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Does Dune Type Regulate Spore Distribution of Arbuscular Mycorrhizal Fungi in Beaches With Different Exposure Regimes?

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Abstract: Community structure of arbuscular mycorrhizal fungi (AMF) in maritime sand dunes is influenced by several biotic and abiotic factors, including temperature, interaction with soil pathogens and soil properties. We assessed the combined effect of dune type and beach exposure regime on the community structure of arbuscular mycorrhizal fungi spores associated with *Spartina ciliata* patches. AMF communities were compared between foredune ridges and stabilised dunes on two beaches in southern Brazil: an oceanic beach and a semi-sheltered beach. In each beach, six sites were sampled to recover, count, and identify AMF spores and for analysis of soil chemical and physical properties. A total of 17 AMF species were recovered belonging to Gigasporaceae, Acaulosporaceae, and Glomeraceae. *Acaulospora scrobiculata* and a *Glomus* sp.1 accounted for 66.22% of the total number of spores. Overall, AMF community structure and species number responded to the interaction between dune type and beach exposure. Mean densities of *Acaulospora scrobiculata*, *Dentiscutata scutata* and *Glomus* sp.1 varied differently among dune habitats at beaches with distinct exposure. Furthermore, environmental variables (organic matter content, nitrogen and phosphorus concentration and mean grain size) showed a strong association both to selected taxa and to sampled sites. We concluded that the combined effect of dune type and beach exposure was an important factor to influence AMF spore densities.

Keywords: spatial distribution, coastal dunes, beach exposure, *Spartina ciliata*, southern Brazil

Resumo. O tipo de duna pode regular o tipo de distribuição dos esporos dos fungos micorrízicos arbusculares em praias com diferentes regimes de exposição?. A estrutura da comunidade de fungos micorrízicos arbusculares (FMAs) em dunas marítimas é influenciada por vários fatores bióticos e abióticos, incluindo temperatura, interações com patógenos do solo e propriedades do solo. Foi avaliado o efeito combinado do tipo de duna e regime de exposição de praias na estrutura da comunidade de esporos de fungos micorrízicos arbusculares associadas à *Spartina ciliata*. As comunidades de FMAs foram comparadas entre dunas frontais e dunas fixas em duas praias do sul do Brasil: uma praia oceânica dominada por ondas e correntes de deriva e uma praia semi-protegida, fortemente dominada por correntes de maré. Em cada praia, seis áreas foram amostras para recuperar, contar, e identificar os esporos de FMAs e para análises químicas e físicas do solo. Um total de 17 espécies de FMAs foram recuperadas pertencentes as famílias Gigasporaceae, Acaulosporaceae, e Glomeraceae. *Acaulospora scrobiculata* e Glomus sp.1 representando 63.22% do número total de esporos. De maneira geral, a estrutura da comunidade e o número das espécies de FMAs responderam à interação entre o tipo de duna e a exposição da praia. Padrões nas densidades médias de *Acaulospora scrobiculata*, *Dentiscutata scutata* e *Glomus* sp.1 entre habitats dunares foram distintos e dependeram do grau de exposição da praia. Além disso, as variáveis ambientais (teor de matéria orgânica, concentração de nitrogênio e fósforo e tamanho médio de grão) exibiram uma forte relação com táxons selecionados e locais amostrados. Conclui-se que o efeito combinado do tipo de dunas e da exposição da praia foi um fator que influencia a densidade de esporos de FMAs.

Palavras-Chaves: distribuição espacial, dunas costeiras, exposição de praias, *Spartina ciliata*, sul do Brasil.

Introduction

Arbuscular mycorrhizas are the most common, ubiquitous and ancestral association between fungi belonging to the phylum Glomeromycota and plant species (Helgason & Fitter 2009). The association provides several benefits to the plant community as it increases plant nutrient uptake, especially of phosphorus, provides protection from pathogens and buffers against adverse environmental conditions (van der Heijden *et al.* 2008, Robinson-Boyer *et al.* 2009). According to van der Heijden *et al.* (2003), communities of arbuscular mycorrhizal fungi (AMF) respond to plant species and edaphic factors, such as nitrogen and phosphorus availability. Similarly, the response of plant species to the association will vary significantly with different AMF community structure, because nutrient acquisition depends on the specific plant–AMF combination (Scheublin *et al.* 2007). Increased AMF diversity can therefore improve plant productivity and diversity (van der Heijden *et al.* 2008).

These benefits are more prominent in low-nutrient environments, such as coastal sand dunes, where the establishment and survival of pioneer plants are hampered by severe abiotic conditions (Yamato *et al.* 2012). Therefore, AMF are essential in dunes because they not only contribute to plant nutrition, but also to dune stabilisation by binding sand grains into wind-resistant aggregates (Gemma *et al.* 1989, Rodríguez-Echeverría & Freitas 2006).

The distribution of coastal dune vegetation is mainly influenced by frequent disturbance events such as sand deposition and erosion (Hesp & Martínez 2007, Miyanishi & Johnson 2007). Environmental conditions on coastal sand dunes are extremely variable and may differ across relatively small scales (Gormally & Donovan 2010), depending on the orientation of the coast, tide regime, sediment grain size (Hesp & Martínez 2007) and other characteristics that are defined by specific beach morphodynamics.

Over time, interactions between environmental processes and dune vegetation determine the different foredune physiographies and associated habitats (Seeliger 1992). In southern Brazil, the main foredune - hereafter referred as the foredune ridge - is characterized by the presence of *Spartina ciliata* Brong, a rhizomatous perennial grass occurring in dunes of the south-western Atlantic coast that shows a pronounced growth and facilitates stabilisation (Cordazzo 2003). This habitat is under constant environmental stress and for this reason invasion by other species is limited. As a consequence of efficient sand binding, a sheltered habitat - hereafter referred to as a stabilised dune - forms in the lee of the foredune ridge (Seeliger 1992). Since this habitat is less influenced by wind and sand accretion, more plant species are able to become established and grow. Therefore, we may find an increased species diversity and vegetation cover in stabilised dunes, with a soil structure that is developed from the accumulated humus (Cordazzo & Seeliger 1993, Maun 2009).

Considering that the vegetation is structured and distributed according to these distinct dune habitats, and that AMF communities respond to changes in plant composition and distribution, we may infer that AMF community structure is also regulated by dune habitats. For example, in stabilised dunes, the increased diversity of plant species leads to an increased diversity of AMF communities because there are more plant roots available to be colonised (Maun 2009).

Arbuscular mycorrhizal fungi are important for maintaining ecosystem functioning (Johnson *et al.* 2003, Heijden *et al.* 2008) and factors controlling their distribution patterns, such as plant species (Scheublin *et al.* 2007), climatic conditions (Koske 1987, Rodríguez-Echeverría *et al.* 2008), seasonality (Gemma & Koske 1988, Gemma *et al.* 1989, Stürmer & Bellei 1994), soil properties (Newbound *et al.* 2012) and dune stabilisation gradient (Koske & Polson 1984, Koske & Gemma 1997, Cordoba *et al.* 2001, Alarcón & Cuenca 2005, Ramos-Zapata *et al.* 2011) have been addressed in many studies conducted in coastal sand dunes. Here we examine spatial patterns of AMF spores at scales ranging from 1 m to tens of kilometres, with the objective of identifying relevant pattern-generating processes.

At larger scales, AMF distribution on coastal dunes may be determined by wave energy and beach morphology while, at smaller scales, dune habitat type, nutrient availability and sediment characteristics may be the major structuring factors. We hypothesised that sporulation of AMF species is influenced by dunes type and beach exposure. We also evaluated the role of environmental variables, such as phosphorus and nitrogen concentrations and mean grain size, in regulating the observed distribution patterns.

Material and Methods

Study area and sampling procedures: Stands of *Spartina ciliata* were selected at the foredune ridge and the stabilised dune in two beaches with different exposure regimes on the Paraná coast, southern Brazil (Fig 1). Deserta beach in Superagui Island (25º24'12.9" S, 48º10'57.4" W) is an oceanic sandy beach completely free from estuarine influence and its dynamic is dominated by waves and longshore drifts (Angulo *et al.* 2006, Gandara-Martins 2007). Deserta beach is a dissipative beach (Dean's $\Omega > 6$) with well-sorted fine sands, a slope of 2.12° and a wave period of approximately 9.5 s (Gandara-Martins 2007, Gandara-Martins *et al*. 2010).

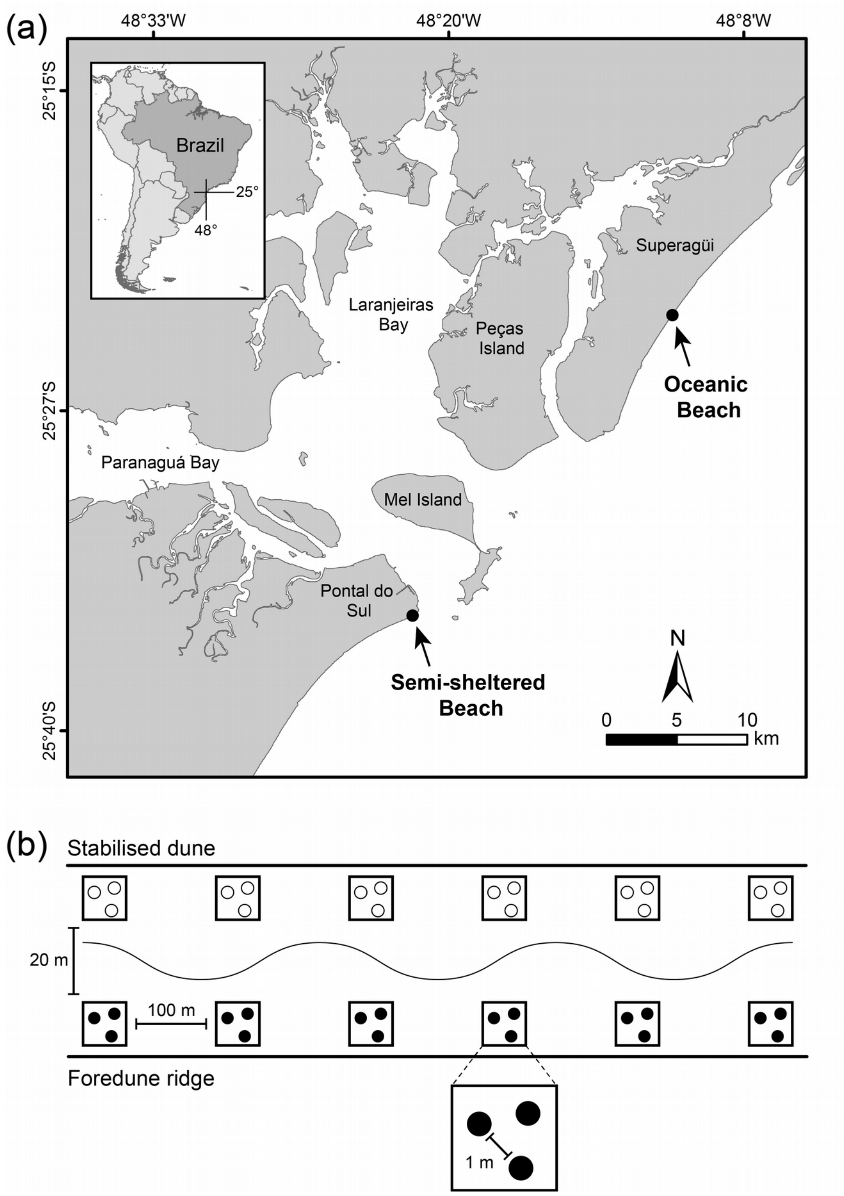

Figure 1. Map of the study area indicating the oceanic and semi-sheltered beaches (a) and schematic arrangement of sites and replicated samples at the foredune ridge and stabilized dune within each beach (b).

Pontal do Sul (25º34'42.3" S, 48º20'57.3" W) is a semi-sheltered, low-energy reflective beach (Dean's $\Omega < 2$), strongly influenced by tidal currents due to its proximity the mouth of Paranaguá Bay (Vieira *et al.* 2016). This beach is characterized by fine sands, a slope of 2.40º and a wave period of 4.90 s (see Vieira et al. 2016 for further physical characteristics). The climate is subtropical with a mean annual precipitation of 2,500 mm (Lana *et al.* 2001).

Rhizosphere samples were collected at a depth of 20–30 cm from stands of *Spartina ciliata*, at six sites (within each beach) approximately 100 m apart (Fig. 1b). At each site, three samples were taken from the foredune ridge and three from the stabilised dune for recovery of AMF spores, and the same number of samples was taken for soil physical and chemical analyses.

AMF spore recovery and identification: Spores were recovered from 100 g of soil by wet sieving (Gerdemann & Nicolson 1963) and sucrose gradient centrifugation (20% and 60%). Spores were transferred to Petri dishes and only those appearing viable under the dissecting microscope were categorised into morphotypes, counted and subsequently mounted permanently in polyvinyl-lacto-glycerol (PVLG) and Melzer's reagent. Spores were identified following the descriptions of the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM). The classification of Redecker *et al.* (2013) was followed in this work.

Soil physical and chemical analyses: For assessment of granulometry and organic matter content, sediments were processed according to Suguio (1973). Granulometric parameters were obtained using the "rysgran" package (Camargo *et al.* 2011). Organic matter content values were determined after burning 5 g of sediment in a muffle furnace for 8 h at 800°C. Total nitrogen and phosphorus concentrations were determined according to the method described by Lana *et al.* (2006).

Statistical analyses: The design comprised spatial scales ranging from 1 m among replicate rhizosphere samples to tens of kilometres between beaches. The largest scale was incorporated in the design in order to verify whether the fungal communities differed between beaches with distinct exposure regime, and the additional scales were adopted because this allowed for unconfounded comparison among beaches and dune types and also verified whether the communities were spatially structured at smaller scales. The linear model used in multivariate and univariate analysis consisted of three factors: dune (fixed, two levels: foredune ridge and stabilised dune), beach (fixed, two levels crossed with dune: oceanic and semi-sheltered beaches) and site (random, six levels nested in the interaction between dune and beach). Independent rhizosphere samples ($n = 3$) were used to quantify within-site variability (i.e. the residual) and avoid pseudoreplication.

Differences in the structure of AMF communities were tested by a permutational multivariate analysis of variance using PERMANOVA software version 1.6 (Anderson 2005). PERMANOVA tests were followed by *a posteriori* pair-wise comparisons on fixed effects in the model that were found to be significant ($\alpha = 0.05$). Non-metric multidimensional scaling (nMDS) was applied to to visualize differences in overall assemblage structure among beaches and dune types. All multivariate analyses were executed using Bray–Curtis similarity coefficients on ln(x+1) transformed data.

Hypotheses concerning numerically dominant and representative taxa were tested by an analysis of variance; heterogeneity (Cochran's test, $p<0.05$) was removed by square root, fourth root or ln(x+1) transformation. The means of significant fixed effects in the model ($\alpha = 0.05$) were compared by Student–Newman–Keuls (SNK) tests. Analysis of variance followed by SNK tests were also used to assess significant differences in the concentration of nitrogen and phosphorus, mean grain size and organic matter content.

A canonical correspondence analysis (CCA) was applied to estimate the relationships between AMF community patterns and environmental variables. Statistical significance of the canonical axes and of the sum of all constrained eigenvalues of the CCA model was assessed with permutation tests (999 permutations under reduced model).

All statistical analyses and graphics were generated in R programming language (R Core Team 2011), using the "GAD" (Sandrini-Neto & Camargo 2011), "sciplot" (Morales 2011) and "vegan" (Oksanen *et al.* 2011) packages.

Results

Distribution of AMF spores: A total of 5,792 spores belonging to 17 different taxa were recovered from both dune systems, belonging to families Acaulosporaceae, Gigasporaceae, and Glomeraceae (Table I). *Acaulospora scrobiculata* and *Glomus* sp. accounted for 36.98 and 26.24% of the total number of spores recovered, respectively, followed by *Gigaspora albida* and Glomus sp2. Gigasporaceae was the family with the highest species richness, being represented by 12 species and 5 genera.

Table I. Mean total abundance and relative abundance (%) of arbuscular mycorrhizal fungi (AMF) associated with *Spartina ciliata* in the study area.

Family / AMF species	Spore abundance (in 100g soil)	Relative abundance (%)
Acaulosporaceae		
Acaulospora foveata	200	3.45
Acaulospora scrobiculata	2142	36.98
Acaulospora sp.	96	1.66
Gigasporaceae		
Gigaspora albida	794	13.71
Gigaspora decipiens	30	0.52
Gigaspora gigantea	21	0.36
Gigaspora margarita	7	0.12
Gigaspora sp.	43	0.74
Cetraspora pellucida	7	0.12
Dentiscutata heterogama	3	0.05
Dentiscutata rubra	1	0.02
Dentiscutata scutata	57	0.98
Racocetra gregaria	101	1.74
Scutellospora calospora	58	1.00
Scutellospora sp.	14	0.24
Glomeraceae		
Glomus sp.1	1520	26.24
Glomus sp.2	698	12.05

A distinct distribution pattern at both beaches was detected based on nMDS (Fig. 2). At the oceanic beach (OC), the stabilised and the foredune ridge showed differences regarding the spatial structure of AMF communities, with a clear separation in the graphical representation, whereas such a pattern was not observed at the semi-sheltered beach (SS).

Figure 2. nMDS plot of arbuscular mycorrhizal fungi (AMF) communities at foredune ridges (○) and stabilised dunes (□) of the oceanic beach, and at foredune ridges (●) and stabilised dunes (■) of the semi-sheltered beach. Numbers from 1 to 6 represent the different sampled sites. Each point represents the sample mean (n = 3 replicates) within each site.

PERMANOVA results corroborated the pattern shown in nMDS ordination (Table II), since differed between the dune habitats at each beach, i.e. interaction between dune and beach (D x B) was significant. The pair-wise *a posteriori* analysis compared dune habitats within each beach and beaches within each dune habitat. Dune habitats were different at the oceanic beach and similar at the semi-sheltered beach. AMF community structure differed between beaches for both dune habitats (Table II).

Differences on mean spore abundances for six separate taxa analysed were observed at all sources of variation (Fig 3, Table III). Spore abundance of *Glomus* sp1, *Acaulospora scrobiculata,* and *Dentiscutata scutata* varied between dune habitats at each beach (D x B). For *D. scutata* and *Glomus* sp1, SNK tests showed a similar pattern of distribution: at the oceanic beach the foredune ridge (FR) had significantly higher densities, whereas at the semi-sheltered beach, densities were greater for the stabilised dune (SD). When comparing beaches based on dune type, differences were only detected for the stabilised dune, for which the semi-sheltered beach presented higher abundances compared to the oceanic beach. Despite the significant dune and beach interaction, *A. scrobiculata* did not vary

Table II. Summary of PERMANOVA (n=3, 9999 permutations) based on Bray-Curtis dissimilarities of ln(x+1) transformed data examining the contribution of Dune, Beach and Site to arbuscular mycorrhizal fungi spores community structure. Significant term of interest (α = 0.05) used in *a posteriori* comparisons is highlighted in bold. For pair-wise *a posteriori* tests: FR, foredune ridge; SD, stabilized dune; OC, oceanic beach; SS, semi-sheltered beach. "≠" indicates p<0.05 and "=" indicates p>0.05

Source	*df*	MS	*F*	*p*
Dune = D	1	1404.851	0.619	0.675
Beach = B	1	29999.298	13.225	<0.001
D×B	1	9576.868	4.222	**0.004**
Site(D×B)	20	2268.309	1.295	0.067
Residual	48	1751.101		
A posteriori tests		**D(B)**	**B(D)**	
		OC: FR ≠ SD	FR: OC ≠ SS	
		SS: FR = SD	SD: OC ≠ SS	

Table III. Summary of analysis of variance for selected taxa. Significant terms of interest (α = 0.05) used in *a posteriori* comparisons are highlighted in bold. For Student-Newman-Keuls (SNK) tests: FR, foredune ridge; SD, stabilized dune; OC, oceanic beach; SS, semi-sheltered beach. "<" or ">" indicates p<0.05 and "=" indicates p>0.05. Type of transform is indicated by: [1] square root; [2] fourth root; [3] ln(x+1).

		Gigaspora albida [1]			*Racocetra gregaria* [1]		
Source	*df*	MS	*F*	*p*	MS	*F*	*p*
Dune = D	1	0.819	0.127	0.725	0.471	0.536	0.472
Beach = B	1	8.875	1.379	0.254	4.144	4.716	0.042
D×B	1	72.826	11.317	0.003	0.337	0.383	0.543
Site(D×B)	20	6.435	3.616	<0.001	0.879	2.142	0.016
Residual	48	1.780			0.410		
SNK		–				–	
		Acaulospora scrobiculata [3]			*Dentiscutata scutata* [2]		
Source	*df*	MS	*F*	*p*	MS	*F*	*p*
Dune = D	1	0.101	0.080	0.780	0.068	0.184	0.672
Beach = B	1	155.295	124.130	<0.001	0.649	1.774	0.198
D×B	1	8.571	6.851	**0.016**	4.211	11.508	**0.003**
Site(D×B)	20	1.251	1.364	0.188	0.366	1.537	0.112
Residual	48	0.917			0.238		
SNK		**D(B)**	**B(D)**		**D(B)**	**B(D)**	
		OC: FR = SD	FR: OC < SS		OC: FR > SD	FR: OC = SS	
		SS: FR = SD	SD: OC < SS		SS: FR < SD	SD: OC < SS	
		Glomus sp.1			*Glomus* sp.2 [1]		
Source	*df*	MS	*F*	*p*	MS	*F*	*p*
Dune = D	1	338.000	1.493	0.236	0.088	0.016	0.901
Beach = B	1	3068.056	13.556	0.002	26.219	4.740	**0.042**
D×B	1	5940.500	26.247	**<0.001**	8.315	1.503	0.234
Site(D×B)	20	226.328	0.562	0.919	5.532	1.237	0.268
Residual	48	402.542			4.473		
SNK		**D(B)**	**B(D)**		**B**		
		OC: FR > SD	FR: OC = SS		OC > SS		
		SS: FR < SD	SD: OC < SS				

between dunes and differences were only detected between beaches. At both the foredune ridge and stabilised dune, densities of *A. scrobiculata* were higher for the semi-sheltered beach (SNK tests, Table II). *Glomus* sp2 was significantly more abundant at the oceanic than at the semi-sheltered beach, and no other spatial scales were found to be significant (Table III). *Gigaspora albida* and *Racocetra gregaria* abundances were patchy at the smallest scale, i.e. their mean abundance varied among sites within each combination of dune and beach [Site (D x B)].

Soil analysis: Soil samples were characterized by fine sand (ϕ < 3) at the oceanic beach and by very fine sand (ϕ > 3) at the semi-sheltered beach (Fig 4). The measured soil properties differed among all sources of variation included (Fig 4, Table IV). Nitrogen concentrations (N) varied between dune

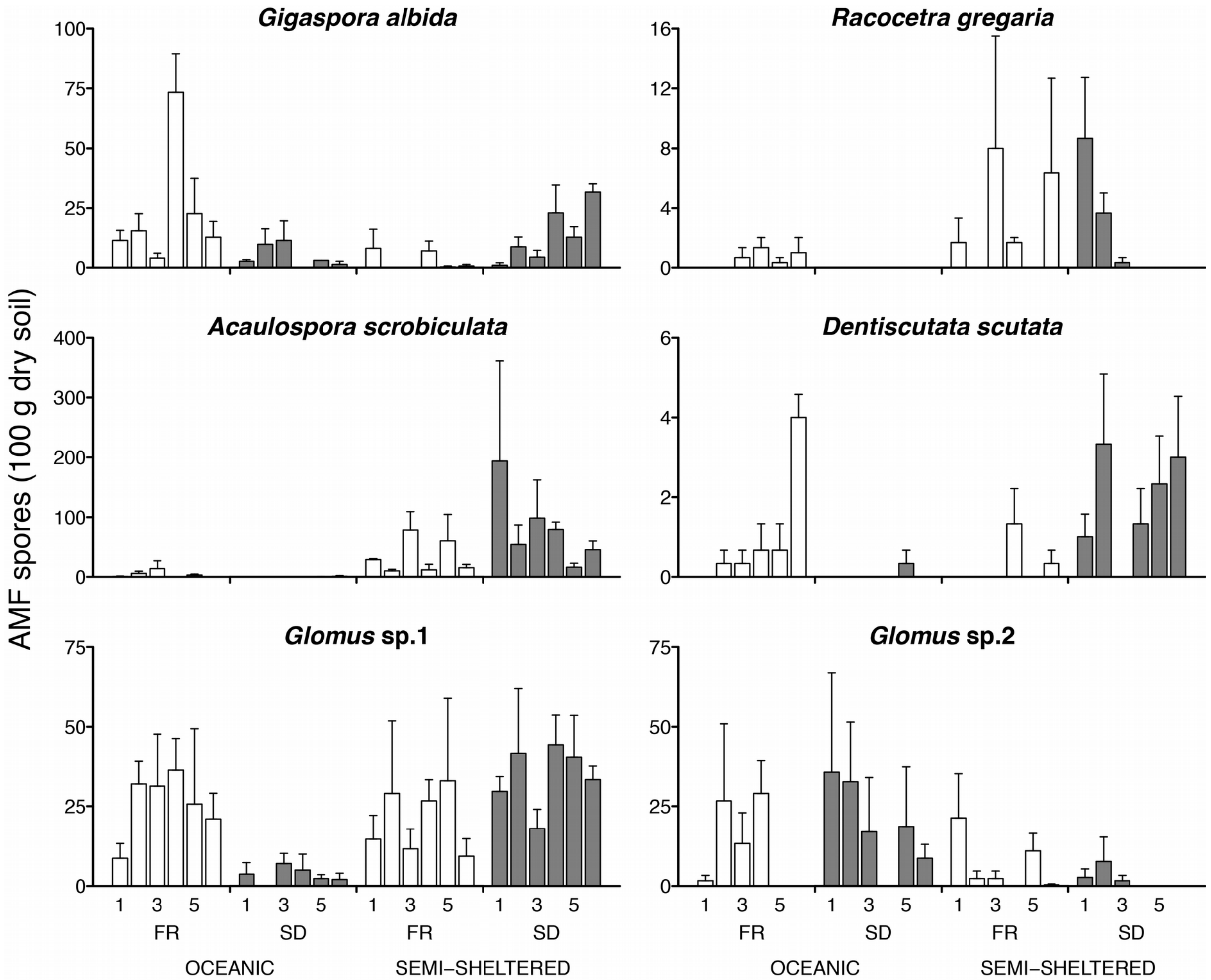

Figure 3. Mean density (+SE) of selected taxa at six sites on foredune ridges (FR, white bars) and stabilised dunes (SD, grey bars) of oceanic and semi-sheltered beaches.

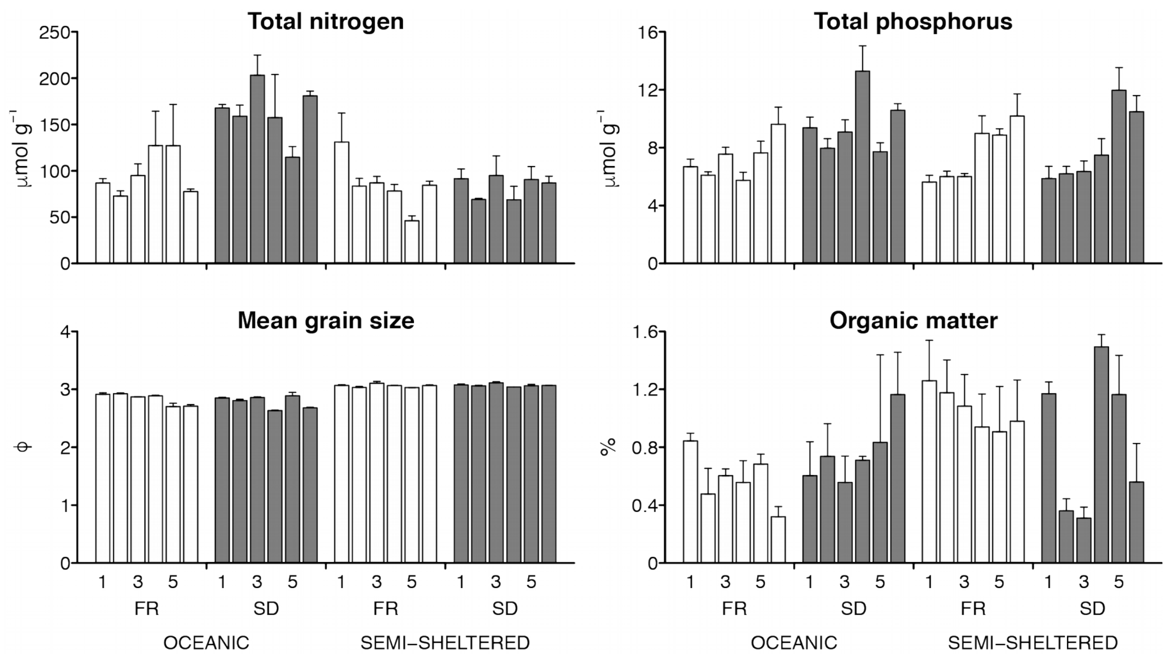

Figure 4. Mean values (+SE) of environmental variables at six sites on foredune ridges (FR, white bars) and stabilised dunes (SD, grey bars) of oceanic and semi-sheltered beaches.

Table IV. Summary of analysis of variance for environmental variables. Significant terms of interest ($\alpha = 0.05$) used in *a posteriori* comparisons are highlighted in bold. For Student-Newman-Keuls (SNK) tests: FR, foredune ridge; SD, stabilized dune; OC, oceanic beach; SS, semi-sheltered beach. "<" or ">" indicates p < 0.05 and "=" indicates p > 0.05. Type of transform is indicated by: [1] square root; [2] ln(x+1); [3] $\sin^{-1}\sqrt{x}$.

		Total nitrogen (μmol g^{-1}) [1]			Total phosphorus (μmol g^{-1}) [1]		
Source	*df*	MS	*F*	*p*	MS	*F*	*p*
Dune = D	1	39.176	10.321	0.004	37.411	3.016	0.098
Beach = B	1	81.423	21.450	< 0.001	6.738	0.543	0.470
D×B	1	39.535	10.415	**0.004**	17.989	1.450	0.243
Site(D×B)	20	3.796	1.692	0.069	12.403	5.194	< 0.001
Residual	48	2.243			2.388		
SNK		**D(B)**	**B(D)**		–		
		OC: FR < SD	FR: OC = SS				
		SS: FR = SD	SD: OC > SS				

		Mean grain size (ϕ) [2]			Organic matter (%) [3]		
Source	*df*	MS	*F*	*p*	MS	*F*	*p*
Dune = D	1	0.0005	0.427	0.521	0.0001	0.151	0.702
Beach = B	1	0.0765	64.851	< 0.001	0.0041	4.545	**0.046**
D×B	1	0.0010	0.846	0.369	0.0023	2.544	0.126
Site(D×B)	20	0.0012	11.710	< 0.001	0.0009	1.770	0.054
Residual	48	0.0001			0.0005		
SNK		–			**B**		
					OC < SS		

habitats at each beach (D x B interaction). At the oceanic beach, stabilised dunes had higher concentrations of N, while no differences were detected at the semi-sheltered beach. When comparing beaches for each level of the factor Dune, nitrogen concentrations were similar for the foredune ridges of both beaches; however, for stabilised dunes the oceanic beach had higher concentrations in comparison with the semi-sheltered beach.

Mean grain size and phosphorus concentrations (P) varied at the smallest scale [Site (D x B)], i.e. these properties were patchily distributed, varying from site to site within each combination of dune and beach (Table III). Organic matter content (OM) responded to beach exposure only, i.e. OM content varied significantly between beaches, with higher concentrations detected by SNK tests at the semi-sheltered beach.

The canonical correspondence analysis (Fig 5) showed that most species were strongly correlated to soil abiotic characteristics and that these are distributed differently amongst sites. *Glomus* sp.2 was associated to higher concentrations of N, especially on the stabilised dunes of the oceanic beach. *Glomus* sp.1 was associated with higher P concentrations at the foredune ridges of the oceanic beach. Occurrence and abundance of *G. albida* were unrelated to concentrations of P, N and organic matter, but it was associated with larger mean grain sizes. However, the occurrence of this species was mainly associated with the foredune ridges of the oceanic beach. The

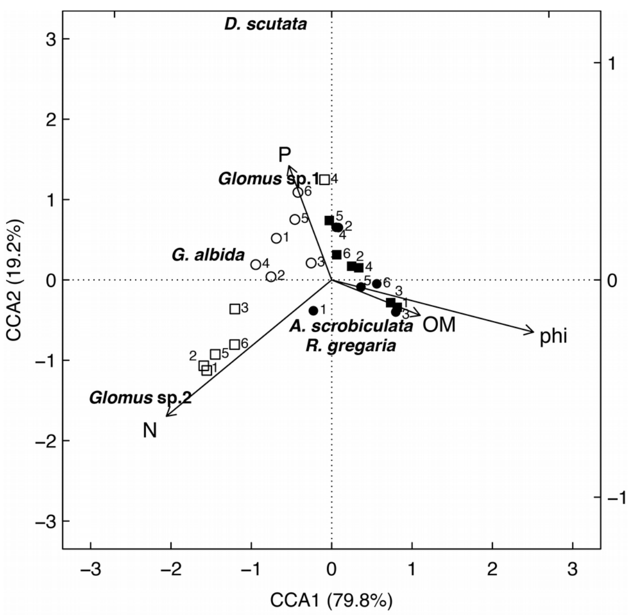

Figure 5. Canonical correspondence analysis (CCA) ordination plot of selected arbuscular mycorrhizal fungi (AMF) taxa, environmental variables and sampling sites. Both axes explained 99% of the total variation present in the samples. "N" nitrogen, "P" phosphorus, "OM" organic matter, "phi" mean grain size. Numbers from 1 to 6 represent sampled sites at foredune ridges (○) and stabilised dunes (□) of the oceanic beach, and at the foredune ridges (●) and stabilised dunes (■) of the semi-sheltered beach. Each point represents the sample mean (n = 3 replicates) within each site.

occurrence of *A. scrobiculata* and *R. gregaria* was strongly influenced by organic matter content, which

was higher for the stabilised dunes of the semi-sheltered beach.
The abundance and distribution of *D. scutata* showed little correlation with the sampled sites, being slightly closer to the higher P concentrations at the foredune ridges of the oceanic beach.

Discussion

Results obtained in this study show that *Spartina ciliata* is commonly associated with AMF and also corroborated our original hypothesis that beach exposure, together with dune type, can influence AMF spore abundance. According to univariate analysis, the interaction between beach and dune has influenced not only the spore abundance, but also the concentration of nutrients. Three of the six species analysed varied between the dune habitats, supporting evidence from previous works that the abundance of AMF species follows a stabilisation gradient (Cordoba *et al.* 2001; Koske & Gemma 1997, Koske *et al.* 2004, Maun 2009).

Further stability of the substrate and the increased diversity of plant species, the main elements of stabilised dunes (Maun 2009), had an effect on *D. scutata* and *Glomus* sp1. However, the high abundances of these species were not only found for the stabilised dunes of the semi-sheltered beach but also for the foredune ridges of the oceanic beach. The reason for this might be that the dynamic of each beach regulates dune formation. The extension of dunes, grain characteristics, nutrient content and vegetation distribution are a direct result of how factors, such as sediment supply, wave and wind regimes and climatic conditions, operate onshore (Angulo 1993, Hesp 2002). The fact that *Glomus* sp2 only varied between beaches, with higher densities found on the oceanic beach, suggests that beach exposure is an essential source of variation and must be included in sampling designs when attempting to compare dune habitats along a stabilisation gradient. *G. albida* and *R. gregaria* exhibited a clumped distribution, therefore showing no direct response either to dune habitats or to beaches, but to site-to-site variation

Our canonical correspondence analysis (CCA) demonstrated that species abundance of fungi in Glomeraceae (*Glomus* sp1 and sp2) were higher at sites where there were greater concentrations of N and P, while members of Gigasporaceae (*i.e., G. albida*) showed a close correlation to foredune ridge sites on the oceanic beach. These findings corroborated those of Cordoba *et al.* (2001), which state that these patterns are probably due to the functional roles of families belonging to Glomerales and the relationship between AMF spores and soil chemical properties. Gigasporaceae species may benefit plants by binding sand grains into large aggregates, and densities tend to decrease with higher P concentration, whereas Glomeraceae species help plants by increasing nutrient uptake and species are predominant where P and N accumulate.

The gradual accumulation of N in soils follows the stabilisation and the age of the dunes (Maun 2009). For this reason, higher N concentrations were found at stabilised dunes on the oceanic beach, since this dune habitat is relatively older than the foredune ridges at both beaches, (Angulo *et al.* 2009). Differences in the abundance and distribution of *Glomus* sp2 between beaches, with higher abundances at the oceanic beach, showed an intimate relationship of this species with N concentration.

The primary source of organic matter added to the beach is the deposition of detritus onshore by waves. Content depends on climate, wave and wind action, nutrient content of the seawater and its productivity (Maun 2009). Higher organic contents were found at the semi-sheltered beach, associated with *A. scrobiculata* and *R. gregaria*. Probably due to the proximity of Pontal do Sul beach (semi-sheltered) to the mouth of Paranaguá Bay, more detritus is deposited onshore. The water flowing from the bay contains high concentrations of nutrients (nitrate, nitrite, particulate organic carbon) and a high productivity, because of the different mechanisms of sink and supply, such as biological uptake, sediment - water interactions and sewage discharge from the city of Paranaguá (Lana *et al.* 2001). Therefore, the relationship between *A. scrobiculata* and *S. gregaria*, based on higher organic matter content, also links these species to the semi-sheltered beach, owing to the input of nutrients. The second most essential nutrient in the sand dunes is phosphorus, and its primary source is the weathering of minerals, such as apatite. It is required in large amounts but is poorly available in soils (Smith & Read 2008, Maun 2009). It is also known that P is patchily distributed in space, which affects the availability to plants and microorganisms (Smith & Read 2008). *Glomus* sp1 was particularly correlated to higher concentrations of P, especially at the foredune ridges of the oceanic beach, which may be evidence of the favoured sporulation of this species at those sites (Cordoba *et al.* 2001).

Our study represents that first report of AMF species occurring in sand dunes for the Paraná state, where AMF community composition was shown to be dominated by members of Gigasporaceae. This pattern was expected as AMF communities in sand dunes along the Atlantic coast of South and North America are dominated by members of Gigasporaceae and this dominance is related to the soil pH (Stürmer *et al.* in press). Gigasporaceae is a family characterized by species differentiating large spores (> 300 μm) and dominance of this family in sand dunes could suggest a relation with grain size. However, our results have shown that mean grain size has very little effect on AMF species distribution, except for *Gigaspora albida* that displayed a preference for soils with larger grain sizes. Six species registered herein are common components of sand dunes in Santa Catarina state (Stürmer & Bellei, 1994; Stürmer *et al.* 2013) in the South region of Brazil.

Our findings have further improved the understanding of mycorrhizal fungi dynamics in a coastal sand dune environment. Due to the natural spatial heterogeneity of AMF species, sampling effort and strategies have a major influence on describing distribution patterns (Whitcomb & Stutz 2007). Placement of samples ranging from metres among replicates to tens of kilometres between beaches, helped us to understand how each source of variation included in the design affects AMF spore abundance. The fact that most patterns of species abundance and nutrients concentrations were in accordance to our predictions shows that beach morphodynamics plays an important role in AMF community structure and that, over time, the interaction with coastal vegetation becomes a definite constraint to spore spatial distribution.

Dunes are of vital importance for coastal protection and wildlife conservation (Seeliger 1992). Since AMF has a fundamental influence on coastal dune vegetation, management approaches must consider not only the aboveground plant community, but also the belowground components, and how they respond to ecological differences within and between dune physiographies and ultimately between morphodynamically distinct coastal environments.

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