Floresta e Ambiente 2018; 25(4): e20170821
https://doi.org/10.1590/2179-8087.082117
ISSN 2179-8087 (online)

Original Article

Conservation of Nature

Natural Regeneration in a Conservation Unit: Subsidy for Restoration Actions

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ABSTRACT

In order to evaluate the regeneration of a forest remnant, we installed 256 2 x 2 m plots for measuring forest species individuals with height ≥ 30 cm and stem diameter ≤ 1 cm. Horizontal structure was evaluated and calculated by the Shannon (H') diversity index and the Pielou (J) evenness index. We performed clustering analysis by the agglomerative and divisive hierarchical method (the variable was the number of individuals); and used the Principal Component Analysis to verify the species distribution. We sampled 3021 individuals, distributed in 26 families and 51 species (H' = 2.78 and J = 0.66). *Allophylus edulis* had the highest values of absolute frequency (63.3) and absolute density (5186 ind.ha-1), while *Ligustrum lucidum* presented greater dissimilarity. The analysis showed the presence of regeneration mechanisms, and evidenced biological invasion problem.

Keywords: exotic species, indicator species, multivariate analysis.

1. INTRODUCTION

Seasonal Forest, a forest typology from Rio Grande do Sul (RS), is the most threatened and least protected phytophysiognomy of the Atlantic Forest Biome (SOSMata Atlântica & INPE, 2015) and therefore, requires more urgent conservation actions. The seasonal forest in RS is located in the upper and middle part of the Uruguay River, in most of the southern slope of Serra Geral, and in dispersed areas around the Ijuí, Jacuí and Ibicuí rivers. This typology occurs as forest disjunctions presenting deciduous stratum (IBGE, 2012).

Information on forest types are necessary to better understand the structure, processes and functions of these ecosystems, aiming at ecological restoration (Santana et al., 2004; Martins et al., 2015). Natural regeneration is an efficient ecological indicator of ecosystem conservation, contributing to the understanding of forest dynamics, and the nucleation effect by colonizing species in ecological restoration (Souto & Boeger, 2011).

Natural regeneration assists biodiversity restoration in anthropogenic environments, initiating the connection between segregated landscapes through plant-animal interaction (Birch et al., 2010). Natural regeneration has a low cost due to the lower level of human intervention, which is an important characteristic to increase large-scale reforestation (Brancalion et al., 2012; Martins, 2013). In addition, regeneration monitoring within conservation units is crucial when elaborating necessary strategies and actions (Pivello, 2011).

Although important, there are few studies analyzing the structure and composition of natural regeneration, mainly due to difficulties in measurement and identification. Considering the current fragmentation of the Seasonal Forest, the present work aimed to characterize the regenerative potential in an area with a recent history of anthropic disturbance, in the Quarta Colônia State Park (*PEQC*), in order to obtain information on floristic and phytosociological composition to support future strategies of forest restoration.

2. MATERIAL AND METHODS

2.1. Characterization of the study area

The study was carried out in a fragment of Seasonal Forest located in the *PEQC*, in the central region of RS. The region is located between the Serra Geral slope and the Peripheral Depression, in the border zone between the Atlantic Forest Biome and the Pampa Biome, constituting an important ecotonal transition zone.

The climate is "Cfa" according to the Köppen classification, humid subtropical with no dry season, an average annual temperature of 22 °C, rainfall varying between 1300 and 1800 mm/year^{-1}, with higher values recorded in the colder season (Alvares et al., 2013).

The study site comprises a forest fragment altered by anthropic actions prior to the installation of the Park, which resulted in clearings of herbaceous and semi-shrub vegetation, predominantly Poaceae, Asteraceae as *Bacharis crispa* Spring and *Campuloclinium macrocephalum* (Less.) DC. Although not a proper ciliary forest, the site is part of the riparian Jacuí river influence domain.

2.2. Sampling the data

A total of 256 sample units of 2 x 2 m were installed in 2011, totaling 1024 m^2 of sample area. The plots were distributed by stratification (Felfili et al., 2011), comprising all individuals with height ≥ 30 cm and collection diameter ≤ 1 cm. Botanical identification was based on the species level, according to the Angiosperm Phylogeny Group IV botanical system (APG IV, 2016). The unidentified botanical material was collected for later identification in the Forest Herbarium of the Forest Science Department (*HDCF*) of the Federal University of Santa Maria (*UFSM*).

Phytosociological parameters of absolute (AF) and relative (RF) frequency, as well as absolute (AD) and relative (RD) density were measured (Finol, 1971). Next, the ecological Shannon diversity (H ‘) index and Pielou Evenness (J) index (Brower & Zar, 1984) were calculated to characterize the horizontal structure of the fragment.

2.3. Data analysis

Data were submitted to multivariate analysis by cluster analysis by Twinspan (Two Way Indicator Species) and Principal Component Analysis (PCA).

The species were grouped by their environmental similarities and/or dissimilarities, using agglomerative hierarchical grouping analysis. For this, we used the Euclidean distance as a measure of similarity or dissimilarity among the species through Statistica 7.0 software.

The analysis by Twinspan was done through PC-Ord software (McCune & Mefford, 1997) to group the study plots, using density of the species according to density by plots. Distribution of the eigenvalues was considered to be relevant when eigenvalues were ≥ 0.30 (30% variance) (Kent & Coker, 1992; Felfili et al., 2007). For the analysis, the standard abundance cut-off level of the PC-ORD program was used (Felfili et al., 2007). Species with less than five individuals were discarded from this analysis, as proposed by Narvaes et al. (2008). To order the species in a system of axes, an ACP was performed through CANOCO version 4.5 software (Ter Braak & Smilauer, 1998), considering species with a total number of individuals greater than 10.

3. RESULTS AND DISCUSSION

3.1. Floristics and horizontal structure

There were 3021 regenerants distributed in 51 species and 26 botanical families, with Lauraceae and Meliaceae (six species), and Myrtaceae (four species) being the most representative. Lauraceae and Myrtaceae are among the most expressive families in the Seasonal Forest (Brito & Carvalho, 2014; Horn Kunz & Martins, 2014; Fávero et al., 2015).

Allophylus edulis showed the highest AD (5186 ind. Ha-1) and AF values (63.28%), occurring in 162 of the 256 evaluated subplots. *A. edulis* presents large seed production, widely dispersed by fauna, fast growth and good regeneration capacity in different types of soil (Abreu et al., 2005). In addition, it does not require fertile soils for development (Almeida-Scabbia et al., 2011). This species is indicated for the recovery of degraded ecosystems, mainly in the surroundings of watercourses (Umeo et al., 2011).

In relation to the floristic diversity of the fragment, an H 'index of 2.78 and a J' of 0.66 was obtained, which can be considered high for an area in restoration. This suggests that the establishment of the regeneration species in the environment. For regeneration of the same typology, an H 'of 1.69 was found by the Continuous Forest Inventory of Rio Grande do Sul (Rio Grande do Sul, 2002). Also for Seasonal Forest in the Central Depression of RS, Sccoti et al. (2011) found an H 'of 2.38 and a J' of 0.61, evaluating a height interval ≥ 30 cm and DAP <1 cm.

The regeneration expression found is possibly due to the proximity to the Plategrass slopes of Riograndense, where the submontane and montane formations of the Seasonal Forest are present. These areas have remained more conserved due to the high slope, and now form a natural continuum, which can act as a source of propagules, even for the flat positions of the Jacuí river valley.

3.2. Hierarchical grouping analysis

The exotic *Ligustrum lucidum* species showed the greatest dissimilarity compared to the other species, uniting with species of other groups at a distance of approximately 74 (Figure 1).

The presence of invasive species within conservation units is contrary to the main objective of creating these areas, which is to preserve natural ecosystems of great ecological relevance (Brasil, 2000). Biological invasion gradually eliminates native species causing an imbalanced ecosystem and often with irreversible consequences. With aggressive and invasive characteristic, *L. lucidum* competes with the native vegetation regeneration, and is able to prevent the growth and even to suppress local species. Working in the same area of study, Hummel et al. (2014) observed that the species is exerting great pressure on the fragment due to the increasing number of regenerants, which represents direct competition for resources with native species. In Argentina, Lichstein et al. (2004) showed that *L. lucidum* dominance limits the recruitment of native seedlings.

In addition, the history of disturbances through which the studied area passes confers a significant fragility to the environment. Hoyos et al. (2010) confirm the rapid spread of *L. lucidum* in deforested areas, currently in the process of secondary succession.

(L.divar = *Luehea divaricata* Mart. & Zucc.; A.salig = *Aiouea saligna* Meisn.; C.ig = *Celtis iguanaea* (Jacq.) Sarg.; H.apicul = *Helietta apiculata* Benth.; S.bompl = *Sorocea bonplandii* (Baill.) W.C. Burger, Lanjouw & Boer; E.rostrif = *Eugenia rostrifolia* D.Legrand; Rubus = *Rubus* sp.; G.urug = *Guettarda uruguensis* Cham. & Schltdl.; I.vera = *Inga vera* Willd.; M.tinctor = *Maclura tinctoria* (L.) Don ex Steud.; P.aduncun = *Piper aduncum* L.; T.catigua = *Trichilia catigua* A. Juss.; Vassobia = *Vassobia breviflora* (Sendtn.) Hunz; P.cattl = *Psidium cattleianum* Sabine;

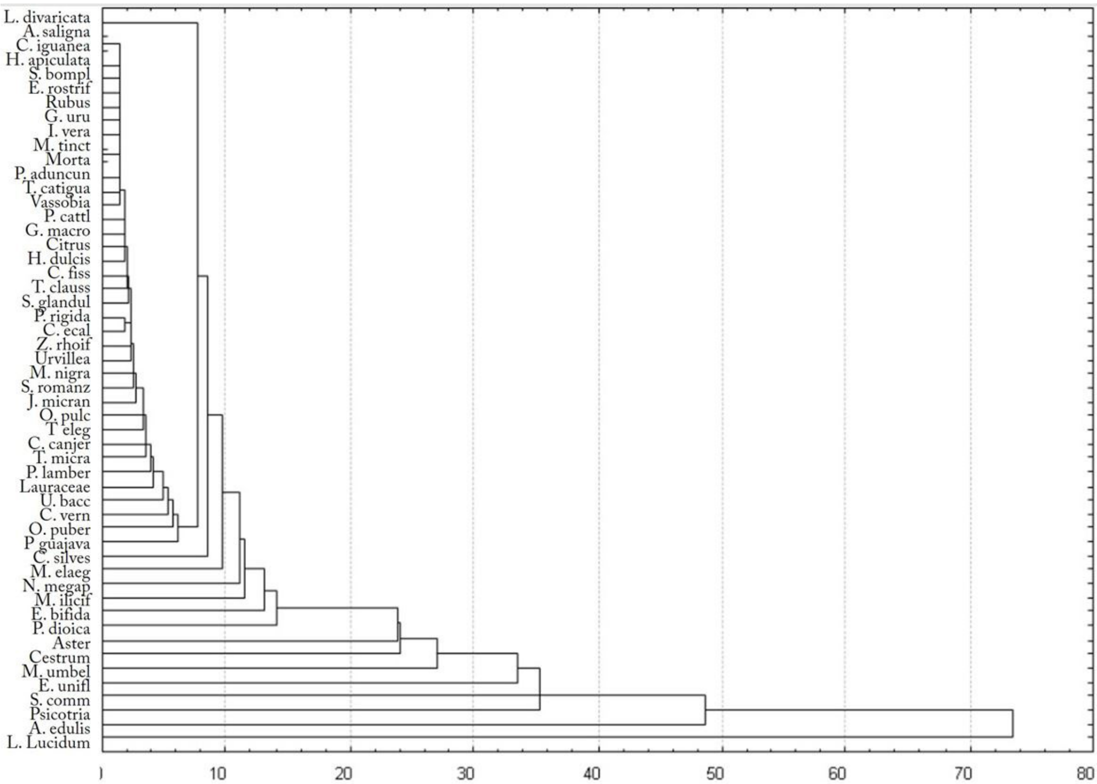

Figure 1. Hierarchical clustering analysis of arboreal and shrub species present in the regeneration stage of the Seasonal Forest, Rio Grande do Sul.

G.macrop = *Guarea macrophylla* Vahl; Citurs = *Citrus* sp.*;* H.dulcis = *Hovenia dulcis* Thunb.; C.fiss = *Cedrela fissilis* Vell.; T.claus = *Trichilia claussenii* C.DC.; S.glandul = *Sapium glandulosum* (L.) Morong; P.rigida = *Parapiptadenia rigida* (Benth.) Brenan; C.ecal = *Cordia ecalyculata* Vell.; Z.rhoif =*Zanthoxylum rhoifolium* Lam.; Urvillea = *Urvillea uniloba* Radlk.; M.nigra = *Morus nigra* L.; S.romanz = *Syagrus romanzoffiana* (Cham.) Glassman; J.micran = *Jacaranda micrantha* Cham.; O.pulc = *Ocotea pulchella* (Nees) Mez.; T.eleg = *Trichilia elegans* A. Juss.; C.canjer = *Cabralea canjerana* (Vell.) Mart.; T.micra = *Trema micrantha* (L.) Blume; LauraceaeNI =Lauraceae não identificada; U.bacc = *Urera baccifera* (L.) Gaudich.; C.vern = *Cupania vernalis* Cambess.; O.puber = *Ocotea puberula* (Rich.) Nees; P.guajava = *Psidium guajava* L.; C.silves = *Casearia sylvestris* Sw.; M.elaeg = *Matayba elaeagnoides* Radlk.; N.megap = *Nectandra megapotamica* (Spreng.) Mez; M.ilicif = *Maytenus ilicifolia* Schwacke; E.bifida = *Escallonia bifida* Link & Otto; P.dioica = *Phytolacca dioica* L.; Aster = Asteraceae; Cestrum = *Cestrum* sp.; M.umbel = *Myrsine umbellata* Mart.; E.unifl = *Eugenia uniflora* L.; S.comm= *Gymnanthes klotzschiana* (Baill.) L.B. Sm. & Downs; Psicotria = *Psychotria myriantha* Müll. Arg; A.edulis =*Allophylus edulis* (A. St.-Hil., Cambess. & A. Juss.) Radlk.*; L.lucidum* = *Ligustrum lucidum* W.T.Aiton).

Psychotria myriantha and *Gymnanthes klotzschiana* joined at a distance of approximately 35 with most other species, and at a distance of 48 with *A. edulis* (Figure 1).

A. edulis and *G. klotzschiana* present an important role in the return of the fauna with the greater yield of fruits, as well as species of the *Psychotria* genus, which are considered important feeding sources for the fauna of both pollinators and dispersers (Teixeira & Machado, 2004). The presence of native pioneer species and initial secondary species that are attractive to the fauna (Rodrigues & Gandolfi, 2000) is essential in order to reconstruct frugivorous-plant interactions in the restoration process. They play a key role as diversity generators and maintainers, in addition to contributing to the genetic exchange with the remnants of the environment.

We also observed the formation of a large group composed of species that showed low occurrence in regeneration (Table 1), constituting linkage groups between *Luehea divaricata* and *Psidium guajava*, clearly separated from a second set formed by the most abundant species. This intermediate cluster had a common characteristic of low frequency in the regeneration.

L. divaricata presented low occurrence, being observed in plots with greater degree of hydromorphy. According to Reitz et al. (1983), the species prefers moist soils.

On the other hand, *P. guajava* was only observed in some subplots of the sample. Because it is an invasive species with fruits that are very appreciated by the fauna, few matrices can give rise to several regenerants, competing with the development of the native vegetation. *P. guajava* prefers areas of agriculture and disturbed areas, soils with good humidity, and places with more light like forest edges. It exerts great impact on the native flora due to the shading that it causes in the dominated areas (Instituto Hórus, 2017).

Table 1. Low occurrence species in the regeneration stage of the Seasonal Forest, viewed by hierarchical clustering analysis.

Scientific Name	Popular Name	Family	Habitat
Aiouea saligna Meisn.	Canela-vermelha	Lauraceae	Tree
Cabralea canjerana (Vell.) Mart.	Canjerana	Meliaceae	Tree
Cedrela fissilis Vell.	Cedro	Meliaceae	Tree
Citrus sp.	Laranjeira	Rutaceae	Tree
Cordia ecalyculata Vell.	Louro-mole	Boraginaceae	Tree
Cupania vernalis Cambess	Camboatá-vermelho	Sapindaceae	Tree
Eugenia rostrifolia D.Legrand	Batinga-vermelha	Myrtaceae	Tree
Guarea macrophylla Vahl.	Catiguá-morcego	Meliaceae	Shrubs
Guettarda uruguensis Cham. & Schltdl.	Veludinho	Rubiaceae	Saplings
Helietta apiculata Benth.	Canela-de-veado	Rutaceae	Tree
Hovenia dulcis Thunb.	Uva-do-Japão	Rhamnaceae	Tree
Inga vera Willd.	Ingá	Fabaceae	Tree
Jacaranda micrantha Cham.	Caroba	Bignoniaceae	Tree
Luehea divaricata Mart. & Zucc.	Açoita-cavalo	Malvaceae	Tree
Maclura tinctoria (L.) Don ex Steud	Tajuba	Moraceae	Tree
Morus nigra L.	Amora-preta	Moraceae	Tree
Ocotea pulchella (Nees & Mart.) Mez	Canela-do-brejo	Lauraceae	Tree
Parapiptadenia rigida (Benth.) Brenan	Angico-vermelho	Fabaceae	Tree
Piper aduncum L.	Matico	Piperaceae	Saplings
Psidium cattleianum Afzel. ex Sabine	Araçá	Myrtaceae	Tree
Psidium guajava L.	Goiaba	Myrtaceae	Tree
Rubus sp.	Framboesa	Rosaceae	Saplings
Sapium glandulosum (L.) Morong	Pau-leiteiro	Euphorbiaceae	Tree
Sorocea bonplandii (Baill.) W.C. Burger, Lanjow & Boer	Cincho	Moraceae	Shrubs
Syagrus romanzoffiana (Cham.) Glassman	Jerivá	Arecaceae	Palm tree
Trema micrantha (L.) Blume.	Grandiúva	Cannabaceae	Tree
Trichilia catigua A. Juss.	Catiguá	Meliaceae	Shrubs
Trichilia claussenii C.DC.	Catiguá-vermelho	Meliaceae	Tree
Trichilia elegans A. Juss	Pau-de-ervilha	Meliaceae	Shrubs
Urera baccifera (L.) Gaudich. ex Wedd.	Urtigão	Urticaceae	Saplings
Urvillea uniloba Radlk.	Cipó-timbó	Sapindaceae	Vines
Vassobia breviflora (Sendtn.) Hunz.	Esporão-de-galo	Solanaceae	Shrubs
Zanthoxylum rhoifolium Lam.	Mamica-de-cadela	Rutaceae	Tree

3.3. Floristic groups

We formed two floristic groups (Figure 2). The first division separated the plots into two groups with an eigenvalue of 0.4430. The left group had the most plots and the right group had 14 plots (97, 140, 148, 159, 170, 187, 195, 206, 212, 223, 224, 227, 230 and 242). The latter formed Group 1, with *Asteraceae* as an indicator species, and *Eupatorium macrocephalum* as the main representative species. In this group, the *Asteraceae* family was classified as indicative and preferential in 14 plots. *Asteraceae* showed aggression in colonization, especially in disturbed areas (Hattori & Nakajima, 2008; Ferreira et al., 2001). According to Chazdon (2008), grasses, herbaceous plants and shrubs dominate recently abandoned areas, but decline in abundance as the forest canopy closes and reduces the availability of light.

The left group presented *G. klotzschiana*, *Eugenia uniflora*, and *Myrsine umbellata* as indicator species, classified as pioneers to early secondary (Carvalho, 2003). Pioneer species play a large role in this type of environment because they improve soil quality by producing biomass, improving compaction, interacting with soil fauna, and creating suitable conditions for the recruitment of late succession species (Rocha et al., 2016). *G. klotzschiana, Nectandra megapotamica*, *E. uniflora* and *A. edulis* are the preferred species. In a study carried out in the same forest formation in the Biological Reserve of Ibicuí-Mirim, central region of RS, Scipioni et al. (2011) found greater abundance of *G. klotzschiana* and *A. edulis* associated to areas near water courses, and *N. megapotamica* associated to the Neosols.

G. klotzschiana presented 302 individuals, which corresponds to the density of 2949 ind. ha^{-1}. With similar characteristics, *E. uniflora* appears with 304 individuals (2969 ind. ha^{-1}) and *M. umbellata* with 84 individuals (1836 ind. ha^{-1}).

The highest number of *G. klotzschiana* individuals can be related to the fruiting of the species. According to Reitz et al. (1983), it blooms during most of the year, producing a moderate amount of seeds and propagules in regeneration.

For the frequency of species in the area, we observed that Group 1 occurred in more than half of the sample

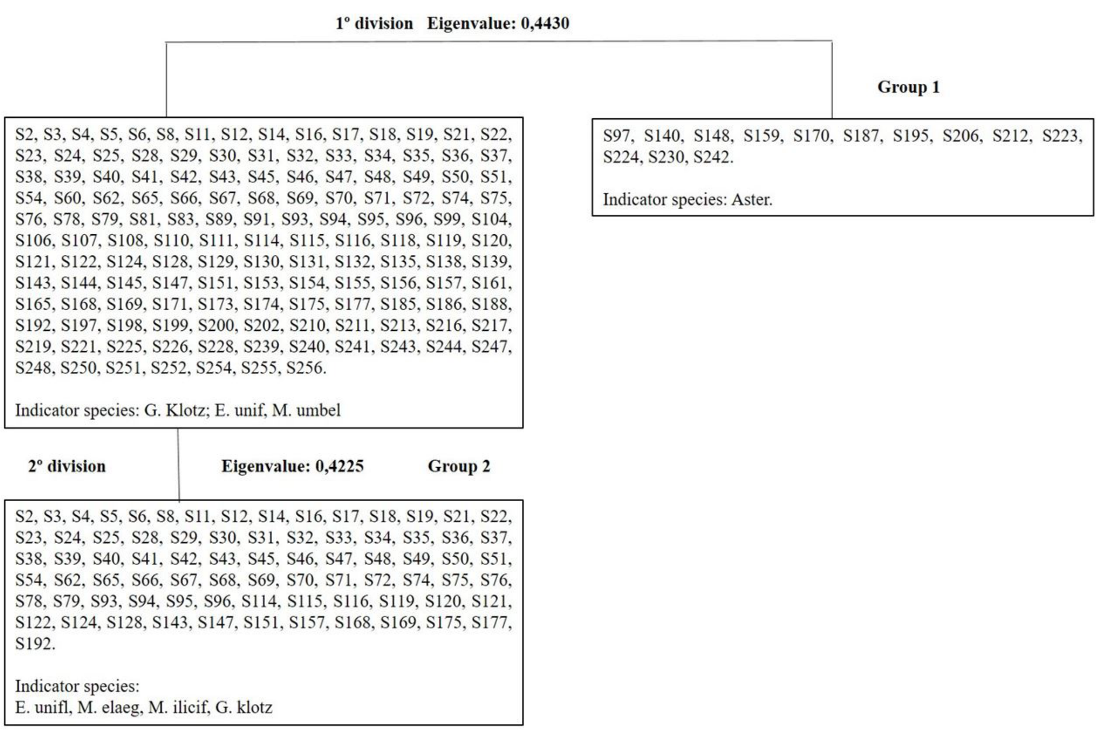

Figure 2. Grouping Analysis (Twinspan) of arboreal and shrub species in the regeneration stage of the Seasonal Forest.

plots. *G. klotzschiana* presented an AF of 42.19%, *E. uniflora* of 43.36% and *M. umbellata* of 32.81%.

The character of these species can be explained by their ecological features, which show that they prefer riparian environments, in addition to being adapted to water saturation in prolonged periods (Giehl et al., 2007). According to Araujo et al. (2004) and Budke et al. (2004), *G. klotzschiana* occurs naturally in forests along rivers and tends to form clusters. *E. uniflora* has a preference for moist soils and *M. umbellata* adapts to a wide range of edaphic conditions.

Abbreviations: (G. klotz = *Gymnanthes klotzschiana*, E.unifl = *Eugenia uniflora*, M.umbel = *Myrsine umbellata*, N.megap = *Nectandra megapotamica,* A.edulis =*Allophylus edulis*, Aster = Asteraceae, M.ilicif = *Maytenus ilicifolia*, M.elaeg = *Matayba elaeagnoides,* C.silves = *Casearia sylvestris,* P.myrti = *Prunus myrtifolia*), P.aduncun =*Piper aduncum*.

The second division generated an eigenvalue of 0.4225and divided the plots into two groups. The left group formed Group 2. In this group, the indicator species are *G. klotzschiana*, *Matayba elaeagnoides*, *Maytenus ilicifolia* and *E. uniflora*, and the preferred species are *G. klotzschiana*, *M. elaeagnoides*, *Casearia sylvestris*, *M. ilicifolia*, *Prunus myrtifolia*, *Piper aduncum and E. uniflora*. Group 3 indicator species show developmental characteristics related to the presence of moist soils (*M. elaeagnoides* and *E. uniflora*) or occurrence in alluvial plains (*G. klotzschiana* and *M. ilicifolia*) (Carvalho, 2008).

3.4. Principal component analysis

The ACP determined three main components, representing a cumulative variance percentage of 63.69% up to the third axis, distributed in 36.62%, 14.89% and 12.18%, for the first, second and third axes, respectively. The relatively low variance values work as an indicator of the environment heterogeneity, typical of riparian vegetation, which is considered one of the most heterogeneous environments in terms of forest typology (Rodrigues & Nave, 2009), and express the discontinuity of vegetation in the area.

As in the hierarchical grouping analysis, the ACP highlighted the behavior of *L. lucidum*, which presented the highest eigenvalue in the first axis (13.701), widely distancing from the other species forming a separate group, and separated from those by axis II (Figure 3).

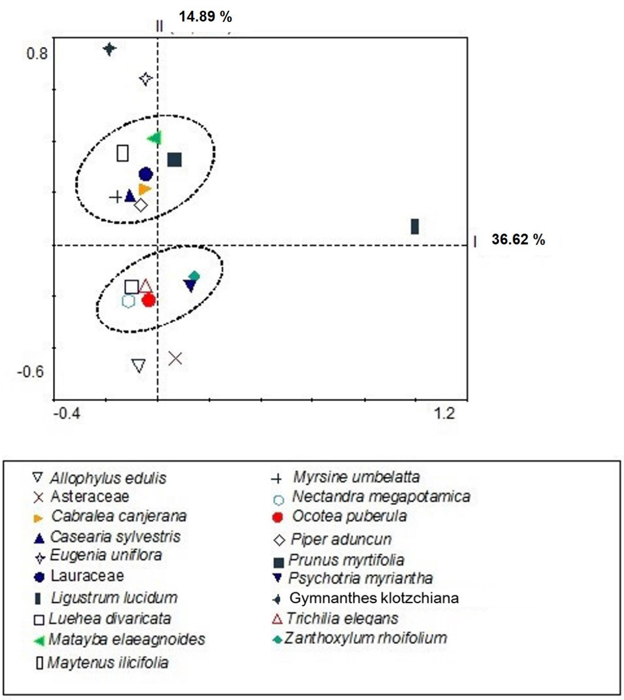

Figure 3. Principal Components Analysis Diagram of forest species with more than ten individuals in the regeneration stage of the Seasonal Forest.

The majority of the species presented higher eigenvalues in the second axis, with L. divarticata, Asteraceae, *N. megapotamica*, *A. edulis*, *P. aduncum* and *P. myriantha* forming a group separated by axis I, presenting *A. edulis* with an eigenvalue of 11.983. The third axis highlighted the behavior of *G. klotzschiana* and *E. uniflora* with eigenvalues of 8.483 and 7.783, respectively.

The appropriate restoration strategy for each area depends on the level of degradation and the desired rate of recovery (Aide et al., 2000). Natural regeneration was confirmed as an efficient strategy for the study site, considering the existence of propagule sources in the environment and the level of local resilience that offers good environmental conditions for the development of the species and expression of diversity.

However, the strong presence of invasive species in regeneration, especially *L. lucidum*, is a worrying factor and should be prioritized in control strategies, mainly because it is a conservation unit. When performed in the initial stages of invasion, the control presents lower costs and greater operational ease, since it does not require the future slaughter of adult individuals.

4. CONCLUSION

1. *Ligustrum lucidum* was the main biological invader in the regeneration phase, and presents a real risk for the maintenance of native species in the area due to the obtained indices;
2. *Gymnanthes klotzschiana, Eugenia uniflora* and *Myrsine umbellata* are the main indicators of the riparian environment in the study area, and may be recommended for restoration actions in the region;
4. *Allophylus edulis* plays an important role in the regeneration of the area due to its abundance and the potential for avifauna attraction, which also makes it a facilitator species in the restoration process;
5. Within the class range used, this analysis efficiently highlighted the biological invasion problem, and demonstrated the importance of regeneration studies and not only the arboreal stratum for forest recovery and conservation projects, but especially when dealing with conservation units.

ACKNOWLEDGEMENTS

The authors thank the National Council of Scientific and Technological Development (*CNPq*) and the State Environment Secretary of Rio Grande do Sul state (*SEMA*).

SUBMISSION STATUS

Received: 31 jul., 2017
Accepted: 14 feb., 2018

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FINANCIAL SUPPORT

Conselho Nacional de Desenvolvimento Científico e Tecnológico.

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