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Evaluation of environmental heterogeneity and its effect on arbuscular mycorrhizal interaction in coastal dunes

Evaluación de la heterogeneidad ambiental y su efecto en la interacción micorrízica arbuscular en la duna costera

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RESUMEN

Antecedentes: La vegetación del matorral de dunas costeras se establece sobre un sustrato que favorece la biodiversidad, pero restringido a pequeños manchones.

Objetivos: Conocer el estatus de los hongos micorrícicos arbusculares (HMA) bajo diferentes presiones antropogénicas y plantear acciones para su conservación y restauración.

Métodos: Se realizó una caracterización de la comunidad vegetal y fúngica a través de la identificación taxonómica de los HMA, su porcentaje de viabilidad, el número de propágulos infectivos (NMP) y el porcentaje de colonización micorrízica de las 12 especies vegetales comunes. En la Reserva de la Biosfera de Ría Lagartos, se identificaron tres sitios contrastantes: C = conservado; PS = perturbación intermedia por industria salinera; PA = perturbación alta de origen antropogénico. En cada sitio se recolectaron las raíces finas y se tomaron muestras de suelo.

Resultados y conclusiones: Se identificaron ocho especies de HMA, en PS se presentaron los valores más altos en porcentaje de colonización 55.43 ± 6.5, NMP = 142.07 ± 91.2 y viabilidad = 27.6 ± 15.21 %, mientras que en PA se encontraron más esporas sanas = 43.6 ± 11.5/50 g. Los propágulos infectivos son indicador de la “salud del ecosistema”; se encontraron en mayor proporción en el sitio conservado.

Palabras clave: esporas, colonización micorrízica, número más probable de propágulos infectivos

ABSTRACT

Background: Coastal dune scrub vegetation is established on a substrate that favors biodiversity, but this is currently restricted to small patches.

Objective: To determine the status of the arbuscular mycorrhizal fungi (AMF) in this vegetation under different anthropogenic pressures and to propose actions addressing its conservation and restoration.

Methods: Plant and fungal community was characterized through taxonomic identification of the AMF and determination of their percentage of viability, number of infective propagules (NIP) and percentage of mycorrhizal colonization. Three contrasting sites were identified in the Ría Lagartos Biosphere Reserve: C = Conserved, IPS = Intermediate perturbation by the salt industry and HPA = high perturbation of anthropogenic origin. In each site, fine roots were collected from 12 common species and compound soil samples were taken.

Results and conclusions: Eight AMF species were identified, in IPS, the highest values were presented in terms of percentage of colonization 55.43 ± 6.5, NIP = 142.07 ± 91.2 and viability = 27.6 ± 15.21 % while, in HPA, a higher number of healthy spores were found 43.6 ± 11.5/50 g. Infective propagules are an indicator of a “healthy ecosystem” and were found at a higher proportion in the conserved site.

Keywords: spores, mycorrhizal colonization, most probable number of infective propagules

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INTRODUCTION

Coastal dunes are complex and dynamic ecosystems that vary along a gradient from the coastline, the species are generally prostrated herbaceous plants of low richness, towards the inland area, a zone of shrub and arboreal vegetation of greater richness is found (Ciccarelli, 2015). Consequently, dunes form key ecosystems for studying the functional bases and responses of the communities to perturbation events (Psuty, 2004; Acosta *et al*., 2007). The environmental factors that influence the community assemblage processes are salt spray, sand burial, substrate instability, hydric stress, high temperature, scarcity of nutrients (Ranwell, 1972; Hesp, 1991; Maun and Maun, 2009) and geosedimentological aspects (Fenu *et al*., 2012; Angiolini *et al*., 2013; Bertoni *et al*., 2014; Ruocco *et al*., 2014).

Human influence is important as a generator of pressure, whether by removal of vegetation to construct touristic complexes, highway infrastructure, holiday homes, etc., or through coastal erosion as a result of the modified currents brought about by these constructions and/or the installation of protective structures (Kerbiriou *et al*., 2008; De Luca *et al*., 2011; Vallés *et al*., 2011; Ciccarelli *et al*., 2012; Santoro *et al*., 2012; Bertacchi and Lombardi, 2014; Ciccarelli, 2014; Malavasi *et al*., 2014). It should be noted the probable scenario for coastal dune habitats under climatic change predicts beach loss as a result of increased tides (Prisco *et al*., 2013).

In Mexico, dunes are one of the coastal environments most impacted by human activity (Guadarrama *et al*., 2012). In the state of Yucatán, more than half of the territorial area of the vegetation that originally bordered the beaches has been lost through processes of urbanization, actions of the salt industry and exploitation of the natural resources of the zone (Durán *et al*., 2010). Coastal dunes in Yucatán can be divided into: 1) the pioneer zone, which begins close to the coastline, creeping plants predominate and exotic species (e.g., *Cocos nucifera* and *Scaevola taccada,* pers. obs.) have become established, with a substrate that is compacted through trampling either by pedestrians or horses or the passage of motor vehicles and, more drastically, through substitution by constructions; and 2) the scrub zone, which is located inland and presents an accumulation of organic material. Shrubs and trees (some of these endemic, such as *Coccothrinax readii*, *Mammillaria gaumeri* and *Pterocereus gaumeri*) (Espejel, 1984; Acosta *et al*., 2007; Carnevali *et al*., 2010) dominate this zone. These species have experienced the greatest loss of plant cover, since they often attract the establishment of real estate developments, palm shelters, clandestine garbage dumps and the activities of the salt industry, which have had the greatest impact through the construction of roads (pers. obs.).

With the increasing loss of coastal dune plant cover, it is necessary to plan appropriate and effective practices of conservation, restoration and reforestation. One novel strategy includes the use of arbuscular mycorrhizal fungi (AMF) to facilitate the establishment and survival of plants. Several studies conducted in coastal dunes show that plants from both the pioneer and scrub zones are colonized by AMF (Koske and Halvorson, 1989; Sigüenza *et al*., 1996; Corkidi and Rincón, 1997; Alarcón and Cuenca, 2005; Guadarrama *et al*. 2012). The AMF have the capacity to increase the capture of phosphorus through their hyphae and to transfer it to the plants (Smith and Read, 2008). Moreover, in coastal environments such as the sand dunes, they are capable of increasing plant tolerance to salt spray, tidal changes, drought and the lack of nutrients (Marshner and Dell, 1994; Augé, 2001; Cantrell and Linderman, 2001). At ecosystem level, the benefits provided by the AMF are related to improved water filtration, efficiency of the biogeochemical cycles (e.g., carbon and phosphorus) and plant nutrition, architecture and diversity (Cuenca, 2015). They also increase physical resistance to erosion through secretion of a cementing glycoprotein by the external hyphae that facilitates the formation of aggregates that act to stabilize the substrate (Bever *et al*., 2001; Vaidya *et al*., 2011). For these reasons, they have been considered as key organisms in terrestrial ecosystems (Brachmann and Parniske, 2006; Gianinazzi *et al*., 2010)

The AMF persist in ecosystems through dispersion of their different propagules, such as spores, which constitute the most permanent source of the fungi in the soil, with fluctuations caused by the interspecific competition to which the species are subjected (Gemma *et al*., 1989). These can initiate colonization of the plants due to their ability to form multiple germ tubes (Koske, 1981; Smith and Read, 2008). However, loss of plant cover leads to the loss of AMF propagules (Torres-Arias *et al*., 2017), including the external hyphal networks that explore the soil and roots. Ecological studies of these endophytes in the coastal dunes of Yucatán have partly focused on quantifying mycorrhizal colonization in the species characteristic of these environments and the availability of infective propagules in the soil, a variable that indicates the potential of the AMF for colonizing new hosts, and involves not only spores with germinative capacity, but also fragments of colonized roots and mycelium (Ramos-Zapata *et al*., 2011), as well as helping to identify the species present in conserved environments and quantify their loss following anthropogenic perturbation (Guadarrama *et al*., 2012). This study evaluated the mycorrhizal colonization of common plant species of the coastal dune scrub vegetation, as well as characterized the AMF community through determination of the abundance of AMF species in the rhizosphere, their germinative capacity and the presence of infective propagules. The study was conducted in contrasting zones of the Ría Lagartos Biosphere Reserve in the state of Yucatán: one conserved zone (C) and two perturbed zones; one with high perturbation close to a settlement, cemetery and garbage dump (HPA), and another with intermediate perturbation, affected by the actions of the local salt industry (IPS).

It was hypothesized that the anthropogenic perturbations that occur in the coastal dunes will have an effect at edaphic level, for which reason changes will be presented in the intraradical AMF community structure, as well as in its extraradical dynamics, through a reduction in the quantity of infective propagules in the rhizosphere.

MATERIALS AND METHODS

Study site

This study was conducted in the Ría Lagartos Biosphere Reserve (RLBR), on the eastern coast of the state of Yucatán, Mexico (Figure 1). According to the Köppen climate classification, the RLBR presents climate type BS_o(h')w(x')iw (the driest of the arid climates) in Ría

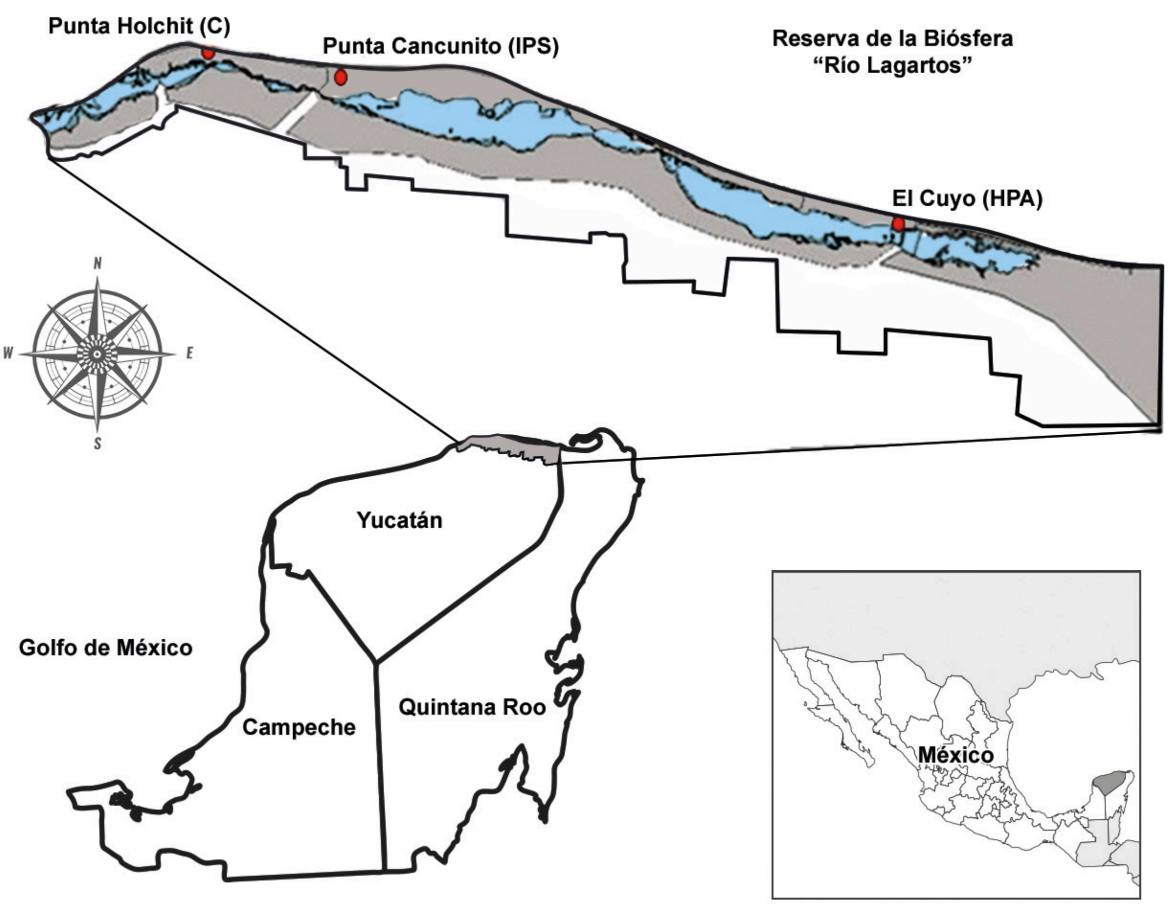

Figure 1. Ría Lagartos Biosphere Reserve. Punta Holchit (C= Conserved). Punta Calcunito (IPS= Intermediate perturbation by the salt industry). El Cuyo (HPA= High perturbation by anthropogenic activities).

Lagartos, and type Ax'(wo)iw (the driest of the subhumid climates) in El Cuyo. Total annual precipitation ranges from 500 to 1000 mm, and the month of April is the driest while September is the most humid (INE, 1999; Orellana *et al*., 2010). Coastal plains formed by marine sediments subjected to the action of the wind are found along the coastline, with elevations of less than 10 masl. The embryo dunes present in the area are vulnerable to the action of the sea, and can thus be flooded, while the dunes farthest from the coastline are more stable and present greater richness and plant diversity, with some vegetal elements of up to 4 m in height, as well as an accumulation of organic material and insects, terrestrial fauna and birds (Bautista *et al*., 2017).

Sampling site selection

Three sampling sites were chosen according to the comparative degree of conservation observed at the site (plant cover and richness) as well as the human activities present:

1) Conserved (C): this site has a high degree of conservation and is located within the core zone of the Reserve (21° 36' 34'' N 88° 02' 12'' W) in a locality known as Punta Holchit. This area has no adjacent urban settlements and access is via a sand road.
2) High perturbation of anthropogenic origin (HPA): this site presents high anthropogenic perturbation and a low degree of conservation. The site is located within the core zone of the Reserve (21° 31' 7.63'' N 87° 41' 16.95'' W) in the area known as El Cuyo, neighboring an urban settlement, cemetery and open-air garbage dump. Exploitation of shrub plant species is permitted in this site.
3) Intermediate perturbation by the salt industry (IPS): this site presents an intermediate degree of conservation due to the presence of a saltworks in the buffer zone of the Reserve (21° 36' 52 '' N 88° 05' 08'' W). The site is adjacent to a touristic zone, access to which is via an asphalt paved road.

Floristic list and sampling of roots and soil

To produce the floristic list, in each site, three transects of 5 m in width and 200 m in length were established (at a distance of 50 m apart), covering the zone of scrub vegetation. Root samples were taken of the 12 plant species present along each transect (roots of at least three individuals of each species per sampling site). A maximum of 50 % of the roots were removed, in order to avoid serious damage to the plant. In addition, three quadrats of 100 x 100 m were established in each site (distance of 100 m apart). On each edge vertex, and in the center of the quadrat, the surface organic material was removed, and approximately 1 kg of soil was collected with a garden spade (for a total of five 1 kg soil samples per quadrat) from the first 20 cm depth of soil. The soil samples collected in each quadrat were mixed in order to obtain compound samples. Three compound soil samples were thus obtained per site. The soil samples were used to quantify the number of spores, the percentage of germination among them and the most probable number of infective propagules (NIP), as well as for soil analysis. All plant and soil samples were taken during the rainy season (in July).

Soil analysis

The soil samples were analyzed in order to determine pH (1:2), electrical conductivity (EC) (direct conductometry), percentage of nitrogen (N) and percentage of available phosphorus (P). Each soil sample was oven-dried (24 hours at 60 °C and 24 hours at 105 °C) and the following parameters recorded: pH in water at ratio 1:2 (potentiometric; Thomas, 1996), electrical conductivity (EC) at ratio 1:5 (potentiometric; Rhoades, 1996), total nitrogen content (N) (Kjeldahl method; Bremner, 1996) and available phosphorous (P) (Olsen method; Kuo, 1996). Electrical conductivity was measured potentiometrically in the units μS/cm and transformed to values of salinity (parts per thousand), according to DAFF (2012).

Percentage of mycorrhizal colonization

The roots of each sample were washed, cleared and stained according to the method of Phillips and Hayman (1970). These roots were then observed under an optical microscope to quantify the level of mycorrhizal colonization according to the method of McGonigle *et al*. (1990).

Taxonomic identification of AMF spores

The spores present in a 50 mL volume of soil collected in each site were dried at ambient temperature and isolated following the wet sieving method proposed by Gerdermann and Nicolson (1963). The spores were placed in permanent preparations in a mixture (1:1; v/v) of polyvinyl-lacto-glycerol (PVLG) with Melzer's reagent (Koske and Tessier, 1983; Brundrett *et al*., 1994).

The AMF were then identified under an optical microscope at 10X, 20X, 40X and 100X magnifications. Taxonomic identification was conducted by comparing and contrasting the morphological characteristics of the spores with specialized descriptions of the genera and species of AMF, many of which are available on the website International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (https://invam.wvu.edu/), the taxonomy of Glomeromycota by Professor Janusz Blaszkowski of the Zachodniopomorski Uniwerytet Technologicznyw Szczecinie (http://www.zor.zut.edu.pl/Glomeromycota/), links provided on the website of Glomeromycota Phylogeny (http://www.amf-phylogeny.com/) and taxonomic documents belonging to this Phyla.

Spore germination

The spores present in a 50 mL volume of soil, collected, dried and isolated as described above, were surface sterilized with a mixture of 100 mg L^{-1} of gentamicin and 200 mg L^{-1} of streptomycin (Becard and Fortin, 1988). To determine the percentage of germination, we selected spores that presented no apparent structural damage (broken, depredated, crushed) or signs of parasitism (perforations) under the stereoscopic microscope, and followed the procedure proposed by INVAM (https://invam.wvu.edu/methods/spores/spore-germination). These were germinated in folded millipore membranes (0.45 µm, 47 mm diameter) in 250 mL containers. During this bioassay, the spores remained in darkness, with moisture content maintained at field capacity and a constant temperature of 26 °C for 21 days. At the end of this period, the spores were placed on slides and stained with trypan blue dye (0.05 %) diluted with distilled water. The spores were then observed under the microscope in order to determine the hyphal growth. The percentage of germination was determined by dividing the number of spores that presented hyphal growth by the total number of spores in the sample, and multiplying by 100.

Most probable number of infective propagules

The most probable number of infective propagules (NIP) was estimated in 50 mL of the soil sample following the method proposed by Porter (1979): briefly, using a fourfold soil dilution series, seven levels of dilution were prepared in 500 mL pots (one non-diluted and six dilutions) with five replicates each. Steam sterilized soil was used to dilute the soil samples. Soil dilutions were performed in dry soil.

Seeds of *Zea mays* (maize) were germinated, for which they were washed in 5 % bleach for a few minutes and subsequently germinated in plastic trays covered with moist paper. On appearance of the radicle (on the third day on average), the seeds were transferred into pots with sterilized soil for 20-30 days, until transplantation. A seedling of *Z. mays* was placed in each 500 mL pot as a trap plant, each pot was filled with 300 mL of steam sterilized soil and 50 mL of the diluted soil. The plants were grown in a greenhouse in sterile soil-sand (50:50) at ambient temperature (19.2-31.8 °C with a photoperiod of 12 hours), with tap water irrigation every two days for a period of six weeks. At the end of this period, the maize roots were cleared and stained following Phillips and Hayman (1970) and observed under an optical microscope in order to determine the presence/absence of AMF colonization.

Statistical analysis

One-way ANOVA and a Tukey multiple comparison test were used to determine significant differences in the level of mycorrhizal colonization and germination of AMF spores among sites. The NIP and confidence interval was calculated with the Fisher and Yates equation (1970). In addition, a non-parametric analysis of variance and Dunn´s multiple comparisons were performed. The analyses were conducted with the software Sigma Stat 3.5 (Systat Software Inc.).

RESULTS

Soil and vegetation analysis

The results indicate high values of pH and low values of EC, N and P in the site with an intermediate degree of conservation (IPS), while the site with a low degree of conservation (HPA) presented the highest values of EC, N and P. The greatest richness of herbaceous plants was presented in HPA. The dominant species per stratum included *Pseudophoenix sargentii* in the conserved (C) and intermediate (IPS) sites and *Coccoloba uvifera* in the site with a low degree of conservation (HPA) (Table 1). Likewise, 12 plant species were identified as common to all three sites: *Bonellia macrocarpa* (Cav.) B. Ståhl & Källersjö, *Caesalpinia vesicaria* L., *Coccothrinax readii* Quero, *Coccoloba uvifera* L., *Gossypium hirsutum* L., *Lantana camara* L., *Malvaviscus arboreus* Cav., *Metopium brownei* Jacq., *Pithecellobium keyense* Britton, *Pseudophoenix sargentii* H. Wendl, *Sideroxylon americanum* Miller and *Thrinax radiata* Scult, et Schult.f.

Mycorrhizal colonization

The site with intermediate perturbation (IPS) generally presented higher values of colonization (55.43 ± 25.95 %) than both the conserved site (C) (35.48 ± 19.63 %) and the site with the highest degree of perturbation (HPA) (39.96 ± 30.15 %). The species analyzed in this study were colonized by AMF of the type Arum and hyphae, vesicles and spores were observed within the roots. The mean percentage of colonization was 43.63 ± 26.78 %. The hyphae were the most abundant structures in the roots (32.87 ± 25.85 %), followed by the vesicles (24.76 ± 23.14 %) and spores (7.77 ± 13.47 %). On analysis of the fungal structures from each site, a greater quantity of hyphae and vesicles was observed in HPA (47.88 ± 4.3 % and 30.66 ± 4.2 %, respectively) than in IPS (hyphae 24.45 ± 4.5 % and vesicles 26.6 ± 4.4 %) and C (hyphae 26.3 ± 3 % and vesicles 16.3 ± 2.5 %); however, the number of spores within the roots was greater in IPS (13.4 ± 3.4 %) than in HPA (6 ± 0.9 %) and C (4 ± 1.4 %).

The species *Metopium brownei* presented the highest colonization (62.52 ± 22.06 %) and *Pseudophoenix sargentii* presented the lowest colonization (21.60 ± 15.42 %). Colonization of the tree and shrub species of the coastal dune scrub varied spatially ($p < 0.05$), depending on species (Table 2). The statistical analyses showed that seven species did not present significant differences related to the site. *Coccothrinax readii* was the only species that presented low colonization values in site C with significant differences, *Bonellia macrocarpa* presented low colonization values in site IPS with significant differences and *Lantana camara* presented significant differences in all three sites, with site C presenting intermediate values. *Pseudophoenix sargentii* presented significant differences in sites IPS and HPA, while *Gossypium hirsutum* presented significant differences in sites C and IPS (Table 2).

Table 1. Climatic, edaphic and herbaceous plant and shrub stratum characteristics of the vegetation in three coastal dune scrub in the Ría Lagartos Biosphere Reserve, Yucatán

Site	Conserved (C)	Intermediate perturbation by the salt industry (IPS)	High perturbation by anthropogenic activities (HPA)
Locality	Punta Holchit	Punta Cancunito	El Cuyo
Degree of conservation	High	Medium	Low
Climate	BSo(h')w(x')iw	BSo(h')w(x')iw	Ax'(wo) iw
Soil			
pH	8.6±0.02	8.7±0.05	8.6±0.05
EC	354.6±18.7	276±41.3	464.3±44.5
N	0.18±0.01	0.16±0.02	0.26±0.007
P	54.3±2.3	50.1±1.7	58.1±4.5
Vegetation			
Richness of herbaceous plant stratum	34	35	50
Richness of shrub stratum	10	8	9
Dominant species in herbaceous plant stratum	*Enriquebeltrania crenatifolia, Ernodea littoralis, Pseudophoenix sargentii*	*Diospyrus veraecrucis, Pseudophoenix sargentii, Thrinax radiata*	*Coccoloba uvifera, Cynodon dactylon, Thrinax radiata*
Dominant species in shrub stratum	*Coccothrinax readii, Pseudophoenix sargentii, Thrinax radiata*	*Metopium brownei, Pseudophoenix sargentii, Thrinax radiata*	*Coccoloba uvifera, Sabal mexicana, Thrinax radiata*

pH: (1:2). EC: Electrical conductivity μS cm^{-3} by direct conductometry. N: % Total nitrogen - modified Kjhendal. P: % available phosphorus - modified Olsen.

Identification of spores

Eight AMF species were identified in the study area. It should be noted that the spores from all of the field sampling sites presented serious damage on their walls, which reduced the number of spores that were mounted in preparations for subsequent identification by more than 70 %. The family Glomeraceae was the most abundant with six species, followed by Gigasporaceae and Acaulosporaceae with one species each (Table 3).

Viability of AMF spores

A total of 16.18 % of the spores isolated from the field samples presented no apparent damage to their walls. On comparison among the sites, the data indicated that those with greater numbers of "undamaged" spores were the lowest degree of conservation HPA (43.6 ± 11.5/50 g) and the conserved site (C) (32 ± 11.5/50 g). The lowest number of "undamaged" spores was presented by the site with an intermediate level of conservation IPS (29 ± 8.1/50 g).

Table 2. Average values of arbuscular mycorrhizal colonization percentage (± S.D) of representative species from coastal dune scrub in three sites of the Ria Lagartos Biosphere Reserve

Family	Species	Site		
		C	IPS	HPA
Arecaceae	*Thrinax radiate*	56.76 ± 17.20	41.12 ± 37.72	86.65 ± 8.34
	Pseudophoenix sargentii	16.86 ± 5.71 ab	9.25 ± 4.97 b	38.68 ± 13.94 a
	Coccothrinax readii	8.44 ± 5.82 b	61.29 ± 15.79 a	57.16 ± 11.01 a
Anacardiaceae	*Metopium brownie*	43.39 ± 25.89	63.36 ± 13.91	80.80 ±5.62
Leguminoseae	*Pithecellobium keyense*	35.93 ± 17.92	54.81 ± 11.90	25.55 ± 7.53
	Caesalpinia vesicaria	44.14 ± 10.97	47.68 ± 44.46	80.20 ± 14.63
Malvaceae	*Gossypium hirsutum*	27.85 ± 23.46 b	91.85 ± 5.37 a	61.00 ± 26.38 ab
	Malvaviscus arboreus	36.92 ± 26.97	28.70 ± 19.78	32.14 ± 10.73
Polygonaceae	*Coccoloba uvifera*	19.31 ± 11.93	10.25 ± 6.48	31.02 ± 21.18
Primulaceae	*Jacquinia aurantiaca*	43.18 ± 11.42 a	7.47 ± 4.17 b	45.75 ± 18.38 a
Sapotaceae	*Sideroxylon americanum*	45.35 ± 15.03	41.16 ± 22.05	43.15 ± 32.86
Verbenaceae	*Lantana camara*	47.74 ± 6.50 b	22.61 ± 2.58 c	83.07 ± 6.34 a

C: Punta Holchit, conserved. IPS: Punta Cancunito, intermediate perturbation by the salt industry. HPA: El Cuyo, high perturbation by anthropogenic activities. Different letters in the same row denote significant differences ($p<0.05$).

Table 3. Species of the AMF site found in the coastal dune scrub of the Ría Lagartos Biosphere Reserve, Yucatán, Mexico

Family	Species
Glomeraceae	*Glomus microcarpum* Tul. & C. Tul.
	G. viscosum T.H. Nicolson
	Rhizophagus fasciculatus Tahxt.
	Funneliformis geosporum T.H. Nicolson & Gerd
	Claroideoglomus claroideum Mukerji, Bhattacharjee & J.P. Tewari
	Sclerocystis rubiformis Gerd. & Trappe
Gigasporaceae	*Scutellospora erythropus* Koske & C. Walker
Acaulosporaceae	*Acaulospora kentinensis* C.G. Wu & Y.S. Liu

On analysis of spore viability, IPS site presented the highest values of germination (27.6 ± 15.21 %), while those of sites HPA and C presented very similar values (7.5 ± 7.78 and 8.6 ± 9.11 %, respectively). However, the statistical analysis did not indicate significant differences among the sites (H = 8.00, p = 0.23) (Figure 2).

Infective propagules

Infective propagules of AMF were found in all three sites analyzed. As with the colonization, the number of infective propagules of AMF varied according to study site; the greatest number of infective propagules was found in IPS (142.07 ± 91.2), followed by sites C (14.99 ± 6.25) and HPA (2.04 ± 0.95). The statistical analysis showed these differences to be significant (H = 5.804, p = 0.025) (Figure 3).

DISCUSSION

Mycorrhizal fungi were present in all three studied environments and in all plant species analyzed. The percentages of colonization did not differ significantly between the conserved and perturbed environments. These results support the findings of Alarcón and Cuenca (2005) in a study of the mycorrhizal colonization in the dominant vegetation of the dunes of the Paraná peninsula in Venezuela, with values of colonization that ranged from 54.3 to 66.2 %. The fact that 100 % of the species studied presented mycorrhizal colonization could be related to the importance of the mycorrhizal fungi in dune stabilization, as demonstrated by Koske and Gemma (1996) who found greater colonization in the most stabilized zones, leading these authors to conclude that stabilization of the sandy substrate is a consequence of the activity of the mycorrhizal fungi.

Although mycorrhizal association has been reported in species of the genera *Thrinax* and *Coccothrinax* (Fisher and Jayachandran, 2005), including *Coccothrinax readii* (Polanco *et al.*, 2013), it should be noted that the species *Thrinax radiate*, *Bonellia macrocarpa* and *Caesalpinia vesicaria* have not been reported previously in association with AMF. Koske and Gemma (1990) reported similar values in the percentage of mycorrhizal colonization in the species *Coccoloba uvifera*. This is of particular importance since this species belongs to the family Polygonaceae, which had hitherto been presumed not to form arbuscular mycorrhizal associations (Smith and Read, 2008).

The interspecific variation in the percentage of mycorrhizal colonization found in the coastal scrub of the RLBR is similar to that reported in other coastal dunes, where most of the species were found within a highly variable range of colonization, in which the variation could even be spatial (Koske and Polson, 1984; Koske and Gemma, 1990; Corkidi and Rincon, 1997; Kulkarni *et al.*, 1997). This could be a consequence of the

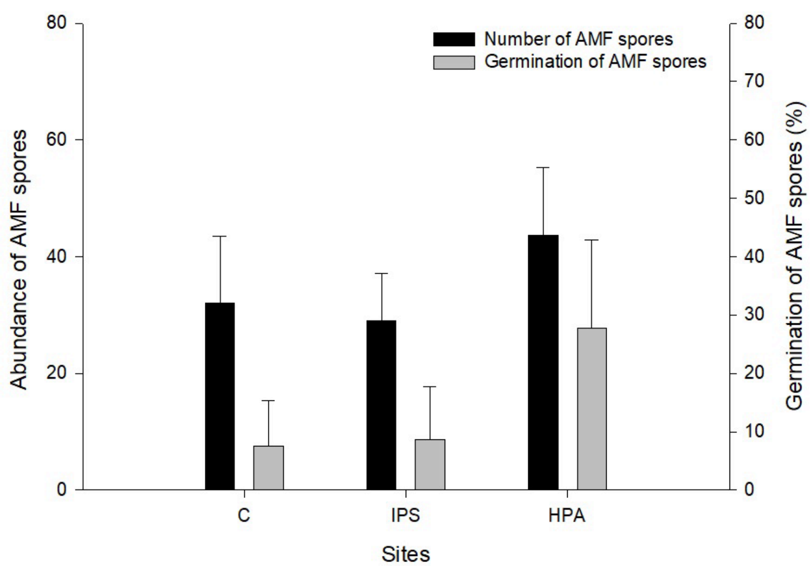

Figure 2. Abundance (black) and percentage of germination (grey) of AMF spores in the coastal dune scrub. C: Conserved. IPS: Intermediate perturbation by the salt industry. HPA: High perturbation by anthropogenic activities.

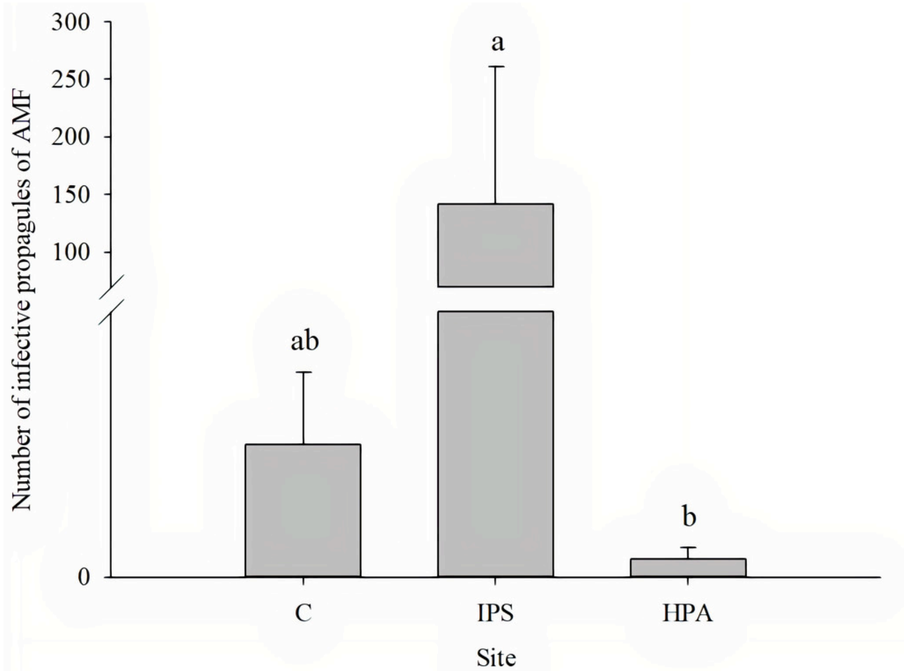

Figure 3. Number of infective propagules of AMF in three coastal dune scrub. C: Conserved. IPS: Intermediate perturbation by the salt industry. HPA: High perturbation by anthropogenic activities.

capacity for regulation of the interaction presented by some plant species depending on the biotic and abiotic conditions at each site. The present study detected a high percentage of colonization, greater quantity of spores in the roots and high viability of spores in the rhizosphere in the site IPS, where the concentrations of N and P were lower, as has been observed in the reduction of the values of colonization when phosphorus (P) is applied in sites with low availability of both P and nitrogen (N) (Treseder and Allen, 2002). However, it must also be considered that the percentage of mycorrhizal root colonization is a relative measurement (Cuenca, 2015) that is influenced by the root growth rate (Allen, 2001). This in turn depends on the physiology of the plant and the environmental conditions (e.g. soil moisture content), which in the tropics can strongly influence the dynamic of fine root production (Green *et al*., 2005).

Eight species of AMF were found in this study, a value lower than that reported in coastal dunes of the state of Yucatán, Mexico, by Guadarrama *et al*. (2012). Koske *et al*. (2004) have indicated that the number of AMF species increases with the successional process and, in the particular case of the coastal dunes of Sisal in the state of Yucatán, Guadarrama *et al*. (2012) found a high species richness in the conserved sites, which decreased in zones with secondary vegetation and was almost null in very perturbed areas. In this study, the high deterioration of the spores impeded their quantification, which could be related to a systemic reduction of the number of spores produced in the rainy season (Cuenca and Lovera 2010), the time at which the samples were taken. This was a consequence of the response of the plants to the arrival of the rains, which initiates their growth and with it the germination of the spores (Cuenca 2015).

With the exception of *Funneliformis geosporus and Sclerocystis rubiformis*, the six other species found represent new records for coastal dune scrub and for the coastal dunes of Mexico. The species richness found in the RLBR is lower than that found in dunes sites in Poland (Blaszkowski, 1994), U.S.A. (Koske and Gemma, 1996) and India (Beena *et al*., 2000), with 35, 31 and 14 species reported, respectively. In contrast, the AMF richness found here, is similar to that found at some other coastal dunes of Mexico (Sigüenza *et al*., 1996) and Venezuela (Cuenca *et al*., 2003). In all cases, including the present study, the family Glomeraceae is the best represented; i.e., it presents the highest number of species.

The present study determined very low levels of viability of the AMF spores compared to that found under controlled conditions with spores of *Gigaspora* sp. and *Acaulospora* sp. (Clark, 1997; Koske, 1981). A great number of the spores found in the present study had apparent structural damage. This reflects that found in other natural environments, i.e. low levels of viability prevail, along with marked fluctuations in the percentage of germination of the spores (Smith and Read, 2008). The spatial variation found in the number of infective propagules of AMF, but not in the percentage of germination, suggests that the spores are not the main source of propagules in these sites. This supports the findings of previous studies that suggest that, even with a significant presence of spores in the soil, previously colonized roots and extra-radical mycelium constitute the main sources of AMF propagules to colonize new hosts (Hepper, 1981; Smith and Smith, 1981; Fisher *et al.,* 1994).

According to Adelman and Morton (1986), the number of infective propagules (NIP) is positively related to the intensity of mycorrhizal colonization. However, this pattern was not observed in the present study. Although more infective propagules were found in the conserved site of Punta Holchit (C), the highest values of mycorrhizal colonization were found in the site IPS. In this sense, the number of infective propagules found was highly variable both within and among the study sites. This could be due to the fact that the sampling area for calculating the NIP and relating it to the colonization of the roots (percentage of colonization) omitted the high spatial heterogeneity with which the AMF propagules were found in the soil (McGee, 1989).

We conclude that the fact that there are plant species shared among the three study sites, two of them that have been subjected to anthropogenic pressures, while presenting mycorrhizal colonization in their roots allows us to consider these species as "rescuers of AMF propagules", thus preventing the disappearance of the scarce inoculum that remains after a perturbation, as indicated by Cuenca (2015). We consider that the NIP is a suitable indicator for estimating the "health of the ecosystem" and is higher in the conserved site, while the other variables analyzed do not differ between the conserved and perturbed environments. Conservation of the coastal dune scrub ecosystems is therefore necessary in order to conserve the ecosystem services they provide, through the knowledge and preservation of positive interactions between the species, as is the case of the arbuscular mycorrhizal interaction that, according to our results, is sensitive to anthropogenic perturbation.

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