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Arbuscular mycorrhizal fungi communities in a coral cay system (Morrocoy, Venezuela) and their relationships with environmental variables

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HIGHLIGHTS

- AMF diversity in a coral cay system in Morrocoy (Venezuela) was very high.
- Environmental factors related to insularity were related with the distribution of AMF.
- Knowledge of AMF in threatened sites is essential for preserving their biodiversity.

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ABSTRACT

Knowledge of the natural diversity of arbuscular mycorrhizal fungi (AMF) and understanding of their biogeographical patterns and what drive them might help to the maintenance and preservation of ecosystems under a changing environment. The objective of this study was to evaluate the contribution of different environmental factors to the determination of the composition of AMF assemblages in representative sites within the Morrocoy National Park (Venezuela). The community structure of the AMF under the canopy of *Coccoloba uvifera* was investigated in four cays (Borracho, Muerto, Peraza, and Paiclás) and one mainland location (Las Luisas). Based on partial sequences of the nuclear small subunit ribosomal DNA gene, the AM fungi in soil samples were divided into 31 operational taxonomic units, grouped in eight families. The canonical correspondence analysis showed that environmental factors related to insularity (the mean annual rainfall, the distance to the mainland coast, and the cay land area) and a soil property related to biological activity (the total carbohydrate content) were significantly related to the distribution of the AMF communities.

1. Introduction

Within the soil microbial community, the arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota (Schüßler and Walker, 2010), are ubiquitous root symbionts which, due to their low host specificity and broad geographical distribution, form intimate associations with the majority of terrestrial plant species (Smith and Read, 2008; Brundrett, 2009; Smith and Smith, 2012). Since AMF provide plants with mineral nutrients in exchange for carbon compounds and protect them from diverse abiotic and biotic stresses (Smith and Read, 2008; Smith and Smith, 2012)the diversity of AMF in the soil and their interactions can drive ecosystem functions such as plant diversity and productivity (van der Heijden et al., 1998, 2003; Wagg et al., 2014). It has been shown that the occurrence of the AM symbiosis and the distributions of AMF species are influenced not only by host plants (Sýkorová et al., 2007; Öpik et al., 2009; Alguacil et al., 2011b; Torrecillas et al., 2012) but also by environmental factors, such as climatic zone, moisture, habitat, land uses, locality, soil temperature, soil fertility, rainfall, and soil type (Öpik et al., 2006, 2010; Kivlin et al., 2011; Lekberg et al., 2011; Hazard et al., 2013, Moebius-Clune et al., 2013; Alguacil et al., 2014; de Oliveira Freitas et al., 2014). Therefore, knowledge of the natural diversity of AMF and of their biogeographical patterns and what drives them might help to the maintenance and preservation of ecosystems under a changing environment.

Target ecosystems in tropical areas can be a good source of knowledge about the environmental factors which influence AMF distribution, due to their variety of habitats. In this sense, coral cay systems can be considered a natural laboratory in which to ascertain the biogeographical patterns of AM fungal communities because of their isolated and relatively

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simple ecosystems: here, environmental variables linked to insularity can influence the occurrence of these symbionts. The Morrocoy National Park in Venezuela stretches over 32,090 ha and comprises both terrestrial and aquatic areas, including diverse and heterogeneous tropical ecosystems, such as coral cays, mangroves, sea grasses, sandy beaches, and estuaries. Morrocoy has been subjected to severe, negative environmental impacts. Among the environmental aspects affecting Morrocoy, of note are the unsustainable tourism practices — both on the mainland and in the cays (Ministry of the Popular Power for the Environment [MPPE], 2011). Thus, to determine and understand the factors that influence species distribution and the assemblage of AMF communities is an important challenge in the ecology of these ecosystems.

We hypothesized that, after eliminating the host factor, the distribution of AMF communities would be linked to other factors related with geographical position and soil characteristics. Therefore, the objective of this study was to evaluate the role of different environmental factors in the determination of the composition, abundance, and diversity of the AMF community in representative sites within the Morrocoy National Park. To do so, soil samples under the canopy of *Coccoloba uvifera* L. were selected from five different sites (four cays and one mainland location) within the park, along a rainfall gradient and differing in their distance to the coast. We selected this plant species for two reasons: (1) it showed the highest percentage of cover in all five sampling sites; and (2) to elucidate biogeographical inferences without the biasing effect of host preferences, AMF surveys across sites need to be carried out using the same plant species.

2. Materials and methods

2.1. Study site and sampling

The experiment was conducted at the Morrocoy National Park (MNP), located on the North-western coast of Venezuela (10° 40′–10° 58′ N; 68° 11′–68° 20′ W). The park soils are recent formations (quaternary/recent Pleistocene geological period), with marine-type base. This substrate is characterized by having very thin materials (silt–clay), organic debris and seashells. The soils have very poor drainage and alkaline reaction, and some areas are affected by salinity (Steyermark et al., 1994).

The climate is tropical with seasonal influence. The precipitation shows a unimodal regime with a dry season from January to April and a rainy season from May to December, with a maximum precipitation peak towards the end of the year (November–December) (Barreto, 2008; Rosell, 2011). The air temperature is relatively constant throughout the year and varies from 24.9 to 29.7 °C and the maximum temperatures occur during the rainy season (Bone et al., 2001). The relative humidity is high (83–90%), with the highest values recorded between May and August (Pérez and Galindo, 2000).

The natural vegetation in the park includes species that are organized in well-defined bands where *Rhizophora mangle* (red mangrove), *Avicennia germinans* (black mangrove) and *C. uvifera* (sea grape) occupy the mainland coastal zone and the coral cays. Among the plant species with the highest percentage of coverage it can be found in *C. uvifera* (42.88%), *Conocarpus erectus* (26.66%), *R. mangle* (9.16%), *Phthirusa stelis* (5.81%) and *Suriana maritima* (5.45%) (Steyermark et al. 1994).

Five sampling stations were selected within the park according to an environmental gradient (north to south) 15 km long: Borracho cay, Peraza cay, Muerto cay, Paiclás cay and Las Luisas (Table 1).

Borracho cay: Sheltered, coralline beach. Area for nesting turtles and coast caiman. Only allowed for the scientific research.

Peraza cay: Exposed, coralline beach. It is the smallest islet in Morrocoy, without any services, so the number of visitors is reduced. The vegetation is abundant and it is surrounded by a coral reef barrier.

Muerto cay: Exposed, coralline beach. This cay contains the largest number of palm trees. There are many services in the cay; restaurants, rent of beach chairs, abundant commerce of food products, and consequently a very intense human pressure.

Paiclás cay: Sheltered, coralline beach. One of the largest cays in the park. Area for nesting turtles and coast caiman. Only allowed for the scientific research.

Las Luisas mainland: Sheltered, bordered by mangrove forests and grasslands. Low intervention area.

Soil texture in all five sites was sandy, with sand percentages between 80 and 86%.

C. uvifera (sea grape) was selected as the plant target species since it was present in all the five chosen areas with the highest percentage of coverage. This tree usually appears in monospecific spots with a dense canopy (Steyermark et al. 1994).

Sea grape typically produces a stout taproot and numerous thin wiry laterals with abundant fine, feeder roots (Parrotta, 1994). The fine roots have been reported to form an association with ectotrophic mycorrhizae (Bandou et al., 2006) but after clearing and staining with trypan blue, typical AMF structures were detected, as is usual for other *Polygonaceae* members (Zhang et al., 2014).

The density of *C. uvifera* was always around 100% in all the sampled areas. Within each area four 5 m^2 plots (under the *C. uvifera* canopy) situated at least 10 m apart were established in a straight line parallel to the coast. We deemed that at the specific spatial scale there would be no spatial effects on AMF communities. One sample per each plot was collected in September 2013. Each sample consisted of six bulked subsamples per replicate plot (100 cm^3 cores), randomly collected at 0 to 15 cm depth and these sub-samples were homogenized putting them in 1000 cm^3 plastic bags and vigorously shaked for 5 min to give one

Table 1
Geographical situation parameters and soil properties of the sites used in this study.

Site	Geographical coordinates	Mean annual rainfall (mm)	Land area (ha)	Distance to mainland coast (km)	Human activity	Organic carbon (g kg^{-1})	Total CH (μg g^{-1})	pH	EC (μS cm^{-1})	Basal respiration rate (μg CO_2 g^{-1} h^{-1})
Cay Borracho	10° 58′ 29″ N 68° 15′ 60″ W	750	7.82	5.0	Protected	24.2 ± 1.61	2890 ± 32.5	7.6 ± 0.1	259 ± 2	18.5 ± 0.5
Cay Peraza	10° 55′ 43″ N 68° 15′ 11″ W	955	1.04	1.5	Restricted	25.4 ± 1.56	2995 ± 39.5	7.9 ± 0.2	182 ± 2	20.1 ± 1.0
Cay Muerto	10° 55′ 41″ N 68° 15′ 41″ W	1115	7.18	1.0	Intense	29.1 ± 2.07	1165 ± 29.9	7.8 ± 0.2	118 ± 1	12.5 ± 0.4
Cay Paiclás	10° 49′ 20″ N 68° 15′ 50″ W	1303	190.70	2.0	Protected	38.4 ± 2.67	2624 ± 48.7	7.6 ± 0.1	88 ± 1	25.4 ± 1.5
Las Luisas	10° 51′ 23″ N 68° 17′ 40″ W	1340	Mainland	0.1	Restricted	31.0 ± 1.98	3919 ± 47.5	7.5 ± 0.1	66 ± 1	26.8 ± 1.8

Means ± standard errors from 4 replicates by site; Total CH: Total carbohydrates; EC: Electrical conductivity; Protected: Human access prohibited, only allowed for the scientific research; Restricted: Only limited visits allowed, without any services, low intervention area.

composite sample per plot. The 20 soil samples (5 sites × 4 replicates) were sieved at 2 mm, frozen and stored until analyzed.

2.2. Soil analyses

Soil electrical conductivity was measured in a 1:5 (w/v) aqueous solution. Total organic C was determined by a C/N Elemental Analyzer LECO Truspec. Total carbohydrates (TCH) extracted with anthrone were determined by the method of Brink et al. (1960) and were measured spectrophotometrically at 630 nm. Soil respiration was calculated as the amount of CO_2 emitted during a 24 h incubation period: 10 g of dry soil was placed in an incubation vessel, the moisture was adjusted to 45% of water-holding capacity and a vial containing 2 mL of KOH (0.1 g of KOH in 50 mL distilled water) was placed inside the incubation vessel for retention of the evolved CO_2. Soil respiration was determined with an automatic analyzer (μ-TRAC 4200, SY-LAB).

2.3. DNA extractions from soils and PCR

Two independent DNA extractions (0.5 g of soil fresh weight) from the 20 soil samples were carried out using a FastDNA™ Spin kit for soil according to the recommendations of the manufacturer (Q-BIOgene, Heidelberg, Germany). DNA extracts were stored at −20 °C.

A dilution (1/50) of extracted DNA was prepared and 2 μL was used as a template. Partial small subunit (SSU) ribosomal RNA gene fragments were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 (White et al., 1990). PCR was carried out in a final volume of 25 μL using the PuReTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM dNTPs and 0.5 μM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles at 94 °C for 30 s, 40 °C for 1 min, 72 °C for 1 min, followed by a final extension period at 72 °C for 10 min).

Two microliters from the first PCR was used as template DNA in a second PCR reaction performed using the specific primers AML1 and AML2 (Lee et al., 2008). PCR reactions were carried out in a final volume of 25 μL using the PuReTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM dNTPs and 0.5 μM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C and 1 min extension at 72 °C, followed by a final extension period of 10 min at 72 °C). Positive and negative controls using PCR positive samples and sterile water respectively were also included in all amplifications. All the PCR reactions were run on a Perkin Elmer Cetus DNA Thermal Cycler. Reaction yields were estimated by using a 1.2% agarose gel containing *GelRed*™ (Biotium).

We pooled the PCR products independently amplified from the two 0.5 g soil DNA extractions per sample, obtaining 20 PCR products in total (one per replicate plot and site).

2.4. Cloning and sequencing

The PCR products were purified using a Gel extraction Kit (Qiagen) cloned into pGEM-T Easy vector (Promega) and transformed into *Escherichia coli* (Xl1 blue). Putative positive transformants were screened in each resulting SSU rRNA gene library, using 0.7 unit of RedTaq DNA polymerase (Sigma) and the supplied reaction buffer to a final volume of 25 μL and a re-amplification with AML1 and AML2 primers with the same cycling parameters described above. Product quality and size were checked in agarose gels as described above. All clones having inserts of the correct size in each library were sequenced using the universal primers SP6 and T7 by Laboratory of Sistemas Genómicos (Valencia, Spain).

102 unique sequences of the clones generated in this study have been deposited in GenBank (http://www.ncbi.nlm.nih.gov) under the following accession numbers HG972863–HG972964.

2.5. Phylogenetical analysis

Sequence editing was done using the program FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). A search for similar sequences to the ones from this study was conducted with the BLAST tool (Zhang et al., 2000) provided by GenBank. Phylogenetic analysis was carried out on the sequences obtained in this study and those corresponding to the closest matches from GenBank and MaarjAM databases (Öpik et al. 2010), as well as on sequences from cultured AMF taxa including representatives of the major groups of *Glomeromycota* from GenBank. All the sequences were aligned, using the multiple sequence comparison program, MAFFT, version 7.0 (available at http://align.bmr.kyushu-u.ac.jp/mafft/software) and the alignment was adjusted manually in BioEdit software version 7.2.5 (Hall, 1999).

Phylogenetic analyses of the aligned sequence data were conducted using the Neighbor-Joining (NJ), and Maximum Likelihood (ML) methods, employing MEGA v.5.05 software (Tamura et al., 2011). Distances for the NJ tree were computed using the default parameters. For the ML analysis, nucleotide data files were first tested to find the best DNA evolution model. Models with the lowest BIC scores (Bayesian Information Criterion) were deemed to best describe the nucleotide substitution pattern. To pick the most appropriate evolutionary model for each analysis, all positions with gaps and missing data were eliminated. Initial trees were generated by the random addition of sequences (10 replicates). The robustness of all trees obtained was evaluated by 1000 bootstrap replications. *Endogone pisiformis* Link and *Mortierella polycephala* Coem, were used as the out-groups.

2.6. Definition of sequence types

Among the *Glomeromycota*, the rDNA gene can have different variants within the same species and even within the same spore. It is therefore not always possible to assign a set of sequences to a particular species. Sequence types, equivalent to operational taxonomical units, were defined as groups of closely related sequences, usually with a high level of bootstrap support in the phylogenetic analysis (80%) and a level of pairwise similarity higher than 96.5%. This cutoff value is equivalent to a similarity of 97%, which in previous studies has been considered a threshold for separating possible AMF species (Helgason et al. 2002) in the analyses of sequence divergences. The pairwise analysis within clusters was carried out using BioEdit software version 7.2.5 (Hall, 1999).

The sequence types were named for the genera to which they belonged (Glo for *Glomus*, Aca for *Acaulospora*, Rh for *Rhizophagus*, etc.), followed by a number, e.g., Glo2. The number relates to different OTUs found and belonging to the same genera. This way of naming clones according to the system used by Helgason et al. (1999) is the one most widely used in field studies of this type.

2.7. Statistical analysis

The Shannon (H') index was calculated as an additional measure of diversity, as it combines two components of diversity, i.e., species richness and evenness. It is calculated from the equation $H' = -\sum p_i(\ln p_i)$, where *pi* is the proportion of individuals found in the $_i$th species.

Rarefaction analyses were applied in order to determine whether the number of tested clones sufficiently represented the AMF diversity in the soil samples (Raup, 1975). The rarefaction curves were produced by plotting the number of OTUs observed against the number of AMF sequences obtained using the freely available Analytic Rarefaction v. 1.3 software (http://www.uga.edu/~strata/software/anRareReadme.html).

We used indicator species analyses to generate a numerical classification of OTUs (Hill et al. 1975). This method uses a reciprocal averaging ordination to classify the OTUs, according to apparently important environmental properties (Hill, 1973). The indicator value (IndVal) index (Dufrene and Legendre, 1997) was used to measure the association between a species and a site group. Finally, the statistical significance

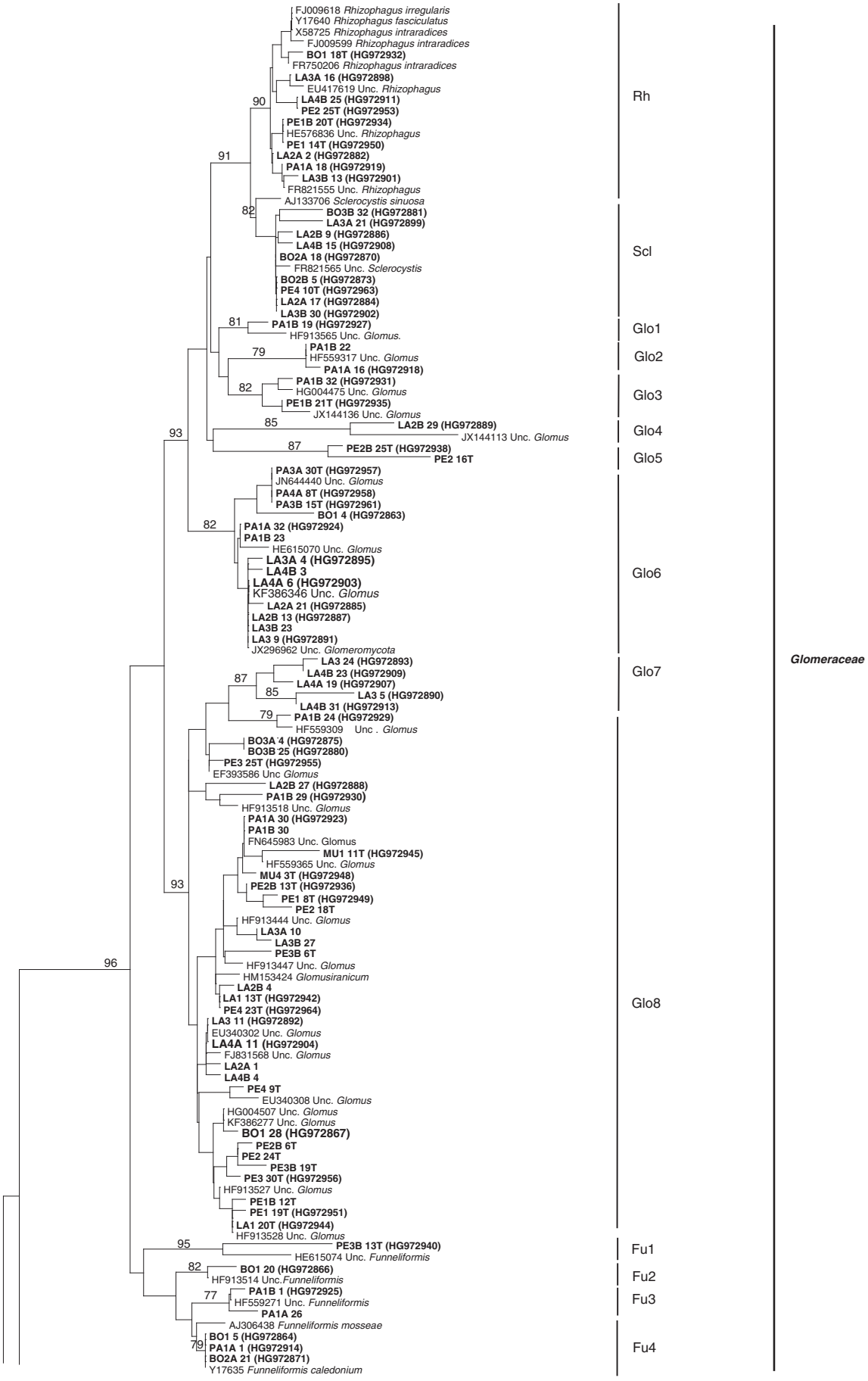

Fig. 1 (*continued*).

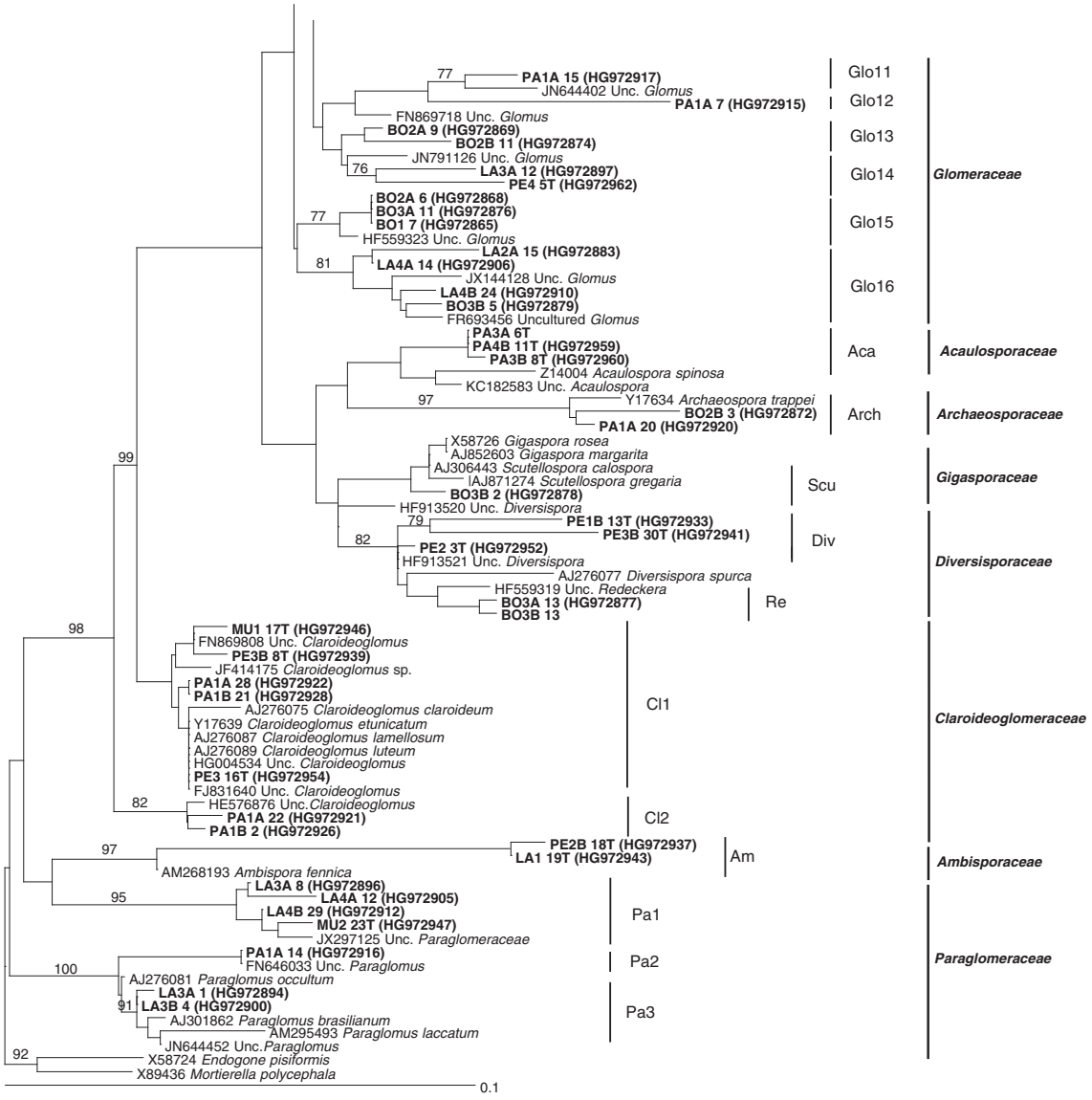

Fig. 1. Neighbor-Joining (NJ) phylogenetic tree showing AM fungal sequences isolated from *Coccoloba uvifera* rhizosphere in the different locations (PA = Paiclás cay, BO = Borracho cay, PE = Peraza cay, MU = Muerto cay, LA = Las Luisas mainland), and reference sequences from GenBank. All bootstrap values >75% are shown (1000 replicates). Numbers above branches indicate the bootstrap values of the maximum likelihood analysis. Sequences obtained in the present study are shown in bold type. They are labeled with the name of site and repetition (1: repetition 1, 2: repetition 2, 3: repetition 3, 4: repetition 4) from which they were obtained and the clone identity number. Group identifiers (for example Glo1) are OTUs found in our study. *Endogone pisiformis* and *Mortierella polycephala* were used as out-groups.

of this relationship was tested using a permutation test with 999 permutations. We performed these analyses using the "indicspecies" package implemented in R (De Cáceres and Legendre, 2009).

The Shannon diversity index and the AMF OTU richness were log-transformed to achieve normality and subjected to ANOVA to test for significant differences between different sites. For the comparisons among means the Duncan's test at $P < 0.05$ was used. Correlation analysis between the soil properties measured, geographic situation parameters and OTU relative abundance was carried out using Pearson's rank correlation coefficients. All the statistical procedures were carried out with the software package IBM SPSS Statistic 19.0 for Windows.

We used a constrained ordination process, canonical correspondence analysis, to seek for the environmental variables that best explain the variation in fungal community composition, in constrained ordination techniques, the matrix of environmental variables conditions the "weight" (eigenvalues), the orthogonality and the direction of the ordination axes, so that the obtained axes explain, as much as possible, the variation in the community composition (Scheiner and Gurevitch, 2001). To elucidate the relationships between the AM fungal community composition, soil properties, geographic situation parameters and sites, multivariate analyses were conducted in CANOCO for Windows v. 4.5 (ter Braak and Smilauer, 2004) using the clone abundance data for each AMF OTUs. Detrended correspondence analysis (DCA) was first applied to the response variable data to estimate heterogeneity in species turnover units throughout the length of the community composition gradients. After confirming the length of composition gradients on the first DCA analysis (>4), canonical-correspondence analysis (CCA) was applied to infer relationships between AM fungal distribution and environmental variables. CCA was performed by scaling inter-species distances, with biplot scaling. Monte Carlo permutation tests were conducted using 499 random permutations. The subsequent forward-selection procedure ranked the environmental variable according to their importance and significance for the distribution of the OTUs. The resulting diagram was visualized using CanoDraw.

3. Results

3.1. Soil properties

In the Borracho, Peraza, and Paiclas cays, the soil biological activity, estimated as the total carbohydrate content and basal respiration rate, was higher than in Muerto cay but lower than in Las Luisas (Table 1). The EC value decreased with the mean annual rainfall; thus, the highest value was registered in Borracho cay and the lowest in Las Luisas. Paiclás cay showed higher organic carbon content with respect to the rest of the sites.

3.2. Molecular analysis of AMF

We successfully amplified AM fungal DNA from all soil samples, with the primer combination AML1/AML2. Twenty clone libraries, coming from five sites and four replicates per site, were created. Approximately 240 clones per site were screened, sequenced, and analyzed phylogenetically. BLAST searches revealed that 715 sequences showed high similarity (97–100% identity) to sequences from AMF taxa and belonged to members of the phylum Glomeromycota. The rest of the sequences were uncultured marine *Eukaryota*, *Chytridiomycota*, *Ascomycota*, *Neocallimastigomycota*, or other *Eukaryota*.

Unique sequences of each OTU were submitted to the EMBL database and are included in bold in the phylogenetic tree shown in Fig. 1.

Thirty-one AM fungal OTUs were detected in this study, grouped to species belonging to eight out of the ten families of the phylum Glomeromycota (Schüßler and Walker, 2010). By a large margin, the most abundant and diverse group in the soil samples was the *Glomeraceae*, with 495 sequences grouped in 20 OTUs, followed by the *Diversisporaceae* and *Paraglomeraceae* groups, represented by two and three OTUs respectively. The *Claroideoglomeraceae* was represented by two OTUs and the *Acaulosporaceae*, *Archaeosporaceae*, *Gigasporaceae*, and *Ambisporaceae* were represented by only one OTU each (Fig. 1).

Seven OTU were nested within a clade of sequences of AMF in culture and could be assigned to recognized species. These included Rh (*Rhizophagus intraradices/irregularis* group), Scl (*Sclerocystis sinuosa*), Fu4 (*Funneliformis caledonium*), Arch (*Archaeospora trappei*), Scu (*Scutellospora gregaria/calospora* group), Cl1 (*Claroideoglomus lamellosum/etunicatum/claroideum* group), and Pa3 (*Paraglomus occultum*). Nine OTU (Glo5, Glo7, Glo10, Glo11, Glo12, Glo13, Aca, Div, and Am) had no closely related reference sequences in the database. The remaining OTU were related to uncultured Glomeromycota sequences available in GenBank. The sequences assigned to each OTU were further confirmed in the MaarjAM databases.

The number of clones sequenced was sufficient to represent the diversity of the AMF communities in the five sites, since all five rarefaction curves reached a well-defined plateau (Fig. 2 and Fig. S1). Therefore, it is highly unlikely that the sequencing of more clones would have revealed more OTUs.

3.3. Correlations between the diversity of the AMF, insularity related parameters, and soil properties

The most abundant and ubiquitous OTU was Glo8, which represented 18.5% of the AMF sequences and was found in all five sites (Table S1). Paiclás cay had the most diverse AMF community, the diversity being significantly higher than in the rest of the sites ($H' = 2.16$) (Table 2). In this cay, 16 of the 31 OTU recovered in this study were found, Aca and Cl2 being significantly associated with the Paiclas cay (IndVal: 0.87 (P-value 0.030)) (Table 3). Borracho cay, Peraza cay, and the Las Luisas mainland showed similar Shannon index values (1.63, 1.73, and 1.82, respectively), without significant differences among them, and similar richness (12, 10, and 11 OTU, respectively). Six OTU were found to be associated with the Las Luisas mainland (Glo7, Am, Glo6, Glo8, Glo4 and Scl), five OTU (Glo13, Re, Scu, Glo11, Fu2) were significantly associated with Borracho cay, and two OTU with Peraza cay (Glo5 and Div). Muerto cay, the only site with intense human activity had (by a statistically-significant margin) the lowest richness (3 OTU) and diversity ($H' = 0.73$) found in this study (Table 2), being exclusive to this cay the OTU Pa (IndVal: 0.85 (P-value 0.015)) (Table 3).

The correlation analysis did not show any significant correlation between the soil properties (organic carbon, total CH, EC, pH, and basal respiration rate) or the geographical situation parameters (rainfall, land area, and distance to the mainland coast) and the AMF species richness or Shannon diversity index (data not shown). However, positive and significant correlations were found between the site land area and some OTUs: Glo4, Glo7, and Pa3 ($r = 0.982$; $P < 0.05$), Glo6 ($r = 0.998$; $P < 0.001$), Glo14 ($r = 0.879$; $P = 0.05$), and Am ($r = 0.966$; $P < 0.01$). The distance to the mainland coast also showed positive, significant correlations with Fu2, Glo11, Glo13, Scu, and Re ($r = 0.926$; $P < 0.05$), Fu4 ($r = 0.918$; $P < 0.05$), and Arch ($r = 0.955$; $P < 0.05$), while Glo8 showed a positive, significant correlation with total CH ($r = 0.905$; $P < 0.05$).

3.4. Analysis of the AM fungal communities and factors affecting assemblages in the five sites

Multivariate analyses were applied to the AMF OTUs that occurred in 20 samples (five sites with four replicates). The length of the AM fungal community composition gradient of the first axis was computed to be 4.99 from DCA. In accordance with this result, CCA was applied as the unimodal ordination method (Fig. 3). The cumulative percentage variance of the species data showed that the first two CCA axes explained 46.8% of the variability in the species data (72.3% with the four significant axes). The eigenvalues of the four significant axes were 0.71, 0.59, 0.38 and 0.33 respectively. The resulting ordination diagram indicated four clearly differentiated AMF communities, those in Peraza cay and Las Luisas being very similar (Fig. 3). The Borracho, Muerto, and Paiclás cays hosted different AMF communities. The Monte Carlo permutation tests on the CCA showed that several quantitative variables – such as distance to the coast, cay land area, mean annual rainfall, and soil total CH content – had significant relationships with the distribution of the AMF OTUs (Table 4).

4. Discussion

We found a significant influence of several environmental variables linked to insularity and soil characteristics on the AM fungal community composition, after selecting five sites (four cays and one mainland

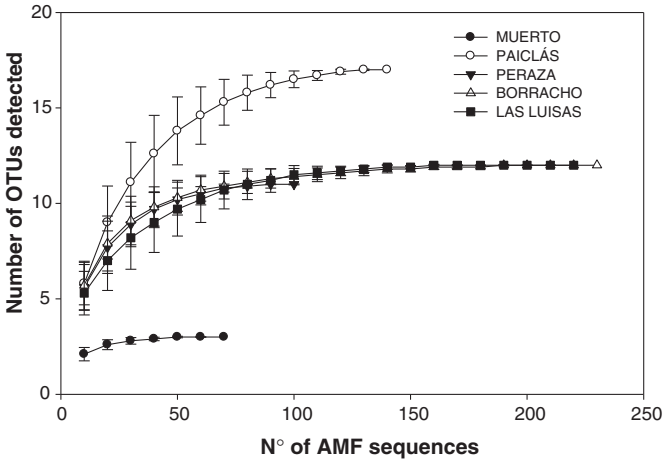

Fig. 2. Rarefaction curves for SSU rDNA libraries from *Coccoloba uvifera* rhizosphere in the different locations. Curves were obtained using the Analytical Rarefaction Program version 1.3 (http://www.uga.edu/~strata/software/anRareReadme.html).

Table 2
OTU richness and Shannon's diversity index in the locations analyzed (n = 4).

	Richness	Shannon's index
Borracho	10bc	1.63b
Peraza	7.25b	1.73b
Muerto	2.5a	0.73a
Paiclás	11.25c	2.16c
Las Luisas	8.75bc	1.82b

Values in columns followed by the same letter do not differ significantly (P < 0.05) as determined by the Duncan test.

location) in a coral cay system (Morrocoy National Park, Venezuela). The sequences analyzed were grouped in 31 AMF OTUs, a species richness that can be considered in line with other studies carried out in several tropical and subtropical protected areas worldwide (Turrini and Giovannetti, 2012). Among these are the tropical rainforest of the Barro Colorado Nature Monument in Panama, where 33 AMF species were detected using molecular methods (Husband et al., 2002a,b); the State Park of Campo de Jordao and the State Park of Alto Ribeira — both sited in a subtropical forest in Brazil with 40 and 24 AMF species, respectively (Moreira-Souza et al., 2003; Moreira et al., 2007); and the El Palmar National Park in Argentina, with 46 AMF species (Velazquez et al., 2010, 2013). However, these studies in Brazil and Argentina were performed with morphological techniques and the results cannot be compared directly with ours.

On the other hand, it should be noted that the OTU richness observed in our study was of similar magnitude to that reported in a coastal ecosystem in Ishikari (Japan), in which Kawahara and Ezawa (2013) found 34 AMF species. In contrast, Yamato et al. (2012) found only 17 AMF species in another coastal ecosystem in Tottori Prefecture, Japan.

In Morrocoy Park, the ordination analyses revealed a differentiation of the AMF community assemblages between most of the cays and the mainland, distinguishing four properly defined AMF communities. The Monte Carlo permutation test showed that several environmental variables related to insularity (mean annual rainfall, distance to the mainland coast, and cay land area) and a soil property related to biological activity (total CH content) had significant relationships with the distribution of OTUs. This suggests that these environmental variables are related with the distribution of AMF species in the different sites. Thus, some OTUs (Glo13, Re, Scu, Glo11, and Fu2) were preferentially associated with Borracho cay and were also positively correlated with the distance to the mainland coast (i.e., the isolation of the site), while other OTUs (Glo7, Am, Glo6, and, Glo4) were preferentially associated with Las Luisas, indicating that they are linked to the mainland position. Several studies have suggested different environmental variables as the main drivers of the AMF structure and diversity. Some authors found a significant variation in the community composition of the AMF with

Table 3
Indicator species analyses.

	OTUs	Indicator value index	P-value
Borracho group	Glo13	1.000	0.005
#sps. 5	Re	1.000	0.005
	Scu	1.000	0.005
	Glo11	1.000	0.005
	Fu2	0.866	0.010
Peraza group	Glo5	0.866	0.020
#sps. 2	Div	0.866	0.015
Muerto group	Pa	0.845	0.015
#sps. 1			
Paiclás group	Aca	0.866	0.030
#sps. 2	Cl2	0.866	0.030
Las Luisas group	Glo7	1.000	0.005
#sps.6	Am	0.941	0.005
	Glo6	0.894	0.005
	Glo4	0.866	0.035
	Scl	0.719	0.010
	Glo8	0.674	0.005

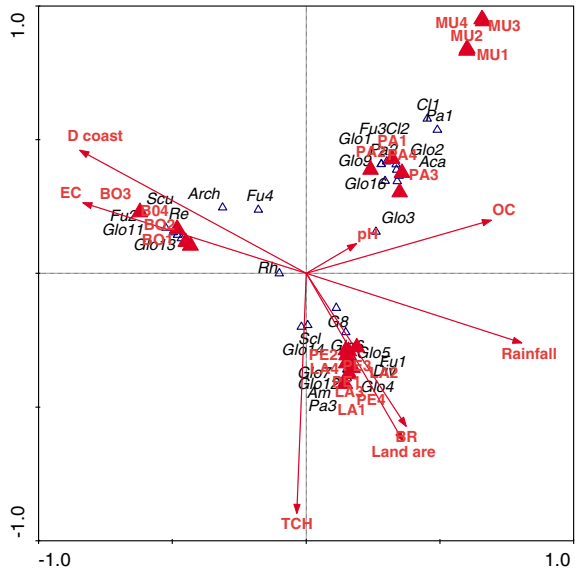

Fig. 3. Canonical correspondence analysis (CCA) of the AM fungal community composition found in the *Coccoloba uvifera* rhizosphere in different locations, soil properties and geographic situation parameters. The eigenvalues of the first and second axes in the two-dimensional ordination diagrams are as follows: CCA1: 0.71 and CCA2: 0.59. The model explained 72.3% of the whole variance. Abbreviation codes: PA = Paiclás cay, BO = Borracho cay, PE = Peraza cay, MU = Muerto cay, LA = Las Luisas mainland, TCH = Total carbohydrates, OC = Organic carbon, EC = Electrical conductivity, BR = Basal respiration rate, D coast = Distance to mainland coast.

geographical distance (Lekberg et al., 2007; van der Gast et al., 2011; Yamato et al., 2012), although to date the extent to which individual AMF species and communities are distributed across the landscape or site is unclear. In fact, a meta-analysis of environmental samples indicated that some AMF sequence types had widespread distribution across continents, while others had been found only at single sites (Öpik et al., 2006). Also, the annual rainfall was observed to influence the richness and diversity of AMF communities, both in spore-based studies (Tchabi et al., 2008; Johnson et al., 2013) and in molecular studies (Torrecillas et al., 2013). Hazard et al. (2013), in a study of AMF community composition at the landscape scale on the mainland, observed that AMF communities were influenced by environmental variables such as rainfall, in accordance with our results. However, they did not find variation in the AMF communities with the geographical distance, suggesting that the variation observed in our study is linked to the isolation factor of the different cays.

Alternatively, Koske and Gemma (1990) in Hawaii found that arbuscular mycorrhizal fungi are most likely to arrive on cays by rafting, and then ocean currents that are unfavorable for raft arrival might have been the major contributor to low fungus diversity in isolated cays. In Morrocoy Park the cays are distributed close to the coast in an open bay, and the influence of ocean currents is minimal, more likely in these cays AMF dispersal could be attributable to other vectors, such as birds and insects (Janos, 1993), whose effectiveness in AMF dispersal could be influenced by distance, rainfall or even human activity.

Table 4
Result of Monte Carlo permutation tests (499 permutations) on CCA for the relationships with environmental variables on AMF community.

	F value	P value
Mean annual rainfall	5.97	0.002
Distance to mainland coast	5.29	0.002
Land area	4.93	0.002
Total CH	5.82	0.002
EC	2.20	0.110
Organic carbon	3.76	0.228
Basal respiration rate	1.99	0.128

Soil properties are important factors influencing the AM fungal community composition (Lekberg et al., 2007; Oehl et al., 2010; Moebius-Clune et al., 2013; de Oliveira Freitas et al., 2014; Jansa et al., 2014). A recent study carried out by Krishnamoorthy et al. (2014), on reclaimed coastal land in Korea, found that the AMF community structure was altered when the soil EC increased. In contrast, we did not find any alteration in the AMF community that was due to the soil EC, probably because the range of EC in our study was not wide enough to mediate this effect. Similarly, Dumbrell et al. (2010) and Hazard et al. (2013) found that AMF community composition was influenced by soil pH, which is in contrast to our results. Of all the soil properties measured in our study, only the total CH content had a significant relationship with the distribution of the AMF community. This soil parameter has been used as an indicator (index) of soil biological activity (García et al., 1997; Alguacil et al., 2005), but not with reference to AMF populations. Except for Muerto cay, the site with the highest human activity and the lowest total CH content, the rest of the sites showed both similar and high values for the total CH content. Therefore, the intense anthropogenic activity exerted in Muerto cay may have resulted in a decline of the AMF diversity and, as a consequence, in the soil biological activity, since it has been shown that reductions in the diversity of soil microorganism communities (mainly AMF, among the most common soil microorganisms) result in the decline of multiple ecosystem functions and processes related with soil biology activity, such as carbon and nutrient cycling (Wagg et al., 2014).

In spite of selecting the same number of clones in the Muerto cay libraries to carry out the screening as in the rest of the sites (240 clones), only 39 sequences belonged to AMF taxa. Muerto cay showed the lowest AMF diversity of this study, with only three OTUs retrieved. We suggest that human pressure could exert some sort of influence on the AMF species distribution and richness. In fact, Muerto cay was the only site that showed intense human activity, unfortunately this is the only cay subjected to an intense touristic activity in the Morrocoy Park, so the lack of a quantified gradient in human activity does not allow establishing clear conclusions. There are reports that show a reduction in AMF diversity in sites experiencing various types of anthropogenic management, compared with natural habitats. For example, natural systems were affected by industrial pollution (Vallino et al., 2006; Bedini et al., 2010), climate warming (Budge et al., 2011), farming practices (Hijri et al., 2006; Alguacil et al., 2008; Verbruggen et al., 2010; van der Gast et al., 2011), or elevated CO_2 concentrations (Klironomos et al., 2005; Drigo et al., 2010). Of the three AMF OTUs found in Muerto cay, it is noticeable that Glo 8 represented 18.5% of the AMF clones recovered in this study and that it was the only OTU found in all five sites — as well as being the most abundant. In other studies, this AMF taxon was found in agricultural systems and natural and revegetated ecosystems from semiarid environments (Alguacil et al., 2011a,b; Torrecillas et al., 2012, 2013), dry Afromontane tropical forest (Wubet et al., 2009), and protected areas such as Kiskunsag National Park in Hungary (Kovács et al., 2007). Thus, this AMF strain could be considered a generalist, since it is highly tolerant of very diverse environmental conditions and is adapted to both many geographical locations and to the human management imposed.

In conclusion, the diversity of AMF communities in a coral cay system was in line with other tropical and subtropical ecosystems, in spite of the limitations in habitat variations and propagule dispersal in these small islands. The distribution of AMF assemblages was significantly linked with environmental factors related to the geographical location and the soil biological activity.

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