

Contrasting Coastal Ecosystems in Cabo Frio, Brazil Alter the Correlations between Plant Species and Arbuscular Mycorrhizal Fungi Community †

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Abstract: Contrasting coastal ecosystems, dune and restinga, in Peró Beach, Cabo Frio, State of Rio de Janeiro altered the correlations between the spatial patterns of the Arbuscular mycorrhizal fungal (AMF) and the diversity of plants. We recorded, during rainy and dry seasons in 2014, a total of 35 plant species. *Ipomoea imperati*, *Stenotaphrum secundatum*, *Hydrocotyle bonariensis* and *Remirea maritima* were the most common. Higher plant species richness was found in restinga over dune in both seasons. Considering that coastal environments occupy large areas around the world, we consider that the AMF-plant relationships evidenced here may contribute to conservation of these environments.

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1. Introduction

Coastal dune ecosystems are found on all continents, except Antarctica, and represent one of the most fragile ecosystems, as they suffer the action of several natural limiting factors and also excessive damage caused by human interference. Known as restinga, Brazilian coastal ecosystems belong to the Atlantic Rainforest biome, with approximately 20,000 plant species, and at least one half of them are endemic [1]. Restinga is characterized by high temperature, high exposure to light, high salinity and difficult retention of water due to sandy soil [2], with the possible formation of coastal dunes.

AMF are beneficial fungi that associate with the roots of most vascular plants. These Glomeromycota fungi are able to absorb scarce nutrients and water from the substrate. Thus, AMFs are important for the development and maintenance of plant diversity [3,4]. From 270 AMF species described thus far worldwide, 119 were recorded in Brazil [5], and 78 were recorded in the Brazilian Atlantic Forest [6]. Regardless of their importance, information on plant-AMF interactions in coastal habitats is scarce. Revegetation practices contributed to diversity enhancement and AMF richness in previously disturbed coastal areas [7–10]. However, there has been a strong theoretical debate about the forces determining plant-AM fungal relationships in natural systems [11].

The AMF diversity in dunes and restinga at Peró Beach was previously shown by da Silva et al. [12]. In our present work, we analyzed the same soil samples, however, from

the point of view of plant diversity and their interaction with AMF. The main goals of this study were (i) to describe the plant community structure and composition present in two adjacent coastal environments (dune and restinga) of Però Beach in Cabo Frio, Rio de Janeiro (Brazil); (ii) to compare the plant communities of two sites (dune and restinga) in two seasons (rainy and dry); and (iii) to correlate plant presence/absence to AMF abundance by multivariate analysis.

2. Materials and Methods

The study was carried out in 2014 at Però Beach (−22.837678, −41.980946 to −22.835992, −41.984550), Cabo Frio, state of Rio de Janeiro, Brazil. The local climate is semiarid, with an average rainfall of 770 mm/year [13]. The experimental area was divided into two plots: “dune site”, with a predominance of frontal and posterior dune, and “restinga site” of shrub-tree components. The soil physical and chemical characteristics were previously described [12]. Plant samples were collected in May, in a rainy season with falling temperature and November 2014, in a dry period and temperature rise. Three transects 400 m long each and at least 51 m of distance were traced in the beach-inland direction. Twenty points were demarcated every 20 m, 10 points at the dune followed by 10 points at restinga for each transect. Finally, all plants present in a 50 cm ray around each point were sampled in the reproductive stage and were herborized. Botanical material identification was carried out with the help of specialists in botanical taxonomy, as well as consultations in the digital collection [14] and specific literature [15,16].

The frequency (F) of plant occurrence was estimated following the equation $F = (F_0/F_1)$, where F_0 is the number of sampling points at which the species occurs and F_1 is the total number of points sampled [17]. The plant species abundance was considered the number of individuals sampled per species. Three diversity indices were calculated using PAST (Version 3.10) software [18]: the Shannon index (H'), the dominance index (D), Equitability (J'). The permutation tests were performed using the tool ‘Past compare diversity’ from PAST (Version 3.10) software [18] (Supplementary Table S2).

Soil AMF spores (glomerospores) were extracted from a 50 g subsample from each soil sample by wet-sieving and identified by spore morphology [12]. To clarify the relationships between all plants sampled and rhizospheric soil fungi, we performed redundancy analysis (RDA) using data from each site (dune and restinga) and season (May and November 2014). The RDAs were performed using CANOCO for Windows (version 4.5) [19] to demonstrate the ordering of plant species × AMF species related to physical (clay, silte and sand percentage) and chemical soil attributes.

3. Results and Discussion

A total of 35 plants species distributed in 23 families were sampled in dune and restinga sites (Table S1). Of all species sampled, eight were dune exclusive occurrences, 19 restricted to restinga and eight were found in both sites (Table S1). Among dune exclusive, *Chamaesyce* sp. and *Bluraparon portulacoides* were more frequent, and in restinga, the species more frequent were *Schinus terebinthifolia* and *Myrsine parvifolia*. The species identified have already been registered in the State of Rio de Janeiro by [15,16]. According to the Brazilian digital collection [14], 30 species are considered native, with *Forsteronia leptocarpa* and *Polygala cyparissias* endemic in Brazil and *Leucena leucocephala* naturalized. The total number of individuals was higher in dune than in restinga in both seasons (Table 1).

The total number of sampled individuals in dune was higher in dry, while in the restinga, the opposite was observed (Table 1). However, in both seasons, restinga diversity was higher than in dunes, as indicated by the richness and Shannon diversity index (H') (Table 1). This result is in line with the expected result since there is ecological succession between the plant species in the direction of the beach towards the interior, being an area of dunes characterized as a pioneer community and a restinga forest as a

secondary community, with the presence of shrub vegetation [20]. At the two sites, it was possible to observe a decay richness for the dry season in comparison with the rainy season, even with an increase in total individuals sampled in dune (from 79 to 92, Table 1). The dominance numbers were low. This result shows that the dominance of a few species above the others did not occur. Equitability index (J') numbers corroborated this observation. The values (0.85 for dune and 0.89 and 0.91 for restinga) show high species composition uniformity.

Table 1. Ecological indices by site (dune and restinga) in the rainy (May) and dry seasons (November) in Però Beach, Cabo Frio, RJ.

Site	Sampling Season	Total of Individuals	Richness	Shannon (H')	Dominance (D)	Equitability (J')
Dune	Rainy (May)	79	16	2.3480	0.1226	0.8468
	Dry (November)	92	10	1.9580	0.1685	0.8505
Restinga	Rainy (May)	71	23	2.7940	0.0811	0.8912
	Dry (November)	45	19	2.6720	0.0913	0.9076

Redundancy analysis showed that in the dune site, the two first axes explained 92% (Figure 2A) and 93% (Figure 2B) of the sample's variation. In May, only three plant species showed a positive relation with the AMF community, represented by the right side of the RDA plot. *Varronia curassavica* (Va. cur, red vectors) was more related to *Gigaspora* sp. (Gi. sp.); *Stenotaphrum secundatum* (St.sec) to the AMF *Rhizophagus clarus* (R.cla) and *Ipomoea pes-caprae* (Ip. pes) to *Racocetra fulgida* (R.ful) (Figure 2A). In November, the dry season, ten plant species were related to AMFs (Figure 2B). [21] observed that these seasonal effects on AM fungal variables are independent of a particular combination between plant species and soil sites, suggesting that seasonality was an important factor in regulating both spore density in soil and changes in AM root colonization morphology in different studied plants. [22] found differences between the dry and wet seasons, with significantly higher colonization percentages in *Ipomoea pes-caprae* during the dry season. In our study, in the dunes during the rainy season, *Ipomoea pes-caprae* (Ip. pes) was positively related to *Racocetra fulgida* (R.ful). This plant species was present at the embryonic dune of Santa Catarina state, southern Brazil [23], where twelve other AMF species have been observed. Ip. pes was also sampled by [24], presenting seven AMF species in its rhizosphere. However, during the whole period of the study developed by these authors, Ip. pes did not present colonization in its roots. In addition, [25] showed that the species normally located in the front line of the sandy coast presented lower averages of glomerospores in their rhizospheres. The rhizosphere of sample points of Però Beach where Ip. pes occurred isolated from the other plant species presented from 7 to 20 glomerospores [12]. Despite the low spore density in the dry and wet seasons, [22] found 31 AMF species in moderately disturbed dunes in the I. pes rhizosphere. Thus, we considered it possible that the association Ip. pes-AMF may be occurring and is important for the stability of the dune ecosystem in Però Beach, but it is necessary to analyze root colonization to confirm specific associations. Symbiosis between plants and AMF is part of a strategy allowing plants to grow under a variety of stress conditions [26]. The plant species dune exclusive more frequent *Blutaparon portulacoides* was previously identified as associative with AMFs in Brazilian dunes [23,27].

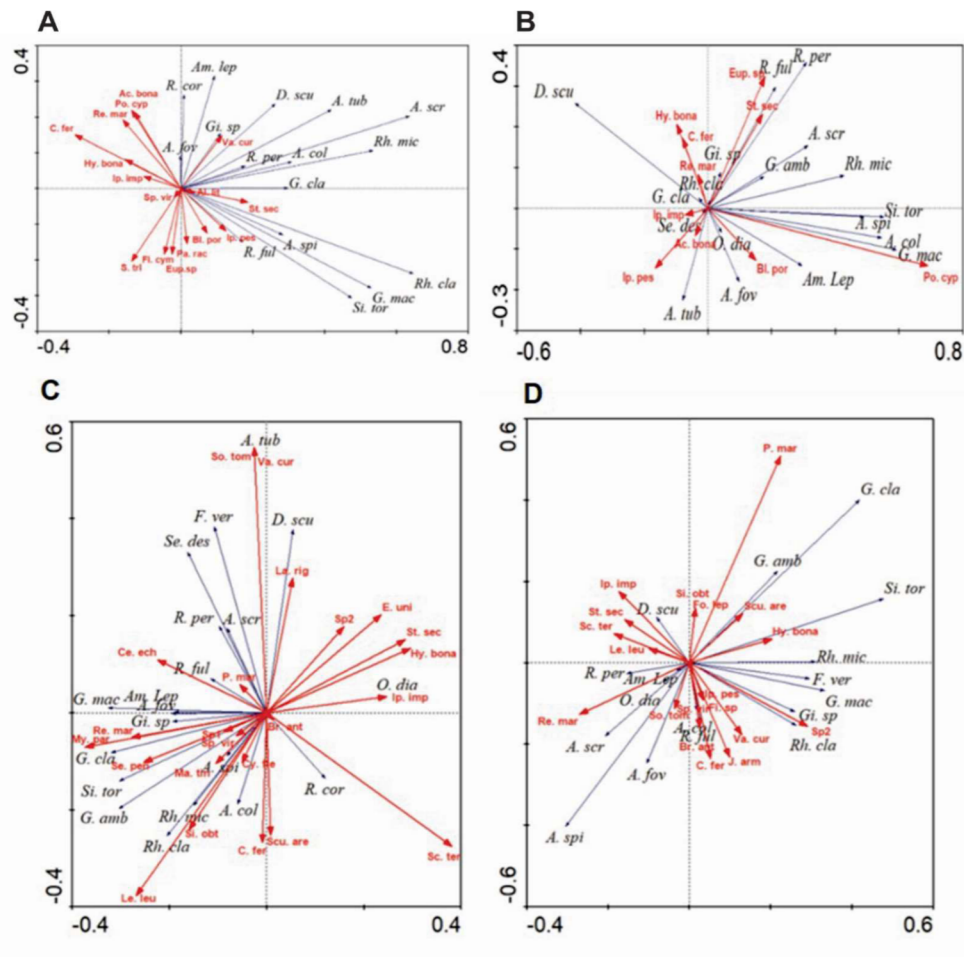


Figure 2. Redundancy analysis (RDA) between the presence and absence of plant species related to the number of glomerospores from each fungal species and soil attributes in the dune site (A,B) and restinga (C,D). (A): dune rainy season, May; (B): dune dry season, November. (C): restinga rainy season, May; (D): restinga dry season, November. In red, the plant species are represented (see abbreviation in Table S1).

For the restinga site, the two first RDA axes together explained 93.7 and 95.9% of the sample variation for the rainy and dry seasons, respectively (Figure 3A,B). Restinga species more frequent and exclusive in our study were *Schinus terebinthifolia* (Sc. ter) and *Myrsine parvifolia* (My. par) (Table S1). According to our redundancy analyses, Sc. ter was related to *Racocetra coralloidea* (R.cor) in the rainy season. In the study conducted by [28], root colonization of Sc. ter when inoculated with *Gigaspora margarita* and *Rhizophagus clarus*. This plant species was also reported to be associated with the AMFs *Claroideoglossum etunicatum* and *Dentiscutata heterogama* [29]. Interestingly, *Schinus terebinthifolia*, known as the Brazilian pepper tree, showed a high spore density of *Glomus* spp. and *Rhizophagus* spp. in Florida and Hawaii (subtropical regions), which is considered a dominant invasive plant [30]. In addition, Sc.ter was shown to have one of the highest responses to AMF infection compared to other pioneer tree shrubs in its native range in Brazil [31].

4. Conclusions

Our data showed that the same plant species was positively related to a fungus in one season and to another fungus species in another season, contributing to evidence that edaphic and environmental factors can govern these associations. AMF species distribution are affected by soil pH in sand dunes on a global scale [32], thus, our results

are in line with Lee et al. [33], who mentioned that associations between AMF and roots are generally considered to be non-specific and do not depend on which AMF is present in the rhizosphere. The development of symbiosis and its efficiency has been shown to be affected by factors such as soil characteristics such as texture fertility, pH and organic matter content [34] and the characteristics of the host plant, such as successional stage, seed size and root morphology [35,36]. However, our study does not allow us to infer symbiotic efficiency. The knowledge of the mutualistic interactions between native plants and soil microbes is important in the scenario of increasing anthropogenic landscape modification. In a future restoration process of native vegetation, success may depend upon reconnecting plants with their fungal symbionts [37]. Despite being a one-off study in only two coastal ecosystems, we hope to have contributed to the discussion about the forces determining plant-AM fungal relationships in natural systems around the world.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1. Table S1: Botanical family frequencies at two sites (dune and restinga) in rainy (May) and dry (November) seasons of a Brazilian restinga ecosystem (Peró Beach, Cabo Frio, Rio de Janeiro State, Brazil). Table S2: Results of the permutation test to compare the mean values of dune (D) and restinga (R) community's richness, dominance, diversity (Shannon) and equitability between rainy (1) and dry (2) seasons.

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