Floristic variation in ecotonal areas: Patterns, determinants and biogeographic origins of subtropical forests in South America

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Abstract We present the first quantification of forest community composition and its relationship with environmental factors in South American subtropical Atlantic Forests. In this region, rain, seasonally dry and mixed forests form an ecotonal zone near the parallel of latitude 30°S. To investigate how well current knowledge on climatic effects and biogeographic distribution apply to subtropical ecotones, we tested the following expectations: (i) there is a floristic longitudinal gradient correlated to altitudinal and climatic gradients; (ii) climatic variables are more important than soil factors in shaping floristic composition; and (iii) there are three floristic regions in the southernmost limit of the Atlantic Forest biome that are expected to be distinct in composition, structure and biogeographical origin. We examined floristic composition and its relationship with environmental factors across 52 1-ha permanent study areas in subtropical Brazil, containing in total 269 tree species \( \geq 9.5 \text{ dbh (diameter at breast height).} \) Climatic data, related to rainfall seasonality and temperature, as well as soil properties, were compiled from published sources or global data banks. Expectations one and two were confirmed, but expectation three was only partially met. Hierarchical cluster analysis divided the southernmost Atlantic Forests into four major groups (Rain, Seasonally Dry, Western Mixed and Eastern Mixed Forests). Overall, the tested environmental variables differed significantly among the four regions. Using indicator species analysis, we distinguished 46 indicator species, which had significant environmental preferences for one floristic region. These species can be used as indicators of environmental conditions or to determine to which floristic region a certain forest belongs. Biogeographic distributions differed between floristic groups, supporting the interpretation that Eastern Mixed Forests are relict forests of a temperate forest of Andean origin that occurred during colder palaeoclimates. Western Mixed Forests represent the main floristic ecotone between Seasonally dry and Eastern Mixed Forests.

Key words: Atlantic Forests, climate, environmental gradient, indicator species, relict communities, soil.

INTRODUCTION

Environmental determinism (niche theory), dispersal limitation (neutral theory) and historical factors interact to shape the spatial structure of the floristic composition of ecological communities (Hubbell 2001; Whittaker et al. 2001; Lortie et al. 2004). In forest communities, the relative importance of these factors is likely to depend on spatial scale (Pyke et al. 2001; Phillips et al. 2003; Butt et al. 2008) and be particularly complex in ecotones (Parmentier et al. 2005). Ecotones represent transitions between ecological communities, generally along environmental gradients, and recent studies indicate that rarity, species richness and abundances tend to peak in ecotonal areas (Kark & van Rensburg 2006). Studies of transitional areas may reveal spatial structure either as a mosaic of clearly identifiable biotic communities (Toledo et al. 2011), or as a continuum of species substitutions (Odum & Barret 2005). It has been suggested that areas of biotic transition should be highly valued as centres of biodiversity, and sound conservation strategies should focus on both biodiversity hotspots and on environmental gradients or ecotones that represent the transition from one habitat type to another, in order to conserve adaptive diversity (Smith et al. 2001).

In subtropical South America, a large transitional area occurs at the southernmost limit of the Atlantic Forest biome, where rainforests and seasonally dry forests, encompassing semideciduous and deciduous...
forests, meet mixed conifer-hardwood forests near the parallel of latitude 30°S (Rambo 1954; Fig. 1), where forests are replaced by the Pampa grasslands. Despite growing efforts to elucidate the links between species composition in forests and explanatory environmental variables in South America, most studies carried out so far have concentrated on core areas within individual biomes and within the tropics (Torres et al. 1997; Oliveira-Filho & Fontes 2000; ter Steege et al. 2000; Pyke et al. 2001; Parmentier et al. 2005; Oliveira Filho et al. 2006; Bohlman et al. 2008; Butt et al. 2008), while studies on subtropical (Jarenkow & Budke 2009) or transitional areas (Mattei et al. 2007; Toledo et al. 2011) remain remarkably scarce. Here we investigated the floristic origins, similarities and dependence on climatic and edaphic factors of South American forests in a large subtropical ecotonal region.

Current understanding of biogeographic relationships in the region may be summarized as follows. Species composition often varies with continuous clines of climatic and edaphic conditions along topographic ecotones (Pyke et al. 2001; Parmentier et al. 2005; Kark & van Rensburg 2006; Toledo et al. 2011). These changes are thought to result from niche specialization and the environmental filtering (Levin 1992; Whittaker et al. 2001; Lortie et al. 2004). Ecological determinants of species abundances are however limited by historical events over long timescales, for example the occurrence of migration routes along river basins (Prado 2000; Ortiz-Jaureguizar & Cladera 2006). These routes are thought to vary with continental and global climate changes, occurring over millennia, and lead to the contraction or expansion of floristic groups and their emergent forest physiognomic types (Pennington et al. 2000; Carnaval & Moritz 2008; Werneck et al. 2011).

The southernmost limit of the Atlantic Forest biome was dominated by grasslands with patchy distribution of deciduous forests during the last Glacial cycle and through much of the Holocene (c. 21 000 years ago, Werneck et al. 2011). From this period on, the region has been colonized by distinct forests originating in the tropical nuclei of the Atlantic Forest. Since the Last Glacial Maximum, the Atlantic rainforests migrated about 1500 km south into subtropical coastal and lower montane areas (Rambo 1950; Behling & Negrelle 2001; Carnaval & Moritz 2008) with wet temperate climate and clayey soils (Streck et al. 2008). Seasonally dry forests, encompassing semideciduous and deciduous forests (Pennington et al. 2000), were restricted to three main refugia during the Last Glacial Maximum (Werneck et al. 2011). These forests experienced a gradual southward expansion from the Misiones glacial refuge, starting in the early Holocene (c. 0.12 Ma, Werneck et al. 2011).

Fig. 1. (a) Geographical situation of the study area in southern Brazil, and the spatial distribution of the studied subtropical forest areas. (b) Topographic map of Rio Grande do Sul, emphasizing the north-eastern coastal plains and lower montane areas (C), the sulriograndense highlands (RH), the Central Depression (CD) and the riograndense Shield (RS).
2011). Finally, the Araucaria mixed conifer-hardwood forest covered the wet and cooler higher elevations of the southern Brazilian highlands (planalto sul-Rio Grandense). It has been suggested that this forest represents a relict of former temperate wet forests (Rambo 1951b) that occurred during colder palaeoclimates and prevailed in the north-eastern Southern South American Mesopotamia (Ortiz-Jaureguizar & Cladera 2006).

Based on the premise that (i) the species occurring in these different forests present distinct adaptations to their past environments (Pennington et al. 2009), and thus distinct biophysical affinities (Philips et al. 2003; Butt et al. 2008); and (ii) species-level ecological preferences are at the basis of broader (biogeographical) patterns (Levin 1992), we tested the following expectations: (i) there is a floristic longitudinal gradient (Souza et al. 2012) correlated to existing altitudinal and climatic gradients (Streck et al. 2008), and to the directions of the Uruguay, Pelotas and Guaíba river basins (Fig. 1), regarded as two key plant migration routes (Rambo 1951a, 1954); (ii) climatic variables are more important than soil factors in shaping floristic composition, because of the closer match between the spatial scales of climatic variables and vegetation types considered (Swaine 1996; Pyke et al. 2001; Toledo et al. 2011); and (iii) based on floristic similarities, there are three floristic regions in the southernmost limit of the Atlantic Forest biome: rain forests in coastal and submontane areas, mixed forests on the highlands and seasonally dry forests in interior lowlands (Veloso et al. 1991; Jarenkow & Budke 2009). The three major forest types are expected to be distinct in composition, structure (Souza 2007; Pennington et al. 2009) and biogeographical origin (Rambo 1951a, 1951b; Sanmartin & Ronquist 2004).

**METHODS**

**Study area**

Data were gathered over an area of about 300 000 km² at the southernmost limit of the Atlantic Forest biome (sensu Oliveira-Filho & Fontes 2000) in Southern South America. The study area lies entirely within the Brazilian state of Rio Grande do Sul, near the parallel of latitude 30°S (Fig. 1). This region is characterized by variation in geomorphology and geological history (Streck et al. 2008), leading to strong gradients in topography and soil characteristics. A basaltic mountain range (the sul-riograndense highlands) forms an arch of descending altitudes from the north-eastern plateau (maximum elevation about 1300 m) to lower south-western undulated terrains (Fig. 1). The Pelotas and Uruguay River valleys circumvent the highlands to the north and west. Abrupt altitudinal changes occur at the easternmost edge of the highlands, where steep slopes mark the transition to a narrow strip of quaternary coastal plains. A North-eastern-South-western (NE-SW) diagonal of flat lowlands of sedimentary origin, known as the Central Depression, borders the inner side of the highland arch. The Central Depression is interrupted to the south by a low-elevation mountain range (maximum elevation about 600 m) of volcanic origin know as the riograndense Shield (Streck et al. 2008). The soils vary largely in fertility, from acid Cambisols in the highlands, to Argisols and Neosols on the slopes of the highlands, Laterosols on the north-western hilly lowlands of the Uruguay river valley, and Chernosols on the Quaternary lowlands of the eastern coast and Guaíba river valley (Streck et al. 2008).

Regional climate is classified as Köppen-Geiger type Cf, a temperate humid climate type, lacking a true dry season (Peel et al. 2007). A Cfa climate, with hot summers (hottest month temperature ≥ 22°C) prevails in most of the studied region, where summer temperatures may reach 40°C. A Cfb climate, with warm summers (hottest month temperature < 22°C), but at least 4 months with average temperature ≥ 10°C, occurs in the eastern and highest areas of the highland arch as well as in elevated patches of the riograndense Shield, where winter temperatures average 9°C and frosts are common (Kuinchtner & Buriol 2001). Annual precipitation varies from 1351 mm to 2091 mm (Hijmans et al. 2005). Subtropical rain, semideciduous and deciduous forests occur on lower elevations, while mixed conifer-hardwood forests, dominated by the conifer _Araucaria angustifolia_, cover the highlands.

**Data sources and characteristics**

We obtained data from two sources. Thirty-eight 1-ha plots were selected from the Rio Grande do Sul Forest Inventory (RSFI) database. The RSFI is a government data bank that contains records from plots located throughout the Rio Grande do Sul State, Brazil, surveyed from 1999 to 2001 using standard protocols (a complete description of the RSFI is available at http://coralux.usf.br/ifcrs/). Plots were 100 × 100 m (1.0 ha) in size, and were located in forest fragments of different sizes after stratification by vegetation type, catchment and geographical district. The diameter at breast height (1.3 m, dbh) and total height of all stems ≥ 9.5 cm dbh in each plot were recorded and each tree was identified to species and received a numbered tag. Voucher specimens were deposited at the Forestry Sciences Department Herbarium (HDCF) of the Santa Maria Federal University. To this data set, data from 14 published forest survey studies were added. Published data were collected in plots ranging from 0.7 to 1.8 ha. Many of these studies used multiple plots to sample vegetation. We pooled data from each study to form a single sample. To avoid confusion regarding the term ‘plot’, hereafter we refer the unique localities from which data were obtained as ‘study areas’. A total of 52 study areas covering 53 ha were used in analyses (Fig. 1; see Appendix S1 for bibliographic sources).

For each study area we obtained the geographical coordinates (UTM), seven climatic variables, interpolated sources). A total of 52 study areas covering 53 ha were selected from the Rio Grande do Sul Forest Inventory (RSFI) databank. The RSFI is a government data bank that contains records from plots located throughout the Rio Grande do Sul State, Brazil, surveyed from 1999 to 2001 using standard protocols (a complete description of the RSFI is available at http://coralux.usf.br/ifcrs/). Plots were 100 × 100 m (1.0 ha) in size, and were located in forest fragments of different sizes after stratification by vegetation type, catchment and geographical district. The diameter at breast height (1.3 m, dbh) and total height of all stems ≥ 9.5 cm dbh in each plot were recorded and each tree was identified to species and received a numbered tag. Voucher specimens were deposited at the Forestry Sciences Department Herbarium (HDCF) of the Santa Maria Federal University. To this data set, data from 14 published forest survey studies were added. Published data were collected in plots ranging from 0.7 to 1.8 ha. Many of these studies used multiple plots to sample vegetation. We pooled data from each study to form a single sample. To avoid confusion regarding the term ‘plot’, hereafter we refer the unique localities from which data were obtained as ‘study areas’. A total of 52 study areas covering 53 ha were used in analyses (Fig. 1; see Appendix S1 for bibliographic sources).

For each study area we obtained the geographical coordinates (UTM), seven climatic variables, interpolated from available data from weather stations, and nine edaphic variables obtained from sampled soils. Climatic variables were downloaded from the World-Clim project at a 30"
(1 km²) spatial resolution (available online at: http://www.worldclim.org/; Hijmans et al. 2005) and included mean minimum, average and maximum annual temperatures, temperature seasonality (standard deviation × 100), annual precipitation and precipitation seasonality (coefficient of variation). To these variables we added the number of dry (< 100 mm) months, an often-used indicator of water limitation for plants (e.g. Butt et al. 2008). Each study area was georeferenced and assigned to a soil category on the Rio Grande do Sul digitized soil map (Streck et al. 2008). Based on quantitative data on soil subtype characteristics (Brasil 1973; Streck et al. 2008), we compiled drainage, depth, organic matter, base saturation, cation exchange capacity, pH, phosphorous, aluminium, and a soil weathering index for each study area.

Data analysis

Because many variables were correlated, we used a principal components analysis (PCA) to identify major trends, separately for the climate and soil data sets (see Appendix S3 for detailed information on PCA). Before proceeding with data analyses, we consolidated a species abundance matrix through extensive taxonomic checking. As the status of accepted species is in constant flux, we corrected spelling errors and resolved synonymy problems through the comparison of every species with its reported status in the Tropicos database of the Missouri Botanical Garden (http://www.tropicos.org/) and in Sobral et al. (2006). Synonymous species were merged with the accepted species, and invalid species names were discarded.Singletons – species only known from a single specimen – were excluded from the analyses (McCune & Grace 2002).

We evaluated the relative influences of climate, soil and spatial autocorrelation (represented as Euclidean distance matrices) on floristic composition (as a Chao-Jaccard similarity matrix) through Multiple Regression on Distance Matrices (Legendre & Legendre 1998), implemented through the MRM function of the ECODIST package version 1.2.2 in R. Spearman (rank) correlations were used. Quadratic terms of each explanatory variable were included in order to account for possible non-linear effects of the explanatory matrices on floristic composition. Significance was assessed through 10 000 permutations. We also estimated spatial autocorrelation of floristic data through standard distance-decay analysis. For this analysis, we employed similarity matrices using the Sørensen similarity index to make results comparable to similar studies (Pyke et al. 2001; Phillips et al. 2003; Bohlain et al. 2008).

To illustrate relationships between geographic coordinates and biophysical variables and species composition (our first, second and fourth expectations) we used ordination techniques. Pairwise floristic similarities among study areas were evaluated through a non-metric multidimensional scaling (NMDS) using the function ‘metaMDS’ of the package ‘vegan’ 1.17-0 in R 2.9.1 (Core Team Development 2008, available at: http://www.r-project.org/). The Chao-Jaccard similarity index was used in the analysis. The Chao-Jaccard abundance-based estimator (Chao et al. 2005) is an abundance-based similarity index that assesses the probability that individuals belong to shared versus unshared species, by accounting for the effect of unseen, shared species. In tropical and subtropical forests, where rare species are frequent and the sampling is incomplete, this index is less biased by sample size, and is therefore more appropriate than other similarity indices commonly used (Chao et al. 2005). In order to account for sample size differences between study areas, species abundances were relativized through Wisconsin double standardization where species are first standardized by maxima, then sites by site totals, and finally subjected to square-root transformation (McCune & Grace 2002). Dimensionality was assessed by examining the change in stress as a function of dimension while stepping down from a six- to one-dimensional solution. We chose the number of dimensions equal to 4 to minimize the stress (maximize the rank correlation between the calculated similarity distances and the plotted distances). The ‘metaMDS’ function standardizes the scaling in the result by a principal components rotation. We used the ‘envfit’ function of the package ‘vegan’ to associate spatial and environmental variables with the NMDS axes. Spatial variables were latitude and longitude (UTM) and environmental variables were the principal components generated in the climatic, soil and forest structure PCAs. The ‘envfit’ function calculates a multiple linear regression of the environmental variable being the dependent variable and site scores on NMDS axes being the independent variables. The function returns normalized regression coefficients, and a coefficient of determination (R²). The coefficient of determination was used as a test statistic, and its null distribution was created by 9999 permutations of the environmental variable.

For the definition of distinct forest community groups (our third expectation) we used hierarchical agglomerative cluster analysis with Ward linkage method (McCune & Grace 2002) and Hellinger-transformed Euclidean distances (Legendre & Legendre 1998), using the VEGAN function ‘hclust’. Indicator species analysis was used as an objective way to determine the number of significant groups in the resultant dendrogram (McCune & Grace 2002). Indicator species are those that are found mostly in a single group and that are present at most of the sites belonging to that group (Legendre & Legendre 1998). We repeatedly compared the average P value of indicator values for the species included in our analyses for different numbers of clusters. The number of clusters that produced the minimum average P value was selected as the optimum pruning level for the dendrogram. Statistical significance of the indicator value was tested by 9999 permutations using the ‘duleg’ function in the ‘labdsv’ package in R. We used the condensed data to perform a direct quantitative assessment of the floristic links between the floristic clusters by plotting the number of shared and exclusive species in Venn diagrams, using the ‘venn.diagram’ function of the ‘VennDiagram’ package in R.

In order to evaluate the distribution of geographic ranges of species found in the studied forests (our fifth expectation), we divided South America into Southern South America, Eastern South America and Amazonia, following Crisci et al. (1991), Olson et al. (2001) and Sanmartin and Ronquist (2004). Southern South America included Argentina, Chile, Uruguay, Paraguay, the Brazilian states of Rio Grande do Sul, Santa Catarina and Paraná, and the
Andean provinces of Bolivia, Peru and Ecuador (Crisci et al. 1991; Morroner 2002). Amazonia included the Brazilian Amazonia (Acre, Amazonas, Mato Grosso, Roraima, Rondônia, Pará, Tocantins and Maranhão), the Guianas, Venezuela, Colombia, and the lowland provinces of Peru and Bolivia. Eastern South America was formed by Brazilian states not included in either the Amazonia or the Southern South America regions. These states roughly correspond to the biogeographic region of the Atlantic Forest, including its semideciduous westward extensions into central Brazil (Oliveira-Filho & Fontes 2000). Each species was classified as belonging to one of the above biogeographic regions or combinations of regions (Appendix S2) by checking its geographical occurrence in Sobral et al. (2006), Tropicos (http://www.tropicos.org), Brazilian Flora checklist (floradobrasil.jbrj.gov.br/2011/index) and Germplasm Resources Information Network (http://www.ars-grin.gov/cgi-bin/npgs/html/taxgenform.pl), with all the online databases being consulted in march 2012. Exotic species were not included in the analysis. We used a series of G-tests to evaluate whether the frequencies of species and individuals were independent of floristic group as identified through cluster analysis (see below) and biogeographic region (Zar 1996).

RESULTS

The PCA on climatic variables produced two significant principal components, the first related to temperature and precipitation, and the second related to precipitation seasonality (see Appendices S3, S4 for further details). The PCA on soil variables produced four principal components, the first of which was mainly related to aluminium and base saturation and the second mainly to cation exchange capacity (Appendices S3, S4). The PCA on forest structure variables produced two principal components, the first related average tree height and basal area, and the second related to tree density (see Appendices S3, S4).

A total of 42,571 trees were measured in the 52 study areas, consisting of 269 tree species, 148 genera and 60 botanical families (see Appendix S2). Climate \( (P = 0.0001) \) was significantly related to floristic composition in the Multiple Regression on Distance Matrices analysis \( (F = 15.29, P = 0.005) \), but neither soil \( (P = 0.24) \) nor space \( (P = 0.91) \) was. The explained variance was, however, very low \( (R^2 = 0.033) \). Distant sites are less likely to share species than close sites (Fig. 2). The increase in species turnover reaches an asymptote for distances more than about 10 km and overall the relationship is rather weak.

The first ordination axis in the NMDS (final solution with four dimensions, stress = 11.9) reflected a clear compositional difference in tree communities between mixed forests concentrated to the right, and the other forest types, concentrated to the left (Fig. 3). Both mixed and deciduous forests were scattered along the second axis, with the two rainforests lying at its lowermost extreme. The temperature-precipitation axis of the climate PCA \( (R^2 = 0.73, P = 0.0001) \), longitude \( (R^2 = 0.69, P = 0.0001) \), and the maturity axis of the forest structure PCA \( (R^2 = 0.51, P = 0.0001) \) were most explained by the first two NMDS axes, although the base saturation \( (R^2 = 0.28, P = 0.0004) \) and cation exchange \( (R^2 = 0.25, P = 0.0011) \) soil PCA axes were also significantly related to these axes. The soil base saturation PCA axis (coefficient = −0.99), temperature-precipitation axis (−0.78) and longitude (−0.66) were negatively associated to NMDS axis 1, while the maturity axis of the forest structure PCA was positively associated to this axis (0.88, Fig. 2). The soil cation exchange axis was negatively associated to axis 2 (−0.99), while longitude (0.75) and the temperature-precipitation PCA axis (0.63) were positively associated to this axis.

Cluster analysis coupled with indicator species analysis as a dendrogram pruning strategy resulted in the identification of four forest communities (Fig. 4, see Appendix S2 for the cluster affiliation of each species). At this grouping level the average \( P \) value of indicator values for each of the species was minimal. The first division in the dendrogram separated mixed forests from deciduous forests. The final four groups highlighted floristic differences linked to gradients identifiable in previous analyses. They corresponded well to the four different regions of the ordination space as depicted by the NMDS, where they formed an arch along the two first ordination axis (Fig. 2). The first floristic group was formed by coastal rainforests and two deciduous forests located in the Central Depression (Fig. 5), characterized by 14 indicator species (20.9% of the group’s species), the strongest one was the palm *Euterpe edulis* (Table 1, Rainforest group hereafter). The second group was formed by...
most deciduous forests sampled (Seasonally Dry Forest group hereafter), and was characterized by 16 species (7.4% of the group’s species), the strongest associations of which were *Casearia sylvestris* and *Chrysophyllum marginatum*. This group formed a dry forest arch linking the Central Depression to the Uruguay River basin (Figs 1,5). Mixed forests were split into two groups. One of these was formed by floristically very distinctive forests, positioned at the far right of the first NMDS axis (Fig. 2). They were located at the easternmost portion of the highlands (Eastern Mixed Forest group hereafter), being indicated by 13 species (18.1% of the group’s species), the strongest of which was *Ilex brevicuspis*. It is worth noting that *A. angustifolia* was the third strongest indicator species within this group. The other group clustered mixed forests scattered through a wider area to the west of the highlands (Western Mixed Forest group hereafter). They presented higher floristic proximity with Seasonally Dry Forests (Fig. 2), and had only three indicator species (2.6% of the group’s species), the strongest of which was *Campomanesia xanthocarpa*. A disjunct patch of Western Mixed Forests occurred to the south on the riograndense
Shield (Fig. 5). Together, the 46 indicator species represented 17.1% of the total studied species. These species can be used as indicators of environmental conditions or to determine to which floristic region a certain forest belongs (Toledo et al. 2011).

Seasonally Dry Forests formed the most species-rich group, with 216 species. It was followed by Western Mixed Forests, with 115 species, Eastern Mixed Forests, with 72 species, and Rainforests, with 67 species (Fig. 6). The four floristic groups did not share high proportions of tree species. The highest floristic overlap occurred between Western and Eastern Mixed Forests, which shared 37.5% of their total richness, and between Western Mixed Forests and Seasonally Dry Forest, with 35.9% of overlap. Seasonally Dry Forests shared 23.0% of their species with Rainforests. The lowest species overlap occurred between Rainforests and both Eastern (7.0%) and Western (15.2%) Mixed Forests. Only seven species (2.9%) occurred in all four floristic groups. Nearly half of the total species (47.6%) were restricted to one of the four floristic groups. Seasonally Dry Forests presented the highest proportion of exclusive species (42.6%), followed by Rainforests (17.9%), Eastern (13.9%) and Western Mixed Forests (12.2%).

Geographical range in South America could be determined for 249 species. Pairwise comparisons between floristic groups revealed that the distribution of individuals across geographical ranges in each forest type was significantly different from all others (data not shown, \( P < 0.0001 \) in all cases, overall test: \( G = 5555.2, \text{d.f.} = 15, P < 0.0001, \) Fig. 7). In the Rain, Seasonally Dry and Western Mixed Forest floristic groups, the majority of trees belonged to species occurring from Eastern to Southern South America. In the Rainforest group this geographical distribution shared strong dominance with that represented by the Broad distribution (i.e. occurring in all considered regions; together, 86.4% of the individuals). In Seasonally Dry Forests, the dominance by these two geographic patterns (Eastern to Southern and Broad) was somewhat reduced because of an increase in the frequency of individuals that belong to species that occur in Eastern South America alone (18.0%). In Western Mixed Forests, the importance of broad and Eastern South America distributions was reduced by a relative increase in the frequency of trees of species that were restricted to Southern South America (12.8%). Eastern Mixed Forests was the only group in which individuals belonging to species restricted to Southern South America were the most abundant group (29.5%), closely followed by the Eastern and Southern South America (27.7%) and Eastern South America (22.9%). The broad distribution was reduced to 12.5% of individuals.
**DISCUSSION**

**Biophysical factors and floristic gradients**

This study is the first quantification of forest community composition and its relationship with environmental factors in South American subtropical Atlantic Forests. Confirming our first expectation, our results indicated the existence of one dominant gradient in tree composition across these forests. This was a gradient correlated to climate running from cooler and wetter areas located on the higher eastern parts of the highlands to western warmer and drier areas scattered through lower montane and lowland areas. The weak explanatory power of climatic variables for the variation in floristic data may have four explanations. First, data on climatic variables such as frost occurrence and precipitation variation between years were not

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</tr>
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EMF, Eastern Mixed Forests; RF, Rainforests; SDF, Seasonally Dry Forests; WMF, Western Mixed Forests.

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doi:10.1111/aec.12051
available but may be important to explain the occurrence of frost- and drought-sensitive species. Second, climatic variation may not be high enough to provoke strong floristic responses, as seen in ecotonal areas marked by extreme climatic transitions (e.g. Toledo et al. 2011). Third, local factors such as disturbance, fine scale edaphic features and local topography may play a role in the production of floristic structure, and our sampling intensity may have been too coarse to detect and quantify these sources of variation. Finally, the forests we studied represent the southern limit of the Atlantic Forests and contain an impoverished subset of the richer flora found at lower latitudes, with species tolerant to climatic variation and lower temperatures than those found in tropical latitudes (Oliveira Filho et al. 2006).

Our second expectation that climatic variables would be the primary driver of species composition at the geographic scale studied was confirmed. Floristic variation was much more related to the climate axes than the soil axes. Soil diversity and structure frequently occur at much finer scales than climate variation (e.g. Clark et al. 1995; Torres et al. 1997; Phillips et al. 2003; Toledo et al. 2011; but see ter Steege et al. 2006), and our study region is no exception to this pattern. This may partly account for the rather diffuse relationship between edaphic variables and floristic variation we found (Swaine 1996).

As is common in tropical and subtropical regions, the autoecology of the majority of the species we found is unknown (with the exception of A. angustifolia). Therefore, the distribution patterns described here could function as working hypotheses about the autoecology of individual species (Oliveira-Filho & Fontes 2000; Pennington et al. 2009). Recent work has found that moisture seasonality influences community composition in a manner that can be related to the life-history trade-off between shade tolerance and pioneer status (Butt et al. 2008). Wet-affiliated genera were correlated with shade tolerance, whereas genera with no rainfall affiliation were often pioneers.

Fig. 6. Venn diagram showing the number of tree species shared by the floristic groups identified through cluster analysis. Number size is proportional to the represented value. RF, Rainforests; SDF, Seasonally Dry Forests; EMF, Eastern Mixed Forests; WMF, Western Mixed Forests.

Fig. 7. Frequency of species and individual trees across geographical ranges in South America. AMAZ, Amazonia; EMF, Eastern Mixed Forests; ESA, Eastern South America; RF, Rainforests; SDF, Seasonally Dry Forests; SSA, Southern South America; WMP, Western Mixed Forests.
and dry-affiliate genera were never dominant. Tree species composition may respond to comparatively small variations in soil, and the distributional patterns of many species segregate by geomorphic unit even within a landscape with comparatively little soil variation (Clark et al. 1995; Phillips et al. 2003). We thus expect that the flora associated with Seasonally Dry Forests includes a high proportion of drought-tolerant, frost- and nutrient shortage-sensitive, pioneer species (Behling & Lichte 1997; Whittaker et al. 2001; Butt et al. 2008; Pennington et al. 2009), while species associated with Eastern Mixed Forests are likely to be sensitive to drought and tolerant to shade, frost and nutrient shortages (Behling & Lichte 1997; Torres et al. 1997; Prado 2000; Whittaker et al. 2001; Butt et al. 2008). While these expectations may be stronger for indicator species, they may be valid for species that were exclusive of these floristic groups as well.

Hubbell’s (2001) neutral theory predicts non-linear distance decay in similarity, and this prediction has been empirically shown in tropical forest landscapes (Pyke et al. 2001; Phillips et al. 2003). Our results confirm this pattern in an ecolonal subtropical region and imply high beta-diversity between forests more than about 10 km apart. However, as in the Panamanian landscape (Pyke et al. 2001), our study region has strong covarying climatic and topographic gradients that make it difficult to quantify the likely drivers of high beta-diversity, which could be related to deterministic factors (climate, geology), dispersal, or both (Duivenvoorden et al. 2002). The overall weak effect of geographical proximity at the landscape scale indicates that the species-rich subtropical forest communities we studied are structured more by in situ processes mediated in a deterministic way by climate and substrate conditions, than they are by spatial processes (Phillips et al. 2003; Parmentier et al. 2005).

Floristic groups and migration routes

Contrary to our third expectation (three floristic regions in the southernmost limit of the Atlantic Forest biome: coastal rainforests, highland mixed forests and inland Seasonally Dry Forests), mixed forests split into two floristic groups, resulting in four distinct southern Atlantic floristic regions. Semideciduous forests did not form a clearly distinct group from deciduous forests. This lends some support to the claim that because of their ecological, structural and floristic similarities, Neotropical seasonally dry tropical forests should be considered together in biogeographic analyses (Pennington et al. 2000). This and the above-mentioned split of Mixed Forests argue for a change in orientation from physiognomy- to floristic-based vegetation classification systems (e.g. the Brazilian system, Veloso et al. 1991), that could rely on the growing body of compositional analyses available (Torres et al. 1997; Oliveira-Filho & Fontes 2000; Oliveira Filho et al. 2006; ter Steege et al. 2006; Jarenkow & Budke 2009).

Among the forests from tropical latitudes, Seasonally Dry Forests were too different from Rainforests (high proportion of exclusive and indicator species) to be regarded as a subset of it, as suggested by Oliveira-Filho and Fontes (2000). This, allied with the distribution of Seasonally Dry Forests along the Pelotas, Uruguay and Guaiaba river basins (Figs 1, 5), support the hypothesis that Rain and Seasonally Dry Forests may represent two parallel migration routes for tree species originating from northern Atlantic Forest nuclei (Rambo 1950, 1951a). Atlantic rainforests are believed to have migrated about 1500 km south into subtropical coastal and lower montane areas after the Last Glacial Maximum (Rambo 1950; Behling & Negrelle 2001; Carnaval & Moritz 2008). The Rainforests we distinguished were marked by species also found at tropical latitudes in the coastal lowlands and seaward mountain slopes (Oliveira-Filho & Fontes 2000).

The higher number of species we found in the Seasonally Dry Forests relative to the other floristic groups is likely the result of the larger area covered by this forest type in our study region. Its floristic distinctiveness, however, may also have historical origins. Subtropical Seasonally Dry Forests are likely the result of the southward expansion of the Misiones glacial refuge from the Holocene onwards (Wernecke et al. 2011). This refuge was likely located in north-eastern Argentina, and was continuous with the Paraguay–Paraná rivers system in south-eastern Paraguay, as well as portions of the Brazilian state of Mato Grosso do Sul (Prado 2000; Werneck et al. 2011). Despite its floristic affinities with south-eastern Brazil’s Atlantic rainforests (Torres et al. 1997; Oliveira-Filho & Fontes 2000), Seasonally Dry Forests far inland extensions seems to have formed old speciation nuclei (Pennington et al. 2009) of which our Seasonally Dry Forests are southernmost representatives. The notorious lack of endemic genera in the southernmost Atlantic Forest region (Waechter 2002) is another indication of the young profile of this slow dispersion flora (Oliveira et al. 2005; Pennington et al. 2009).

Cluster analysis split mixed forests into two different groups, which we named Eastern and Western Mixed Forests. Although statistically significant, it is worth noting that this separation suffers from the spatial aggregation of study areas grouped in the Eastern Mixed Forest group (10 1-ha plots within several kilometres of each other, Appendix S1). Although this spatial bias in the data may weaken the
case for a distinction between two different mixed forest communities, there is ecological and biogeographical evidence in its favour. Western Mixed Forests cover the majority of the *sulriograndense* highlands and are clearly transitional between the Eastern Mixed Forests and Seasonally Dry Forests. By transitional, we do not mean just spatially intermediate, but also floristically intermediate, as evidenced by the reduced number of indicator and exclusive species and by the large proportion of species (73.4%) shared with either Eastern Mixed or Seasonally Dry Forests. This extended floristic gradient between Eastern Mixed and Seasonally Dry Forests was noted before by botanists (Rambo 1954; Klein 1960), and was responsible for the recently detected association between longitude and composition (Souza et al. 2012). In its westernmost parts, Western Mixed Forests are basically Seasonally Dry Forests whose mixed status derives from the presence of the conifer *A. angustifolia*, a long-lived pioneer and emergent (Souza 2007) that is physiognomically diagnostic of mixed forests in Brazil (Veloso et al. 1991). The same phenomenon has been detected by Jarenkow and Budke (2009) at lower latitudes in the Paraná highlands. The proximity between these two forests is also apparent in the disjunct track of Western Mixed Forest that covers part of the *riograndense* shield to the south-eastern portion of the study region. Discovered by earlier workers on field survey grounds (Reitz et al. 1983; Carlucci et al. 2011), its quantitative confirmation here lays ground for a southward expansion of the formal distribution of Mixed Forests in general and *A. angustifolia* in particular.

Located at the highest parts of the *sulriograndense* highlands, Eastern Mixed Forests receive elevated amounts of rainfall (c. 2000 mm per year), and are subjected to low winter temperatures with occasional frosts or snow. They were most correlated with forest height and basal area, supporting our third expectation (structural differences between floristic groups). The elevated biomass is attributable to well-conserved areas where the large-bodied and dominant *A. angustifolia* has not been cut (Souza 2007; Souza et al. 2012), but also to higher productivity. It is believed that an abundant water supply plays a large role in promoting species richness and forest complexity compared with seasonal forests (Pennington et al. 2000). Eastern Mixed Forests are also distinctive in regards to the geographical range of its species. Regarding biogeographic origin, our third expectation (Mixed Forests would be dominated by species restricted to Southern South America) was only partially met as only Eastern Mixed Forests showed increased frequency of trees of Southern South American distribution. This result lends partial support to the interpretation (Rambo 1951b; Prado 2000) that Mixed Forests are relict forests of a temperate forest of Andean origin that occurred during colder palaeoclimates and prevailed in the Southern South American Mesopotamia (Ortiz-Jaureguizar & Cladera 2006). It is worth noting that despite the dominance by species restricted to Southern South America, the majority of individuals and species occurring in Eastern Mixed Forests present a wide geographical distribution. This result suggests that these forests are not just relictual. In fact, they should be more properly regarded as communities of mixed origins, as species of Austral-Antarctic origin (Sannmartin & Ronquist 2004) coexist with species originating from mountainous areas in south-eastern Brazil. This result was even more pronounced in the other floristic groups, all of which showed a remarkable lack of geographically restricted species. The frequency of trees whose species’ ranges included Eastern South America reinforces the above-mentioned migration routes for Rainforest and Seasonally Dry Forest species (Rambo 1951a).

In the present work we have explored the complex floristic relationships in a large transitional zone in the southernmost South American Atlantic Forest. While there is a steep ecological gradient along the coastal Rainforest-highland Eastern Mixed Forest ecotone, a broader gradient links Seasonally dry, Western Mixed and Eastern Mixed Forests to the west. Floristic patterns proved to be particularly complex in ecotones (Parmentier et al. 2005; Kark & van Rensburg 2006). At the scale studied, the spatial structure of the floristic groups we identified consists of a mosaic of identifiable biotic communities at the extremes of the biophysical gradients, and a continuum of species substitutions at intermediate parts of these gradients, thus mixing the two classic ecolonal community structures (Odum & Barret 2005). Finally, if conservation strategies are to include ecotones in order to conserve both landscape and adaptive diversity (Smith et al. 2001), the design of conservation networks should explicitly consider the geographic scale of floristic complexity, and the establishment of nature reserves should cover both distinctive and transitional floristic groups.

**ACKNOWLEDGEMENTS**

Financial support was provided by CAPES to ETG through a Mater degree scholarship. We are grateful to the Rio Grande do Sul State Government for authorization to access the RSFI database, and to Doádi Brena and Solón Jonas Longhi for facilitating this access. We are grateful to Iuri Buffon for help in the preparation of maps and access to climatic data. João André Jarenkow, Juliano M. Oliveira, Gabriel. C. Costa, Anastasia Rahlin and Kyle Dexter read an
earlier version of the manuscript and contributed with valuable comments. Kyle Dexter and Anastasia Rahlin kindly revised the English.

REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Appendix S1.** General characterization of the study plots.

**Appendix S2.** List of tree species sampled.

**Appendix S3.** Principal components analyses of 52 forest plots.

**Appendix S4.** Principal components analysis ordination diagrams of 52 subtropical forest sampling plots based on climatic, soil, and forest structure variables.