margarita Island, Nueva Esparta state, Venezuela. Over the last 20 years, sand has been extracted for construction purposes in many sectors of this 30 ha area. There is a marked seasonality in rainfall with the dry season spanning from January to June. Mean annual rainfall ranges between 326 and 522 mm with the highest peak in August. In June 2009, the soil samples were collected and mycorrhizal analysis took place. At that time, three months had passed without rain, and the mean temperature for this month was 28.9° C. The peninsula has several vegetation types among which predominate: semi-deciduous tropical forest with *Spondias mombin* (Anacardiaceae) and *Lonchocarpus violaceus* (Fabaceae) as the most abundant species; semi-deciduous gallery forest dominated by *Bulnesia arborea* (Zygophyllaceae) and *Piscidia carthagenensis* (Fabaceae) and deciduous xerophytic shrubland with species such as *Bourreria cumanensis* (Boraginaceae) and *Parkinsonia praecox* (Caesalpiniaceae) (González, 2007).

In May 2005, we planted five native tree species (*Tecoma stans*, *Bulnesia arborea*, *Piscidia carthagenensis*, *Prosopis juliflora* and *Parkinsonia praecox*) and quantified their growth and survival under eight treatments following a factorial design, with combinations of hydrogel, fertilizer and water as the factors considered. The hydrogel and water factors were considered important as the study area was located in a semi-arid region where water plays an essential regulatory role. The hydrogel is a copolymer of acrylamide/acrylic acid, potassium salt and crosslinked. Each gram of hydrogel absorbs until 300 ml of water. After six months in a nursery, 640 seedlings (40 per plot) were planted in 16 field plots of 100 m^2 each; two per treatment. After six years, more of the saplings grown under the hydrogel treatment had survived and were taller than untreated controls (mean height 6 m *vs* 2 m, respectively). Detailed results can be found in Fajardo et al. (2013).

Four years later, for the present study, we selected four plots established in the restored area (8 x 12.5 m each plot) (Fajardo et al., 2013): two of these plots had previously been treated with hydrogel (henceforth R2 and R2') and the other two were left as untreated (controls, henceforth R1 and R1'). In addition, we selected an abandoned (non-restored) area from which sand was also extracted up to four years ago. In this abandoned area, we established four plots of a similar size to those in the restored area. Two of these (NR1 and NR1') had a graminoid physiognomy with some scattered shrubs, while the other two plots (NR2 and NR2') showed a more species rich plant community, which led us to suppose that they could have been favored by soil water accumulation. The plant communities of all these plots are described in detail in Fajardo, Cuenca, Arrindell, Capote and Hasmy (2011).

Soil analysis: Four composite soil samples, each made up of samples randomly taken (0-20 cm deep) from the two plots under the same treatment, were taken and transported to the laboratory. Samples were air dried, homogenized and passed through a 2 mm sieve. They were then sent to the service unit of soil-water-plant analysis at the National Center for Agricultural Research (CENIAP, Spanish acronym) for chemical and physical analyses as follows. For each collected soil sample total N was measured by the micro-Kjeldahl method, available P was determined according to the Olsen method standardized by Gilabert de Brito, López de Rojas and Pérez de Roberti (1990), and the exchangeable cations (K^+, Na^+, Ca^{+2} and Mg^{+2}) were extracted and quantified following Olsen and Morgan (Gilabert de Brito, López de Rojas, & Pérez de Roberti, 1990). Soil pH was measured in a 1:2.5 soil-water mix (Jackson, 1976), and organic matter was assessed by the Walkley-Black method according to Jackson (1976). Soil texture type was evaluated by the Bouyoucos method (Day, 1965).

To calculate the percentage of moisture in the soil, we determined the fresh weight of ten intact soil samples from each of the four treatments. They were dried at 60 °C to constant weight and water content was calculated using the difference between wet and dry weight.

AMF community composition: To characterize the AMF communities, we randomly collected six soil samples (0-10 cm) per treatment and combined them to form one composite sample giving a total of four composite samples. To assess AMF richness and composition, a subsample of 200 g was taken from each composite sample and AMF spores were isolated by the wet sieving and decanting method followed by sucrose centrifugation, according to Sieverding (1991). Isolated spores were preserved in 0.05 % sodium azide until their analysis. Spores with similar morphologies were separated and counted under a dissecting microscope and mounted on permanent slides with polyvinyl alcohol-lactic acid-glycerol (PVLG) and (or) PVLG-Melzer, following Morton, Bentivenga and Wheeler (1993). They were then examined under an optical microscope with a Normanskii interference contrast system. Species identification was based on spore color, size, surface ornamentation and wall structure using the specialized literature available and the INVAM (http://www.invam.caf.wvu.edu), Blazkowski (http://www.agro.ar.szczecin.pl/~jblaszkowski/) and Schüssler (http://www.lrz.de/~shuessler/amphylo/) web sites. Morphotypes were photographed with an automatic Leica MPS 60 camera. Voucher specimens of each morphotype were deposited at the Instituto Venezolano de Investigaciones Científicas (IVIC) Glomeromycota Herbarium. Henceforth, in this text, the term "species" is used to refer to these morphological entities or morphotypes. We used the most recent taxonomic consensus proposed by Redecker, Schüssler, Stockinger, Stürmer, Morton and Walker, (2013).

Trap cultures: To complement the AMF species inventory, we prepared three AMF trap cultures for each treatment, as follows. Field soil was placed in 4 L pots and pregerminated seedlings of *Vigna luteola* were planted in each one to act as the host plants. This species is a wild legume, highly mycotrophic, the germination is quick and easy and it is used routinely in our laboratory. After six months in the greenhouse, spores were isolated using Sieverding's method (1991) as described previously. This long-term culture technique has the advantage that it enables the detection of seasonal and late successional sporulating AMF species (Oehl et al., 2010).

Mycorrhizal colonization: To assess the extent of mycorrhizal colonization, five soil cores (5 x 15 cm) were collected from each plot (ten soil cores per treatment). Fine roots (< 2 mm) were extracted by hand from core samples, washed under tap water, patted with absorbent tissue to remove excess moisture and weighed. Root samples were divided into two aliquots: one of these was used to measure root length using the WinRhizo software, version

2003b, and the other was prepared for the assessment of mycorrhizal colonization: roots were stained following the method of Phillips and Hayman (1970) and the mycorrhizal, arbuscule and vesicle colonization percentages were determined according to McGonigle, Miller, Evans, Fairchild, and Swan (1990).

The Shannon and the Simpson indexes were used to calculate AMF species diversity from both field and trap culture samples. Due to the absence of homogeneity of variances, the Kruskall-Wallis non parametric test was used to evaluate differences in spore number, soil moisture content, root biomass (RB), total root length (TRL), AM colonized root length (AMRL) and specific root length (SRL) among treatments. Data on AM colonization percentages were transformed using the arcsin $x^{1/2}$ to fulfill assumptions of normality and homogeneity of variances. These transformed data were used to conduct one-way ANOVA to test for significant differences in AM colonization and structures (arbuscules and vesicles) between treatments. The least significant difference (LSD) was applied as an *a posteriori* test. The relationship between AM colonization and spore density was determined by Pearson's correlation analysis. This analysis was also applied to evaluate the relationship between AM colonization and vegetation (Importance value, IVI; Fajardo et al., 2011).

The ANOVA and Kruskall Wallis tests were conducted with the statistical software STATISTICA version eight and diversity indexes and cluster analysis were done using the Past programme, version 2.03.

RESULTS

Soil analysis: Soils tended towards alkalinity (Table 1). In general, available P, total N and organic matter content improved in restored soils in comparison with non-restored soils, except in NR1, which showed similar or even higher values of these variables than restored soils. Soil texture ranged from sandy to loamy sand in non-restored soils and in restored untreated (R1) soils. However, in the restored hydrogel treated (R2) soils, soil texture was sandy loam. The moisture content of the R2 soils was the highest among treatments (Table 1).

AMF community characterization: A total of 41 AMF species were identified in the field and trap cultures. Of these, 32 species were recorded from field samples belonging to five families and ten genera (Table 2), with the Glomeraceae family contributing the largest number of species (18 spp., 56 %). There were three species (9 %) that we could not even identify to genus. Of the 32 AMF species found in the field samples, *Claroideoglomus etunicatum* and *Rhizophagus intraradices* were present in all treatments (Table 2). *Diversispora spurca* and *Funneliformis geosporum* were, however, only found in non-restored plots (Table 2).

Regarding the trap cultures, we were able to distinguish 25 species belonging to seven families and 11 genera (Table 2). Again, the Glomeraceae family contributed with the largest number of species (13 spp., 52 %). Five species collected in the trap cultures were

TABLE 1
Soil analysis of the restored (R) and non-restored (NR) treatments in the Macanao peninsula, Margarita Island, Venezuela

Treatment	pH	OM[1]	N_{TOT}	P	($cmol_c$/kg)				Sand	Clay	Silt	Texture[2]	Moisture
	(H_2O)	(%)	(%)	(mg/kg)	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	(%)	(%)	(%)		content (%)
NR1	7.3	2.30	0.11	6	9.42	1.22	0.12	0.41	83	3	14	LS	0.44 ± 0.06 ab
NR2	8.0	0.54	0.04	3	4.41	1.37	0.11	0.19	90	3	7	S	0.33 ± 0.01 b
R1	7.4	1.32	0.06	6	5.68	1.34	0.07	0.27	79	5	16	LS	0.34 ± 0.04 b
R2	7.6	1.15	0.08	12	8.00	2.23	0.12	0.49	70	7	23	SL	0.51 ± 0.04 a

[1] OM, organic matter.
[2] S: sandy soil, LS: loamy sand, SL: sandy loam.
Different letters indicate significant differences between treatments ($P < 0.05$).

TABLE 2
AMF species found in field samples and trap cultures taken from the restored (R) and non-restored plots (NR) in the Macanao peninsula, Margarita Island, Venezuela

No	AMF family/species	TREATMENTS/SAMPLE ORIGIN[1]			
		NR1	NR2	R1	R2
	Acaulosporaceae				
1	*Acaulospora mellea*				T
2	*Acaulospora undulata*		F	F	FT
3	*Acaulospora infrecuens*				T
4	*Acaulospora* sp. 1	F			
5	*Acaulospora* sp. 2	F			
6	*Acaulospora* sp. 3	T	T	T	T
	Claroideoglomeraceae				
7	*Claroideoglomus etunicatum*	FT	FT	F	FT
	Diversisporaceae				
8	*Diversispora spurca*	FT	FT	T	T
	Gigasporaceae				
9	*Gigaspora* cf. *albida*				T
10	*Gigaspora* sp. 1		F		
11	*Gigaspora* sp. 2			F	T
12	*Gigaspora* sp. 3	FT		T	
13	*Cetraspora gilmorei*			F	
14	*Scutellospora* sp. 1			F	
15	*Scutellospora* sp. 2		FT		T
16	*Scutellospora* sp. 3	T	T	T	
	Glomeraceae				
17	*Funneliformis geosporum*	FT	F	T	T
18	*Funneliformis mosseae*				T
19	*Rhizophagus aggregatum*				T
20	*Rhizophagus intraradices*	FT	FT	FT	FT
21	*Rhizophagus manihotis*				T
22	*Glomus microcarpum*	F		F	F
23	*Glomus minutum*		FT		
24	*Glomus rubiformis*			F	
25	*Glomus tortuosum*	F			
26	*Glomus* sp. 1	FT	T	T	T
27	*Glomus* sp. 2	F	F		FT
28	*Glomus* sp. 3	F	T	F	F
29	*Glomus* sp. 4	T			FT
30	*Glomus* sp. 5				F
31	*Glomus* sp. 6	F		F	F
32	*Glomus* sp. 7				FT
33	*Glomus* sp. 8		F		
34	*Glomus* sp. 9			F	
35	*Glomus* sp. 10			F	
36	*Septoglomus constrictum*	FT	FT	FT	T
37	*Septoglomus deserticola*	FT			FT
	Paraglomeraceae				
38	*Paraglomus occultum*				T
39	Unknown 1				F
40	Unknown 2		F		
41	Unknown 3		F		

[1]F: species collected from field samples, T: species isolated from trap cultures, FT: species found both in field samples and trap cultures.

TABLE 3
AMF species richness (S), the Shannon diversity index (H') and Simpson index (1-D) calculated for the field and trap culture samples from restored (R) and non-restored (NR) treatments

Treatment	Field samples			Trap culture samples			Total			
	S	H'	1-D	S	H'	1-D	S	H'	1-D	TCS[1]/FS[2]
NR1	15	2.2a	0.92a	11	1.9a	0.90a	18	2.4a	0.94a	0.7
NR2	13	2.1a	0.92a	9	1.9a	0.90a	17	2.4a	0.94a	0.7
R1	13	2.1a	0.92a	7	1.6a	0.88a	19	2.4a	0.94a	0.5
R2	12	2.0a	0.92a	21	2.6b	0.95a	26	2.7a	0.96a	1.8

[1]TCS: richness of trap culture sample; [2] FS: richness of field sample.
Different letters in the same column indicate significant differences between treatments ($P < 0.05$).

found in all of the treatments (Table 2). Both the richness and diversity of AMF species between different treatments were similar in the field samples (Table 3); however, in the trap cultures, the restored hydrogel treated soils (R2) showed the higher number and diversity of AMF species, according to Shannon index (Table 3). This last was also true if both sample types (field and trap cultures) were taken into account, although the difference was not significant (Table 3). It is important to point out that the soils under the restored treatments contained a higher number of AMF species belonging to the Gigasporaceae family (7) than non-restored soils (4) (Table 2).

Spore density and mycorrhizal colonization: Soil of the NR1 treatment showed the highest spore density compared to soils under the other three treatments (Table 4). Regarding mycorrhizal colonization there were no significant differences in the colonization percentages between treatments (Table 4). Neither there was any significant relationship between AM colonization and spore density ($r = 0.06$; $P = 0.88$). We did find a positive and significant correlation ($r= 0.77$; $P = 0.02$), between AM-colonization and the presence of four plant species: *Cnidoscolus urens* (Euphorbiaceae), *Ipomoea incarnata* (Convolvulaceae), *Capraria biflora* (Scrophulariaceae) and an unidentified shrub.

Root biomass: Due to high variability found within treatments, there were no significant differences in total root length (TRL), AM colonized root length (AMRL), specific root length (SRL) and root biomass (RT) among treatments (data not shown).

TABLE 4
Number of spores in 100 g of soil and AM-colonization percentages (%) found in non-restored (NR) and restored (R) treatments at the Macanao península, Venezuela

Treatment	Number of spores (± SE)	% Colonization (± SE)
NR1	817 ± 132 **a**	36.4 ± 5.3 **a**
NR2	143 ± 44 **b**	48.4 ± 6.3 **a**
R1	280 ± 76 **b**	46.6 ± 3.8 **a**
R2	95 ± 31 **b**	36.0 ± 4.2 **a**

Different letters in the same column indicate significant differences between treatments ($P < 0.05$).

DISCUSSION

Only a few studies have explored the behavior of mycorrizal communities in actively restored sites within degraded tropical dry ecosystems. This study thus represents an important contribution to our knowledge on these processes.

Although the differences were not significant, AMF species richness and diversity was higher in restored soils, especially those treated with hydrogel (R2). The highest contribution came from the AMF species found in the trap culture samples, according to the Shannon index. This suggests that more developed vegetation is associated with a greater AMF

species richness, including species with different life-history strategies (i.e a slow growth rate), as has been reported previously (Hart, Reader, & Klironomos, 2001). Additionally, we found that AMF species composition was different between non-restored and restored treatments, which may be associated with the change of herbaceous to arboreal vegetation (Leal et al., 2013). *C. etunicatum* and *R. intraradices* were found in all treatments, which is consistent with the report by Oehl et al. (2010) who considered these ubiquitous species as "generalists", strongly associated with highly disturbed sites. In another ecosystem type (the Brazilian Atlantic Forest), Aidar, Carrenho and Joly (2004) found that *C. etunicatum* occurred in all successional phases and seasons in field samples. On the other hand, two AMF species were collected exclusively in the non-restored plots (*D. spurca* and *F. geosporum*). It is likely that several plants such as *Axonopus* sp., found in non-restored treatments (Fajardo et al., 2011) stimulated the sporulation of these particular AMF species, as noted by Bever, Morton, Antonovics and Schultz (1996). Another aspect to highlight is that the highest number of species belonging to Gigasporaceae family, which are considered particularly susceptible to disturbance, was found in the restored plots (Lovera & Cuenca, 2007; Picone, 2000). This result could imply that the restored condition represents a less stressful environment.

We decided to evaluate AMF species composition using trap cultures in addition to field samples in order to obtain a more complete AMF inventory for each treatment and to correct for the possible absence of some species at the time of sampling. Leal, Stürmer and Siqueira (2009) pointed out that the trap culture technique yields a large number of healthy, readily identifiable spores that provides a useful supplement for the assessment of local species diversity in different ecosystems. The higher richness and species diversity of AMF under the R2 treatment was only significant for trap culture samples. Our results support Stutz and Morton's (1996) suggestion that in arid ecosystems many AMF species may be nonsporulating in the field.

A total of 41 AMF species were detected in the field and trap culture samples. This result is similar to the richness reported for other tropical dry ecosystems during different successional stages. Gavito et al. (2008) identified a total of 39 AMF species in primary forest, secondary forest and pastures in dry forest ecosystems in the Chamela region, Jalisco state, Mexico. A large number of AMF species (61) have also been reported from other tropical ecosystems encompassing pristine forest, young and old secondary forest, agroforestry systems, croplands and pasture (Stürmer & Siqueira, 2011). In this study of disturbed areas we have reported the presence of many unidentified AMF species (22). Nevertheless, it is now widely recognized that although the morphological homogeneity of the glomoid spore has resulted in the description of relatively few species, there are probably many more AMF species than published descriptions would suggest (Helgason & Fitter, 2005).

Mycorrhizal colonization was similar in both restored and non-restored treatments. Nevertheless, although differences between treatments were not significant, mycorrizal colonization was slightly lower in R2 compared to the other two treatments (NR2 and R1). Several contrasting results related to the excess and deficit of soil water and their effect on the mycorrhizal colonization of plant roots, as well as soil infectivity, have been reported (Auge, 2001; Li et al., 2007b). Drought stress may cause a decrease in colonization in mycorrhizal plants (Borowicz, 2010). However, in some cases, excess water may also lead to a reduction in AM-colonization (Schreiner, Tarara, & Smithyman, 2007). Interestingly, in our investigation, R2 soils showed the highest moisture content. In an environment where water is a limiting factor, as at our study site, an increase in soil humidity may facilitate a greater availability and mobility of nutrients in the soil (Marshner, 1997). Under these conditions, plants may access water and nutrients through their own root systems, rather than

via AMFs, since the plant-AMF association demands a significant portion of the energy fixed by the host (Jakobsen, Smith, & Smith, 2002). Indeed, no arbuscules were observed in the roots of plants under any of the treatment regimens (data not shown).This could explain the lack of differences in AM colonization found between treatments.

Soils under the NR1 treatment showed the highest AMF spore numbers, which is consistent with the results from other studies where stressful environmental conditions (high temperatures and light intensity) promote higher AMF sporulation (Li et al., 2007b). In fact, in general, our results regarding spore number were similar to those reported by other studies conducted in arid regions (Li et al., 2007b; Li & Zhao, 2005). In addition, sampling was done during the dry season when the highest spore density could be expected (Guadarrama & Álvarez-Sánchez, 1999; Li et al., 2007b). It is important to mention, however, that an enhanced abundance of AMF spores is not necessarily related to increased root mycorrhizal colonization (Guadarrama, et al., 2008).

To summarize, restoration management affected AMF species richness and diversity, particularly in restored soils treated with hydrogel. However, the similar AM intraradical colonization percentage between restored and nonrestored treatments, suggests that in a system strongly limited by water availability, AM percentage is not a sensitive indicator of the ecosystem recovery. In the neotropics, most investigations have compared the mycorrizal status of sites undergoing natural regeneration with those found in mature forest. However, few studies have assessed what occurs in actively restored sites. The lack of such data makes it difficult to draw conclusions about the role of AMF in the restoration of degraded areas. Future work on the dynamics of AMF communities in this study area will evaluate mycorrhizal status in plots with natural vegetation. This, in order to clarify whether this important biological interaction may be used as a criterion to measure restoration success by providing information about the resilience of the restored ecosystem.

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RESUMEN

Caracterización basada en morfotipos de las comunidades de hongos micorrízicos arbusculares en un bosque seco tropical restaurado, Isla de Margarita-Venezuela. Pocos estudios han evaluado el estado micorrízico de áreas tropicales áridas y semi-áridas sujetas a restauración o rehabilitación, en particular después de actividades de minería. Evaluamos y comparamos la presencia de hongos micorrízicos arbusculares en una pequeña área de un bosque seco restaurado que había sido destruido por la extracción de arena, con un área no restaurada de edad similar en la península de Macanao, Isla de Margarita (Venezuela). En Mayo 2005 fueron plantadas cinco especies de árboles nativos (*Tecoma stans*, *Bulnesia arborea*, *Piscidia carthagenensis*, *Prosopis juliflora* and *Parkinsonia praecox*) en las áreas deforestadas, con la aplicación de hidrogel como tratamiento más exitoso en términos de sobrevivencia y crecimiento de las plantas. En el presente estudio fue evaluado el estatus micorrízico de cuatro parcelas restauradas (8 x 12.5 m) y cuatro parcelas no restauradas de edad similar. Dos de las parcelas restauradas habían sido tratadas previamente con hidrogel (R2 y R2') y a las otras dos no se les agregó hidrogel (R1 y R1'). Las parcelas no restauradas (NR1 y NR1') tenían una fisonomía graminoide con algunos arbustos dispersos, mientras que las otras dos parcelas (NR2 y NR2') mostraron una comunidad de plantas más diversa. Los resultados indican que aparentemente la restauración promueve una mayor riqueza y diversidad de hongos micorrízicos arbusculares (HMA), particularmente en los suelos restaurados donde fue empleado un hidrogel (tratamiento R2). El suelo del tratamiento NR1 (con un alto componente herbáceo) presentó la mayor densidad de esporas comparado con los suelos de los otros tres tratamientos, lo cual es consistente con los resultados de otros estudios donde las condiciones ambientales estresantes (alta temperatura e intensidad lumínica) promueven una mayor esporulación de HMA. La composición de especies de HMA difirió entre suelos no restaurados y restaurados. *Clareidoglomus etunicatum* y *Rhizophagus intraradices* fueron encontrados en todos los tratamientos, *Diversispora spurca* y *Funneliformis geosporum* fueron encontrados sólo en las parcelas no

restauradas, mientras que especies pertenecientes a la familia Gigasporaceae, una familia que está asociada con sitios poco alterados, fue observada en mayor proporción en los suelos de las parcelas restauradas. La colonización micorrízica fue similar en las áreas restauradas y no restauradas, y es un indicador menos sensible de la recuperación del ecosistema. La mayor riqueza y diversidad de los HMA en las parcelas restauradas con el hidrogel, sugiere que esta estrategia de manejo podría contribuir con la aceleración de la regeneración natural en un ecosistema donde el agua juega un papel esencial.

Palabras clave: bosque seco tropical, colonización micorrízica, restauración ecológica, riqueza de hongos micorrízicos arbusculares, sucesión natural.

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