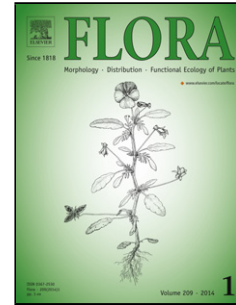# Journal Pre-proof

Functional traits reveal coastal vegetation assembly patterns in a short edaphic gradient in southern Brazil

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Functional traits reveal coastal vegetation assembly patterns in a short edaphic gradient in southern Brazil

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Graphical abstract

Highlights

- Do functional traits explain plant community organization in a short edaphic gradient?

- Four plant communities were influenced by organic matter, sodium and moisture

- Trees/shrubs showed a conservative strategy investing in permanent aerial organs

- Herbs invested in root reserves showing different ecological strategies along the gradient

- Anatomical traits were important to differentiate subtropical coastal plant communities

Abstract

The relationship between plant functional traits and soil variables is useful for understanding plant community composition and circumscribing plant functional groups to highlight their adaptations to environmental conditions. The principal aim of this study was to explain assembly patterns of coastal vegetation using functional traits along a short edaphic gradient. The work was carried out on the pioneer zone in the coastal lowland vegetation (foredune) in southern Brazil. We selected 40 functional traits related to the morphology and anatomy of leaves, stems and roots for 60 species recorded in 25 vegetation plots positioned along three transects from the shoreline to slacks. In each plot, floristic and soil data were collected, and functional traits measured. We analysed the relationships between species functional traits and soil factors through RLQ and fourth-corner analyses. Salinity and organic matter content were the most significant edaphic factors in the differentiation of foredune vegetation, while the most significant traits to explain plant adaptations to coastal environments were plant height, sclerenchyma, spongy parenchyma and reserves of inulin in the root. Two functional groups of plants were circumscribed: a conservative group formed by trees and shrubs dominated the Woody Community, with low values of SLA (specific leaf area), thick cuticles, high frequencies of phenolic compounds and crystals, woody stems and great plant heights, which tended to invest in permanent aerial organs; and a more heterogeneous group of herbaceous plants (found in Beach Community, Non-floodable, and Wet Communities) with acquisitive characteristics (high SLA values) or conservative strategy (rhizome and xylopodium). Finally, our results suggested that root and stem functional traits, which are infrequently taken into consideration, were useful to differentiate subtropical coastal plants and, in general, to study plant adaptations to environmental conditions in depth.

1. Introduction

Dune plant communities are a sparse vegetation highly adapted to stress caused by salinity, burial, and the nutritional restriction that occur in coastal sandy regions throughout the world (Doing, 1985; Sýkora et al., 2004; Isermann, 2005; Lane et al., 2008). Factors such as climate, geographic location and physiography can imprint differences on dune vegetation among regions (Rozema et al., 1985; Hesp, 1991; Maun, 1997; Gornish and Miller, 2010). At a local scale, the zonation of plant communities from the beach to the inland margin is well recognised (Dech and Maun, 2005). Usually, three to six different plant community types can be identified on the dune complex (Maun, 2009): the dynamic dunes (typically halophilous community and perennial herb community), semifixed dunes (presence of shrubs interspersed with herbaceous vegetation), slacks (wet communities) and fixed dunes (dominated by tree vegetation).

In southern and southeastern Brazil, the coastal lowland vegetation is characterised by a mosaic of herbaceous, shrubby and arboreal physiognomies, regionally named "restinga", which is an associated ecosystem of the Atlantic Forest Biome (Marques et al., 2011). In this mosaic, the coastal dune communities are represented by a more or less defined zonation from shoreline to inland, where a pioneer vegetation formed by halophilous herbaceous (foredune) and scrubby communities occurs sequentially (Menezes-Silva and Britez, 2005). The pioneer vegetation in the foredune usually presents less diversity, productivity and structural complexity (Scarano, 2002) due to the extreme environmental conditions (Rozema et al., 1985; Hesp, 1991; Maun, 1997; Gornish and Miller, 2010). Phenotypic plasticity is often reported for Atlantic Lowland Forest species (Melo-Júnior and Boeger, 2016; Bächtold and Melo-Júnior, 2015). The leaves are amphistomatic (Arruda et al., 2009), xeromorphic, compact, thick and sclerified (Rôças and Scarano, 2001; Rosado and Matos, 2007) with low SLA (specific leaf area) values (Boeger and Wisniewski, 2003). Remarkably, the functional responses of the leaves were convergent in relation to the availability of water and organic matter

(from herbaceous vegetation to the forest) (Rosado and Matos, 2007; Melo-Júnior and Boeger, 2015). There is also evidence that physiological traits such as maintaining high water potential during the drought period may favour the dominance of certain species (Rosado and Matos, 2010). Differences in soil characteristics that occur on a broad scale (Melo-Júnior and Boeger, 2015; Rosado and Matos, 2007) and that influence the arrangement and distribution of species in coastal vegetation (Santos-Filho et al., 2013) may also be present on a finer scale, that is, in the pioneer zone (foredune), as there are visible variations in the physiognomy of the vegetation in this peripheral area of the Atlantic Lowland Forest (Menezes-Silva and Britez, 2005).

Plant functional traits impacting plant species fitness affecting growth, reproduction, resource use and establishment (Violle et al., 2007; Garnier and Navas, 2012) mediate the response of plants to the environment and influence ecosystem functioning and have also been useful to infer assembly processes in plant communities (Grime, 1977; Lavorel and Garnier, 2002), particularly along natural stress gradients, which have been extensively studied in coastal sand dunes (Feagin and Wu, 2007; Gallego-Fernandez and Martinez, 2011; Chelli et al., 2019). Although morphological, anatomical and physiological traits of Atlantic Lowland Forest plants are well documented, few studies have evaluated the vegetation through a functional trait approach, despite the importance not only in connecting plant traits and environmental factors, but also among properties of plant and community structure (Rosado and Matos, 2007, 2010). The coastal vegetation is strongly influenced by edaphic factors (Santos-Filho et al., 2013). The analysis of functional groups in coastal communities is a valuable tool that helps interpreting community assembly and functioning at a local scale (García-Novo et al., 2004), which has not yet been explored, in pioneer zone of the Atlantic Lowland Forest, with the use of anatomical traits of root, stem and leaf.

Although Ilha do Mel has well-preserved pioneer zones (Praia Grande) with well-known vegetation (Menezes-Silva and Britez, 2005), there are no studies with a functional approach that could explain the distribution of species along the coastline. In this way, our hypothesis was that

functional morpho-anatomical traits of the root, stem and leaf may explain the organization of the plant community in the pioneer zone (foredune) of the Atlantic Lowland Forest in a short edaphic gradient, that is, from shoreline to slacks. This research addressed the following questions: (1) Can we identify a sequence of plant communities that show different functional traits along the foredune gradient? (2) Are there any relationships between species abundance, trait occurrence / values and soil variables?

2. Materials and methods

2. 1. Study area

This research was conducted in the coastal plain of Paraná state in southern Brazil, more precisely in an area of pioneer formations with marine influence (foredunes) of Praia Grande in Ilha do Mel State Park (25° 32' 7" S, 48° 19' 52" W) (Fig. 1). The soil consists predominantly of medium to fine sand (Giannini et al., 2004). The climate of the island is transitional, between tropical and subtropical (Maack, 1981). Annual average rainfall is 1959 mm, with the highest monthly rainfall average from January to March (272–286 mm) and the lowest one in July and August (72–73 mm). The mean annual temperature is 21.1 °C with the highest average temperature in February (25 ºC) and the lowest one in July (17 ºC) (Marques and Britez, 2005).

>>insert Fig. 1 here

2. 2. Data collection

Three transects, about 100 m long, equidistant 100 m and perpendicular to the shoreline, were traced across a mosaic of open herbaceous vegetation (Fig. 1). Plots of 2 m × 2 m were positioned along each transect starting from the shoreline until the end of the herbaceous vegetation. The distances between plots varied from 5 to 15 m, that is, closer when the vegetation was distinct between the plots and more distant in regions with similar vegetation. The plots were positioned

following the soil profile (from the shoreline to slacks) and the most significant vegetation features (pioneer vegetation of drift lines, herbaceous and shrub vegetation of shifting dunes, and vegetation of wet slacks). Each plot was identified by a capital letter and two numbers, the first number identifies the transect and the second number identifies the plot (e.g. B12 = plot 2 of transect 1). The plots nearest to the sea was number one and the most distant were eight and nine (see Table A1). Soil samples were collected in each plot, mixing three random subsamples of the first 15 cm of the soil. Each sample consisted of approximately 400 g of soil, which was subjected to the following chemical analysis: quantification of gravimetric moisture, pH, P (extracted with Mehlich 1), Na, N (total), SB (sum of bases = Ca+Mg+Na+K), CTC (transfer capacity = SB+H+Al), V (saturation of bases = SB $\times$ 100/CTC) and OM (organic matter) (Table A1). All soil analyses followed the methodologies of Pavan et al. (1992). Vegetation sampling along with the soil and functional traits data were collected from each of 25 plots (Table A1). The fieldwork took place between May and June 2017. Regarding vegetation sampling, the percentage cover of every plant was visually estimated in each plot as the vertical projection of the aerial part of the plants onto a horizontal surface. Plant nomenclature followed the Flora do Brasil 2020 (http://floradobrasil.jbrj.gov.br, page under construction, accessed: Oct 30. 2019) and The Plant List (http://www.theplantlist.org).

All the species occurring within the plots were considered for functional trait analysis. Forty morphological and anatomical traits of the leaves, stems and roots were measured (Table 1). The thickness of the epidermis and the external periclinal wall of the epidermis were measured only on the adaxial face of the leaf. If the same species was present in several plots, we collected plant material of that species in each plot and considered them different sampling. Up to ten individuals from each species were collected per plot. Rhizomatous or stoloniferous species were considered subpopulations within the same plot. Leaves fully expanded and with no evidence of herbivory, between the 3rd and 6th nodes from the apex, were collected. At least fifteen leaves per species in each plot were collected, with at least 10 leaves for morphological analyses and three for anatomical

analyses (Pérez-Harguindeguy et al., 2013). Stem samples were collected near the base of the herbaceous species, and branches of approximately 1 cm in diameter were collected from the woody plants. Samples of whole roots were collected to obtain the basal region (near the stem) and small-calibre lateral roots (the woody plants were not sampled).

>>insert Table 1 here

The collection and storage of samples and their processing for the measurement and analysis of the functional traits followed Pérez-Harguindeguy et al. (2013) and Ciccarelli et al. (2016). Plant materials intended for use in anatomical analyses were fixed in FAA 50 (Johansen, 1940) and stored in 70% ethanol. For the morphological analyses, the materials were moistened, placed in sealed zip-lock bags, and transported in a thermal box to the laboratory, where they were rehydrated in the dark at 4 °C for approximately 6 h (Ciccarelli, 2015).

Anatomical sections were made by hand with a disposable razor and submitted to staining or reactions with safranin and astra blue (Kraus and Arduin, 1997) to evidence the cell wall, sudan III (Sass, 1951) for lipids, lugol (Johansen, 1940) for starch and ferric chloride (Johansen, 1940) for phenolic compounds. To identify inulin crystals, the samples were fixed, stored in absolute ethanol and observed under a polarisation microscope. The blade was made semi-permanently with glycerinated gelatine (Kaiser, 1880). The sections were transversal, in the middle third of the limb (between the median rib and the edge), the stem base or branches, root base and absorption zone (when available). Measurements were made under a microscope with an ocular fitted with a calibrated scale via a Zeiss 5 + 100/100 micrometric blade.

2. 3. Data analysis

We performed a cluster analysis using average-linkage clustering and the Bray-Curtis similarity index for a square-root transformed species abundance matrix (25 plots per 60 species) to explore the main patterns of the plant communities along the shoreline–inland gradient. The same

resemblance matrix was used to perform non-metric multidimensional scaling (NMDS), which is a technique that represents samples in a low-dimensional space by optimising the correspondence between the original dissimilarities and the distances in the ordination (Økland, 1996). Moreover, the contribution of each plant species to the characterisation of the plant communities was quantified using similarity percentage analysis (SIMPER; Clarke, 1993). SIMPER analysis, based on the Bray-Curtis similarities, was used to identify the species that contributed most to the average between-group dissimilarity for pairs of plots grouped according to the plant community types. These analyses were performed using PRIMER v.7 (PRIMER-E, Plymouth; Clarke and Warwick, 2001).

We simultaneously analysed the relationships between species abundance, 40 trait occurrence and environmental variables using RLQ analysis (Dolédec et al., 1996) and fourth-corner analysis (Legendre et al., 1997). First, we checked for correlations among the species traits using Spearman's rank correlation coefficient. Four traits exceeded the chosen 0.7 threshold (abaxial cuticle thickness, buliform cell, endoderm, thickness of palisade parenchyma); thus, we excluded them from the analyses. Spearman's test was also used to check for correlations among environmental variables. In this case, five variables (CTC, N, P, SB, and V) were significantly correlated, so they were excluded from the analyses. Second, three matrices were assembled: species traits ($\mathbf{Q_{(q \times m)}}$, 60 species by 36 functional traits), environmental variables ($\mathbf{R_{(n \times p)}}$, 25 plots by four soil variables), and species abundance ($\mathbf{L_{(n \times q)}}$, 25 plots by abundance of 60 species). RLQ is an extension of co-inertia analysis that searches simultaneously for linear combinations of the variables in $\mathbf{Q}$ and linear combinations of the variables in $\mathbf{R}$, maximising covariance and weighting per $\mathbf{L}$ matrix (Dolédec et al., 1996). The preliminary step of RLQ analysis is to analyse each matrix separately. Correspondence analysis (dudi.coa function in R) was applied to the species abundance matrix $\mathbf{L}$. The species traits matrix $\mathbf{Q}$ contained both quantitative and categorical variables. In this case, we used the Hill and Smith analysis (dudi.hillsmith function in R), which can perform a multivariate analysis with mixed quantitative variables and factors. For the environmental data, all of the variables were quantitative, and thus, we

applied a principal component analysis (dudi.pca function in R). Fourth-corner analysis was used to test the associations between individual traits and environmental variables in a $\mathbf{D_{(m \times p)}}$ matrix. We computed a permutation test using 999 re-samples to evaluate significant relationships in matrix $\mathbf{D}$ (Dray et al., 2014). The species scores on the first two axes of the RLQ analysis were used to perform a cluster analysis to obtain information on how functional groups are distributed along environmental gradients (Kleyer et al., 2012).

The RLQ and fourth-corner analyses were performed using R statistical software (R Core Team, 2019) using the ade-4 package (dudi.coa, dudi.hillsmith, dudi.pca, rlq and fourthcorner functions).

3. Results

3. 1. Plant communities along the shoreline-inland gradient

A total of 60 vascular plant taxa were recorded in the 25 plots along pioneer vegetation in the Ilha do Mel, distributed in 53 genera and 25 families (Table A.2).

The dendrogram obtained by cluster analysis showed four plant community types along the shoreline–inland gradient, however Non-floodable Community and Wet Community were quite similar (Fig. 2). The cluster analysis classified the plots into four groups with a similarity value of 17.13%. The Woody Community group was composed of only 3 plots corresponding to the tree / shrub community type present in taller dune ridges; the most frequent species were *Guapira opposite* (Vell.) Reitz*, Ocotea pulchella* (Nees & Mart.) Mez, and *Psidium cattleianum* Afzel. ex Sabine. The second cluster could be divided into Beach Community group dominated by herbaceous plants in the upper beach; the most frequent species were *Sporobolus virginicus* (L.) Kunth*, Hydrocotyle bonariensis* Comm. ex Lam., and *Sphagneticola trilobata* (L.) Pruski, and Non-floodable Community, which was more heterogeneous and was made by herbaceous communities interspersed

with the Woody Community in the highest and driest parts of the dune with *Smilax campestris* Griseb.*, Varronia curassavica* Jacq., and *Hydrocotyle bonariensis*, and Wet Community in temporary wet slacks, with *Ischaemum minus* J.Presl*, Stylosanthes viscosa* (L.) Sw., and *Tibouchina clavata* (Pers.) Wurdack. The results obtained with the cluster analysis were confirmed by NMDS (Fig. 3) with an excellent stress index (= 0.06) that attests to the reliability of the segregation of the plots. Finally, the results of the SIMPER analyses have shown that the species differed in their contributions to the similarity of each plant community type (Table 2).

>>**insert Fig. 2 here**

>>**insert Fig. 3 here**

>>**insert Table 2 here**

3. 2. Relationships between species traits and environmental factors

The species traits, environmental factors and species abundances were highly correlated (RLQ, P < 0.01). The major variation was captured by the first RLQ axis (62%), reflecting the distribution of the trait states and values along the edaphic gradients (Table 3, Fig. 4). The organic matter content was highly negatively correlated with both RLQ axes, while Na was only positively correlated with RLQ 1. There were no significant correlations between the moisture or pH and the RLQ axes (Table 3).

Regarding species traits, maximum height and root inulin were significantly associated with both axes; the presence of sclerenchyma was significantly associated with axis 1, while the spongy parenchymal thickness and root starch were significantly associated with axis 2 (Table 3). There was an increase in plant height, spongy parenchymal thickness, woody growth form, leaf crystals, and presence of sclerenchyma in plots with the highest amount of organic matter (negative RLQ 1 in Fig. 4). These plots belonged to Woody Community, which represented the community of tree / shrub plants) (Fig. 2) with hypostomatic leaves and low values of SLA. The highest Na content was related

to a perennial herbaceous community in the upper beach as it belonged to beach vegetation group (Beach Community) (Fig. 2). This group was characterised by species with root inulin and the presence of leaves generally fleshy and not sclerified (Table 4). The second RLQ axis explained considerably less variance (25%) and ordered the plots along a gradient linked especially to the organic matter content (Table 3). These plots showed the highest content of soil moisture and represented the Wet Community (Fig. 2) in the slack zone which was associated with the presence of sclerenchyma in the leaf (Table A.3).

>>**insert Table 3 here**

>>**insert Table 4 here**

The cluster analysis of the matrix based on species scores of the first two RLQ axes classified the taxa into two functional groups (Fig. A.1): the first functional group was made up of plants belonging mainly to the woody plant communities (*Calophyllum brasiliense* Cambess., *Chiococca alba* (L.) Hitchc., *Clusia criuva* Cambess., *Dalbergia ecastaphyllum* (L.) Taub., *Guapira opposita*, *Myrcia parviflora* A.R. Lourenço & E. Lucas, *Myrsine umbellata* Mart., *Ocotea pulchella*, *Psidium cattleianum*, and *Schinus terebinthifolia*), while the other species were segregated into a larger functional group that included the annual and perennial herbaceous plants scattered across the foredune, from the shoreline to slack. This analysis considered simultaneously the relationships between plant functional traits and soil variables in the study area.

The woody functional group was characterised by secondary growth stems rich in phenolic compounds for 91% of the species (Fig. 5A, B); leaves with low SLA values due to cell compaction and high mesophyll thickness (Fig. 5C), abundant sclerenchyma (Fig. 5D), thick external periclinal walls of the epidermis and cuticle (Fig. 5E), hypostomatic leaves (Fig. 5F), dorsiventral mesophyll with thick spongy chlorophyllous parenchyma (Fig. 5C, F, G) and the presence of crystals (Fig. 5G) (Table A.3).

The herbaceous functional group was composed of plants that often had a subterranean or ground resistance structure, such as a rhizome and xylopodium (Fig. 5H, I), with starch or inulin reserves (Fig. 5J, K). The roots characteristically had exodermis with thickened walls such as in the Poaceae and Cyperaceae and a cortical aerenchyma (Fig. 5L). The leaves showed higher SLA values and thinner mesophyll (Fig. 5M) with sclerenchyma and bulliform cells in the Poaceae and Cyperaceae (Fig. 5N, O) and without sclerenchyma in annual herbaceous and fleshy species such as *Acicarpha spathulata* R. Br.*, Hydrocotyle bonariensis* (Fig. 5P) and *Ipomoea pes-caprae*, which dominated Beach Community. Herbaceous leaves also stood out due to their lower cuticle thickness and stomata on both leaf faces (Fig. 5Q, R, Table A.3).

In summary, the two functional groups obtained analysing species scores of the first two RLQ axes were linked to the four distinct plant communities found in the study area. The woody functional group was mainly concentrated in the Woody Community, distributed in the highest portions of the dunes, except for *Dalbergia ecastaphyllum* which also occurred in the Beach Community. The more heterogeneous herbaceous functional group was scattered through the three herbaceous communities (Beach, Non-floodable and Wet Communities) that showed different ecological strategies along the edaphic gradient (reserve of inulin, thickened exoderm and aerenchyma at the root, higher SLA, frequent amphistomatic leaves, protected stomata, trichomes, frequent C4 photosynthetic pathway, higher incidence of cryptophytes or geophyte, among others) (see Fig. 6, Table A.3).

4. Discussion

In the present study, we did not find an ordered sequence of phytocoenoses along the shoreline-inland gradient in the foredunes, but a mosaic of plant communities (Fig. 6), dominated by herbaceous vegetation with intermixed shrubs and wet vegetation due to the geomorphological features of the Ilha do Mel (Menezes-Silva and Britez, 2005). In Praia Grande the foredunes vary

from 2–4 m forming undulations (Marques and Britez, 2005) with higher regions (dune ridges) presenting higher concentration of organic matter and lower regions periodically flooding (Fig. 6). The higher concentration of organic matter in the dune ridges is related to greater plant cover and consequently greater leaf deposition in the soil. This spatial distribution of the plant communities of coastal sand dunes differ slightly from one region to another in southern Brazil, but they do share several species, such as *Hydrocotyle bonariensis, Ipomoea pes-caprae, Microstachys corniculata, Smilax campestris, Stylosanthes viscosa, Varronia curassavica*, and *Dalbergia ecastophyllum* (Martins et al., 2008; Melo-Júnior and Boeger, 2015; Castelo and Braga, 2017).

The plots characterised by the highest organic matter contents were dominated by the Woody Community vegetation characterised by shrubs and small trees which showed high values for their maximum height. The increased thickness of the spongy parenchyma is a plastic feature usually present in Atlantic forest plants occurring in coastal dune habitats (Melo-Júnior et al., 2017). These species can modulate the thickness of the spongy parenchyma depending on the environmental conditions (Boeger and Wisniewski, 2003; Melo-Júnior and Boeger, 2016; Castelo and Braga, 2017). The greater thickness of the leaf and palisade parenchyma also maximises the photosynthetic capacity, while preventing excessive water loss in an environment with intense radiation and wind. Under these conditions, more robust leaves with a large mass per area are associated with long-lived leaves species in a variety of habitats (Westoby et al., 2002). Calcium oxalate crystals occur in angiosperms, most often in the Atlantic forest species (≈70%) (Medina et al., 1990; Boeger and Wisniewski, 2003), which dominated our plots with the highest organic matter contents. The presence of calcium oxalate crystals in the coastal dune plants was thought to show no clear ecological relation (Arruda et al., 2009). Nevertheless, the selection of species with crystals seems strategically advantageous for the shrub plants with long-lived leaves that are associated with phenolic compounds and high sclerophylly. These characteristics have a protective function against inclement weather and herbivory (Westoby et al., 2002; Franceschi and Nakata, 2005). Moreover, the presence of

sclerenchyma in shrubs is essential to maintain leaf structure in cases of drought and to mitigate the risk of cell damage caused by wilting (Cunningham et al., 1999; Turner, 1994). The Woody Community was composed of many species that had the ability to colonise different habitats (Scarano, 2002) due to their physiological and phenotypic plasticity (Lüttge, 2006; Bächtold and Melo-Júnior, 2015). Woody plants are generally competitive or stress-tolerant species with conservative characteristics (Grime, 1977).

Herbaceous species were distributed along the whole shoreline-inland gradient forming three distinct communities: some predominated in the upper beach (Beach Community) with higher levels of sodium; others in soil with low level of organic matter and moisture (Non-floodable Community); and some in slacks with greater soil moisture (Wet Community). They were mostly hemicryptophyte or geophytes (Table A.3) with buds very close to or below the ground according to Raunkiaer's classification (Raunkiaer, 1934). These plants showed resistance structures such as rhizomes, lignotubers or xylopodium and reserves in the root that promoted regrowth during favourable periods (Canadell and López-Soria, 1998), i.e. when rainfall is higher. These structures are typical of grassland plants that occur in southern Brazil (Fidelis et al., 2009), which constantly lose the aerial part due to grazing. Similarly, in the foredune environment there might be rapid loss of the aerial part due to drought stress, high salinity, radiation and mechanical damage (wind and abrasion by sand). This group included many perennial or short-lived herbs with acquisitive strategies (*Hydrocotyle bonariensis*, *Ischaemum minus, Porophyllum ruderale* (Jacq.) Cass.*,* and *Spartina densiflora*), or others with conservative strategies, such as with rhizome (*Smilax campestris*) and xylopodium (*Microstachys corniculata*).

The great majority of the perennial herbaceous species had amphistomatic leaves, which is a morphological trait frequently found in coastal plants all over the world (Arruda et al., 2009; Perrone et al., 2015). Our data showed the greatest amphistomatism and the lowest hypostomatism (Table A.3) in the species prevalent in the Beach community, where there is greater sand burial. The presence

of stomata on both the adaxial and abaxial surfaces of the leaves could be interpreted as an adaptation to sand burial, which is a frequent event in maritime sand dune ecosystems (Ciccarelli et al., 2009). Moreover, plants with amphistomatic leaves have increased photosynthetic efficiencies that lead them to develop more rapidly and to better contend against sand burial (Ciccarelli et al., 2009).

This functional group showed higher values of SLA with respect to those found in the Woody Community plants. In general, it was due to their non-sclerified leaves, which can be characterised by the presence of aquiferous parenchyma or multi-stratified chlorophyll parenchyma typical of the Beach Community (i.e. *Hydrocotyle bonariensis*, *Ipomoea pes-caprae*, and *Sphagneticola trilobata*). In the herbaceous group, Wet Community had the lowest SLA value, because it was dominated by Poaceae and Cyperaceae that had sclerified leaves, but also the highest percentage of plants with aquiferous parenchyma. These two characteristics and the presence of C4 photosynthesis, and aerenchyma in the root of most plants (Table A.3) defined the Wet Community as a phytocoenosis that resists both temporary flooding and drought periods. The species that avoid drought stress with short life cycles, such as the eudicot herbaceous species analysed here, tend to show higher SLA values and grow mainly during periods with higher rainfall (Bermúdez and Retuerto, 2014). These species mainly dominated the Non-floodable Community that occurred dispersed among the Woody Community in the highest parts of the dunes and with drier soil (see Fig. 6 and Table A.3).

Moreover, many succulent halophytes of the foredunes (Boeger and Gluzezak, 2006; Arruda et al., 2009) showed a low dry weight value (Boeger and Gluzezak, 2006) and consequently a relatively high SLA value. As demonstrated in Mediterranean coastal sand dunes, the SLA values of psammophytes were lower in the upper beach and embryonic dunes, while they increased along the sea–inland gradient, especially in the backdunes where the environmental conditions were less harsh (Ciccarelli, 2015). The coastal dunes of Ilha do Mel are characterised by a subtropical climate without a dry season or frost, which is probably the reason for the higher SLA values than those found in Mediterranean environments with a hot and dry season. Among the perennial herbaceous species, we

found a group of Asteraceae characterised by the presence of inulin crystals in the root. These crystals are produced when there are favourable conditions for CO_2 fixation, such as long and sunny days and when the plants are not growing because of low temperatures, dry conditions or low nitrogen concentrations (Raccuia and Melilli, 2010). Such climatic conditions, which are very common in coastal environments, favour inulin accumulation, which functions as an alternative reserve of carbohydrates with respect to starch (Hendry, 1987). The reserve of starch and inulin that is stored in underground organs and is used rapidly during springtime (Raccuia and Melilli, 2010) can be considered a conservative characteristic in some species (e.g. *Hydrocotyle bonariensis*, and *Sphagneticola trilobata*) which were representative of the Beach Community, but also occurred in other herbaceous communities throughout the whole transect.

The presence of a root exodermis with thickened walls in herbaceous communities (see Fig. 5L, Table A.3) is frequent and common among the Poaceae and Cyperaceae. It is well known that the exodermis plays an important role in regulating the flow of water and ions from the epidermis to the stele and from the stele to the soil (Dickison, 2000; Enstone et al., 2003). In general, roots characterised by an exodermis with thickened and lignified walls are more resistant in stressful environments, since it has greater capacity to retain water and is an effective barrier against the diffusion of oxygen from the root to the flooded soil (Enstone et al., 2003). This is in line with our results, which showed a positive correlation between a thickened exodermis and soil moisture, because the coastal dunes of Ilha do Mel are characterised by high rainfall from January to March (272–286 mm) and during the rest of the year they experience moderate rainfall or occasionally drought stress (72–73 mm). The great majority of the ubiquitous perennial herbaceous plants showed the presence of aerenchyma in their roots. This feature could be explained by the fact that coastal areas may be flooded by both saltwater and rainwater accumulation in the interdunal regions, which cause frequent decreases in soil oxygen. Thus, the traits of the root (aerenchyma and thickened exodermis) together with different leaf functional traits seem to favour the herbaceous communities

to a variety of stresses typical of the foredunes (water and hypoxia in Wet Community, saline and burial in Beach Community and drought in Non-floodable Community).

5. Conclusion

In conclusion, we could answer that: along the short edaphic gradient in a pioneer vegetation of the Atlantic Lowland Forest there was not an ordered sequence of phytocoenoses but a mosaic of plant communities with a predominance of herbaceous taxa intermixed with trees and shrub community. We found strong relationships between species abundance, trait occurrence and soil variables which could be linked to the specific environmental features operating at local scale. Each plant community type showed different and peculiar functional traits linked to the foredune gradient, but the greatest variety of strategies was registered in the herbaceous group.

The organic matter content and salinity were the most significant edaphic factors in the differentiation of the subtropical pioneer vegetation of Ilha do Mel. The most significant traits to explain the plant adaptations to coastal environments were plant height (as a consequence of the plant life form among other environmental characteristics), spongy parenchyma, sclerified leaves and low SLA for the Woody Community, which colonised soils with high values of organic matter content; the presence of sclerenchyma in the leaves, root aerenchyma, and exodermis with thickened walls (especially due to the high frequency of Poaceae and Cyperaceae) for the Wet Community found in soil with high levels of moisture; reserves of inulin in the roots and thickened exodermal walls for the Beach Community in plots with high salinity and burial stress; and several traits such as high SLA value, little sclerified leaves, trichomes and resistance structures present in most species were common features in Non-floodable Community, which colonised soils with low levels of moisture and medium values of salinity and organic matter content.

Different functional traits play a role in the assembly patterns of coastal plant vegetation: root inulin and the presence of generally fleshy and not sclerified leaves were dominant in Beach Community; annual herbaceous with trichomes, thin cuticle and aerenchyma at the root were predominant in Non-floodable Community; shrubs and small trees standing out for the highest height and spongy parenchymal thickness, hypostomatic leaves and low values of SLA characterised Woody Community; species with sclerified leaf, medium-thickness cuticle, aerenchyma at the root and little resistance structure dominated Wet Community.

Moreover, the present study highlighted the importance and utility of anatomical traits in functional studies. Attributes regarding roots and stems, which are seldomly taken into consideration, were highly important to differentiate the subtropical coastal plants from a functional point of view and to describe their adaptations to the environmental conditions of the coastal ecosystems in depth.

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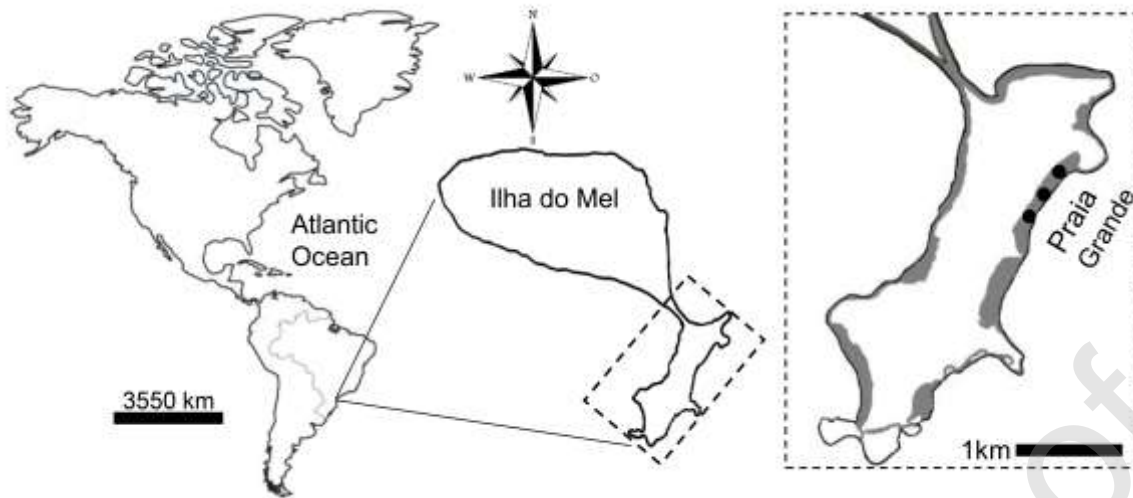
Fig. 1. Geographical position of the transects (●) in the pioneer vegetation of the Praia Grande, Ilha do Mel, coast of Paraná, Brazil. The beach areas are identified by grey.
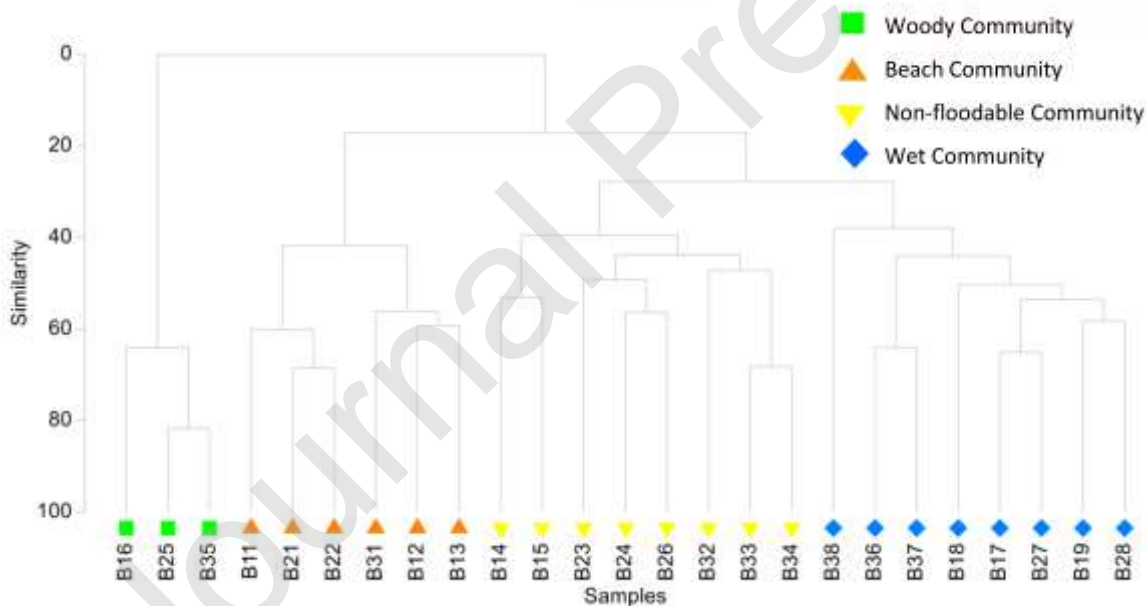
Fig. 2. Dendrogram obtained by average-linkage cluster analysis based on the Bray-Curtis distances of the abundance matrix species × plots. Abbreviations of the sampling localities: B11–B19, plots 1–9 of transect 1; B21–B28, plots 1–8 of transect 2; B31–B38, plots 1–8 of transect 3. Woody Community is represented by plots 5 and 6, in the middle region of the dune; Beach Community is

distributed in plots 1–3, closest to the sea; Non-floodable Community is distributed between plots 2–6; Wet Community occurs in plots 6–9, furthest from the sea.

Fig. 3. NMDS diagram based on the similarity (measured by the Bray-Curtis index) occurring among the plots. Groups obtained with the CA were superimposed into the diagram and are indicated with different symbols and colours. The excellent stress index (= 0.06) attests to the reliability of the segregation of the plots. Woody Community is located in the middle region of the dune; Beach Community is distributed closest to the sea; Non-floodable Community is distributed between plots 2 to 6; Wet Community is located farthest from the shoreline.

Fig. 4. Ordination graphics along the RLQ axes of (A) 25 plots, (B) 60 species of vascular plants, (C) four soil variables and functional traits that are highly correlated to soil parameters. Abbreviations: Moi = soil moisture, MO = organic matter content, Na = sodium content, pH. Abbreviations of the sampling localities: B11–B19, plots 1–9 of transect 1; B21–B28, plots 1–8 of transect 2; B31–B38,

plots 1–8 of transect 3. The plant species abbreviations and functional trait codes are given in Table A.2 and Table 1, respectively.

Fig. 5. Anatomy of the woody (A–G) and herbaceous (H–R) functional groups. (A, B) *Calophyllum brasiliense* woody stem. (A) Phloem (ph), xylem (xy) and parenchyma (pa). (B) Ferric chloride test

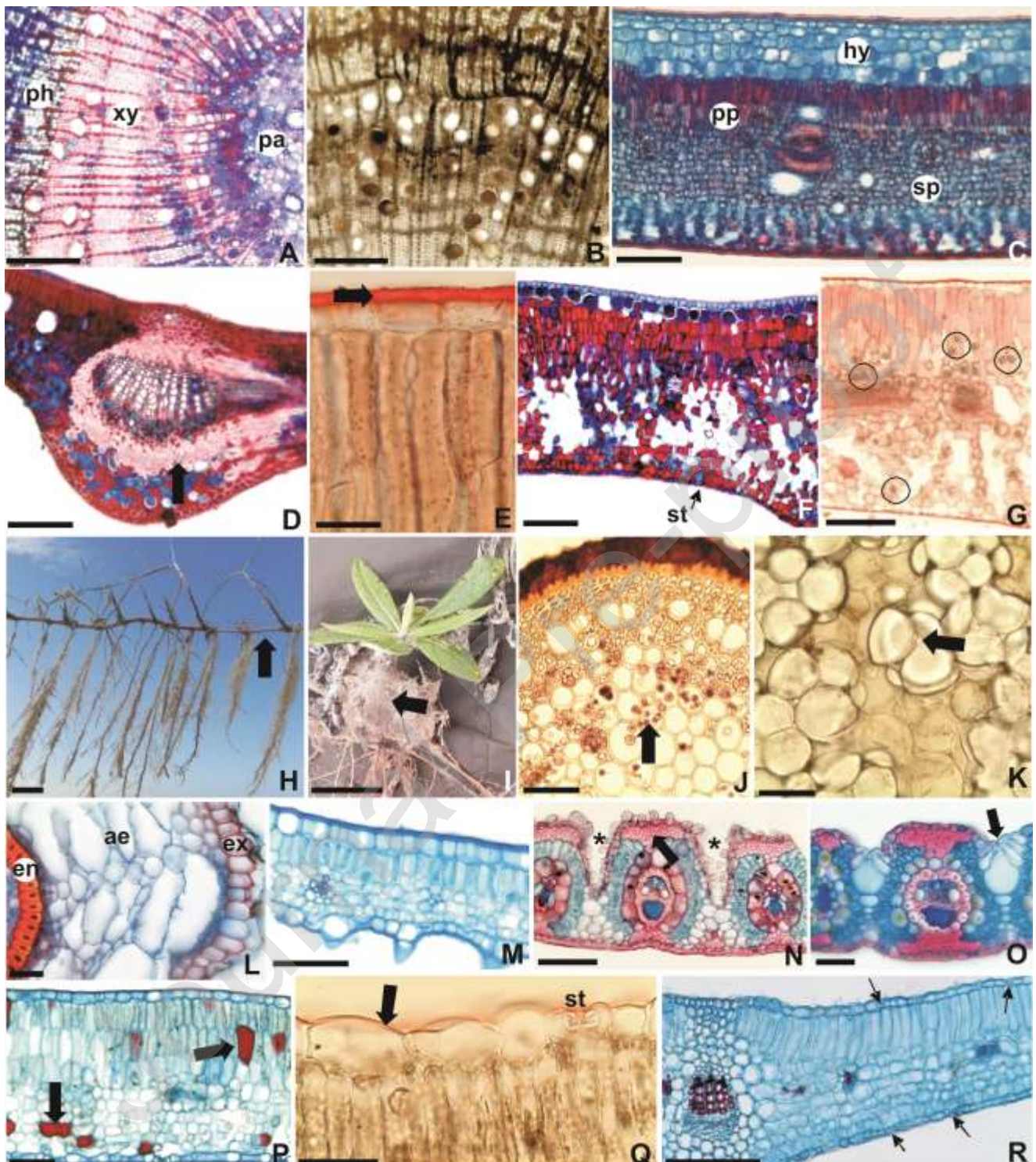identifying phenolic compounds (brown and black). (C) *Clusia criuva,* hypostomatic dorsiventral leaf evidencing hypodermis (hy) on the adaxial side, palisade (pp) and spongy (sp) chlorophyllic parenchyma. (D) *Ocotea pulchella*, leaf with sclerenchyma (arrow) surrounding the vascular tissue. (E) *Myrcia parvifolia*, leaf with thick cuticle (arrow), reaction with sudan III. (F) *Psidium cattleianum*, hypostomatic leaf, stomata (st). (G) *Myrsine umbellata*, leaf with crystals (circles), reaction with sudan. (H) *Sporobolus virginicus*, geophyta with rhizome (arrow). (I) *Pterocaulon lorentzii*, geophyta with xylopodium (arrow). (J) *Smilax campestris*, rhizome with starch (arrow). (K) *Sphagneticola trilobata*, root with inulin crystals (arrow). (L) *Fimbristylis cymosa*, root with thickened endoderm (en) and exoderm (ex) and aerenchyma (ae). (M) *Aristolochia trilobata*, C3 leaf without sclerenchyma. (N) *Sporobolus virginicus*, leaf with Kranz anatomy (C4), sclerenchyma (arrow) and stomatal crypts on the adaxial face (*). (O) *Eragrostis ciliaris*, C4 leaf with bulliform cells (arrow). (P) *Hydrocotyle bonariensis*, succulent leaf with phenolic compound idioblast (arrow). (Q) *Microstachys corniculata*, leaf with thin cuticle (arrow) and stomata (st) on the adaxial face. (R) *Porophyllum ruderale*, amphistomatic leaf (arrows indicate stomata). All sections are transversal. Scale bars: (A–D, F, G, R) 200 µm, (J, K, M, N, P) 100 µm, (E, L, O, Q) 50 µm, (H, I) 5 cm.
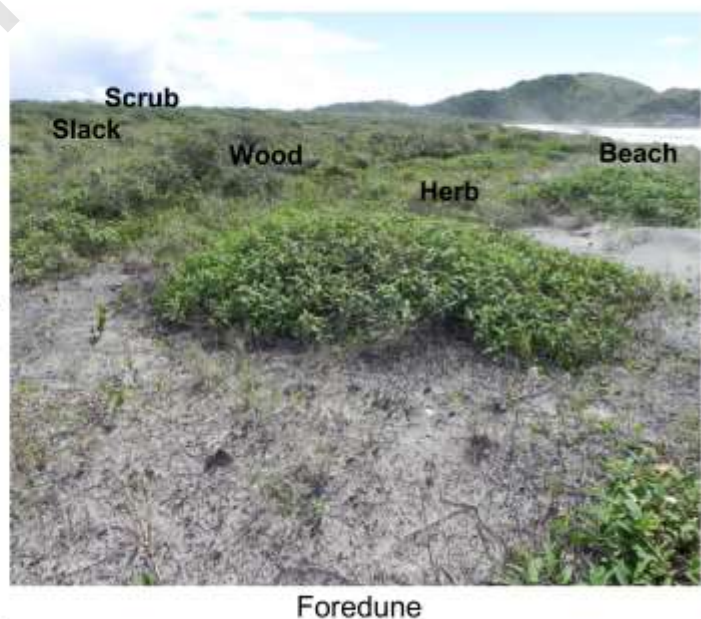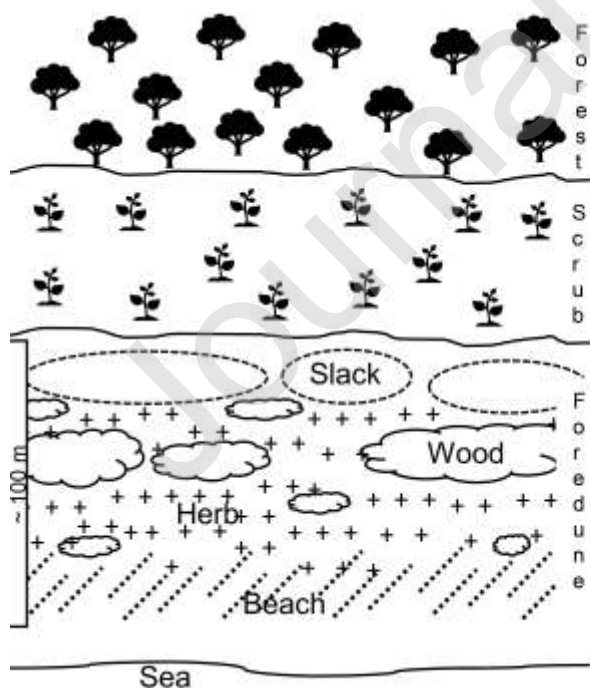
Fig. 6. Diagram (left) and image (right) showing the distribution of coastal lowland vegetation in Praia Grande, Ilha do Mel, southern Brazil along the sea / inland gradient. (Forest) coastal lowland vegetation forest (*Restinga* forest); (Scrub) scrub *restinga*, (Beach) Beach Community, species with root inulin and the presence of leaves generally fleshy and not sclerified; (Herb) Non-floodable Community, with a predominance of annual herbaceous with trichomes, thin cuticle and aerenchyma at the root; (Wood) Woody Community, shrubs and small trees that stand out for the highest height and spongy parenchymal thickness, hypostomatic leaves and low values of SLA; (Slack) Wet Community, species with sclerified leaf, medium-thickness cuticle, aerenchyma at the root and little resistance structure. The scale on the left represents the position of the transects.

Table 1 Traits measured in subtropical coastal dune species and their ecological correlate. Scales of measurement were originally binary (bin), categorical (cat) or continuous (cont)

Traits	Scales	Description of classes	Ecological correlate
Plant height (Hmax)	cont	m	Growth, relation to light, competition, reproduction, ability to withstand such events as fire, storm, grazing (Pérez-Harguindeguy et al., 2013)
Plant life forms (Plf)[a]	cat	1=Ph, 2=Ch, 3=He, 4=Ge, 5=Hel, 6=Th	Competition, exploratory resource skills, persistence after unrest (Pérez-Harguindeguy et al., 2013)
Annual herbaceous (anherb)	bin	0=absent, 1=present	Growth form: Ecophysiological adaptation, maximizing photosynthetic production, sheltering from severe climatic conditions (Pérez-Harguindeguy et al., 2013)
Perennial herbaceous (peherb)	bin	0=absent, 1=present	
Creeping or climbing (creep)	bin	0=absent, 1=present	
Woody (wood)	bin	0=absent, 1=present	
Resistance structure (resist)	cat	0=not identified, 1=rhizome, 2=tuber or other	Strategy of preservation of the vegetative body during the unfavorable periods (Montserrat et al., 2012)

LEAF TRAITS

Traits	Scales	Description of classes	Ecological correlate
Specific leaf area (sla)	cont	$cm^2 . g^{-1}$	RGR, photosynthetic rate, N concentration in the leaf, longevity and investment in secondary compounds (Turner, 1994; Pérez-Harguindeguy et al., 2013)
Leaf dry matter content (ldmc)	cont	$mg . g^{-1}$	Related to decomposition resistance, physical disturbances such as wind, herbivory and fire (Pérez-Harguindeguy et al., 2013)
Trichomes (tri)	cat	0=absent, 1=little, 2=much	Radiation protection (Jordan et al., 2008)
Limb thickness (lim)	cont	µm	Water loss reduction and radiation, nitrogen deficiency (Dickison, 2000)
Sclerenchyma (scl)	cat	0=absent, 1=little, 2=much	Resistance to water loss and damage (Turner, 1994)
Adaxial cuticle thickness (adcut)	cont	µm	Resistance to water loss and radiation, nitrogen deficiency (Dickison, 2000)
Abaxial cuticle thickness (abcut)	cont	µm	Resistance to water loss and radiation, nitrogen deficiency (Dickison, 2000)
Wall epidermis thickness (wall)	cont	µm	Resistance to water loss, predation, protection UV-B radiation, nitrogen-deficient (Dickison, 2000)
Epidermis thickness (epithick)	cont	µm	Protection from solar radiation (Turner, 1994), nitrogen deficiency (Dickison, 2000)
Distribution of stomata (stom)	cat	0=epi-, 1=hypo-, 2=amphistomatic	Improving photosynthetic efficiency in relation to environmental conditions (Smith et al., 1998)

Trait	Type	Values	Description
Stomatal protection (stompro)	cat	0=not, 1=semi, 2=very protected	Protection against water loss (crypts) or repel water and prevent stoma occlusion (trichomes) (Jordan et al., 2008)
Thickness of palisade parenchyma (pp)	cont	µm	Photosynthesis efficiency in relation to environmental conditions (Smith et al., 1998)
Thickness of spongy parenchyma (sp)	cont	µm	Maximization of photosynthetic rate (Rôças and Scarano, 2001)
Photosynthetic pathway (pho)	cat	0–C3, 1–C4, 2–CAM	Photosynthesis efficiency in different type of environment (Bresinsky et al., 2013)
Phenolic compounds (pheL)	bin	0=absent, 1=present	Protection against UV-B radiation (Kofidis et al., 2003), response to stress (Turner, 1994)
Crystals (crysL)	bin	0=absent, 1=present	Calcium regulation and defense (Franceschi and Nakata, 2005)
Aquifer tissue (aqui)	bin	0=absent, 1=present	Water conservation, salinity tolerance (Dickison, 2000)
Secretory structure (secL)	bin	0=absent, 1=present	Defense against herbivores and pathogens (Bresinsky et al., 2013)
Hypodermis (hyp)	bin	0=absent, 1=present	Protection from solar radiation, retention of water, salinity tolerance (Turner, 1994)
Aerenchyma (aereL)	cat	0=absent, 1 = some times, 2=present	Resistance to submersion; storage and transportation of oxygen (Dickison, 2000)
Bulliform cell (bul)	bin	0=absent, 1=present	Related to leaf winding and protection against water loss (Dickison, 2000; Turner, 1994)

STEM TRAITS

Trait	Type	Values	Description
Stem-specific density (ssd)	cont	mg.mm^{-3}	Importance for the stability, defense, architecture, hydraulic, C gain and growth of plants (Pérez-Harguindeguy et al., 2013)
aerenchyma (aereS)	bin	0=absent, 1=present	Resistance to submersion; storage and transportation of oxygen (Dickison, 2000)
Secretory structure (secS)	bin	0=absent, 1=present	Defense against herbivores and pathogens (Bresinsky et al., 2013)
Starch (starS)	bin	0=absent, 1=present	Ability to sprout after disturbance. Water conservation (Canadell and Lòpez-Soria, 1998)
Inulin (inuS)	bin	0=absent, 1=present	Rapid growth and facilitation of water absorption (Ritsema and Smeekens, 2003)
Phenolic compounds (pheS)	bin	0=absent, 1=present	Defense against herbivores, response to stress (Turner, 1994)

ROOT TRAITS

Trait	Type	Values	Description
Aerenchyma (aereR)	cat	0=not identified, 1=absent, 2=present	Resistance to submersion; storage and transportation of oxygen (Dickison, 2000)
Starch (starR)	cat	0=not identified, 1=absent, 2=present	Ability to sprout after disturbance (Canadell and Lòpez-Soria, 1998)
Inulin (inuR)	bin	0=absent, 1=present	Rapid growth and facilitation of water absorption (Hendry, 1987; Raccuia and Melilli, 2010)
Phenolic compounds (pheR)	bin	0=absent, 1=present	Response to stress (Turner, 1994)

Endoderm (endo)	cat	0=not identified, 1=thin, 2=thick	Resistance of water movement from the stele to soil, barrier to apoplastic inflow of ions (Enstone et al., 2003)
Exoderm (exo)	cat	0=not identified, 1=thin, 2=thick	Barrier to apoplastic inflow of ions (Enstone et al., 2003)

[a] Life forms according to Raunkiaer (1934): phanerophytes (Ph), chamaephytes (Ch), hemicryptophytes (He), geophytes (Ge), helophytes (Hel) or therophytes (Th).

Table 2 Results of similarity percentages analysis (SIMPER), showing the contribution of each plant species in determining 90% of the average similarity percentage within each community type obtained by cluster analysis. s.d. – standard deviation. The species are listed in decreasing order of contribution

Species	Average Abundance	Average Similarity	Similarity/s.d.	Contribution%	Cumulative Contribution%
A – Woody Community					
Guapira opposita	2.74	18.44	5.69	26.35	26.35
Ocotea pulchella	2.65	17.01	6.15	24.30	50.65
Psidium cattleianum	1.56	9.32	6.15	13.31	63.95
Myrcia parviflora	1.49	8.10	9.43	11.57	75.52
Schinus terebinthifolia	1.41	7.61	6.15	10.87	86.39
B – Beach Community					
Sporobolus virginicus	4.08	12.90	5.46	26.27	26.27
Hydrocotyle bonariensis	3.77	11.78	3.64	23.98	50.26
Sphagneticola trilobata	4.38	8.38	0.72	17.06	67.31
Spartina densiflora	3.10	8.29	1.34	16.88	84.19
C – Herbaceous Communities					
C1 – Non-floodable Community					
Smilax campestris	3.68	9.08	5.64	20.51	20.51
Varronia curassavica	2.70	6.09	2.78	13.76	34.27
Hydrocotyle bonariensis	2.55	5.43	3.30	12.27	46.54
Microstachys corniculata	2.24	5.07	5.13	11.45	57.99
C2 – Wet Community					
Ischaemum minus	5.21	9.71	2.42	20.74	20.74

Stylosanthes viscosa	4.27	8.88	2.45	18.98	39.72
Tibouchina clavata	3.43	6.34	2.23	13.56	53.28

Table 3 Summary of RLQ analysis showing correlations of both axes with environmental factors and species traits. Significance levels: (**) p < 0.01, (*) p < 0.05

	RLQ1	RLQ2
Eigen value	1.78	0.72
Variance explained (%)	62	25
Moisture	-0.67	0.22
Organic matter	****-0.79**	****-0.47**
Na	***0.58**	-0.65
pH	0.32	-0.51
Adaxial cuticle thickness (leaf)	-0.64	0.03
Plant height	***-0.63**	***-0.38**
Leaf dry matter content	-0.62	0.37
Limb thickness	-0.24	-0.26
Specific leaf area	0.50	0.40
Thickness of spongy parenchyma (leaf)	-0.15	***-0.67**
Stem-specific density	-0.21	0.09
Wall epidermis thickness (leaf)	-0.58	0.12
Epidermis thickness (leaf)	0.21	0.28
Annual herbaceous	0.05	0.26
Perennial herbaceous	-0.04	0.59
Creeping or climbing	0.05	0.03
Woody	-0.26	-0.55
Resistance structure	0.04	-0.19
Photosynthetic pathway	-0.13	0.75
Crystals (leaf)	-0.43	-0.33
Phenolic compounds (leaf)	-0.33	0.17
Starch (leaf)	-0.02	-0.06
Aerenchyma (leaf)	-0.19	0.08
Aquifer tissue (leaf)	-0.21	0.10
Distribution of stomata (leaf)	0.60	-0.20
Stomatal protection (leaf)	-0.06	0.22

Sclerenchyma (leaf)	**-0.67	0.61
Trichomes (leaf)	0.27	-0.24
Hypodermis (leaf)	-0.28	0.05
Secretory structure (leaf)	0.32	-0.52
Phenolic compounds (stem)	0.18	-0.04
Starch (stem)	-0.36	0.27
Inulin (stem)	0.50	-0.59
Aerenchyma (stem)	0.43	-0.07
Secretory structure (stem)	0.40	-0.53
Aerenchyma (root)	0.32	0.31
Starch (root)	-0.10	*0.46
Inulin (root)	*0.55	*-0.50
Phenolic compounds (root)	-0.09	-0.27
Exoderm (root)	0.12	0.60
Plant life forms	0.18	0.21

Table 4 Matrix D($_{mxp}$) generated by fourth-corner analysis showing correlations between environmental factors and species traits. Significance levels: (**) $p < 0.01$, (*) $p < 0.05$

	Moisture	Organic matter	Na	pH
Adaxial cuticle thickness (leaf)	0.09	0.18	-0.16	-0.05
Plant height	0.09	**0.58	-0.06	-0.03
Leaf dry matter content	0.14	0.16	-0.20	0.01
Limb thickness	-0.11	0.11	0.01	0.03
Specific leaf area	0.09	*-0.20	0.11	-0.09
Thickness of spongy parenchyma (leaf)	-0.08	**0.29	0.07	0.06
Stem-specific density	0.06	0.05	-0.06	-0.02
Wall epidermis thickness (leaf)	0.06	0.08	-0.12	-0.03
Epidermis thickness (leaf)	0.13	-0.02	-0.02	-0.08
Annual herbaceous	-0.04	-0.06	-0.14	-0.09
Perennial herbaceous	0.09	*-0.17	-0.10	-0.02
Creeping or climbing	0.19	0.04	0.17	-0.04
Woody	0.01	**0.35	-0.15	-0.05
Resistance structure	-0.16	*-0.17	0.04	0.15
Photosynthetic pathway	0.12	-0.10	-0.08	-0.07
Crystals (leaf)	0.09	*0.27	-0.21	-0.01
Phenolic compounds (leaf)	0.07	0.03	-0.16	-0.04
Starch (leaf)	-0.09	0.02	0.08	0.06
Aerenchyma (leaf)	0.05	0.05	-0.06	-0.02
Aquifer tissue (leaf)	0.02	0.06	-0.19	-0.11
Distribution of stomata (leaf)	-0.18	**-0.24	0.24	0.15
Stomatal protection (leaf)	0.06	-0.10	-0.00	0.09
Sclerenchyma (leaf)	**0.31	**0.22	*-0.32	-0.18
Trichomes (leaf)	-0.14	-0.14	0.05	0.13
Hypodermis (leaf)	0.02	0.11	-0.09	-0.08
Secretory structure (leaf)	-0.15	0.00	0.15	-0.01
Phenolic compounds (stem)	-0.11	*-0.19	0.13	-0.05

Starch (stem)	0.10	0.04	-0.04	0.07
Inulin (stem)	-0.10	-0.08	0.26	0.09
Aerenchyma (stem)	0.08	-0.09	0.21	0.01
Secretory structure (stem)	-0.11	-0.06	0.17	0.08
Aerenchyma (root)	0.00	****-0.26**	0.08	0.02
Starch (root)	0.15	***-0.24**	-0.17	-0.08
Inulin (root)	-0.13	-0.09	***0.41**	0.05
Phenolic compounds (root)	0.18	0.16	-0.02	0.01
Exoderm (root)	0.08	***-0.20**	0.07	-0.04
Plant life forms	-0.05	****-0.27**	0.02	0.02